UNIVERSIDADE FEDERAL DE MINAS GERAIS
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SOCIAL DYSFUNCTION AND DEPRESSIVE-LIKE BEHAVIOUR IN AN ANIMAL MODEL OF EARLY PARKINSON'S DISEASE

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Dissertação apresentada ao Programa de Pós-graduação em Neurociências da Universidade Federal de Minas Gerais, como requisito parcial à obtenção do título de Mestre em Neurociências

Orientador: Cleiton Lopes Aguiar

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FOLHA DE APROVAÇÃO

DISFUNÇÃO SOCIAL E COMPORTAMENTO DEPRESSIVO EM UM MODELO ANIMAL DE DOENÇA DE PARKINSON EM ESTÁGIO INICIAL

BEATRIZ LAGE ARAUJO SCHWEIZER

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RESUMO

Os sintomas depressivos, que podem incluir anedonia, são alterações não-motoras comuns na doença de Parkinson (DP). A disfunção do comportamento social também é observada em pacientes com DP, de forma independente ou em associação com depressão ou outros distúrbios neuropsiquiátricos. Essas alterações não-motoras podem estar presentes juntamente ou mesmo antes do início dos sintomas motores clássicos da doença. O tratamento dos sintomas não-motores, especialmente nas fases iniciais da doença, pode ter um impacto positivo na qualidade de vida do paciente e no prognóstico da doença. No entanto, pouco se sabe sobre os seus mecanismos subjacentes e a sua associação com outros sintomas, resultando na falta de intervenções eficazes. A perda de neurônios dopaminérgicos na substância negra pars compacta (SNc) e seus terminais na região dorsal do estriado é uma alteração característica da DP e pode ser suficiente para induzir sintomas não-motores nos estágios iniciais da doença. Para testar esta hipótese, ratos com lesão bilateral parcial no corpo estriado dorsolateral foram avaliados quanto a comportamentos associados à anedonia e disfunção social. A lesão foi induzida por infusão de 6-hidroxidopamina (6-OHDA), seguindo um método previamente descrito para geração de modelo de DP precoce. Os animais foram submetidos ao teste de preferência à sacarose (na primeira e terceira semana pós-lesão) e ao teste de interação social (na terceira semana pós-lesão). A avaliação da função motora também foi realizada (na terceira e quarta semana pós-lesão) por meio do teste da pegada e do campo aberto. Os déficits hedônicos só foram aparentes na primeira semana após a lesão, mas não na terceira. O grupo 6-OHDA apresentou uma redução significativa no comportamento social. Não foram observadas deficiências motoras grosseiras na locomoção ou na marcha que pudessem afetar os resultados em nenhum dos dois momentos investigados. As mudanças no comportamento social podem refletir um comprometimento do comportamento motivado, dependente dos circuitos frontoestriatais, ou uma disfunção em outras regiões associadas tanto aos comportamentos sociais quanto aos sintomas depressivos, que podem ser prejudicados como resultado de uma disfunção no sistema nigroestriatal (ex.: sistema límbico). No geral, os resultados sugerem que o comprometimento da via nigroestriatal é suficiente para induzir mudanças no comportamento social, o que poderia refletir o comportamento de retraimento social observado em pacientes com DP. Estudos futuros devem investigar se esse modelo também apresenta alterações comportamentais associadas a outros sintomas não motores comumente observados em pacientes com DP, como alterações no ciclo sono/vigília e comprometimento cognitivo nas funções executivas.

Palavras-chave: doença de Parkinson; 6-OHDA; estriado dorsolateral; sintomas não motores; depressão; função social; anedonia.

ABSTRACT

Depressive symptoms, which can include anhedonia, are common nonmotor changes in Parkinson's disease (PD). Social dysfunction is also observed in PD patients, either independently or in association with depression or other neuropsychiatric disorders. These nonmotor changes may be present at the time of, or even before, the onset of the cardinal motor symptoms. Treating nonmotor symptoms, particularly in the very early stages of the disease, can have a positive impact on the patient's quality of life and the disease's prognosis. However, little is known about their underlying mechanisms and their association with other symptoms, resulting in a lack of effective interventions. The loss of dopaminergic neurons in the substantia nigra pars compacta (SNc) and their terminals within the dorsal striatal region is a key feature of PD and may be sufficient to induce nonmotor symptoms early in the disease. To test this hypothesis, rats with a partial bilateral lesion within the dorsolateral striatum were assessed for behaviours associated with anhedonia and social dysfunction. The lesion was induced by infusion of 6-hydroxydopamine (6-OHDA), following a previously described method for generating a model of early PD. The animals underwent the sucrose preference test (in the first- and third-week post-lesion) and the social interaction test (in the third week post-lesion). Assessment of motor function was also conducted (in the third- and fourth-week post-lesion) using the footprint and open field test. Hedonic deficits were only apparent in the first week following the lesion, but not in the third. The 6-OHDA group showed a significant reduction in social behaviour. No gross motor impairments in locomotion or gait that could confound the results were observed at either of the two time-points investigated. The changes in social behaviour may reflect an impairment of motivated behaviour, dependent on frontal-striatal circuits, or dysfunction in other regions associated with both social behaviours and depressive symptoms, that might be impaired as a result of dysfunction in the nigrostriatal system (i.e. limbic system). Overall, the results suggest that the early impairment in the nigrostriatal pathway is sufficient to induce changes in social behaviour, which could reflect the behaviour of social withdrawal observed in PD patients. Future studies should investigate whether this model also exhibits behavioural changes associated with other non-motor symptoms commonly observed in PD patients, such as changes in the sleep/wake cycle and cognitive impairment in executive functions.

Keywords: Parkinson's disease; 6-OHDA; dorsolateral striatum; non-motor symptoms; depression; social function; anhedonia.

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LIST OF ABBREVIATIONS AND ACRONYMS

BG Basal ganglia

BDNF Brain derived neurotrophic factor

DA Dopamine

DAT Dopamine transporter

GPi Globus pallidus internal

GPe Globus pallidus external

MFB Medial forebrain bundle

MSN Medium spiny neurons

NA Noradrenaline

NAc Nucleus accumbens

OFT Open field test

PD Parkinson's disease

RDB REM sleep behaviour disorder

SEM Standard Error of Mean

SD Standard deviation

SI Social interaction

SNc Substantia nigra pars compacta

SPT Sucrose preference test

ToM Theory of Mind

VTA Ventral tegmental area

5-HT Serotonin

6-OHDA 6-hydroxydopamine

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1 LITERATURE REVIEW

1.1 Parkinson’s Disease

1.1.1 Epidemiology

Parkinson’s disease (PD) stands as the second most common neurodegenerative disorder after Alzheimer’s disease. It is estimated that in 2019 there were 8.5 million individuals living with PD world-wide, representing a 155.5% surge compared to 1990 (OU et al., 2021). In the United States alone, nearly 90,000 new cases are reported annually (WILLIS et al., 2022). PD tends to affect males more frequently than females and predominantly manifests in individuals over the age of 60. The increase in PD cases is in alignment with the growth of the elderly population, with the most substantial rise occurring among those aged over 80 (OU et al., 2021).

Indeed, ageing is a primary risk factor for many neurodegenerative disorders, including PD. Alongside the ageing process, genetics, and environmental factors, like exposure to pesticides, oils, and metals, have also been identified as significant risk markers for PD. Conversely, certain lifestyle factors have been associated with protective effects, including the consumption of coffee, smoking, and engagement in physical activity (BELVISI et al., 2020).

1.1.2 Key pathophysiological features

Over two centuries have passed since James Parkinson's initial account of the condition that would later be termed Parkinson's disease (PD). Since that time, key discoveries have unveiled the current known features of the disease’s pathology, namely the aggregation of alpha-synuclein into fibrillar masses known as Lewy bodies and the progressive degeneration of dopaminergic neurons within the substantia nigra pars compacta (SNc) (MCDONALD et al., 2018; POEWE et al., 2017).

The SNc comprises tyrosine-expressing neurons that send dopaminergic projection to the striatum (mainly its dorsal region) via the medial forebrain bundle (MFB), constituting the nigrostriatal system (LANCIEGO; LUQUIN; OBESO, 2012). Consequently, the loss of dopaminergic neurons results in the depletion of their terminals within the striatal region, a phenomenon that has recently gained increased attention in PD diagnosis (TAGLIAFERRO; BURKE, 2016). In this context, dopamine transporter (DaT) imaging has become a widely adopted technique to detect changes

in DaT expression, which suggests striatal presynaptic dysfunction in PD patients. This imaging technique has proven to be a useful tool not only for the differential diagnosis of PD but also for assessing the disease's progression (HENG et al., 2023; IKEDA et al., 2019).

It is estimated that striatal DaT activity loss is around 35–45% at the time of diagnosis and gradually increases to around 62% after more than 5 years (HENG et al., 2023). Notably, recent observations pointing to the early and predominant involvement of axons of the dopaminergic system in PD have led to a current hypothesis suggesting that retrograde axonal degeneration may precede the loss of SNc neurons (TAGLIAFERRO; BURKE, 2016). Substantiating this hypothesis is a study by Caminiti et al. (2017), where they conducted a regional analysis of DaT in the structures of the nigrostriatal and mesolimbic systems, along with assessing dopamine network connectivity in early-stage PD **(Figure 1)**. Their findings revealed a more pronounced neurodegeneration within the afferent axonal projections within the dorsal putamen region of the striatum, prompting the authors to propose that the presynaptic terminals of the nigrostriatal dopaminergic system are the principal site for vulnerability in PD (CAMINITI et al., 2017).

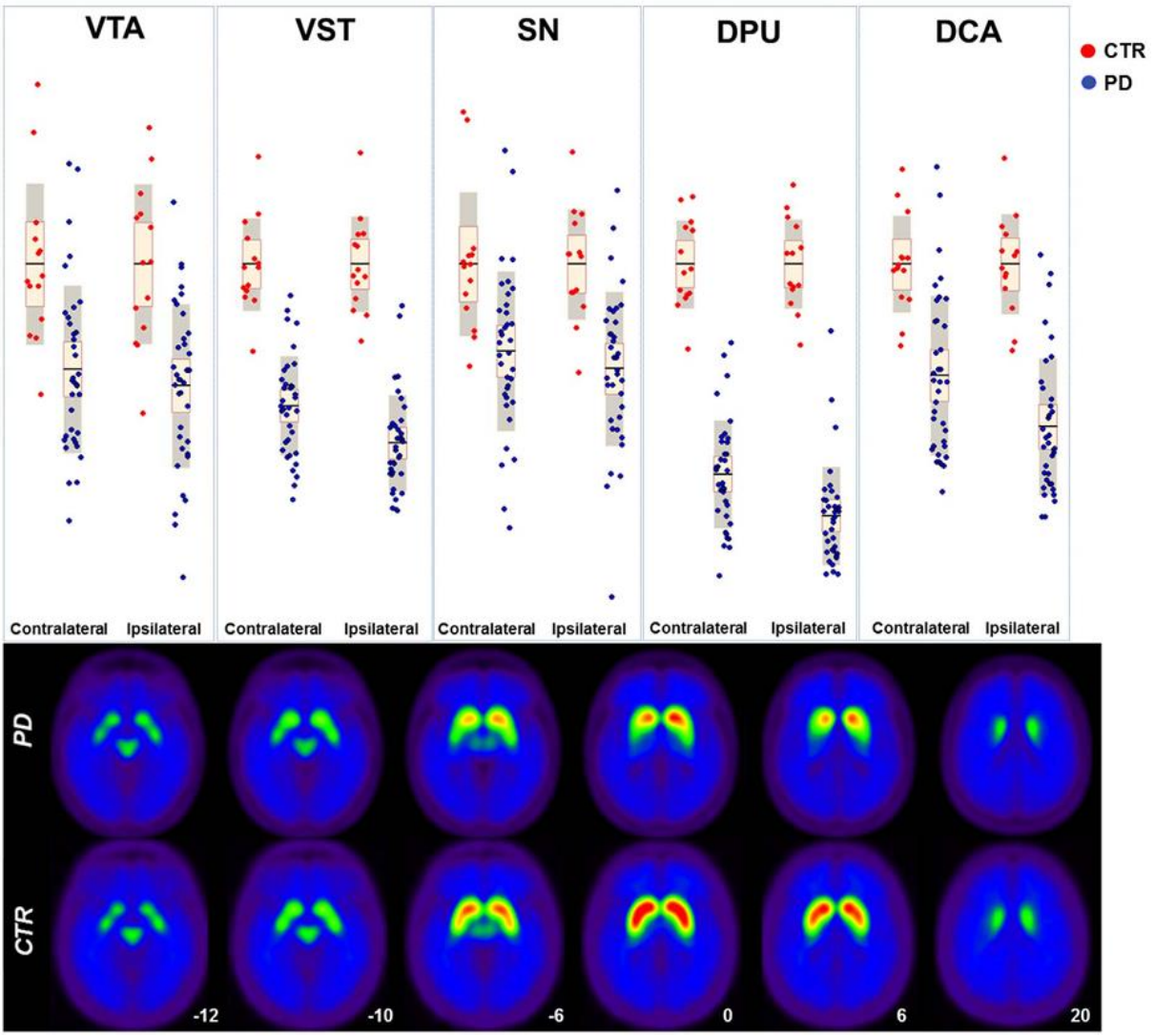


Figure 1. Analysis of DaT expression in PD and healthy control. a box-scatter plot representing standardised uptake value ratio (SUVr; top-panel) and images of averaged [^{11}C]FeCIT PET (bottom panel) used to measure presynaptic DAT activity in the nigrostriatal and mesolimbic systems in PD and CTR. **Abbreviations:** PD: Parkinson's disease patients; CTR: healthy control subjects; SN: substantia nigra; DPU: dorsal putamen; DCA: dorsal caudate; VTA: ventral tegmental area; VST: ventral striatum (modified from CAMINITI et al., 2017)

PD pathology is not limited to the nigrostriatal structures, however. Alpha-synuclein appears to diffuse in a prion-like fashion and to follow a temporal progression. Deposits of this protein have been detected in numerous cortical and subcortical non-dopaminergic structures and cell groups, extending beyond the boundaries of the central nervous system (VISANJI et al., 2013). Furthermore, akin to other neurodegenerative conditions, PD involves multiple molecular mechanisms that can both result from, and contribute to, alpha-synuclein pathology; ultimately culminating in cell death **(Figure 2)**. These mechanisms encompass the impairment of protein degradation systems, mitochondrial dysfunction, oxidative stress, and

neuroinflammation (POEWE et al., 2017). In essence, PD presents as a multifaceted, multisystem disorder.

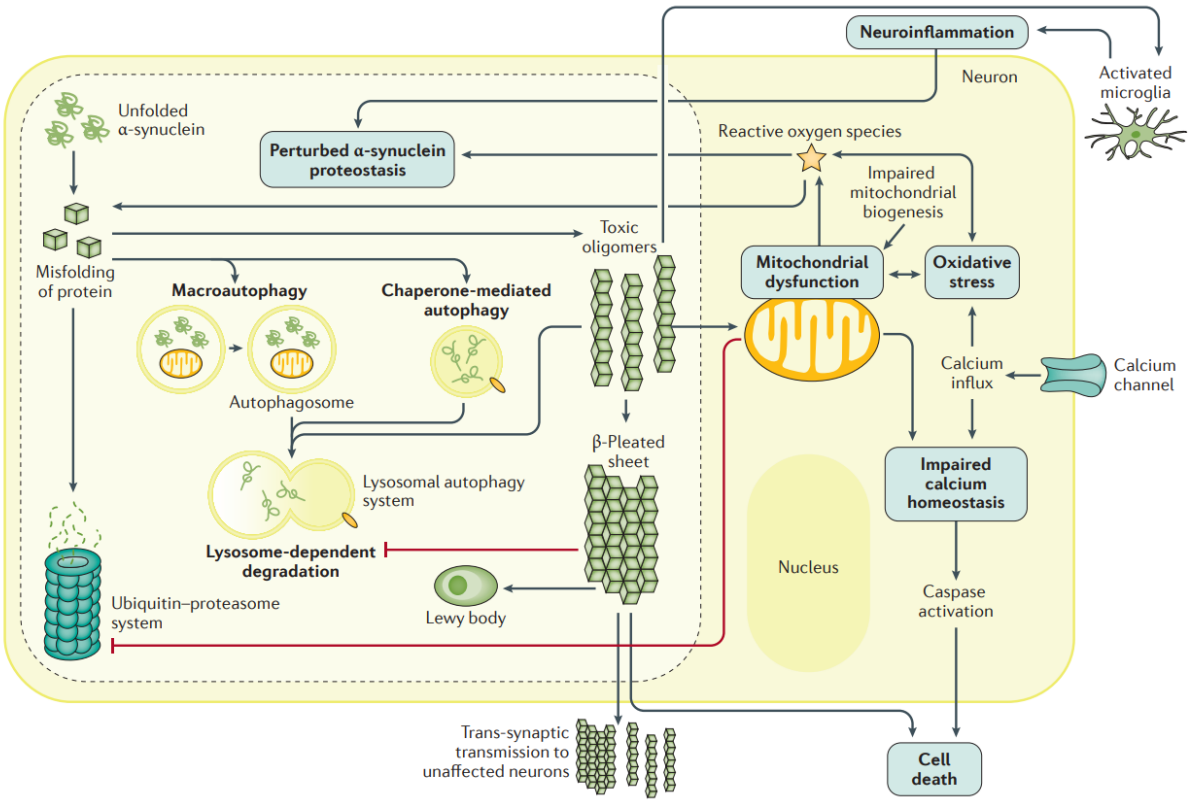


Figure 2. Schematic representation of the molecular mechanisms involved in PD. Different major molecular pathways that are believed to interact and to play a role in the pathogenesis of the disease, contributing to the process of neurodegeneration (adapted from POEWE et al., 2017)

1.1.3 Clinical phenotypes and progression

PD has long been associated with the presence of motor symptoms; a syndrome referred to as parkinsonism. Clinical diagnosis relies on the presence of bradykinesia (slowness of movement) in combination with either rest tremor or rigidity (increased muscle tone). Postural instability and gait difficulties, also characteristic of parkinsonism, may also develop during the course of the disease (POSTUMA et al., 2015). However, PD is not limited to motor symptoms, as many non-motor symptoms can be prevalent and even dominate the clinical presentation. Interestingly, a few non-motor manifestations were already described by James Parkinson in 1817, though they remained underrecognized for many years. Currently, there is an increasing recognition of these symptoms, particularly regarding their impact on quality of life and the increased focus on the prodromal stage of the disease, which is a crucial period for early diagnosis and intervention (HEINZEL et al., 2019; MCDONALD et al., 2018).

Common non-motor symptoms encompass cognitive impairment, neuropsychiatric disorders, disturbances in the sleep-wake cycle, pain, anosmia, and other autonomic dysfunctions (HUSTAD; AASLY, 2020; ZHOU et al., 2023). These symptoms can emerge at various stages in the disease course, whether after, concomitantly, or even decades before the onset of motor symptoms (in the prodromal phase) (HUSTAD; AASLY, 2020; POEWE et al., 2017) **(Figure 3)**. However, the clinical phenotype of PD varies greatly among patients, even in the prodromal phase. Certain symptoms may manifest in some individuals but not in others, and they can occur at different stages of the disease. Genetic predisposition, environmental factors, presence of comorbidities, direction of α-synuclein propagation, level of cognitive reserve and variability in the individual's degenerative thresholds are all factors that can influence this heterogeneity (BERG et al., 2021).

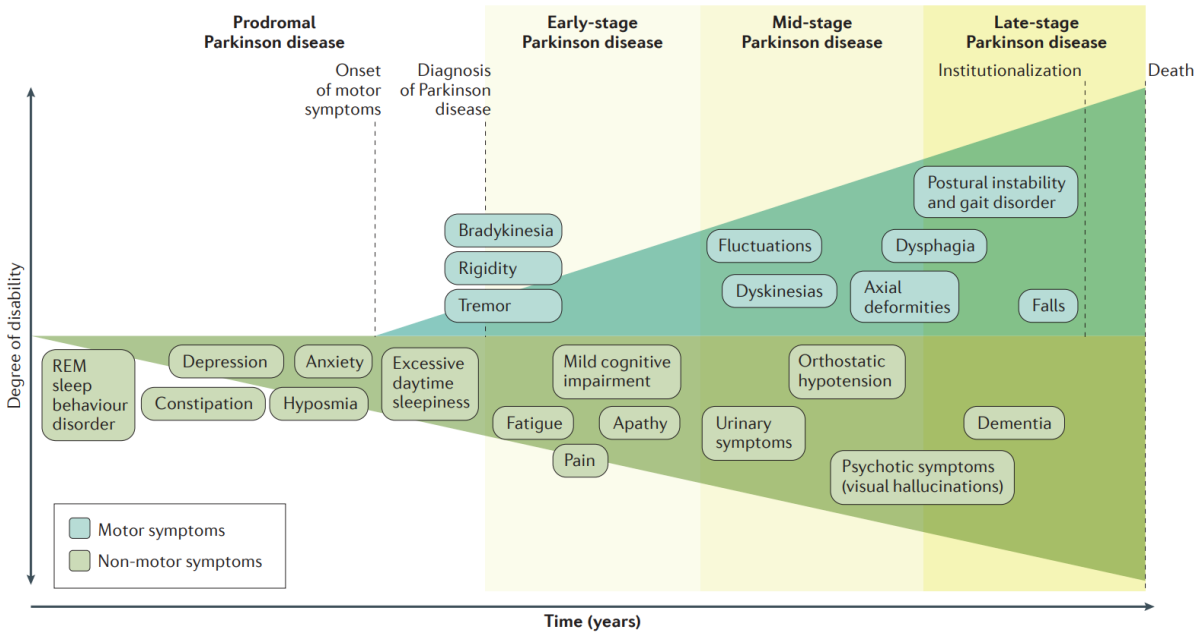


Figure 3. Clinical progression of Parkison's disease. The image depicts the motor and nonmotor symptoms commonly observed in different stages of PD. The clinical diagnosis is made with the onset of the classical motor symptoms. The incidence of nonmotor symptoms and their temporal presentation varies greatly between patients. A few can occur years before the onset of motor symptoms (prodromal phase). Overall, the symptoms tend to become more prevalent and increase in severity with the disease's progression. Motor complications may also appear in the mid-stages as a consequence of treatment with L-DOPA (i.e. fluctuations and dyskinesias). Adapted from POEWE et al., 2017.

Despite the complexity of PD, some distinct patterns have emerged, and efforts have been made to categorise potential disease subtypes through multi-domain phenotyping, based on the interplay of various factors, including genetics, age of onset, predominant motor symptoms, incidence and extent of nonmotor alterations, and the rate of disease progression. One such subtype, associated with a poor prognosis, is

referred to as the 'Diffuse Malignant Subtype'. It typically manifests in GBA-mutant patients, with early presence of the rapid eye movement (REM) sleep behaviour disorder (RBD) and is linked to late-onset, postural instability and gait-dominant phenotype, a high non-motor symptom burden, and rapid disease progression. In contrast, the 'Motor Benign Subtype' tends to occur in LRRK2-mutant, RBD-negative patients and is associated with young age of onset, a tremor-predominant phenotype, low non-motor symptom burden, and a slower disease progression (BERG et al., 2021) **(Figure 4)**.

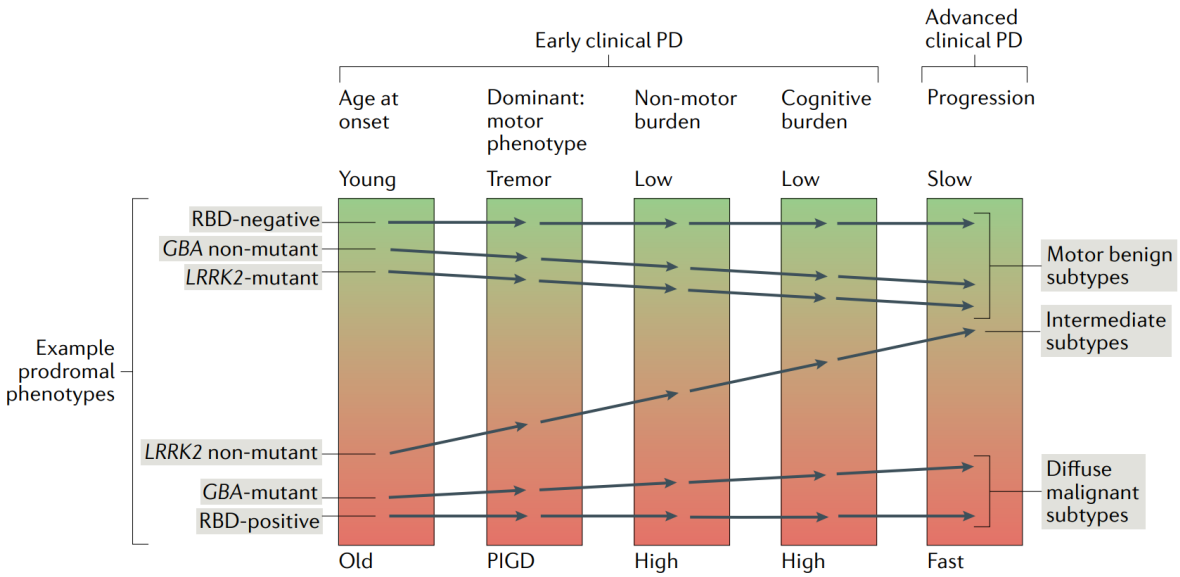


Figure 4. Prodromal subtypes of PD. Schematic representation of the clinical markers and trajectory of the different prodromal subtypes with their associated clinical phenotypes. **Abbreviations**: PIGD: postural instability and gait disturbance; RBD: REM sleep behaviour disorder (adapted from BERG et al., 2021)

Regarding the different progression trajectories of α-synuclein spread, a recent hypothesis posits two possible contrasting routes: the first involves the emergence of α-synuclein pathology in the limbic system or entrance via an olfactory route, followed by its descent through the brainstem and into the periphery (referred to as "brain-first"). In the second, α-synuclein aggregation begins in the periphery, in the peripheral autonomic nervous system, and then spreads to the spinal cord and brainstem (termed "body-first") (HORSAGER et al., 2020). These two routes provide a framework that could help explain both the temporal and phenotype heterogeneity of PD **(Figure 5)**. For instance, REM sleep behaviour disorder (RBD), often associated with brainstem dysfunction, can manifest in both subtypes. However, in the "body-first" subtype, RBD is more likely to occur and may manifest very early, in the prodromal phase. Conversely, in the "brain-first" subtype, it is less common and tends to emerge after

the onset of motor symptoms (parkinsonism), which often coincide with the involvement of the SNc (BERG et al., 2021; HORSAGER et al., 2020). This hypothesis finds support in a recent study that employed machine-learning techniques and data-driven methods to identify PD subtypes based on distinct trajectories of clinical and neurodegenerative events (ZHOU et al., 2023).

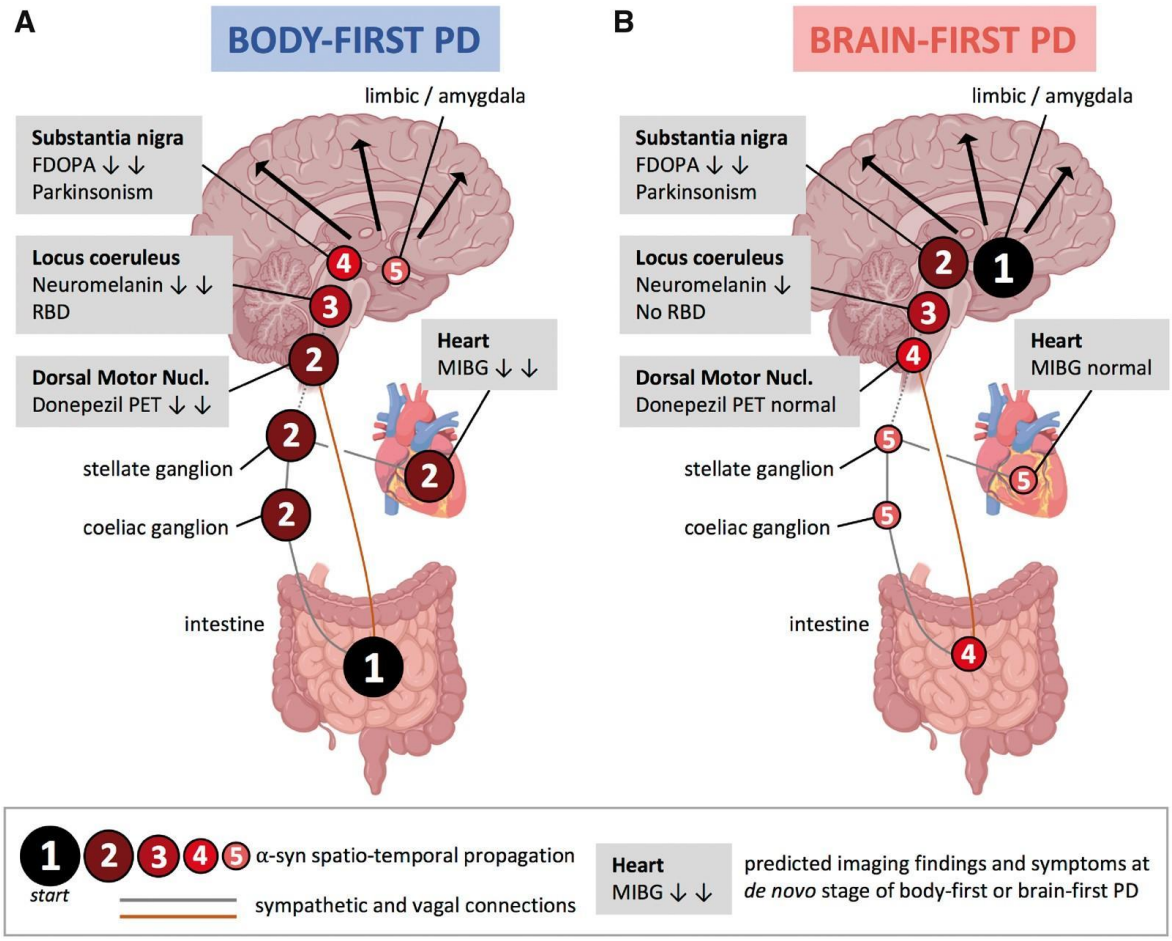


Figure 5. Body-first and brain-first subtypes of PD. Two contrasting spreading routes of the hypothetical (A) body-first and (B) brain-first subtypes of Parkinson's disease (PD) and their predicted symptoms and imaging findings at the time of clinical diagnosis are summarised in the boxes. The numbered circles indicate the starting point and subsequent propagation of pathology in the two subtypes (adapted from HORSAGER et al., 2020)

1.1.4 The striatum and basal ganglia circuitry

The striatum is the largest structure of a cluster of nuclei called the basal ganglia (BG). In humans, it is generally divided into the dorsal striatum (which includes the caudate nucleus and putamen) and ventral striatum (corresponding to the nucleus accumbens; NAc). It serves as the main input region of BG. The striatum receives glutamatergic afferents from the thalamus and the entire cerebral cortex, as well as dopaminergic innervation from the SNc (in the dorsal striatum) and the ventral tegmental area (VTA; primarily in the ventral striatum) (LANCIEGO; LUQUIN; OBESO, 2012).

In the classic model of the BG circuit, cortical inputs into the striatum project through GABAergic efferent from the striatum's medium spiny neurons (MSN) onto either the globus pallidus internal (GPi) or the external (GPe) (known as 'direct pathway' and 'Indirect pathway', respectively) and then to thalamic nuclei and back to the cortex. These striatal circuits are modulated by dopaminergic projections from the SNc via contrasting actions. MSNs projecting to the GPi in the direct pathway express the D1-family of dopamine receptors, which are excited by dopamine; while MSNs projecting to the GPe in the indirect pathway express the D2-family, which are inhibited by dopamine (ALBIN; YOUNG; PENNEY, 1989; LANCIEGO; LUQUIN; OBESO, 2012).

In PD, dysfunction in the nigrostriatal system results in an imbalance in favour of the indirect pathway, which is associated with the common akinetic-rigid syndrome of PD (OBESO et al., 2014) **(Figure 6)**. Indeed, the basal ganglia circuitry was initially thought to be primarily involved in motor function. However, growing evidence suggests its role extends to other domains, including cognitive functions, psychiatric symptoms, and sleep regulation (HASEGAWA et al., 2020; MACPHERSON; HIKIDA, 2019). Cortico-striatal connections are segregated in different regions of the striatum, forming various functionally distinct parallel cortico-striatal 'loops'. These loops can be broadly categorised into motor, associative, and limbic/emotional domains, based on their primary cortical projections and possible associated functions **(Figure 7)** (MACPHERSON; HIKIDA, 2019).

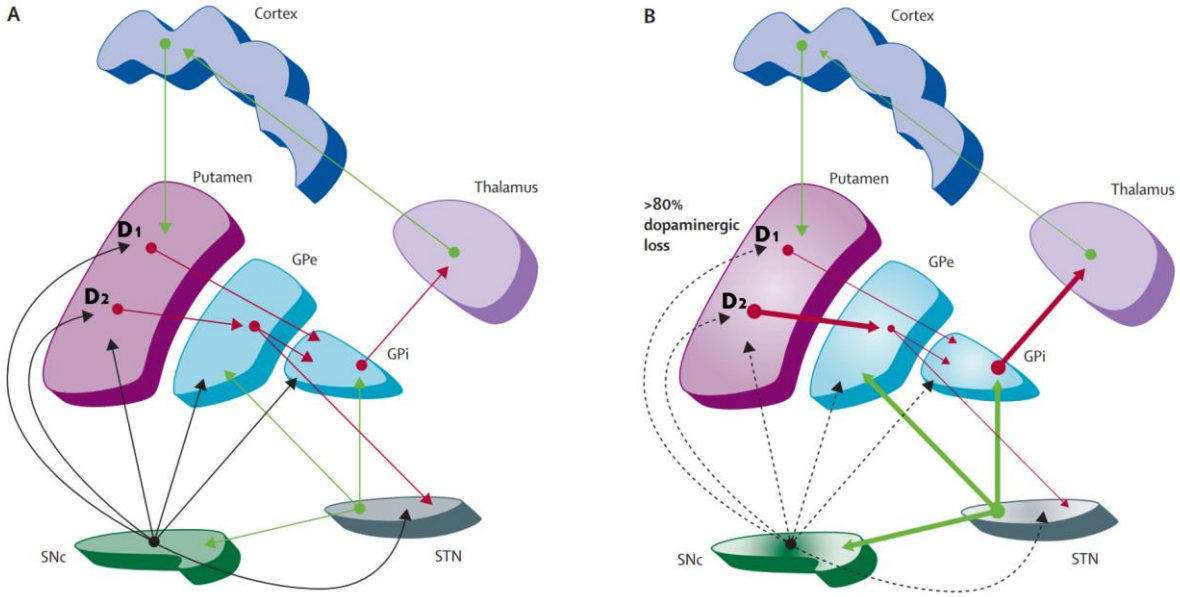


Figure 6. Classic model of the basal ganglia motor circuit in normal and PD patients. The basal ganglia circuitry (A) in the normal state and (B) in the parkinsonian state. Red arrows indicate inhibitory GABA-ergic projections, green arrows represent excitatory glutamatergic projections, and black arrows indicate dopaminergic innervation. The thickness of the arrows represents the strength of transmission (modified from OBESO et al., 2014)

Additionally, there is evidence of cross-talk between these specialised striatal circuits (HABER, 2014, 2016). For instance, zones of converging inputs from all the prefrontal regions have been estimated in some areas within the striatum of non-human primates, suggesting its role as a possible 'information hub' (AVERBECK et al., 2014) **(Figure 8)**. Connectivity across different striatal subdivisions has also been suggested to play a role in integrating information across the parallel loops. Importantly, impairments in such connectivity have been reported following dopaminergic deficits, further suggesting a crucial role of the SNc in modulating striatal function (BELL et al., 2015).

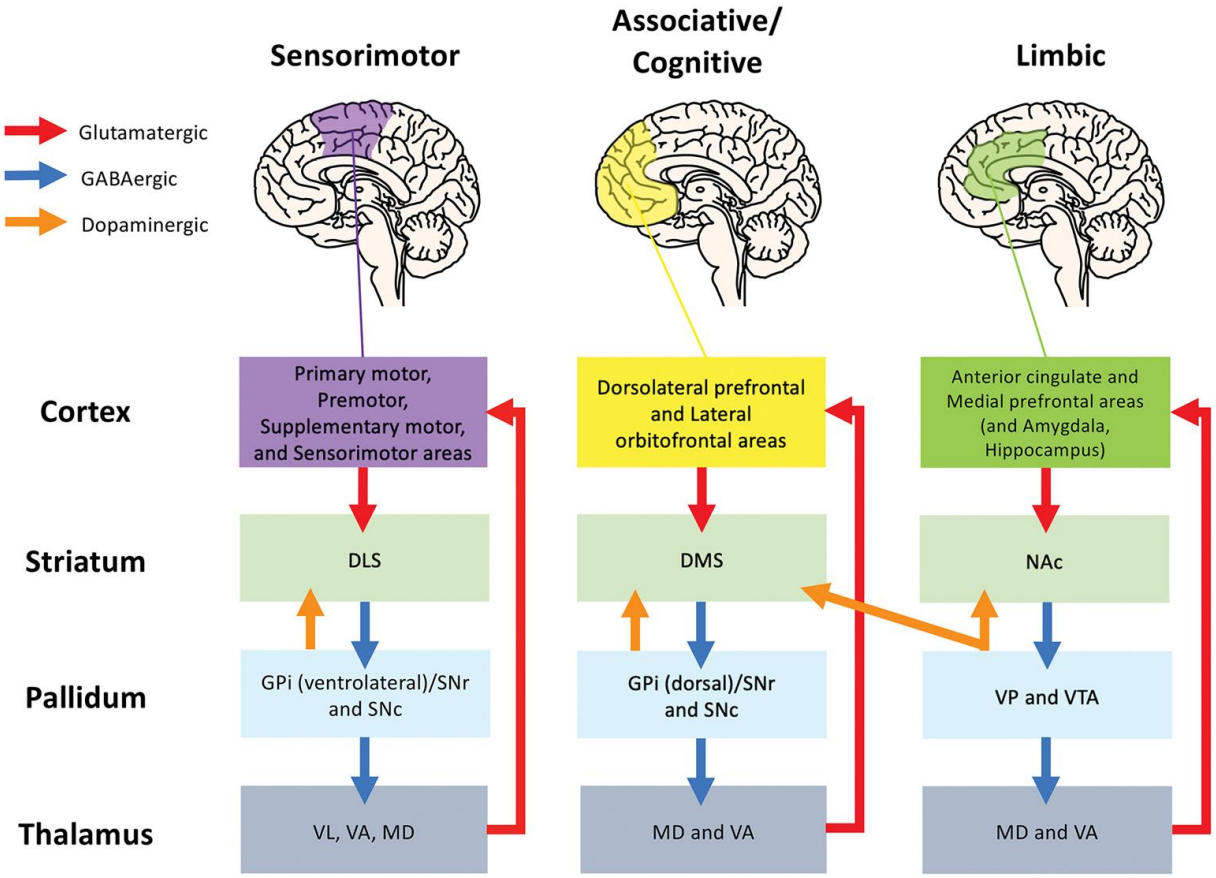


Figure 7. Basal ganglia neurocircuits. Parallel cortico-striatal 'loops' distinguished according to their associated functions. **Abbreviations**: DLS: dorsolateral striatum; DMS: dorsomedial striatum; GPi: globus pallidus internal section; MD: medial dorsal thalamus; NAc: nucleus accumbens; SNc: substantia nigra pars compacta; SNr: substantia nigra pars reticulata; VA: ventral anterior thalamus; VL: ventrolateral thalamus; VP: ventral validum; VTA: ventral tegmental area (adapted from MACPHERSON & HIKIDA, 2019)

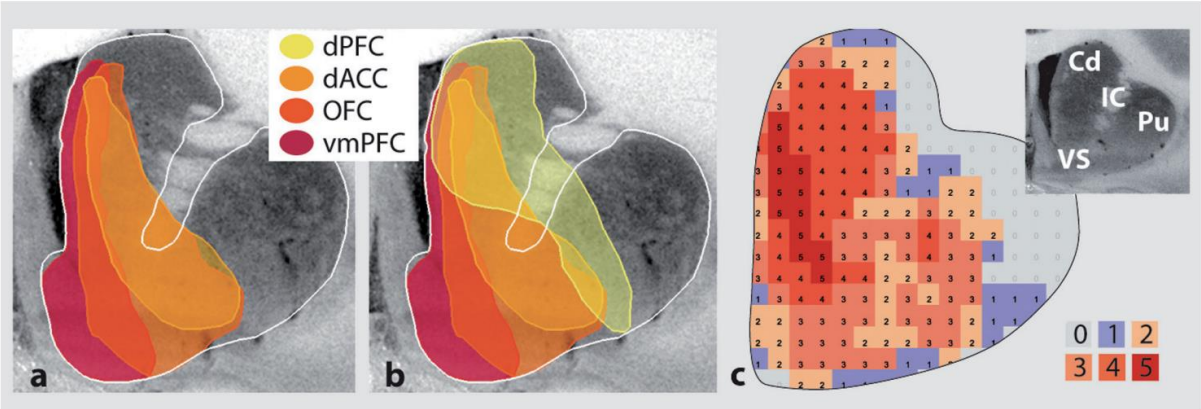


Figure 8. Estimations of zones of convergence of cortical projections within the striatum in a non-human primate. Convergence between projections (a) from vmPFC, OFC, and dACC; and (b) from vmPFC, OFC, dACC, and dPFC. (c) Map of areas of convergence. Colour in each section indicates voxels that receive projections from 0 to 5 prefrontal cortical regions (ie, vmPFC, OFC, dACC, dPFC, vlPFC). **Abbreviations**: Cd: caudate; dACC: dorsal anterior cingulate cortex; dPFC: dorsal prefrontal cortex; IC: internal capsule; OFC: orbitofrontal cortex; Pu: putamen; vmPFC: ventromedial prefrontal cortex; VS: ventral striatum (adapted from HABER, 2016)

1.2 Depression in Parkinson’s Disease

1.2.1 Epidemiology and clinical manifestations

Depression is a significant non-motor symptom and the most common neuropsychiatric disturbance in PD, with a profound negative impact on the individual's quality of life (SU et al., 2021). Its clinical presentation typically involves low mood, a marked decrease in interest or pleasure (anhedonia), and resultant impairment in various aspects of life functioning, such as sociability and the ability to cope with the disabilities associated with PD (AMERICAN PSYCHIATRIC ASSOCIATION; AMERICAN PSYCHIATRIC ASSOCIATION, 2013; MARSH, 2013).

Diagnosing depression in the context of PD is a complex task due to the overlap of many symptoms between the two conditions. Somatic symptoms of depression, such as sleep disturbances and fatigue, are common in PD, irrespective of the presence of depressive symptoms. Additionally, apathy, anxiety, and cognitive impairment can also be present independently in PD or directly associated with the depression phenotype, making it challenging to distinguish them (AARSLAND et al., 2012; AARSLAND; MARSH; SCHRAG, 2009; PRANGE et al., 2022).

Like other non-motor symptoms, depressive disorders can emerge at any point during the disease course, even before the onset of motor symptoms (HEINZEL et al., 2019; HUSTAD; AASLY, 2020; POSTUMA et al., 2015) **(see Figure 3)**. The development of depression in the early stages of PD is indicative of greater disability and a less favourable prognosis overall (AHN; SPRINGER; GIBSON, 2022; JELLINGER, 2022; PONTONE et al., 2016; SU et al., 2021). Furthermore, depressive symptoms may precede cognitive decline, and its severity are related to worse cognition (AARSLAND et al., 2021; JONES et al., 2019; MENG et al., 2023).

A recent review and meta-analysis of 129 studies reported a prevalence of depression in 38% of 38304 PD patients. In line with previous reports, depression in this study was associated with longer disease duration and severity (as measured using the Hoehn-Yahr scale stages); and more severe behavioural symptoms, including fatigue, apathy, anxiety, daytime somnolence, and cognitive decline (lower Mini-Mental State Examination; MMSE scores). Postural instability and gait difficulty (but not tremor) were also more strongly linked to depression (CONG et al., 2022).

1.2.2 Possible pathophysiological mechanisms

The underlying pathophysiology of depression in PD remains largely unknown. Depressive symptoms in major depressive disorders have been linked to various morphological and molecular biochemical changes, many of which are present in the context of PD pathology and the neurodegenerative process (DUJARDIN; SGAMBATO, 2020; JELLINGER, 2022).

Given that depression is often attributed to monoaminergic deficiency, it is likely that the dysfunction of both dopamine (DA) and non-dopaminergic (non-DA) systems in PD are involved (DUJARDIN; SGAMBATO, 2020). Neuronal loss in the SNc and widespread degeneration of dopaminergic terminals in the striatum have been strongly associated with depressive symptoms in PD patients (JELLINGER, 2022; YOO et al., 2019). Additionally, prior evidence has confirmed the efficacy of dopaminergic agonists like pramipexole in alleviating depressive symptoms in these patients (HEINZEL et al., 2019; JI et al., 2022). Selective serotonin reuptake inhibitors (SSRIs) are widely used to treat depression in the general population and are also a common treatment option for depression in PD (HEINZEL et al., 2019). Indeed, there is a suggestion that abnormal serotonergic neurotransmission plays a role in depression in the context of PD (JELLINGER, 2022; POLITIS et al., 2010)**.** However, there is some conflicting evidence regarding its involvement in early PD, with a few studies proposing that it may be more implicated in the aetiology of parkinsonian tremors rather than nonmotor changes (QAMHAWI et al., 2015)**.** Finally, progressive loss of the locus coeruleus (LC) signal is observed early in PD patients and has also been associated with the occurrence of depressive symptoms as well as with other non-motor symptoms, such as sleep disorders and cognitive impairment (JELLINGER, 2022; PAREDES-RODRIGUEZ et al., 2020).

The monoamine hypothesis has long been the prevailing explanation for depression and has served as the foundation for numerous antidepressant treatments. However, this hypothesis has limitations. For example, it cannot explain the delay in response after treatment initiation or the cases of treatment resistance (BOKU et al., 2018). Presently, there is increasing recognition that depression may involve several major systems beyond monoamines, such as neuroplasticity, neurotrophins and hippocampal neurogenesis, mitochondrial dysfunction, inflammation, and the gut-brain axis, among others (BOKU et al., 2018; FRIES et al., 2023).

While these systems have been investigated to a lesser extent in the context of depression in PD, many of them have indeed been associated with depressive symptoms. These associations encompass changes in brain-derived neurotrophic factor (BDNF) (WANG et al., 2017), impaired neurogenesis (LIM; BANG; CHOI, 2018), inflammation, stress hormones and alterations in gut microbiota. Collectively, these findings suggest depression in PD might include the combined involvement of monoamine dysregulation, immunomodulatory and neurotrophic factors **(Figure 9)**, which underscore their potential as targets for antidepressant treatments in the context of PD (AARSLAND et al., 2012; JELLINGER, 2022).

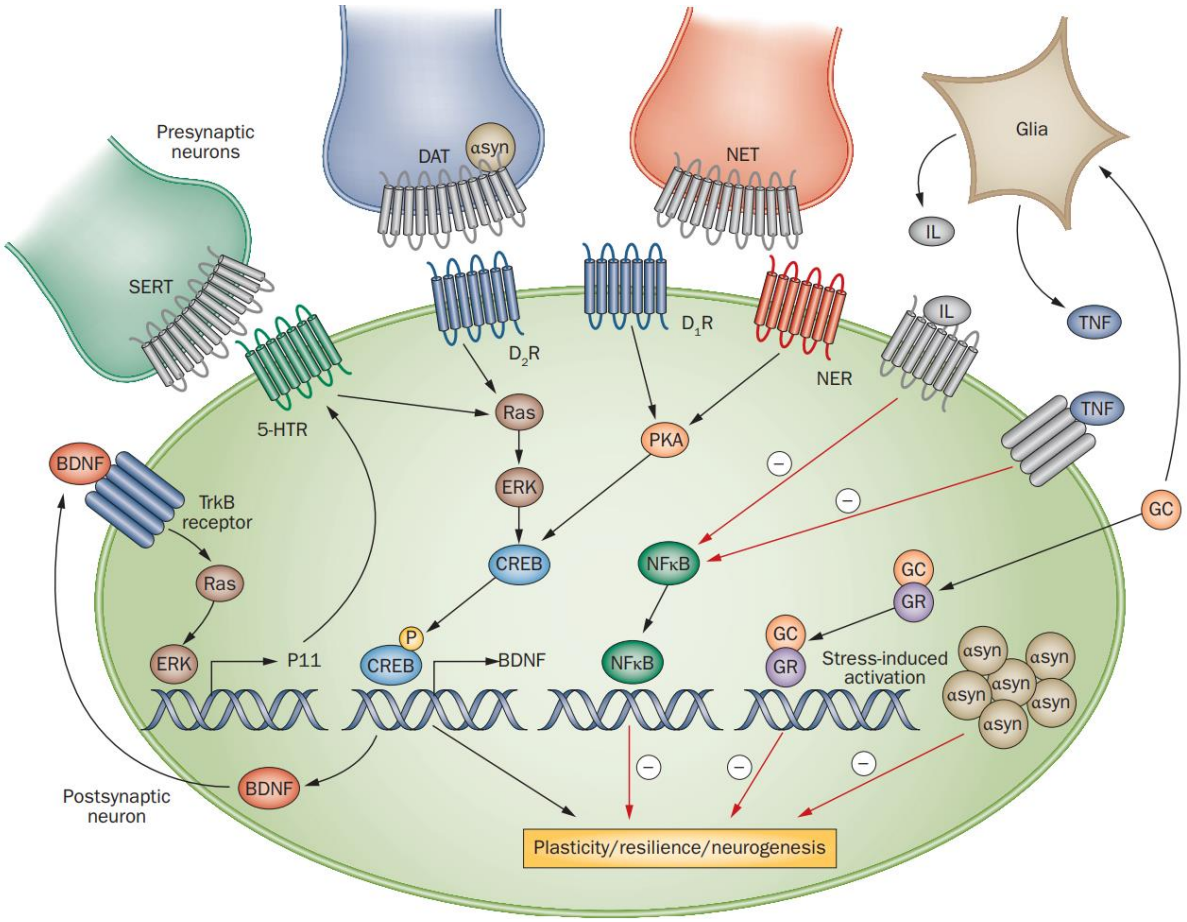


Figure 9. Molecular alterations and signalling cascades implicated in depression in PD. PD is characterised by a downregulation of monoamines. Monoamines receptors activate several signalling cascades, resulting in increased levels of BDNF. BDNF in turn, drives the expression of the monoamine receptor adaptor protein p11, which increases the efficacy of some 5-HT receptors. BDNF also supports important processes for cellular survival, plasticity and neurogenesis. Excessive amounts of glucocorticoids and inflammatory markers released from glial cells are also observed in PD. They can contribute to depressive symptoms by acting via transcription factors (i.e. glucocorticoid receptor and NFκB), exerting a negative influence on neuronal plasticity, resilience and neurogenesis. Similarly, increased α-synuclein load could increase the risk of depression by also negatively affecting monoamine transmission, cellular plasticity, and survival. **Abbreviations:** 5-HT: 5-hydroxytryptamine; 5-HTR: 5-HT

receptor; αsyn: α-synuclein; BDNF: brain-derived neurotrophic factor; CREB: cyclic AMP response element binding protein; DAT: dopamine active transporter; D1R: dopamine receptor D1; D2R, dopamine receptor D2: ERK, extracellular signal-related kinase; GC: glucocorticoid; GR: glucocorticoid receptor; IL: interleukin; NER: norepinephrine receptor; NET: norepinephrine transporter; NFκB: nuclear factor κB; PD: Parkinson disease; PKA: protein kinase A; SERT: sodium-dependent serotonin transporter; TNF: tumor necrosis factor; TrkB: BDNF/NT-3 receptor (adapted from AARSLAND et al., 2012).

On a larger scale, depression is often described as a 'disconnection syndrome' due to the observed alterations in various brain circuitry — including cortical-cortical, limbic-cortical, and cortico-striatal circuits (LIAO et al., 2013; PAUL et al., 2023) — and overall imbalanced communication among large-scale brain networks (KAISER et al., 2015). Likewise, depression in PD has been associated with dysfunction of the limbic cortico-basal ganglia circuit, linked to decreased functional connectivity between the limbic systems, cingulate cortex, striatum and thalamus; and to abnormal functional connectivity in multiple networks (DAN et al., 2017; JELLINGER, 2022). In particular, dysfunction in the salience network's cortico-striato-thalamo-cortical loop has been linked to both the occurrence and severity of depression in PD (LIU et al., 2022).

1.3 Social dysfunctions in Parkinson's Disease

1.3.1 Clinical manifestations

A decline in social engagement is frequently noted in PD and has been linked to diverse social and disease-related factors. Specifically, it can be intentional, stemming from feelings of embarrassment or stigmatisation linked to the evident disease symptoms; or it may result from a diminished desire and/or increased challenges in socialising, often connected to motor, cognitive, and psychiatric symptoms (AHN; SPRINGER; GIBSON, 2022; PRENGER et al., 2020). Depression, in particular, appears to be a significant predictor of social withdrawal and avoidance in PD, although a causal relationship is not clear. Moreover, the inverse is also true, with social avoidance often considered a predictor of depression in PD (AHN; SPRINGER; GIBSON, 2022).

In addition to its prevalent psychiatric symptoms, a range of emotional and communicative alterations, which can significantly disrupt social functioning and contribute to social withdrawal, are present in PD. Examples include difficulties in displaying emotional facial expressions and emotional speech. Moreover, impairments

in social cognition, involving difficulties in recognising both verbal and nonverbal emotional cues of others and Theory of Mind (ToM), which involves contemplating one's own or others' mental states, have been noted in the early stages of the disease, even in the absence of other cognitive deficits. Notably, only the patients' performance on the Frontal Assessment Battery has shown a connection to such social cognitive deficits in recent studies, with no influence from factors such as age, education level, disease severity, mood or dopamine replacement therapy (CZERNECKI et al., 2021; SANTANGELO et al., 2012). Regarding ToM, impairments in both cognitive and affective aspects of ToM, which correspond respectively to ability to infer others' beliefs or intentions and appreciate others' emotional states, have been reported in nondemented, nondepressed individuals with early PD (SANTANGELO et al., 2012).

1.3.2 Possible neurobiological correlates

Social cognition and behaviour involve the amalgamation of diverse cognitive processes, which encompass salience of social stimuli, evaluation, motivation and seeking of rewards, and understanding oneself and others. Similarly, the networks in the brain associated with social function exhibit comparable complexity. Contemporary notions of the social brain network commonly portray it as constituting a hierarchical system of circuits engaged in more fundamental, automated processes (i.e. salience of social stimuli), alongside partially overlapping circuits involved in motivation and higher order processes, such as ToM (IKE et al., 2020; PORCELLI et al., 2019; REDCAY; SCHILBACH, 2019).

An evolutionary highly conserved neural network associated with social decision-making, has been proposed, which functionally integrates the intimately interconnected limbic structures (e.g. hippocampus, amygdala, and various thalamic and hypothalamic nuclei) and the motivational reward circuitry, with added top-down modulatory control by cortical structures and ascending midbrain nuclei. Importantly, this network is known to be susceptible to changes induced by external and internal factors, such as stress, infection, genetics and pathological process of many neuropsychiatric disorders, including major depressive disorders, which may lead to their common manifestation of social withdrawal (IKE et al., 2020).

The underlying neural circuitry of motivational and rewarding aspects of social behaviour is hypothesised to be similar to that of reward and addiction to non-social

stimuli. In other words, to involve the reward circuit in the basal ganglia, mediated by dopamine – albeit with the added complexities associated with social stimulus (BÁEZ-MENDOZA; SCHULTZ, 2013; KRACH, 2010). The striatum, with its connections to relevant limbic structures and cortical regions, has been proposed to contribute to the integration of social stimuli into coding of social reward and behaviour (BÁEZ-MENDOZA; SCHULTZ, 2013; BICKS et al., 2015). Although not specific to Parkinson's Disease, dysfunctions in the reward systems as well as changes in amygdala activity, and altered balance of excitation and inhibition within the PFC, have been reported as common focal points among different neuropsychiatric disorders that share social withdrawal as a symptom. These disorders include schizophrenia, Major Depressive Disorder (MDD), and Alzheimer's Disease (BICKS et al., 2015; CZERNECKI et al., 2021; IKE et al., 2020; PORCELLI et al., 2019) **(Figure 10)**.

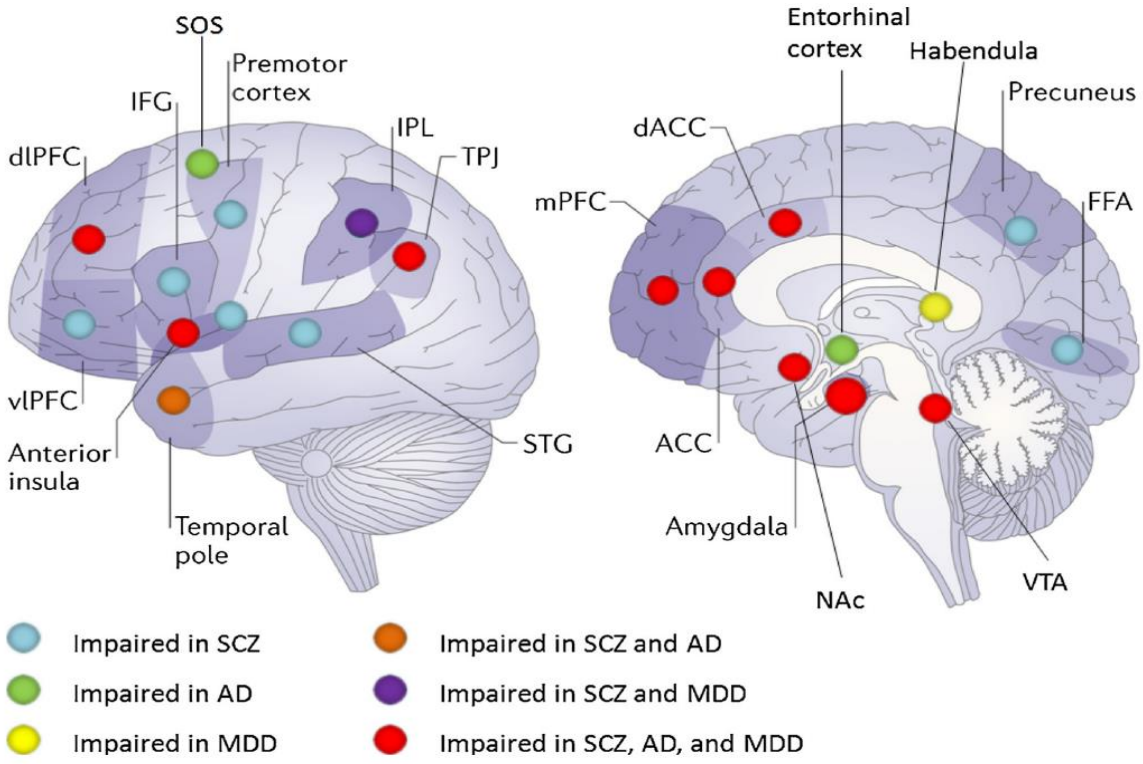


Figure 10. Brain regions linked social dysfunction in neuropsychiatric disorders. **Abbreviations**: FFA: fusiform face area; STG: Superior temporal gyrus; IFG: inferior frontal gyrus; IPL: inferior parietal lobule; ACC: anterior cingulate cortex; TPJ: temporo-parietal junction; PFC: prefrontal cortex; VTA: ventral tegmental area; NAc: nucleus accumbens; SOS: superior orbital sulcus; SCZ: schizophrenia; AD: Alzheimer's disease and MDD: major depressive disorders (modified from PORCELLI et al., 2019)

Regarding the complex processes of social cognition, the medial PFC, in particular, has been proposed to have a diverse role (in both humans and rodents),

with its more dorsal regions associated with perception of self and others and its ventral region with social reward and motivation (BICKS et al., 2015) **(Figure 11)**. In PD, frontal dysfunction may occur following impairment in BG structures connected to these regions via frontal-striatal circuitries, which in turn is likely to underline changes in social cognition. This assumption is supported, for instance, by observations that deficits in the cognitive aspects of ToM in early PD, were associated with performance in the Frontal Assessment Battery score, which is thought to involve prefrontal areas. (SANTANGELO et al., 2012).

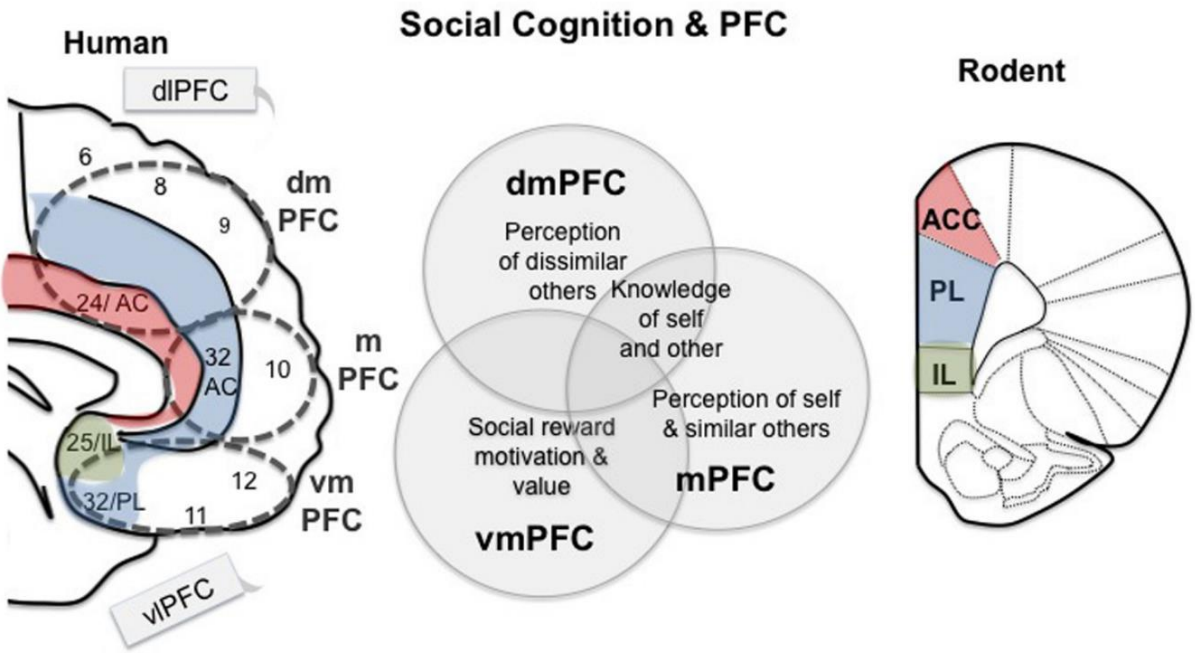


Figure 11. Prefrontal regions involved in social cognition in humans and rodents. Lateral regions of the PFC (i.e dlPFC and vlPFC), can be active during social tasks, but have not been related to any specific social behaviour, as opposed to the medial regions of the PFC. The colours depict different representations of some of the areas of these PFC divisions in humans that share homology with the rodent's PFC. These areas are believed to play a shared role in social cognition across mammalian lineages (modified from BICKS et al., 2015)

1.4 Modelling PD in rodents

1.4.1 6-hydroxydopamine (6-OHDA) models

Animal models play a crucial role in the comprehensive experimental study of distinctive characteristics and underlying pathologies of diseases like PD and also serve as essential tools for testing therapeutic interventions. Currently, there is a wide range of PD animal models available, each capable of replicating specific features of

the disease. These models encompass pharmacological, toxin-induced, genetic, and α-synuclein-based models (LAMA et al., 2021). Among the toxin-induced models, one of the widely employed methods involves the stereotaxic injections of 6-hydroxydopamine (6-OHDA) into regions within the nigrostriatal pathway of rodents. This approach was described by Urban Ungerstedt in 1968. He observed an anterograde degeneration of the entire nigrostriatal pathway following infusion of this neurotoxin into the SNc and called attention to its potential use in future studies (UNGERSTEDT, 1968). Since then, animal models based on 6-OHDA infusion have been extensively used and have contributed significantly to our understanding of the disease (MAGNARD et al., 2016; MASINI et al., 2021; MEDEIROS et al., 2019).

The mechanisms by which 6-OHDA infusion leads to cellular dysfunction and death are still not fully understood. However, it is likely related to the resulting formation of potentially toxic products, the generation of reactive oxygen species (ROS), and mitochondrial dysfunction (VAREŠLIJA et al., 2020). This neurotoxin can be taken up by the dopamine transporter and can therefore induce a selective loss of catecholaminergic neurons. The extent of this loss varies depending on the lesion site and infusion concentration (HERNANDEZ-BALTAZAR; ZAVALA-FLORES; VILLANUEVA-OLIVO, 2017; KIRIK; ROSENBLAD; BJÖRKLUND, 1998; VAREŠLIJA et al., 2020) **(Figure 12)**.

Therefore, although the 6-OHDA model does not replicate the α-synuclein and Lewy bodies pathology observed in PD, it effectively reproduces the degeneration of the dopaminergic nigrostriatal tract, a key pathological feature of idiopathic PD (MASINI et al., 2021)**.** Additionally, other pathogenic features of PD are observed in this animal model, including neuronal dysfunction outside the nigrostriatal tract, mitochondrial dysfunction, oxidative stress, autophagy and proteasomal dysfunction, and neuroinflammation (LAMA et al., 2021).

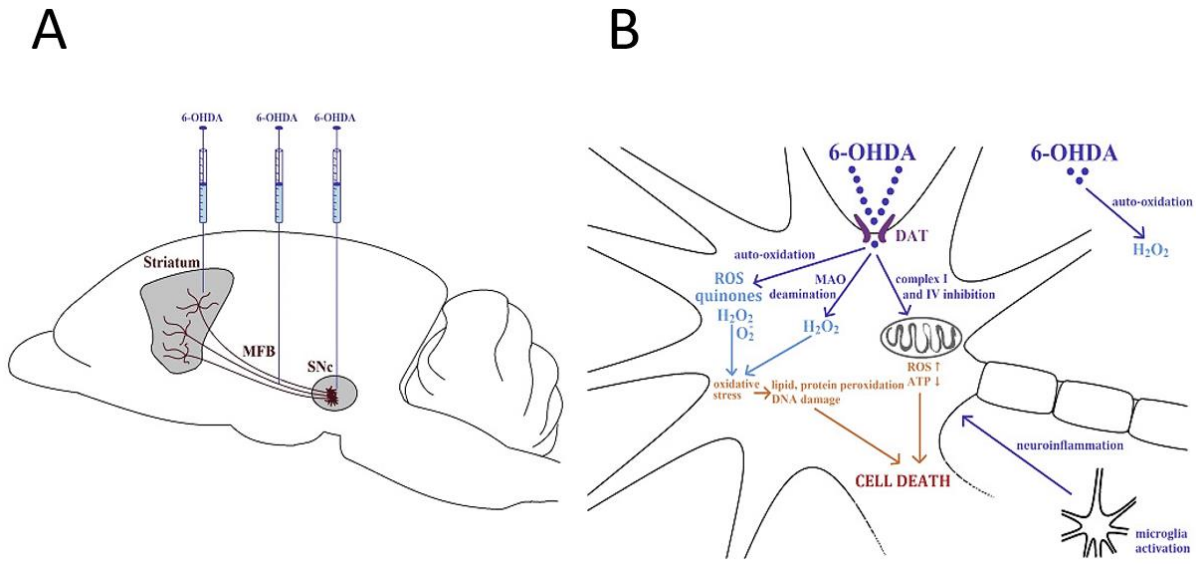


Figure 12. 6-OHDA infusion sites and mechanism of action. The figure depicts the **(A)** three common infusion sites of 6-OHDA infusion to generate the lesion within the nigrostriatal pathway in animal models, and **(B)** 6-OHDA supposed mechanisms of action. The neurotoxin rapidly auto-oxidises forming ROS. It can be taken up by DaT and, in the intercellular space, 6-OHDA is believed to culminate in cell death through a combination of oxidative stress and mitochondrial respiratory dysfunction. **Abbreviations**: MFB: medial forebrain bundle; SNc: substantia nigra pars compacta; DAT: dopamine transporter; ROS: reactive oxygen species (modified from KACZYŃSKA; ANDRZEJEWSKI, 2020)

Regarding behavioural changes, 6-OHDA models have successfully replicated both motor and non-motor deficits associated with PD. Non-motor alterations observed in 6-OHDA models are diverse and include cognitive dysfunction in different memory paradigms (BONITO-OLIVA et al., 2014; HERRERA et al., 2020; MASINI et al., 2017; MATHEUS et al., 2016a) and executive functions (LHOST et al., 2021)*;* depressive-like behaviour (BONITO-OLIVA; MASINI; FISONE, 2014; MARQUES et al., 2019; MATHEUS et al., 2016b); circadian perturbations (HUNT et al., 2022; MASINI et al., 2017; REQUEJO et al., 2020) and significant changes in sleep microstructure, comparable to those observed in PD patients (CIRIC et al., 2019; VO et al., 2014; ZHURAKOVSKAYA et al., 2019).

Importantly, the amount of toxin infused can be adjusted to generate a partial lesion of the nigrostriatal pathway, enabling the simulation of earlier or prodromal phases of PD, with non-motor manifestations, while minimising the potential influence of motor confounding factors (LAMA et al., 2021; MAGNARD et al., 2016). Nonetheless, it's important to recognise that there is significant variability between protocols used to establish these models, including variations in the coordinates of infusion, the amount of 6-OHDA administered, the species used, and other factors, all of which may impact research results.

1.4.2 Overview of 6-OHDA models assessing nonmotor symptoms

To have a better, more comprehensive, overview of the various approaches to the 6-OHDA lesion and what to expect in terms of both DA cell loss and behavioural outcomes, a literature search was conducted in our lab (on 9 September 2022), for experimental studies using 6-OHDA infusion to establish a PD model in rodents that focused on assessing non-motor symptoms. The search was conducted in the following electronic databases: Scopus, MEDLINE (PubMed), and Web of Science. It resulted in 254 studies after excluding duplicates of which 75 were selected and further 60 were added from other sources **(see Appendix A, Figure A1).**

In studies using 6-OHDA rodent models to assess nonmotor symptoms, rats have been the most commonly used animals, primarily from the Wistar and Sprague-Dawley lineages**.** Of all the 135 studies analysed, only nine utilised females, and an additional six used both male and female animals **(Appendix A, Figure A2)**. The three main sites for toxin infusion are the dorsal striatum (constituting 38% of studies), the medial forebrain bundle (MFB, 32%), and the substantia nigra (SN, 25%). In the dorsal striatum and SN, infusions were carried out both unilaterally and bilaterally in similar proportions, whereas in the MFB, unilateral infusions were more common **(Appendix B, Figure A3).** The specific coordinates for 6-OHDA infusion within each region, as well as the amount of 6-OHDA administered, show significant variations among studies **(Appendix B, Figure A4).**

Infusions in the SN or the dorsal striatum are generally preferred over MFB infusions when assessing nonmotor functions (BONITO-OLIVA; MASINI; FISONE, 2014; DEUMENS; BLOKLAND; PRICKAERTS, 2002; MAGNARD et al., 2016), as lesions in the MFB often result in a more extensive dopamine loss (>80%), which is higher than what is observed in early PD patients (HENG et al., 2023). Infusion in the striatum leads to a rapid depletion of local dopamine innervation, followed by a more gradual reduction in the number of dopamine cell bodies in the SN. This is in contrast to the rapid onset of neuronal loss observed in direct infusion into the SN (MASINI et al., 2021). Considering previous observations of retrograde axonal degeneration early in the disease (CAMINITI et al., 2017; TAGLIAFERRO; BURKE, 2016), infusions in the striatum supposedly better reflect the early PD pathology in patients and are considered more relevant models of PD. Moreover, the larger size of the striatum is advantageous, as it allows for better control of the infusion and more selective targeting

of the nigrostriatal dopaminergic pathway (CAMINITI et al., 2017; DEUMENS; BLOKLAND; PRICKAERTS, 2002).

While early PD may present with unilateral motor features, it's important to note that striatal depletion occurs in both hemispheres, albeit to varying degrees, in these patients (HENG et al., 2023). Therefore, bilateral infusions of toxins in animal models help ensure a more accurate representation of the disease pathology and also mitigates compensation from the contralateral hemisphere and potential interference from any asymmetrical movements that might occur in animals with unilateral lesions (DEUMENS; BLOKLAND; PRICKAERTS, 2002; MASINI et al., 2021).

In 6-OHDA models, depressive-like behaviours, which encompass defensive behaviour or learned helplessness, social isolation, and anhedonia, have been among the most extensively assessed nonmotor changes, followed by memory and anxiety **(see Appendix A, Figure 4A).** In models generated by bilateral striatal lesions, depressive-like behaviours have been reported; however, there are some conflicting results, and not all findings have been consistently replicated **(as illustrated in Figure 13)**. Therefore, additional studies are necessary to determine the consistency of previously reported findings, assess the robustness of the results, and consider potential confounding factors that may contribute to variations in the outcomes.

Given that depressive symptoms have been previously reported in 6-OHDA models, it can be hypothesised that dysfunction of the nigrostriatal pathway, as induced by this model and observed in PD patients, may be sufficient to induce nonmotor symptoms early in the disease.

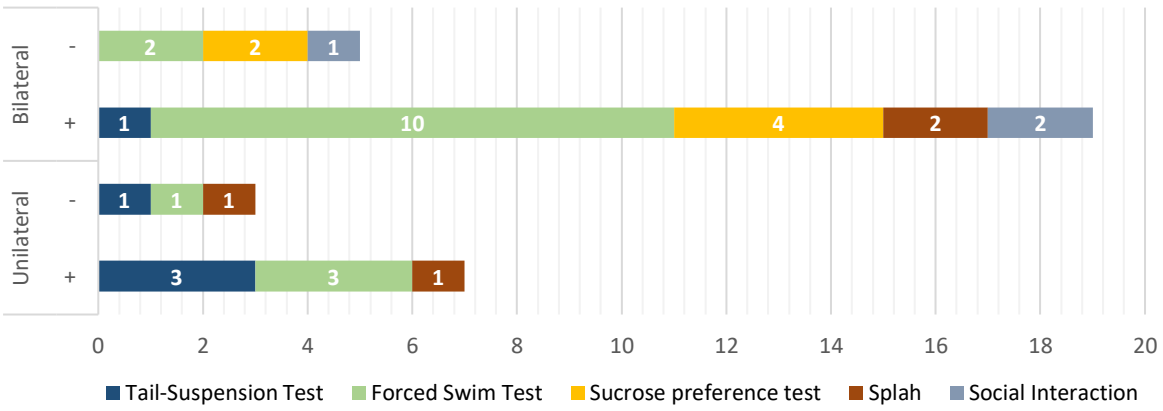


Figure 11. Depressive-like behaviours in rodents infused with 6-OHDA into the dorsal striatum, either unilaterally or bilaterally. The number indicates how many studies used each test to assess depressive-like behaviour. The signs (+) and (-) indicate when change in the studied behaviour was or was not present, respectively, for each test.

1.5 Significance of the study

Depressive symptoms are prevalent in early PD and have a significant impact on patients' quality of life (CONG et al., 2022). They can influence the individual's ability to cope with PD and are related to worse disease prognosis (MARSH, 2013; PONTONE et al., 2016). Additionally, social withdrawal is also present in the PD and is highly associated with depression, though it can also occur independently and/or reciprocally influence depressive symptoms (AHN; SPRINGER; GIBSON, 2022).

The fact these symptoms often manifest early in the disease further underscores the importance of studying them, since it is a stage where interventions are most likely to be effective (POEWE et al., 2017). Moreover, given their observed association, it is reasonable to assume that treating depression may also positively impact sociability as well as other non-motor manifestations, such as cognitive abilities (AARSLAND et al., 2012). However, the development of effective interventions depends on a thorough understanding of the pathobiological mechanisms of such symptoms and, unfortunately, despite increasing attention to non-motor symptoms in general, still very little is known about their underlying pathologies.

Clinical studies with early PD patients provide valuable insights, but they have limitations and are susceptible to various confounding factors (JELLINGER, 2022). In this context, well-validated rodent models, particularly 6-OHDA models generated by infusions into the dorsolateral striatum, offer a valuable complementary tool. These models replicate a key pathological feature observed early in the disease, allowing researchers to study whether and how the specific pathological changes in this system alter behaviour as well as the identification of biomarkers, and testing potential interventions (LAMA et al., 2021; MASINI et al., 2021).

Of note, it's important to recognise that the lack of reproducibility and translation of animal research is a major issue in the current scientific research. In this context, the replication of previous findings and the assessment of potentially conflicting results are fundamental steps to determine the value and validity of an animal model (see SPANAGEL, 2022).

2. OBJECTIVE

2.1 General objective

This study aims to investigate possible changes in social behaviour and the presence of anhedonia in a previously established model of early PD.

2.2 Specific objectives

- To generate the early PD model by bilaterally infusing 6-OHDA into the dorsolateral striatum of Wistar rats.
- To assess any potential motor changes following infusion.
- To quantify behavioural changes associated with hedonic deficits and social withdrawal, by using the sucrose preference test and social interaction test, respectively.

3 METHODOLOGY

3.1 Animals

A total of 32 adult male Wistar rats (weighing on average 289.5 ± 26.68g at the time of surgery) were used in this study. The rats were housed in groups of two per cage (41 × 34 × 18 cm) in a temperature-controlled room (23 ± 1 ◦C), with free access to food and water, and with a 12 h light/12 h dark cycle (lights on at 7:00 am). The procedures described here have been submitted to and approved by the Ethical Committee for the care and use of laboratory animals at the Federal University of Minas Gerais (CEUA PP0154/2022).

3.2 Experimental protocol

The animals were injected with either 6-OHDA (6-OHDA group) or with ascorbic saline as a vehicle solution (Control group). After the stereotaxic surgery, two independent groups of animals were assessed for anhedonia and/or social dysfunction, using the sucrose preference test (SPT) and social interaction test (SI), respectively; and for motor function, using the footprint and open field (OFT) test. The first group (consisting of 8 6-OHDA-infused animals and 8 controls), underwent testing in the footprint, OFT and SI during the third-week post-lesion (days 21 to 25). They were also tested in SPT at two time points: in the first- and third-week post-lesion. After

completing the behavioural evaluation, the animals were sacrificed and transcardially perfused for immunohistochemical quantification of tyrosine hydroxylase (TH) in the substantia nigra and dorsolateral striatum. The second group (consisting of 6 6-OHDA-infused animals and 10 controls) were also tested in the SI and OFT tests during the third-week post-lesion; and in the footprint and in the OFT in the fourth-week post-lesion (days 30 or 31) **(Figure 14)**.

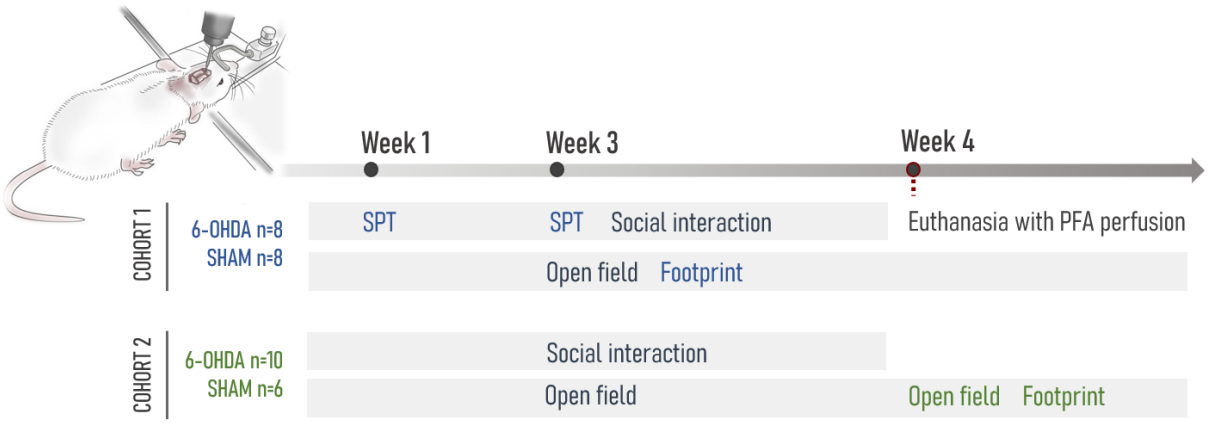


Figure 14. Schematic representation of the experimental protocol. Abbreviation: DLS: dorsolateral striatum; SPT: sucrose; PFA: paraformaldehyde.

3.3 Surgical procedures

3.3.1 Anaesthesia

For general anaesthesia, the animals were injected with ketamine (10% ketamine hydrochloride, diluted in q.s. sterile vehicle) and xylazine (2% xylazine hydrochloride, diluted in q.s. vehicle) intraperitoneally and intramuscularly. The dosage of drug received per animal was calculated according to its weight (1mL/Kg for ketamine and 0.5mL/Kg for xylazine), with the initial dose being 0.7 mL/Kg of ketamine and 0.35 mL/Kg of xylazine (70% of the dose) intraperitoneally. After 10 minutes of initial sedation, a supplement of 0.5 ml/kg of ketamine and 0.25 ml/kg of xylazine (50% of the dose) was administered intramuscularly. The animals' sedation was verified by observing the lack of response after clamping the animal's paw or tail. Throughout the surgery, supplementary doses of 30% ketamine were applied according to the animal's response.

3.3.2 Stereotaxic surgery and 6-OHDA infusion

After checking the degree of anaesthesia by respiratory rate and absence of tail pinch reflex, the animals were placed in the stereotaxic framework (KOPF Instruments; USA). Body temperature was maintained between 37±0.5°C with a heating blanket placed under the animal throughout the procedure. Asepsis of the scalp with iodine and hydrogen peroxide and subcutaneous injection of local anaesthetic (2% lidocaine in 0.15 M NaCl) was performed for scalp incision and exposure. Sterile saline (0,9%) was applied as needed (from the moment the animals lost the motor reflex) to prevent corneal desiccation and the analgesic status was checked regularly.

To generate the parkinsonian animal model, the animals were injected with 3 µL of 6-OHDA (10 µg/3 µL dissolved in saline solution with 0.1% ascorbic acid) at a rate of 0.5 µL/min, using a glass micropipette coupled to an infusion pump **(Figure 15).** The infusion was done bilaterally into the dorsolateral striatum, in the following coordinates from Paxinos and Watson (2009): AP +0.2 mm; ML ±3.5 mm; DV −4.8 mm. In the control groups, 3 µL of the vehicle (saline solution with 0.1% ascorbic acid) was injected instead. After the infusion, the micropipette was left in place for 5 minutes before being slowly retracted to allow complete diffusion of the drug. The concentration of 6-OHDA infused and infusion sites were determined following previous studies by Matheus et al. (2016).

During the post-anaesthetic period, the animals were placed individually in clean cages. Ocular lubrication was continued as needed until complete recovery from anaesthesia. All the animals were given Banamine (flunixin meglumine; 0.5 ml diluted in 10 ml of sterile saline, subcutaneously) at the end of surgery and every 12 hours for 72 hours for pain control; and provided easy access to supplemental food by placing it on the floor of the cage. During the 7 days recovery period, their health status was monitored daily: they were checked once or twice a day for signs of pain, stress, dehydration, illness and/or infection.

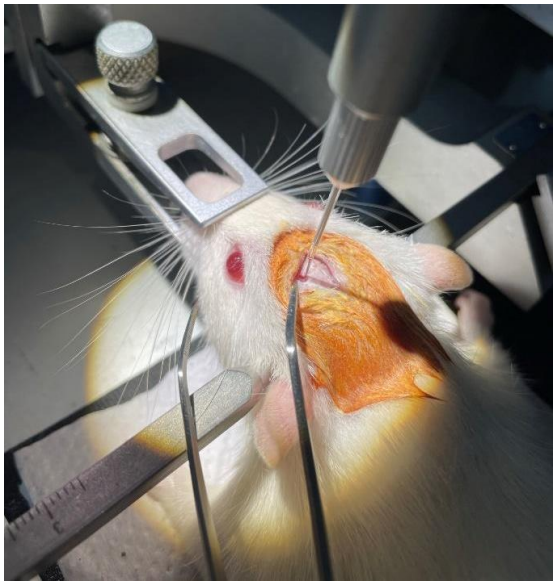


Figure 15. 6-OHDA infusion using a glass micropipette. Image showing Infusion of 3 µL of 6-OHDA, via a glass micropipette, into the left dorsal striatum of a fully anaesthetised rat, secured to the stereotaxic framework.

3.4 Behavioural testing

All behavioural tests were performed between 8:00 and 12:00h by the same experimenters, in an observation room with sound attenuation, where and with whom the rats had been previously habituated (for at least a week). All equipment was cleaned with 20% ethanol between trials and with 70% ethanol before and at the end of testing sessions (to avoid odour signs). Except for the footprint test, all tasks were recorded by a video camera positioned above the devices and subsequently analysed by a blinded experimenter.

3.4.1 Footprint Test

The footprint test was conducted to assess changes in the animals' gait. For this, an illuminated wooden runway (70cm × 10cm × 10cm) connected to a dark wooden box was used. As training for the task, the rat was first placed on the proximal end of the runway, facing the goal box and allowed to walk towards it. For the test, a strip of graph paper was placed on the wooden runway and the rat's paws were immersed in nontoxic ink (forelimbs in red ink and hindlimbs in black ink). Immediately after, the rats were placed on the proximal end of the runway, facing the goal box and allowed to walk towards it (**Figure 16**). If the rat stopped on the paper, they were gently

pushed to the goal box. The test was repeated 1-3 times or until a satisfactory recording of uninterrupted locomotion of at least six paw placements was achieved. Steps just before the entry to the wooden box were not included since rats often slowed down and made smaller steps.

For gait analysis, measurements of stride lengths and base widths of forelimbs and hindlimbs were obtained with a ruler from foot-printed paper and averaged (**Figure 16**). Stride length is the distance between the same parts (e.g., paw pad or toe) of two subsequent paws, for this, the average of the individual averages of the left and right strides were used. Base width is the distance from the left hind or front paw to the right front or hind paw print (FERNAGUT et al., 2002; SUGIMOTO; KAWAKAMI, 2019).

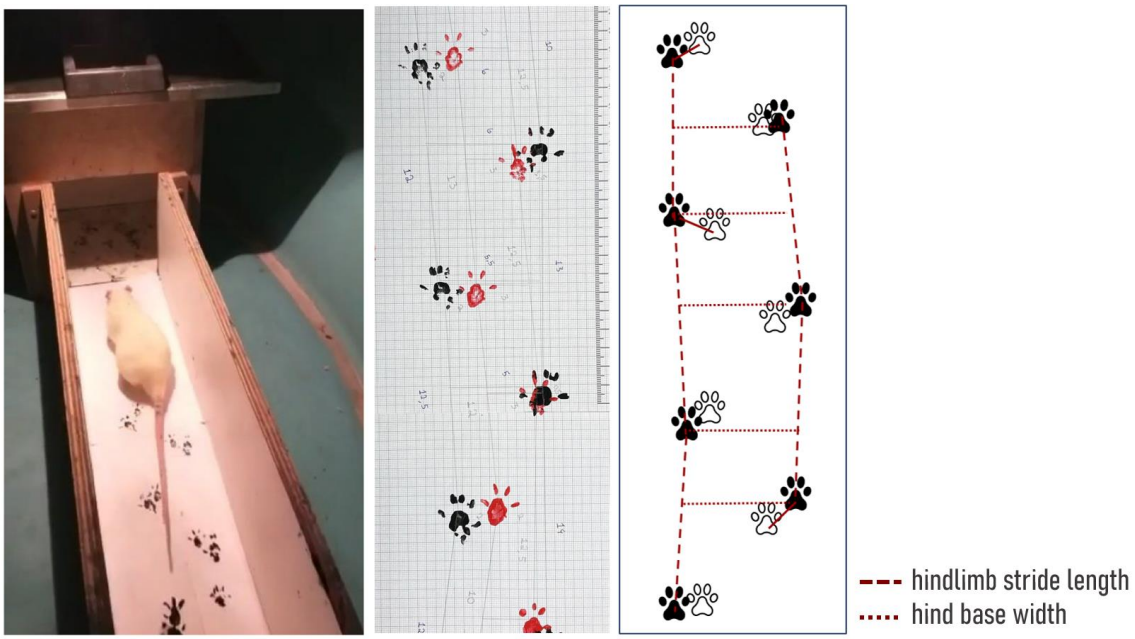


Figure 16. Footprint test. From left to right: Illustrative photo of the wooden runway and footprint test; picture of paw prints in the graph paper (forelimb in red and hindlimb in black) compared to schematic representation of the measured parameters: stride length (dashed vertical lines) base width (dotted horizontal lines).

3.4.2 Open Field Test

The open field test consists of placing the rats in the centre of a dimly lit (~40 lux) wooden arena (100 × 80 cm, grey walls, gridded grey floor) and allowing them to explore it freely for 15 min. Assessment of the recording was performed using the Bonsai software **(Figure 17;** the Bonsai workflow used can be found in the following link: https://github.com/fgmourao/Behavior_Analysis**).** Total distance covered and average speed of movement, were extracted as indicators of the animal's spontaneous locomotor activity (MATHEUS et al., 2016b).

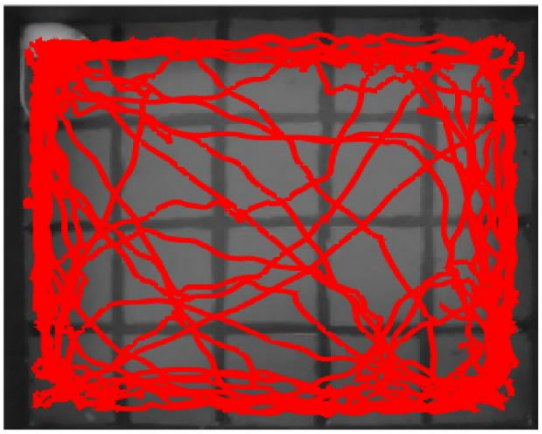


Figure 17. Open field test. Animal tracking using the Bonsai Software. Red lines illustrating the animals' movements in the open field during 15 min.

3.4.3 Sucrose Preference Test

For the sucrose preference test, each rat was placed alone in its standard housing cage and provided with two bottles of water during 24 hours to adapt the rats to drinking from the two bottles (smaller, 125ml, drinking bottles were used to avoid leaking). After the 24h-habituation, the liquid from a bottle was changed to a 1% (wt/vol) sucrose solution and left again for 24h. During the test period, the bottles were checked for complementary analysis and inverted, after 1, 5 and 7 hours after beginning the test. The consumption of water and sucrose solution was estimated by weighing the bottles before and after the test. The level of sucrose preference was estimated by the percentage of sucrose intake. This was calculated using the formula: sucrose intake × 100/total intake. Lack of or significant decrease in sucrose preference is considered an indicator of anhedonia (SANTIAGO et al., 2014).

3.4.4 Social Interaction Test

In the social interaction test, two weight-matched rats, housed in different cages, but of the same experimental group, were placed on opposite sides of a dimly illuminated wooden arena (100 × 80cm), where they had been previously habituated. They were allowed to interact for 10 min. To differentiate the animals, one of each pair was marked by dying their dorsal coat with nontoxic black ink. The time spent in different behaviours were scored manually by an experimenter blind to the animal's experimental group **(Figure 18)**.

The timing of each behaviour of interest was carried out using the open-source VIA software (VGG Image Annotator) **(Figure 19)** and the data of the total time on each behaviour was extracted using Matlab 2022 (MathWorks®). The ethogram **(Table 1)** was adapted from (BRANCHI et al., 2008; MANFRÉ et al., 2018; PETERS et al., 2016).

Table 1. Ethogram. Descriptions of all behavioural classifications used to score the social interaction test

Behavioural category	**Behavioural element**	**Description**
Social interaction	Sniffing /nose contact	The animal makes contact or near contact with the body part of the other animal
	Allogrooming	The animal grooms its partner
	Approaching / Following	The animal moves directly towards the other animal, rapidly decreasing the space between them or by following the other animal.
Other behaviours	Aggressive/Dominant	Fighting, nape attacking, biting/pulling, boxing/kicking or pinning/supine the other animal to the ground
	Submissive/Passive	When the animal is being sniffed, picked up or mounted on and is immobile
Social avoidance	Moving away	The animal moves away from the other animal after interacting or when the other animal approaches
	Defence	During an interaction, the animal repositions its body or pushes the other away
Non-social	Cage exploration	The animal carries out individual actions, such as rearing, and exploring the cage.
	Self-grooming	---
	Quite-awake	The animal stands still/immobile

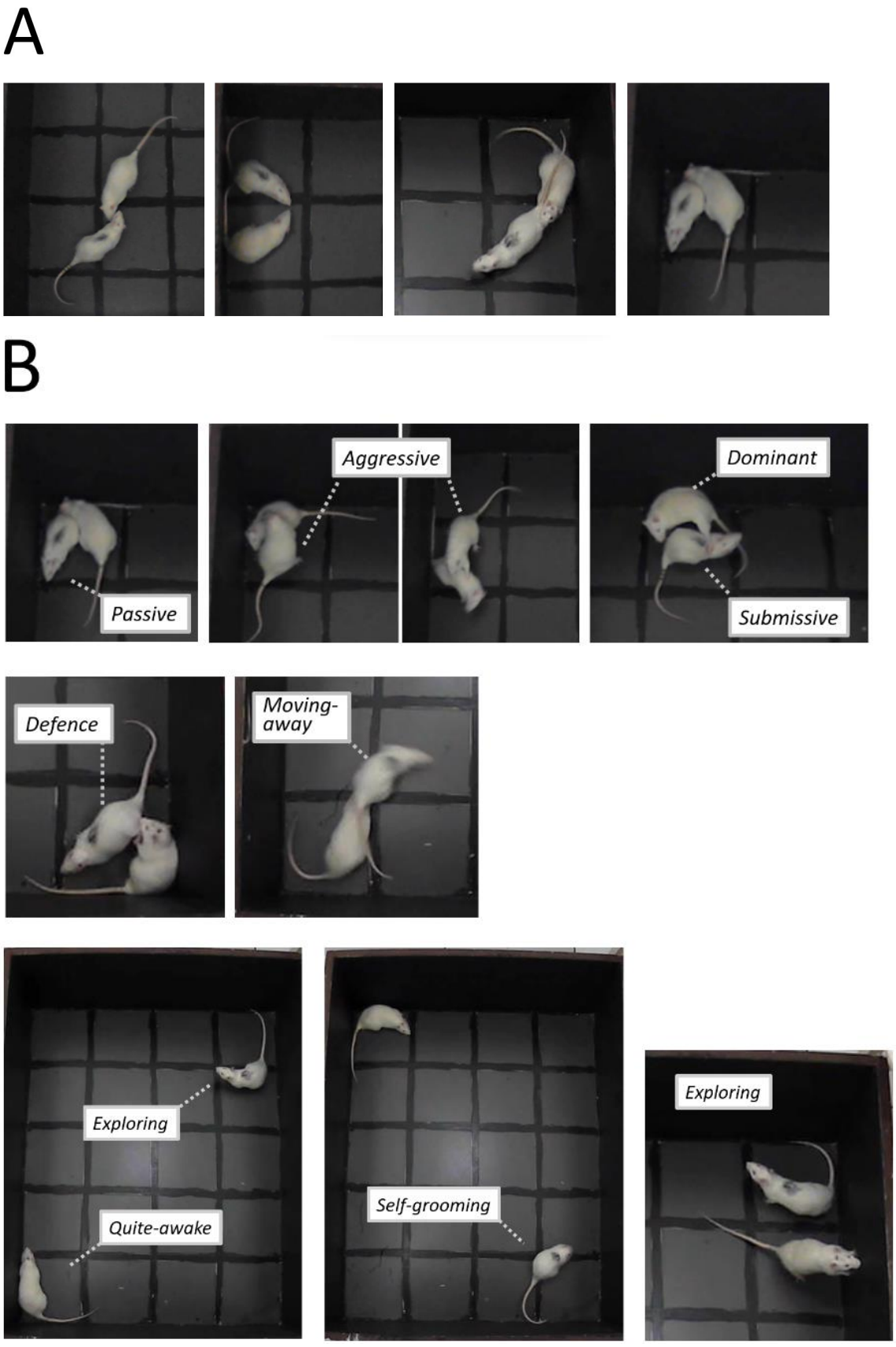


Figure 2 Illustrative examples of the scored behaviours in the social interact test. (A) Example of behaviours of social interaction of the non-marked animal. (B) Other behaviours scored for complementary analysis.

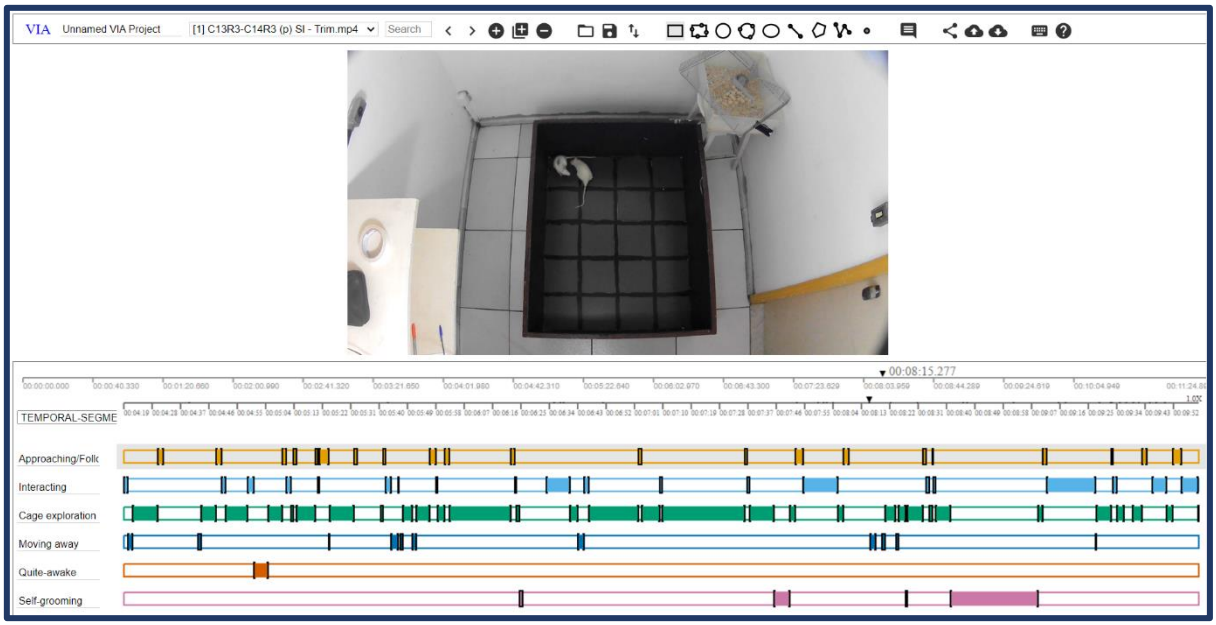


Figure 19. Social behaviours scored on the VIA software. The image depicts an example of behaviour scoring using VIA. On the centre of the screen there is the recording of two animals interacting during the social interaction test. The animal on the left is marked with non-toxic black ink. At the bottom portion of the page is the video's timeline and the temporal segments created representing the time the focal animal spent on each of the behaviours (listed on the left).

3.5 Brain extraction and sectioning for immunohistochemistry

Following the behavioural experiments, a cohort of animals from both groups were deeply anaesthetized with ketamine/xylazine and perfused, through the left cardiac ventricle, with phosphate-buffered saline (PBS) 0.01 M (250 mL/L; to clean the tissues), followed by 4% paraformaldehyde (PFA) in 0.1 M phosphate buffer (300 mL/L; to fix the tissue). The brains were extracted and fixed in the same 4% PFA solution. After 48h, the cryoprotection protocol began: the brains were immersed in progressive sucrose solutions of 10, 20 and 30% each day, for three days. The brains were then frozen by submersion in isopentane (~40s) and stored in an ultrafreezer at 80 °C.

Subsequently, the brains were transported (in dry ice; CO2) to the University of São Paulo (USP) in Ribeirão Preto (RP), where the experimenters underwent training and carried out the sectioning of the samples using a freezing microtome (Leica CM1850; Leica Microsystems, Wetzlar, Germany). Coronal sections, 50 µm thick, of the motor cortex (M1), striatum and the SNc were collected in labelled Eppendorf tubes containing 2 ml of antifreeze solution. The right hemisphere was marked by a small hole in the tissue, outside the regions of interest. The start and end point of collection

for each region was defined according to their bregma coordinates (PAXINOS; WATSON, 2007) **(Figure 20)**. An approximate total of 24 and 44 sections were collected in 4 tubes, from the M1 and SNc, respectively; and approximately 60 sections from the striatum were distributed in 8 tubes. The sections were collected in blocks: each consecutive section was placed in a different tube, in a sequential order, so that each tube ended up with the same number of non-contiguous serial sections (roughly 200 µm apart from each other). **(See Appendix B for the protocol used).** Different protocols for immunohistochemical quantification of tyrosine hydroxylase (TH) were tested and optimised by an experienced professor. The samples were then transported back to UFMG, where the histochemical analyses will be conducted.

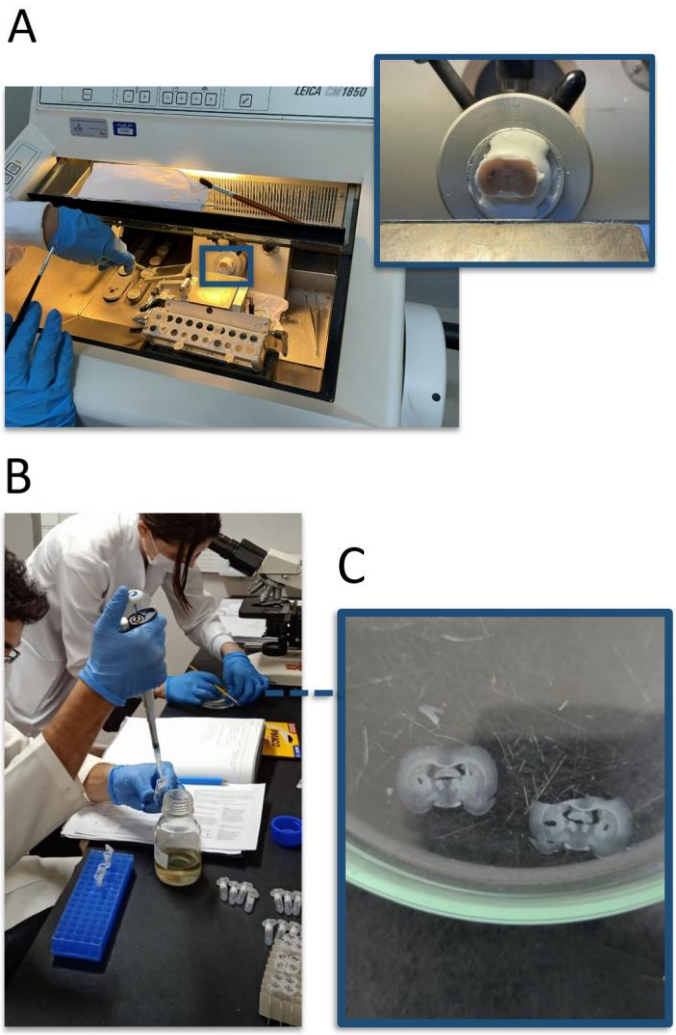


Figure 3. illustrative images of the process of tissue sectioning. (A) Thes brains were sectioned using a freezing microtome. **(B)** Eppendorf tubes were labelled and filled with anti-freeze coolant. **(C)** A few sections were collected in PBS to be analysed under a microscope to determine their bregma coordinate.

3.6 Statistical Analysis

Statistical analysis of all collected data was performed using GraphPad Prism version 9.00 (GraphPad Software, Inc,). The homogeneity of variance of all variables was first assessed using the Shapiro-Wilk test. Student's t-test was used to analyse variables between the control and 6-OHDA groups, when the data had a normal distribution, and the Mann-Whitney U test was used otherwise. Two-way analysis of variance (ANOVA) was also performed to assess differences between the groups in average weight during the first week following surgery; and in locomotion in the open field test at different time points during the test. The accepted level of significance was $p \leq 0.05$. When a significant difference between groups was observed the effect size was calculated using Cohen's d to indicate the magnitude of the observed difference between groups (SERDAR et al., 2021).

4 RESULTS

4.1 Post-operative care

A total of 11 animals died or had to be euthanized due to complications during surgery (i.e. anaesthesia overdose) or during the postoperative recovery period (i.e. stitches opened or showed signs of pain without improvement). The remaining rats showed no significant difference in body weight on the day of surgery or during the following week (**Figure 21**). Specifically, mixed effect analysis two-way ANOVA revealed a significant effect for time ($F(2.585, 69.8) = 40.76$, $p < 0.0001$), but not for treatment ($F(1, 27) = 0.027$, $p=0.87$) on the animals weight on the day of surgery and throughout the first week. No statistically significant interaction between the effects of time and treatment ($F(4, 108) = 1.279$, $p=0.283$) was found either.

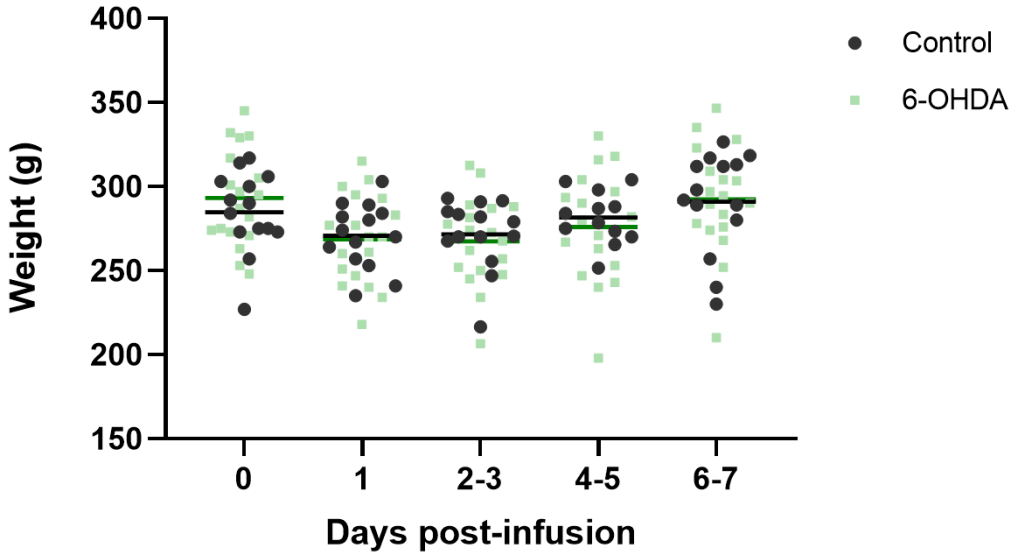


Figure 21. Animals' weight. Average weight of the rats on the day of surgery (day 0) and during the first week. Data are expressed as mean ± SEM from 14 animals of the control group and 18 from the 6-OHDA group.

4.2 Behavioural Analysis

4.2.1 Animals with 6-OHDA lesions showed no gait changes in the footprint test.

This study employed the Footprint test to evaluate potential changes in gait at three and four weeks post-lesion. It is common for animal models of Parkinson's disease to exhibit a decrease in stride length, which is similar to the shorter steps observed in patients with Parkinsonian gait. Additionally, an increase in base width has been previously reported as an indicator of dynamic postural instability (Hsieh et al., 2011; Madiha et al., 2017). However, as this is a model of early PD, we hypothesised that the animals would not exhibit any gross impairments in motor function, including changes in gait.

As expected, the 6-OHDA and control groups did not differ significantly in the average stride length of their forelimb (Mann-Whitney U test; U=29.5, p= .81), or hindlimb (two-sample t-test; t(14)=0.24, p = .82; **figure 22A**). Forelimb (two-sample t-test; t(14)=1.71, p=.11) and hindlimb (two-sample t-test; t(14)=0.16, p=.87), base width were also not statistically different between the two groups (**figure 22B**).

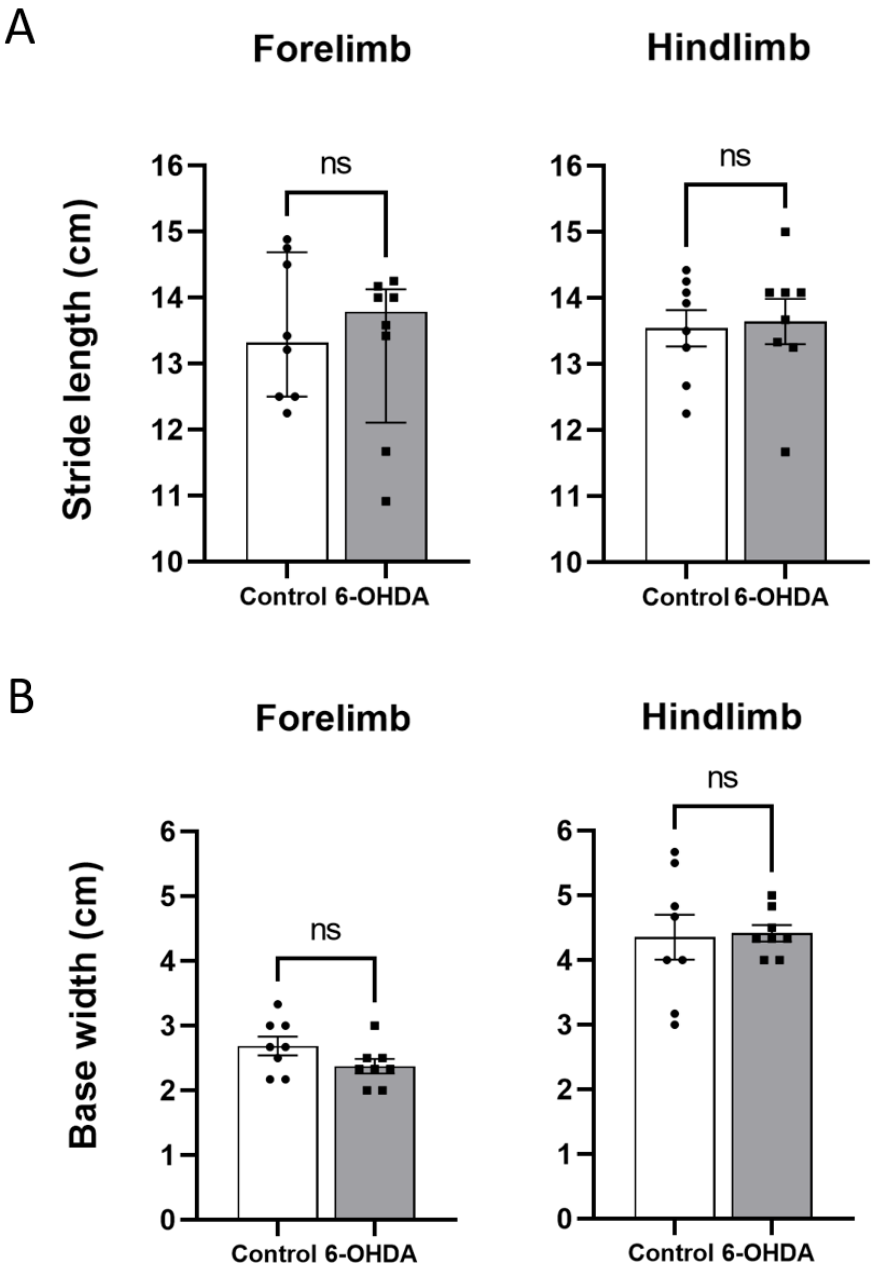


Figure 22. Gait parameters assessed in the footprint test three weeks post-lesion. (**A**) Comparison of average forelimb and hindlimb stride length of the two groups. For the forelimb, data is expressed as median and interquartile from 8 animals of the control and 8 from 6-OHDA group. Mann Whitney test; ns: $P > 0.05$. For the hindlimb, data are expressed as mean ± SEM from 6 animals of the control and 10 from 6-OHDA group. Student's t-test; ns: $P > 0.05$. (**B**) Fore-base and hind-base width of the control and 6-OHDA group. Data are expressed as mean ± SEM from 8 animals of the control and 8 from 6-OHDA group. Student's t-test; ns: $P > 0.05$.

A two-sample t-test and a Mann-Whitney U test were performed to compare the stride length and base width, respectively, between the groups in the fourth week post-lesion. At this time point, there was still no statistically significant difference between

the average forelimb, t(14)=0.51, p=.61, and hindlimb, t(14)=0.49, p=0.63, stride lengths (**figure 23A**); nor of forelimb, U=30, p>.99, or hindlimb, U=26.5, p=.72, base width between control and 6-OHDA groups (**figure 23B**).

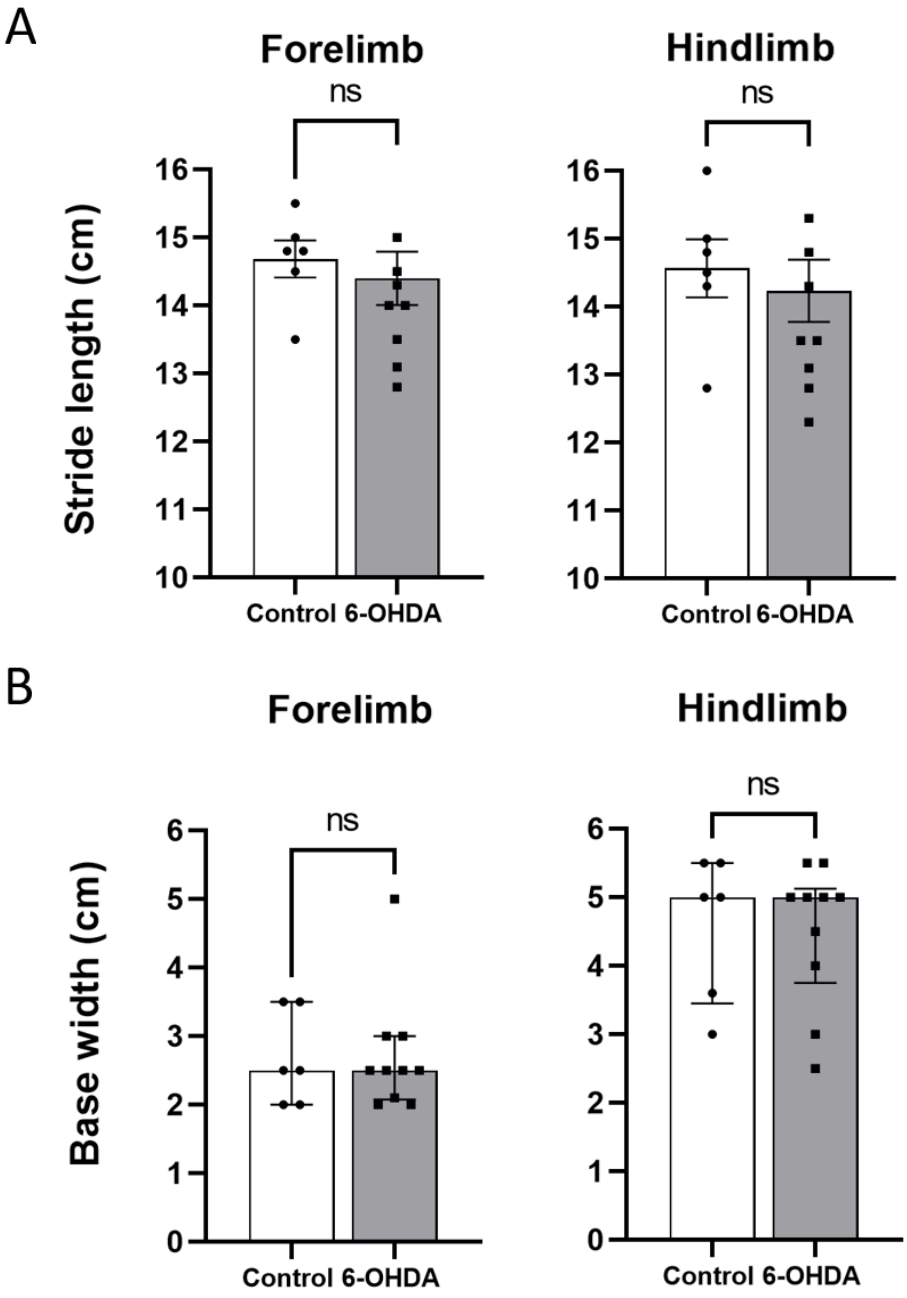
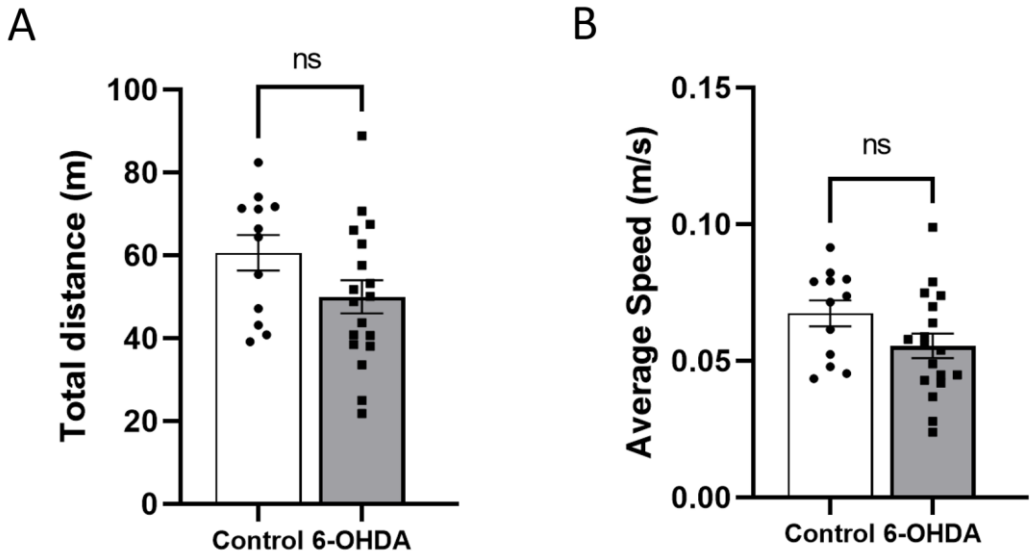


Figure 23. Gait parameters assessed in the footprint test four weeks post-lesion. (**A**) Comparison of average forelimb and hindlimb stride length of the two groups. Data are expressed as mean ± SEM from 6 animals of the control and 10 from 6-OHDA group. Student's t-test; ns: P > 0.05. (**B**) Fore-base and hind-base width of the control and 6-OHDA group. Data is expressed as median and interquartile from 6 animals of the control and 10 from 6-OHDA group. Mann Whitney test; ns: P > 0.05.

4.2.2 The 6-OHDA-lesioned animals exhibited preserved spontaneous locomotor activity during the open field test.

The Open field test was used to assess spontaneous locomotor activity in the third- and fourth-week post-lesion. The total distance covered and average speed of the control and 6-OHDA groups were compared by employing a two-sample t-test. In the third week post-lesion, no significant difference in total distance, t(28)=1.759, p=.089 (**Figure 24A**) and average speed, t(28)=1.750, p=.091 (**Figure 24B**) were found between the two groups.

A two-way ANOVA was further performed to analyse the effect of time and treatment on distance travelled per minute (**Figure 24C**). Simple main effects analysis showed a significant effect for time, F(14, 420) = 24.53, p <0.0001, but not treatment, F(1, 30) = 1.19, p=.284, on distance travelled. No statistically significant interaction between the effects of time and treatment, F(14, 420) = 1.235, p=.247, was found.



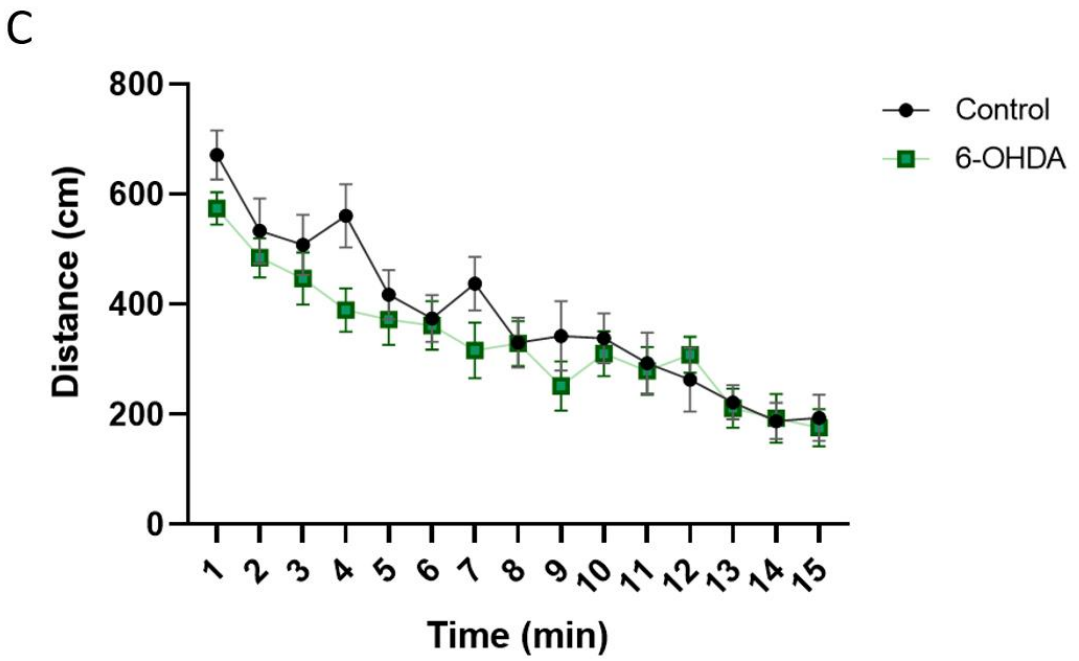


Figure 24. Spontaneous locomotor activity assessed in the open field test in the third week post-lesion. (**A**) Total distance (m) travelled; **(B)** Average speed (m/s). Data are expressed as mean ± SEM from 12 animals of the control group and 18 from 6-OHDA. Student's t test; ns: P > 0.05. **(C)** Distance travelled (cm) per minute. Data are expressed as mean ± SEM.

These results remained consistent in the fourth week post-lesion. No significant difference in total distance, t(13)=0.487, p=.634 (**Figure 25A**), and average speed, t(13)=0.469, p=.647 (**Figure 25B**), was found between control and 6-OHDA groups. Two-way ANOVA analyses revealed no significant interaction between the effects of time and treatment, F(14, 420) = 1.235, p=.247, on distance travelled per minute (**Figure 25C**). Simple main effects analysis showed a significant effect for time, F(14, 182) = 6.7, p <0.0001, but not treatment F(1, 13) = 0.237, p=.634.

Two animals from the control group, which were tested in the third week, and one animal from the 6-OHDA group, which was tested in the fourth week, were excluded from the analyses due to technical issues with their video recordings.

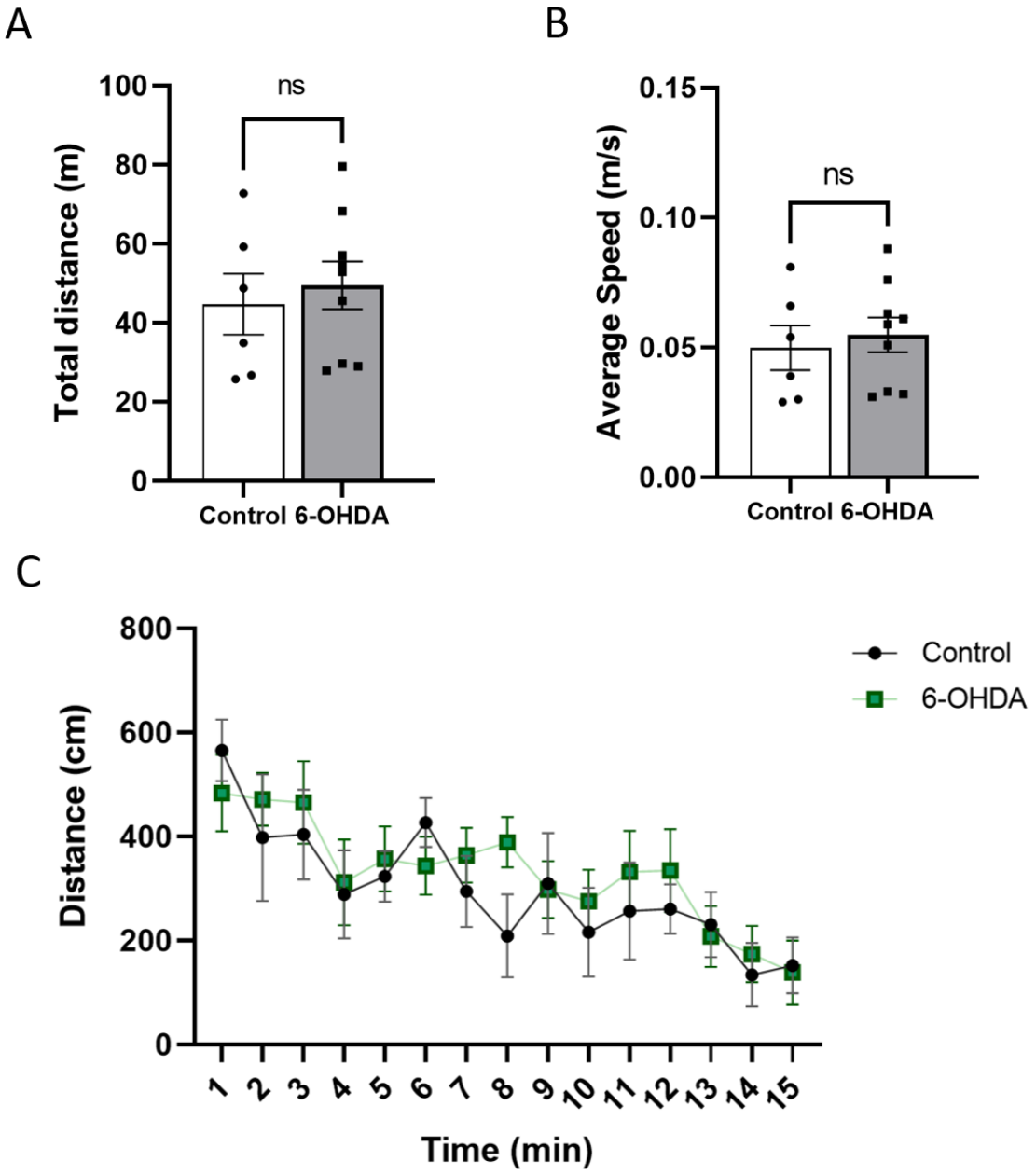


Figure 25. Spontaneous locomotor activity assessed in the open field test in the fourth week post-lesion. (**A**) Total distance (m) travelled; **(B)** Average speed (m/s). Data are expressed as mean ± SEM from 6 animals of the control group and 9 from 6-OHDA. Student's t-test; ns: $P > 0.05$. **(C)** Distance travelled (cm) per minute. Data are expressed as mean ± SEM.

4.2.3 Hedonic deficits in the 1st but not in the 3rd week post lesion in the 6-OHDA group

The sucrose preference test was used to investigate the presence of depressive-like behaviour, specifically anhedonia, at two time points: in the first and third week post-lesion. A one-tailed two-sample t-test was conducted to compare the percentage of sucrose preference between the control and 6-OHDA groups in the first week post-lesion **(Figure 26A)**. The 6-OHDA group exhibited a reduced sucrose preference compared to the control group, t(14)=2.507, p=0.013, which may indicate hedonic deficits. The effect size, as measured by Cohen's d, was d = 1.25 (95% CI for Cohen's d: 0.18, 2.3).

To evaluate whether the groups also differed in sucrose preference in the third week post-lesion, a one-tailed Mann-Whitney U test was performed **(Figure 26B)**. At this later time-point, the results showed no significant difference in sucrose preference between control and 6-OHDA groups, U=20, p=.12.

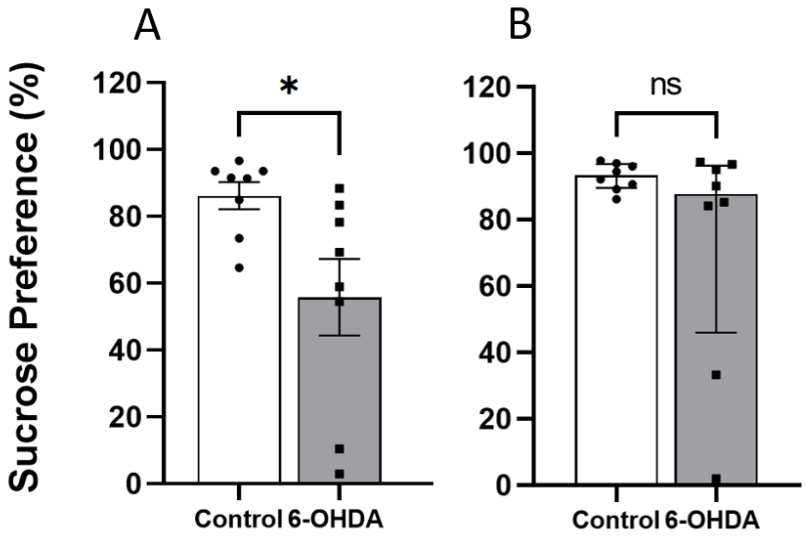


Figure 26. Sucrose preference assessed in the SPT. (**A**) Percentage of sucrose preference 1-week post-lesion. Data is expressed as mean ± SEM from 8 animals of the control and 8 from 6-OHDA group. Student's t-test, one-tailed. *P ≤ 0.05; ns: P > 0.05. (**B**) Percentage of sucrose preference 3 weeks post-surgery. The distribution of data is expressed as median and interquartile from 8 animals of the control and 8 from 6-OHDA group. Mann Whitney test, one-tailed; ns: P > 0.05.

4.2.4 Change in social behaviour in the 3rd week following lesion in the 6-OHDA group

The social interaction test was used to measure potential changes in social behaviour among the animals in the third week post-lesion. This was done to identify any lack of social interest or behaviour similar to social withdrawal, which may be associated with a depressive-like phenotype. A one-tailed two-sample t-test, was conducted to compare the total time spent in behaviours associated with social interest/interaction (i.e sniffing, playing, allogrooming, approaching and following, etc) between the control and 6-OHDA groups. The results indicate the 6-OHDA group exhibited a significant reduction in total time engaging in social interaction when compared to the control group (t(28)=2.769, p=.005; **Figure 27**). The effect size, as measured by Cohen's d, was d = 1.03 (95% CI for Cohen's d: 0.26, 1.81).

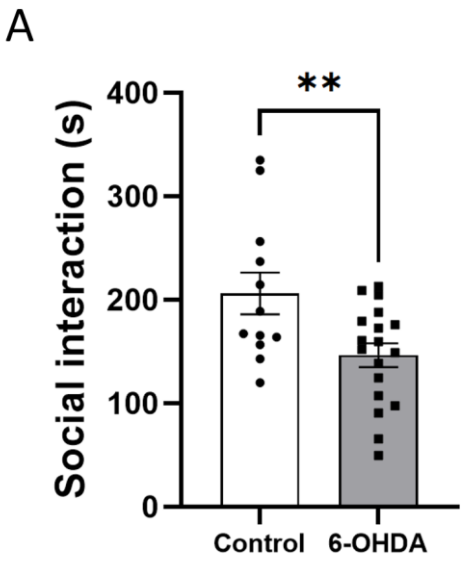


Figure 27. Time spent in social interaction in the social interaction test. Data are expressed as mean ± SEM from 12 animals of the control group and 18 from 6-OHDA. Student's t-test, one tailed ** P ≤ 0.01.

We also examined the time spent by each animal on other behaviours. No significant differences between groups were observed for time spent in non-social behaviours, including cage exploration (two-sample t-test; t(28)=0.0004, p=.1, **figure 28A**), self-grooming (Mann Whitney test; U=93, p=.55, **figure 28B**) and quiet-awake (Mann Whitney test; U=78.5, p=.22, **figure 28C**); in other behaviours associated with social avoidance (two-sample t-test; t(28)=0.507, p=.62, **figure 28D**); nor in time spent in submissive/passive behaviour (Mann Whitney test, one-tailed; U=96, p=.63, **figure 28E**). Only a few animals from both groups displayed aggressive behaviours, 4 from

the control group and 6 from the 6-OHDA group. Collectively, these results suggest that the observed reduction in social interaction in the 6-OHDA group was not a consequence of an increase in time spent by these animals in any other specific behaviour analysed here.

A pair of animals from control group were not marked with black ink prior to the experiment. As a result, they could not be distinguished in the video and were excluded from the analysis.

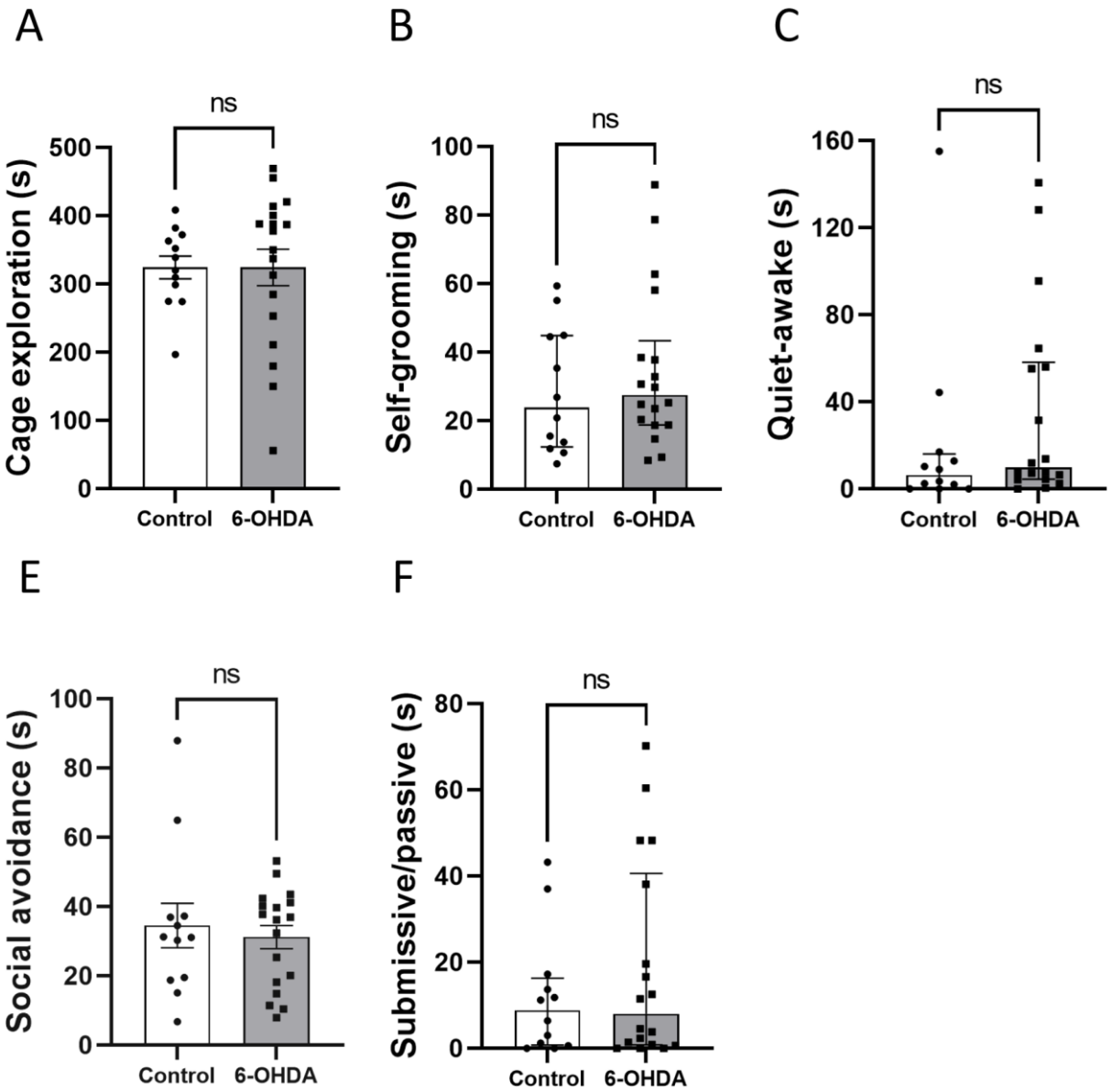


Figure 28. Time spent in other behaviours in the social interaction test. (A) (B) Data are expressed as mean ± SEM from 12 animals of the control group and 18 from 6-OHDA. Student's t-test; ns: P > 0.05. **(C) (D) (E)** Data expressed as median and interquartile from 8 animals of the control and 8 from 6-OHDA group. Mann Whitney test; ns: P > 0.05.

5 DISCUSSION

In this study, we investigated whether the loss of dopaminergic neurons in the SNc and their terminals within the dorsal striatal region, which make up this pathway, would be sufficient to induce non-motor symptoms in an animal model of PD. Specifically, we tested the animals for anhedonia and social dysfunction. To achieve this, we induced a partial lesion in the nigrostriatal pathway of rats by bilaterally infusing 6-OHDA into the dorsal striatum, following a previously described protocol. Our results indicate that the lesion condition was associated with changes in the animals' social behaviour, as evidenced by a decrease in the time spent interacting with their partner during the social interaction test. Hedonic deficits were observed in the first week but subsided by the third week. No changes in locomotion that could affect the results of the social interaction test were observed in the 6-OHDA group.

5.1 Motor function and DA depletion

Dysfunction of the nigrostriatal pathway is associated with some of the classical motor symptoms of PD (OBESO et al., 2014). However, these symptoms do not manifest immediately. Instead, there is an initial period characterised by the presence of only non-motor alterations (HEINZEL et al., 2019). It is hypothesised that compensatory mechanisms come into play in this context. These might include elevated DA biosynthesis and release by the remaining dopaminergic neurons, as well as increased postsynaptic DA receptor density and/or sensitivity. Collectively, these mechanisms may help to maintain motor functions and only as the degenerative process in the nigrostriatal system progresses significantly do motor dysfunctions become prominent (DEUMENS; BLOKLAND; PRICKAERTS, 2002).

The methodology employed in this study to create the PD model has been previously reported to cause a partial loss of DA cells and cell terminals in the SNc and CPu (BRANCHI et al., 2008; MATHEUS et al., 2016b). Specifically, it has been previously observed that the lesion induces a stable partial reduction in TH immunoreactivity in the dorsal striatum (around 65%) and a progressive decrease in the SNc — from approximately 50% in the first week to 68% in the third week post-lesion (MATHEUS et al., 2016b). This level of depletion has been shown to be

insufficient to induce gross motor impairment in rodents, accurately mirroring the very early or even prodromal stages of the disease.

Accordingly, this study did not reveal any changes in gait patterns in the footprint test among the 6-OHDA group. It's conceivable that subtle gait changes might have gone undetected, since manual assessment, as employed here, may have some limitations compared to automated gait analysis. However, such subtle changes are unlikely to have a significant impact on locomotion (BONITO-OLIVA; MASINI; FISONE, 2014). No effects of lesion on distance travelled and average speed in the OFT were found in this study either. This aligns with the results obtained from the footprint test and is consistent with previous reports (BRANCHI et al., 2008; MARQUES et al., 2019; MATHEUS et al., 2016b). Importantly, these results suggest that the 6-OHDA group did not exhibit any motor dysfunction that could confound the results in the social interaction test.

Moreover, the lack of observable changes in the footprint and OFT in the fourth week post-lesion, suggests that the lesion has not progressed sufficiently to cause motor symptoms at this time point either. Indeed, a study that infused a similar amount of 6-OHDA (10.5 µg/3 µl) in the same striatal coordinates, only observed motor changes in the OFT 9 weeks following the lesion (BRANCHI et al., 2008). Conversely, those that infused higher concentration of 6-OHDA (20 µg/3 µl), already reported motor changes in the third and fourth weeks, concomitantly with greater TH depletion (approximately 80%) (HERRERA et al., 2020; MATHEUS et al., 2016a).

5.2 Depressive-like behaviours and social interaction dysfunction

Depression is a complex diagnostic concept encompassing a range of different symptoms; not all of which can be directly observed or measured in other species in the same way as in humans. Nevertheless, it is reasonable to assume that certain features of depression, both in terms of neurobiology and symptomatology, may be conserved across species. In the context of rodent models, a 'depressive-like phenotype' has been defined, which includes behaviours associated with depressive symptoms observed in the clinical setting, including: anhedonia, apathy, despair and hopelessness, social withdrawal and even anxiety, cognitive impairment and sleep changes (VON MÜCKE-HEIM et al., 2023).

In this study, the animals were assessed for a depressive-like phenotype with a focus on anhedonia and social dysfunction, to investigate whether this model presents with behavioural changes akin to the depressive symptoms observed in PD patients.

The behavioural changes observed in this study and their possible underlying neuropathological mechanisms, are discussed in this section by taking into consideration findings from both other animal studies and clinical studies with early-stage PD patients. For the studies using animal models, a focus was given for those that generated a partial lesion in the nigrostriatal pathway by bilateral infusion of 6-OHDA either in the dorsal striatum or the SN.

5.2.1 Hedonic deficits

Anhedonia is generally regarded as a central symptom of depression in PD patients, though it has also been linked with apathy as well as regarded as a specific mood disorder associated with decreased DA signalling in the reward pathway (ASSOGNA et al., 2011; LOAS et al., 2014). In rodents, hedonic deficits are often assessed using the sucrose preference test (SPT). Specifically, this test is thought to assess to the capability of experiencing reward or pleasure in a pleasurable activity (i.e., drinking the sweeten solution), known as ‘liking’ or ‘consummatory behaviour’ (DER-AVAKIAN; MARKOU, 2012; MAGNARD et al., 2016).

In this study, animals that received 6-OHDA infusions apparently exhibited insensitivity to the rewarding properties of the sucrose solution in the first week, evidenced by decreased percentage of consumption. However, this was no longer observed in the third week post-lesion. Interestingly, this same temporal pattern was previously observed in a study by Matheus et al. (2016). Furthermore, in other studies that performed 6-OHDA infusions in the same striatal coordinates, hedonic deficits were also observed in the first week (MARQUES et al., 2019), but not at a later time point (7 weeks post-lesion) (BRANCHI et al., 2008).

Previous studies using rat models of PD with bilateral infusion of 6-OHDA into a more ventrolateral region of the dorsal striatum – relative to the dorsolateral infusion carried out in this study – have also reported reduced sucrose preference in the first week post-lesion (SILVA et al., 2016; TADAIESKY et al., 2008). However, in the study by Silva et al., (2016) the changes in the SPT persisted at two and three weeks after surgery. Moreover, in 6-OHDA PD models with bilateral infusions in the SN, this

behaviour change has also been identified at specific time points following lesion, although the results are inconsistent: few studies observed a decreased preference in the SPT at 7 and 21 days, but not at 14 days post-lesion (SANTIAGO et al., 2010, 2014); while in others, it was observed at 14 and 21 days post-lesion but not 7 days (GRADOWSKI et al., 2013; ILKIW et al., 2019). Additionally, change in sucrose preference has also been reported consistently throughout all 6 weeks following the lesion (VECCHIA et al., 2018, 2021). Overall, this variability raises questions about whether and how the rodent's brain response differs to infusions into different sites within the nigrostriatal pathway and whether this difference might be associated with the observed behavioural changes. Moreover, differences in protocols (i.e., sucrose concentration; previous food/water deprivation, etc) might influence the results and should also be taken into consideration.

Assessments of the neurobiological mechanisms of hedonic deficits have generally focused on the role of dopaminergic neurotransmission (DER-AVAKIAN; MARKOU, 2012). In animal models, a significant reduction in DA, and its metabolites — 3,4-dihydroxy-phenylacetic acid (DOPAC) and homovallinic acid (HVA) — have been reported, already in the first week following partial lesion of the nigrostriatal system (SANTIAGO et al., 2014; SILVA et al., 2016; TADAIESKY et al., 2008). Interestingly, Silva et al., (2016) observed that reduction in striatal DOPAC persisted throughout the second and third week, as did changes in sucrose preference in this study; while Santiago et al., (2014) reported normal levels of DOPAC specifically at 14 days post-lesion (not at 7 or 21 days), the same time point where hedonic deficits were not observed. A positive correlation between DA levels and sucrose preference has also been found (SANTIAGO et al., 2014), further suggesting a role for this neurotransmitter system in hedonic deficits. Nonetheless, analyses of the DA striatum levels alone seem insufficient to explain the difference in temporal presentation of this symptom. More specific findings come from the study by Matheus et al (2016), where they observed that hedonic deficits at 7 days post-lesion were associated with a selective increase in the levels of both dopamine receptors (D1 and D2) in the striatum, which could constitute a compensatory response by the striatum to the decreased levels of DA. They also carried out electrophysiological recordings, which revealed a disruption in presynaptic dopaminergic control and increased dopaminergic sensitivity in the striatum. Importantly, such responses were not observed 21 days after the

injection of 6-OHDA, when the hedonic deficits were no longer apparent (MATHEUS et al., 2016b).

Although the data is inconsistent, a few studies suggest that the prevalence of anhedonia tends to be lower in the early stages relative to the advanced stages of the disease and that it is linked to more severe depression and motor symptoms (LOAS et al., 2014). By taking this assumption into account, it seems counterintuitive that hedonic deficits would be present only at an earlier time-point after lesion. However, when considering the temporal presentation of symptoms in the lesioned animal, it is crucial to consider that the 6-OHDA model has a limitation when it comes to accurately reflecting the progressive, age-dependent changes observed in PD (DEUMENS; BLOKLAND; PRICKAERTS, 2002; EL-GAMAL et al., 2021; PENTTINEN et al., 2016). While striatal lesions in this model do result in a progressive cell loss in the SNc within the first weeks (MATHEUS et al., 2016b), the acute nature of the lesion's damage could lead to more severe dysfunction in the striatum in the short-term following 6-OHDA infusion, which may subsequently improve to some extent as compensatory mechanisms come into play (DEUMENS; BLOKLAND; PRICKAERTS, 2002). This raises the question of whether the appearance of hedonic deficits in this model, specifically in the first week, could be due to potentially more severe striatal dysfunction in response to the lesion at this time point, before the system has had a chance to adapt.

Regions of the reward system, encompassing the NAc and orbitofrontal cortex, as well as the opioid system have been suggested to particularly contribute to the perception of pleasure, as assessed by the SPT (DER-AVAKIAN; MARKOU, 2012). However, a specific role for dysfunction in these regions/systems in this PD model is not clear. Considering the common infusion site (nigrostriatal pathway), most studies only report a reduction in DA cell and cells fibres in the SNc and dorsal striatum, making it unclear whether the 6-OHDA lesion-related pathology/dysfunction extends to mesolimbic system; and, importantly, whether there is a temporal association in accordance with the presentation of hedonic deficits. Interestingly, direct infusion of 6-OHDA in the VTA of rats in a previous study, which led to partial depletion of TH in both the VTA and NAc regions, was not sufficient to reduce the level of sucrose preference three weeks following infusion, suggesting that dysfunction of this region alone does not seem to induce hedonic deficits (DRUI et al., 2014). Nonetheless, whether and how the NAc is affected and its possible role in the lesion progression is

worth considering as it has been suggested that, despite being functionally independent, different regions within the striatum (i.e. NAc and dorsolateral) can compensate for one another when there is a disruption of function (BURTON; NAKAMURA; ROESCH, 2015).

Specific changes in other, extra-striatal, regions, such as the mPFC and hippocampus, have also been previously reported following 6-OHDA infusion (MATHEUS et al., 2016b; SANTIAGO et al., 2014; TADAIESKY et al., 2008). Such dysfunctions in remote regions beyond the nigrostriatal pathway are expected following the lesion, considering the complexity of the circuitry formed by the striatum along with all the BG nuclei (OBESO et al., 2014); and the direct modulatory role of DA in various brain regions beside the striatum and BG circuitry (DI DOMENICO; MAPELLI, 2023; EDELMANN; LESSMANN, 2018; TESSITORE et al., 2002). Evidence from pharmacological intervention in a model based on striatal lesions have suggested a possible contribution of hippocampal mitochondrial membrane potential disruption to the behavioural changes in the SPT (MARQUES et al., 2019). Moreover, a positive correlation between hippocampal 5-HT levels and sucrose preference in the first week post-lesion was also found in a model generated by 6-OHDA infusion into the SNc (SANTIAGO et al., 2014). Nonetheless, a few studies observed changes in the hippocampus and PFC following striatum lesion (i.e. reduced monoamine levels and DAT expression) only at a later time-point following the striatal lesions (MATHEUS et al., 2016b; TADAIESKY et al., 2008); and there is still limited evidence overall for a direct role of dysfunction in these regions in the observed hedonic deficits in the first week.

5.2.2 Social withdrawal

Although hedonic deficits do not seem to persist beyond the first week following bilateral 6-OHDA infusion, at the third week post-lesion, animals infused with 6-OHDA in this study showed a behaviour change, which may be comparable to the symptom of social withdrawal seen in PD. Namely, they spent less time engaging in social behaviours in the social interaction test. This is in line with previous findings by Matheus et al (2016), using the same model, and other studies involving bilateral 6-OHDA lesions in the SN (CHIU et al., 2015; DRUI et al., 2014).

However, these results have not always been consistently replicated, even in studies with infusions at the same striatal coordinates. For instance, Marques et al

(2019) observed no change in social interaction and Chen et al (2011) reported a somewhat opposite behaviour, in which the 6-OHDA animals spent more time investigating their partner, which the authors associated with anxious behaviour. Notably, this latter conflicting result may be attributed to changes in the test protocol, as the authors indicated that the rats were not habituated to the apparatus and were tested under bright lighting conditions (CHEN et al., 2011). Additionally, another study observed reduced offensive behaviour in 6-OHDA-infused animals, but increased propensity to interact socially, although the assessment was made at a later time point (7 weeks post-lesion) (BRANCHI et al., 2008). The inconsistency of results may be attributed to differences in behavioural categorisation and approaches to quantifying the animal's behaviour. Even when the same pre-defined behavioural categories/ethograms are used in different studies, the complexity of its quantifying method (i.e., correctly identifying and categorising the different possible behaviours), could make this test more susceptible to subjective influence/bias.

Social withdrawal is often associated with depressive symptoms in PD (AHN; SPRINGER; GIBSON, 2022). Indeed, depressive disorders are often accompanied by a socioemotional change, regardless of the depression phenotype, whether it includes the symptom of anhedonia or is predominantly characterised by sadness**.** As such, reduction or non-beneficial changes in social behaviours have been regarded as a proxy for the 'depressive-like' phenotype in rodents — even though it can also occur independently of other depressive symptoms (ELMER; STADTFELD, 2020; VON MÜCKE-HEIM et al., 2023). Interestingly, another commonly assessed depressive-like behaviour, namely behaviour despair or learned hopelessness, has also been reported at the same time-point (approximately three to six weeks) following bilateral lesions in the dorsal striatum. This behaviour is often inferred by increased immobility in the forced swimming (FST) or tail suspension (TST) tests (BONITO-OLIVA; MASINI; FISONE, 2014; BRANCHI et al., 2008; MARQUES et al., 2019; MATHEUS et al., 2016b). Furthermore, treatment with commonly prescribed antidepressants, fluoxetine and bupropion, have been found to effectively counteract the decrease in social interaction and behavioural despair in animals infused with 6-OHDA (MATHEUS et al., 2016b).

The mechanisms underlying depression and social dysfunction are likely to overlap. The pathological process of many neuropsychiatric disorders, including depression, as well as specific external and internal factors, such as stress, infection

and genetics, are all believed to induce changes in the ‘social decision-making’ neural network that may result in the common manifestation of social withdrawal (IKE et al., 2020). Dysfunction of regions and systems which are part of the social network, including extra-striatal regions modulated by DA inputs (such as the amygdala and the hippocampus) and the frontal-striatal circuit, have been identified and suggested to underline the depressive-like behaviour in rodents — including changes in social behaviours (BÁEZ-MENDOZA; SCHULTZ, 2013; CHEN et al., 2011; DRUI et al., 2014; FETCHO et al., 2023; MATHEUS et al., 2016b).

5.3 Possible pathological mechanisms underlying symptoms

5.3.1 Is behavioural change linked to cortico-striatal circuit dysfunction?

The medial prefrontal cortex (mPFC) has been identified as a vital neural substrate for exerting control over social interaction in both humans and rodents, including behaviour of social approach (BICKS et al., 2015; KINGSBURY et al., 2019; KO, 2017; LEE et al., 2016). PFC activity is modulated by DA activity, which is thought to contribute to optimal regulation of the excitation/inhibition balance in the PFC, by acting on both pyramidal neurons and inhibitory interneurons (DI DOMENICO; MAPELLI, 2023). Both reduced and increased activity in the mPFC appear capable of diminishing sociability in rodents and DA modulation of the mPFC might have a key role (PORCELLI et al., 2019; SOTOYAMA et al., 2022). Transient dopamine release in the dorsomedial PFC is triggered by social stimuli and is positively correlated with the duration of social interactions in rats. Notably, this effect is dependent on the pattern of DA activity, as a sustained increase has been shown to have an opposite, negative, correlation with sociability in rats in the same study (SOTOYAMA et al., 2022).

At the specific time-point of 21 days following 6-OHDA infusion, when both reduced social interaction and behavioural despair were apparent, Matheus et al (2016) observed a reduction in DAT levels in both the striatum and the mPFC of 6-OHDA animals, as well as a decrease in dopamine-induced inhibition of excitatory transmission in the mPFC specifically (MATHEUS et al., 2016b). Other changes in the PFC have also been reported three weeks following bilateral infusion of 6-OHDA into the dorsal striatum and SN, including decreased monoamine levels — DA, NA and 5-

HT — reduced staining of TH as well as mitochondrial dysfunction (decrease in aldehyde dehydrogenase 2 enzyme) (HERRERA et al., 2020; MATHEUS et al., 2016a; TADAIESKY et al., 2008). Collectively, these findings suggest that dysfunction from nigrostriatal lesions extends to the frontal-striatal network, potentially providing a neural substrate for the behavioural changes observed in this study.

Reduction in social interaction time of the 6-OHDA group could reflect a lack of motivation or reduced goal-directed behaviour (i.e. apathy-like behaviour), which is mediated by cortico-striatal loops and DA activity (DE WAELE; CRAS; CROSIERS, 2022; MAGNARD et al., 2016). It has been suggested that dysregulation of the frontal-striatal networks is implicated in both motivational disorders, such as apathy, and cognitive impairments in executive function in PD patients. Namely, patients exhibiting apathy displayed diminished functional connectivity primarily within left-sided circuits and involving limbic striatal, and frontal regions, relative to both healthy controls and non-apathetic patients (BAGGIO et al., 2015); and an association between apathy and dysexecutive syndrome, as well as with anhedonia and social cognition, has been reported in patients in the early stages (SANTANGELO et al., 2012, 2015). Moreover, it has been previously suggested that social withdrawal may result from a lack of motivation to actively seek out social engagement, rather than the absence of pleasure in participating in social activity (KRACH, 2010).

DA appears to be indeed more involved in aspects of the reward process related to ‘wanting’ or anticipation for the reward; motivation (the drive to attain the reward) or effort (the capacity to maintain the necessary energy expenditure to attain it), rather than the ‘liking’ or hedonic response per se (BERRIDGE, 2007; RIZVI et al., 2016). DA output from the nigrostriatal system may play a role particularly in motivation or effort, as evidenced in a study by Drui et al (2014). The authors observed a reduction in both social interaction and sucrose consumption of SNc-lesioned rats, however, the latter was only observed when they employed an operant self-administration procedure. This procedure required rodents to press a lever at an exponential number of times for an amount of sucrose solution to be delivered, in other words, it involved an extra effort for the reward as compared to simply choosing between the two bottles to drink from. Importantly, the reduction in instrumental responses of the lesioned animals was not attributed to motor deficits, impairment in instrumental learning, or insensitivity to the rewarding properties of SPT (as no change in sucrose preference was observed in the two-bottle paradigm of the SPT). Lack of response to the sucrose solution as well as

decreased sociability, were therefore supposed to be reminiscent of dysfunction in the preparatory component of motivated behaviour (DRUI et al., 2014; MAGNARD et al., 2016).

5.3.2 Is the hippocampal hypofunction a contributing factor?

When considering social changes and mood disorders, attention should also be given to the limbic system. The hippocampus in particular has become a focal point in depression research, with the neuroplasticity and neurogenesis hypotheses highlighting the morphological, molecular, and functional changes in this brain region associated with depressive symptoms (BOKU et al., 2018). Such hippocampal changes are often related to the maladaptive response to stress or dysregulation of the HPA axis, and the state of chronic inflammation, which are thought to induce many signs of depression, among which is social withdrawal (HASSAMAL, 2023; PORCELLI et al., 2019).

In drug-naïve, early, PD patients, higher depressive symptoms were linked to reduced hippocampal subfield CA2–CA3 volumes (GYÖRFI et al., 2017). Impaired hippocampal neurogenesis has also gained attention for its possible implication in the pathophysiology of, and as a promising therapeutic target for, PD-associated depression (CALABRESI et al., 2013; LIM; BANG; CHOI, 2018). Additionally, concerning the hippocampus' role in social behaviour specifically, it has been observed, for instance, that chronic dysfunction of NMDA receptors in the dorsal CA3 region in mice decreases behaviour of social approach (FINLAY et al., 2015) and optogenetic manipulation of projections from the basolateral amygdala to the ventral hippocampus also results in changes in social interaction levels (FELIX-ORTIZ; TYE, 2014).

Interestingly, several pathobiological changes within the hippocampus linked to depression have also been observed following partial bilateral lesions of the nigrostriatal pathway. Namely, decreased neurogenesis (CHIU et al., 2015) and impaired long-term potentiation in the dentate gyrus (BONITO-OLIVA et al., 2014; HERRERA et al., 2020); as well as decreased catecholamines levels (BONITO-OLIVA et al., 2014; BRAUN et al., 2012), mitochondrial dysfunction (HERRERA et al., 2020; MARQUES et al., 2019) and changes in astrocytes reactivity (HERRERA et al., 2020).

Of note, when considering these extra-striatal changes, it's crucial to account for whether the animals were pretreated with the noradrenaline uptake inhibitor,

desipramine. Pretreatment with desipramine has been shown to reduce the decrease in TH immunoreactivity induced by striatal infusion of 6-OHDA in the hippocampus but not in the striatum. It also prevents the effect of 6-OHDA on NA, but not on DA levels, in both regions (BONITO-OLIVA et al., 2014; TADAIESKY et al., 2008). Moreover, many of these hippocampal changes have been investigated in association with cognitive impairments in memory also observed in this model, with less focus given to this structure on mood disorders (BONITO-OLIVA et al., 2014; HERRERA et al., 2020; MATHEUS et al., 2016a).

The hippocampus receives DA innervation mainly from the VTA, although other sources have been suggested, which include the Locus coeruleus (LC), NAc and SNc (EDELMANN; LESSMANN, 2018). DA is believed to modulate long-term potentiation and synaptic plasticity in different regions of the hippocampus and to play a role in the hippocampal-related functions of memory and emotional regulation (CALABRESI et al., 2013; EDELMANN; LESSMANN, 2018). However, whether and how nigrostriatal DA dysfunction observed in PD patients and PD models might lead to hippocampal impairment is not entirely clear. Besides changes in DA transmission, inflammatory reactions and changes in neurotrophic factors may play a role (CALABRESI et al., 2013; LIM; BANG; CHOI, 2018).

The dopamine D2/D3 receptor agonist pramipexole has shown positive effects on motor and depressive symptoms in PD patients, including anti-anhedonic properties (ASSOGNA et al., 2011; SEPPI et al., 2019). In PD animal models, pramipexole has also been effective in counteracting depressive-like behaviours evidenced in the SI, FST and SPT tests (BERGHAUZEN-MACIEJEWSKA et al., 2014; BONITO-OLIVA; MASINI; FISONE, 2014; CHIU et al., 2015). Interestingly, its antidepressant activity has been associated with the normalisation of the decreased neurogenesis in the hippocampal dentate gyrus caused by a bilateral 6-OHDA lesion in the SN (CHIU et al., 2015).

Treatment with the nucleoside guanosine (GUO), which was found to protect the hippocampus against the mitochondrial membrane potential disruption induced by striatal lesion, significantly attenuated both hedonic deficits and behaviour despair in the 6-OHDA animals (MARQUES et al., 2019). Hedonic as well as cognitive deficits were also contracted by ketamine in a model generated by partial bilateral lesion of the SN (VECCHIA et al., 2018, 2021). Low-dose Ketamine has gained recognition as a fast-acting antidepressant in treatment-resistant individuals with depression (BOKU et

al., 2018) and as a promising intervention against levodopa-induced dyskinesia in PD (PHARMATHER HOLDINGS LTD., 2021). Its antidepressant mechanism are not fully understood, but have been associated with neuroplastic mechanisms, marked by an increase in BDNF mRNA expression both in the prefrontal cortex and the hippocampus, and changes in sleep EEG (BOKU et al., 2018; DUNCAN; ZARATE, 2013).

Overall, biochemical analyses and findings from pharmacological interventions strongly suggest the involvement of both striatal and extra-striatal regions in depressive-like behaviours observed in this model. It can be speculated that in the case of this PD model, and possibly in PD patients as well, these dysfunctions are, at least in part, a consequence of the dysfunction of the nigrostriatal pathway. The PFC and hippocampus, seem to be particularly – though not exclusively – involved in the observed non-motor symptom of social withdrawal.

5.3 Limitations of the study and future directions

One important limitation of this study is that, at the time of writing, there is still no data from the immunohistochemical analyses confirming the extent of DA loss between animals and the proportional loss between the two hemispheres. Instead, potential outliers were identified based on observations of their behaviours during the post-operative period and in both motor and nonmotor tests. Moreover, no biochemical analyses were conducted to elucidate the mechanisms underlying the depressive-like behaviours observed in this study. The discussion was therefore largely speculative, based on previous evidence from other studies with, the same or similar, 6-OHDA models. This study results suggest a role of the nigrostriatal dysfunction in non-motor symptoms in PD. Now more studies are needed to better characterise which neurobiological changes in the level of synapses, cells, and circuits, in both the nigrostriatal pathway and extra striatal structures, relate to different behavioural changes observed in this model, at different time-points post-lesion. Different interventions, with promising clinical applicability, for early-stage PD should also be investigated in future studies.

Concerning this study's methodology, another important limitation is the use of male rats exclusively. In the literature, there is a preference for using males in research **(see Appendix B)**. However, it's worth noting that studies with 6-OHDA models which included both biological sexes have found differences in the evaluated behavioural parameters, and a potential protective effect of sex hormones has even been suggested (BETANCOURT et al., 2017; SOMENSI et al., 2021). Differences in the prevalence of PD and depressive symptoms in male and female PD patients have also been reported (CONG et al., 2022; POEWE et al., 2017), which further underscore the importance of considering both sexes in research. Therefore, future studies in our laboratory will aim to investigate whether females exhibit a similar depressive-like phenotype using this model.

Importantly, this study's results are in line with previous findings from studies that used the same model and a similar study design, indicating its consistency and reproducibility. Notably, the 6-OHDA group exhibited the same behavioural changes and temporal presentation as previously reported. Therefore, despite its limitations, this study's findings are valuable in addressing the major issue in the current research landscape of lack of reproducibility and the necessity of an appropriate and reliable animal model for investigating nonmotor changes in PD.

Future studies can use other experimental paradigms to assess social dysfunction and depressive-like behaviours more thoroughly in this same animal model to better characterise these nonmotor symptoms. For instance, evaluating sucrose preference using the operant sucrose self-administration test could detect possible changes in aspects of reward processing, such as motivation or effort, as previously observed in a different model (DRUI et al., 2014). Social approach/avoidance behaviour could also be tested using the social preference test with the three-chamber apparatus for further assessment of possible differences in preference for social vs non-social stimuli, while excluding the influence of the other animal's behaviour (IKE et al., 2020).

Beside further assessment of depressive-like behaviours, the next steps should also involve investigation of other non-motor symptoms in this animal model that might be related to depressive symptoms. In particular, sleep and circadian disturbances are important nonmotor manifestations of PD, which were already described by James Parkinson in 1817, but have generally remained underrecognized (HUNT et al., 2022; MCDONALD et al., 2018).

5.4. Future Perspectives

5.4.1 Targeting the BDNF/TrkB Pathway

For future investigations, attention should be specially given to the role of neurotrophic factors, such as BDNF, which have been recognised as promising disease-modifying agents and therapeutic targets for both depression and neurodegenerative diseases, including PD (NAGAHARA; TUSZYNSKI, 2011; PALASZ et al., 2020; YANG et al., 2020). Their potential use for intervention stems from their broad action in neuroplasticity, maintaining neuronal activity and neuroprotective properties. Specifically, the neuroprotective effects of physical exercise in both PD patients and animal models, including DA neuronal survival and/or improvements in motor and cognitive symptoms, have been attributed to the accompanying enhancement of BDNF levels (PALASZ et al., 2020; REAL et al., 2013). Moreover, augmentation of BDNF in the nigrostriatal pathway — through gene delivery into the SN; transplantation of epigenetically induced BDNF-secreting human mesenchymal stem cells; or direct BDNF infusion — has been sufficient to counteract the MPTP-induced DA neuron loss; increase innervation of DA fibres in 6-OHDA lesioned rodents; and/or to attenuate the accompanying motor deficits as well as cognitive impairment in this animal model (CHANG; WANG, 2021; PALASZ et al., 2020).

Regarding treatment of depressive symptoms, although the therapeutic properties of Ketamine are indeed promising, it has important limitations for clinical use (i.e., inducing psychotic symptoms and abuse). Therefore, a preferable approach is to substitute its use with new treatments developed based on ketamine's specific antidepressant mechanisms (BOKU et al., 2018). Interestingly, both the fast-acting Ketamine and the typical, selective serotonin reuptake inhibitor, fluoxetine — which has been effective in counteracting both reduced social interaction and behaviour despair following striatal lesion (MATHEUS et al., 2016b) — have been suggested to exert their antidepressant effect via direct binding to the tyrosine kinase receptor 2 (TrkB), the binding site of BDNF's mature form (CASAROTTO et al., 2021; CASAROTTO; UMEMORI; CASTRÉN, 2022). Therefore, testing the effects of novel therapeutics specific to the BDNF/TrKB pathway (i.e. BDNF mimickers) or of augmentation of BDNF via infusion of AAV-BDNF, on nonmotor symptoms in early-

stage PD or PD animal models is a promising avenue of future research (HASSAMAL, 2023; NAGAHARA; TUSZYNSKI, 2011; PALASZ et al., 2020).

5.4.2 Understanding sleep's role in Parkinson's Disease

Sleep is a crucial physiological process essential for cognitive function and overall brain health. It is widely considered to play a disease-modifying role in PD, as it appears to have a strong association with disease progression and the worsening of symptoms (LATREILLE et al., 2015; MAGGI et al., 2023; YANG et al., 2021; ZAHED et al., 2021). Although the number of studies investigating changes in the sleep/wake cycle is relatively small in comparison to other nonmotor symptoms, a few animal studies using 6-OHDA have indeed identified circadian perturbations (HUNT et al., 2022; MASINI et al., 2017; REQUEJO et al., 2020) and changes in sleep, similar to those observed in PD patients (CIRIC et al., 2019; VO et al., 2014; ZHURAKOVSKAYA et al., 2019).

Sleep and depression have a complex, bidirectional, relationship, with sleep disturbances being recognised as both symptoms and risk factors for this psychiatric disorder (see (PANDI-PERUMAL et al., 2020). Associations between depression and sleep have also been reported in the context of PD. For instance, excessive daytime sleepiness (EDS) is often observed alongside depression in PD patients (BERG et al., 2021; CONG et al., 2022); and overall, the presence of comorbid sleep disorders, such as EDS, RBD and insomnia, is linked to more depressive symptoms in the course of PD as well as poorer cognitive function (BERG et al., 2021; MA et al., 2022; ZHOU et al., 2023). Moreover, some interesting findings following pharmacological interventions in animal studies have hinted at an association between sleep disturbances and other nonmotor signs. Namely, in a mouse model of PD with a unilateral lesion in the MFB, pramipexole (a dopamine receptor agonist effective in treating depressive symptoms in PD), was found to also counteract EDS (MEDEIROS et al., 2019). Additionally, treatment with thioperamide, a selective H3R antagonist, was able to reverse the disruptions of endogenous circadian rhythms following bilateral striatal lesion in mice as well as deficits in long-term recognition memory and anxiety-like behaviour (MASINI et al., 2017).

To the best of our knowledge, disturbances of the sleep/wake cycle have not yet been investigated in this model of dorsal striatal lesion in rats, as employed in this

study; and questions persist regarding whether there is a casual relationship between sleep and depressive symptoms in PD. Therefore, future studies in our laboratory will also aim to explore whether this model displays similar dysfunction in circadian rhythms and pathological EEG changes during sleep, and its possible correlations with the observed depressive-like behaviours. Investigating the potential association between the observed symptoms in PD models, along with electrophysiological and molecular analyses and chemogenetic and/or pharmacological interventions, could provide valuable insights into the interplay between non-motor symptoms and the development of better therapeutic interventions.

6 CONCLUSION

This study was carried out to assess whether a model of early PD, based on bilateral infusion of 6-OHDA into the dorsal striatum of rats, would lead to a depressive-like phenotype comparable to what is observed in PD patients, with a focus on behaviours suggestive of anhedonia and social dysfunction.

The results indicated that three weeks after the lesion, the 6-OHDA animals showed a decrease in time spent in social interactions, which could reflect social withdrawal, a symptom commonly associated with depression in PD patients. However, no hedonic deficits were observed at this same time point. Such deficits were only apparent one week after surgery. Additionally, the animals did not present with any motor impairments, which suggests that the resulting lesion within the nigrostriatal region of 6-OHDA animals was indeed partial, as reported by previous studies.

In the context of the multifaceted PD, early impairment of the nigrostriatal pathway is not likely to be the sole cause of the observed symptoms. However, considering that the nigrostriatal system is the primary site of lesion in this animal model, the results suggest its implication in social withdrawal, and possibly other depressive symptoms. It can be speculated that loss of DA cells and fibres in this region may lead to dysfunction extending to other regions commonly associated with mood disorders and social dysfunction (i.e. PFC and hippocampus). Further research is needed to fully understand the relationship between striatal impairment and neuropsychiatric symptoms as well as how they relate to other non-motor changes; and the effects of promising interventions aiming to treat such symptoms.

REFERENCES

AARSLAND, D. et al. Depression in Parkinson disease—epidemiology, mechanisms and management. **Nature Reviews Neurology**, v. 8, n. 1, p. 35–47, jan. 2012.

AARSLAND, D. et al. Parkinson disease-associated cognitive impairment. **Nature Reviews Disease Primers**, v. 7, n. 1, p. 47, 1 jul. 2021.

AARSLAND, D.; MARSH, L.; SCHRAG, A. Neuropsychiatric symptoms in Parkinson's disease. **Movement Disorders**, v. 24, n. 15, p. 2175–2186, 15 nov. 2009.

AHN, S.; SPRINGER, K.; GIBSON, J. S. Social withdrawal in Parkinson's disease: A scoping review. **Geriatric Nursing**, v. 48, p. 258–268, nov. 2022.

ALBIN, R. L.; YOUNG, A. B.; PENNEY, J. B. The functional anatomy of basal ganglia disorders. **Trends in Neurosciences**, v. 12, n. 10, p. 366–375, 1 jan. 1989.

AMERICAN PSYCHIATRIC ASSOCIATION; AMERICAN PSYCHIATRIC ASSOCIATION (EDS.). **Diagnostic and statistical manual of mental disorders: DSM-5**. 5th ed ed. Washington, D.C: American Psychiatric Association, 2013.

ASSOGNA, F. et al. Anhedonia in Parkinson's disease: A systematic review of the literature. **Movement Disorders**, v. 26, n. 10, p. 1825–1834, 15 ago. 2011.

AVERBECK, B. B. et al. Estimates of Projection Overlap and Zones of Convergence within Frontal-Striatal Circuits. **Journal of Neuroscience**, v. 34, n. 29, p. 9497–9505, 16 jul. 2014.

BÁEZ-MENDOZA, R.; SCHULTZ, W. The role of the striatum in social behavior. **Frontiers in Neuroscience**, v. 7, 2013.

BAGGIO, H. C. et al. Resting-state frontostriatal functional connectivity in Parkinson's disease–related apathy. **Movement Disorders**, v. 30, n. 5, p. 671–679, 2015.

BELL, P. T. et al. Dopaminergic basis for impairments in functional connectivity across subdivisions of the striatum in Parkinson's disease. **Human Brain Mapping**, v. 36, n. 4, p. 1278–1291, abr. 2015.

BELVISI, D. et al. Risk factors of Parkinson disease. **Neurology**, v. 95, n. 18, p. e2500–e2508, 3 nov. 2020.

BERG, D. et al. Prodromal Parkinson disease subtypes — key to understanding heterogeneity. **Nature Reviews Neurology**, v. 17, n. 6, p. 349–361, jun. 2021.

BERGHAUZEN-MACIEJEWSKA, K. et al. Pramipexole but not imipramine or fluoxetine reverses the "depressive-like" behaviour in a rat model of preclinical stages of Parkinson's disease. **Behavioural Brain Research**, v. 271, p. 343–353, set. 2014.

BERGHAUZEN-MACIEJEWSKA, K. et al. Alterations of BDNF and trkB mRNA Expression in the 6-Hydroxydopamine-Induced Model of Preclinical Stages of Parkinson's Disease: An Influence of Chronic Pramipexole in Rats. **PLOS ONE**, v. 10, n. 3, p. e0117698, 4 mar. 2015.

BERRIDGE, K. C. The debate over dopamine's role in reward: the case for incentive salience. **Psychopharmacology**, v. 191, n. 3, p. 391–431, 2 mar. 2007.

BETANCOURT, E. et al. The impact of biological sex and sex hormones on cognition in a rat model of early, pre-motor Parkinson's disease. **Neuroscience**, v. 345, p. 297–314, mar. 2017.

BICKS, L. K. et al. Prefrontal Cortex and Social Cognition in Mouse and Man. **Frontiers in Psychology**, v. 6, 2015.

BOKU, S. et al. Neural basis of major depressive disorder: Beyond monoamine hypothesis. **Psychiatry and Clinical Neurosciences**, v. 72, n. 1, p. 3–12, 2018.

BONITO-OLIVA, A. et al. Cognitive Impairment and Dentate Gyrus Synaptic Dysfunction in Experimental Parkinsonism. **Biological Psychiatry**, v. 75, n. 9, p. 701–710, maio 2014.

BONITO-OLIVA, A.; MASINI, D.; FISONE, G. A mouse model of non-motor symptoms in Parkinson's disease: focus on pharmacological interventions targeting affective dysfunctions. **Frontiers in Behavioral Neuroscience**, v. 8, 27 ago. 2014.

BRANCHI, I. et al. Nonmotor symptoms in Parkinson's disease: Investigating early-phase onset of behavioral dysfunction in the 6-hydroxydopamine-lesioned rat model. **Journal of Neuroscience Research**, v. 86, n. 9, p. 2050–2061, jul. 2008.

BRAUN, A. A. et al. Dorsal striatal dopamine depletion impairs both allocentric and egocentric navigation in rats. **Neurobiology of Learning and Memory**, v. 97, n. 4, p. 402–408, maio 2012.

BURTON, A. C.; NAKAMURA, K.; ROESCH, M. R. From ventral-medial to dorsal-lateral striatum: Neural correlates of reward-guided decision-making. **Neurobiology of learning and memory**, v. 0, p. 51–59, jan. 2015.

CALABRESI, P. et al. New experimental and clinical links between the hippocampus and the dopaminergic system in Parkinson's disease. **The Lancet Neurology**, v. 12, n. 8, p. 811–821, ago. 2013.

CAMINITI, S. P. et al. Axonal damage and loss of connectivity in nigrostriatal and mesolimbic dopamine pathways in early Parkinson's disease. **NeuroImage: Clinical**, v. 14, p. 734–740, 2017.

CASAROTTO, P. C. et al. Antidepressant drugs act by directly binding to TRKB neurotrophin receptors. **Cell**, v. 184, n. 5, p. 1299, 3 mar. 2021.

CASAROTTO, P.; UMEMORI, J.; CASTRÉN, E. BDNF receptor TrkB as the mediator of the antidepressant drug action. **Frontiers in Molecular Neuroscience**, v. 15, 2022.

CHANG, E.; WANG, J. Brain-derived neurotrophic factor attenuates cognitive impairment and motor deficits in a mouse model of Parkinson's disease. **Brain and Behavior**, v. 11, n. 8, ago. 2021.

CHEN, L. et al. Alterations of emotion, cognition and firing activity of the basolateral nucleus of the amygdala after partial bilateral lesions of the nigrostriatal pathway in rats. **Brain Research Bulletin**, v. 85, n. 6, p. 329–338, jul. 2011.

CHIU, W.-H. et al. Long-term treatment with l-DOPA or pramipexole affects adult neurogenesis and corresponding non-motor behavior in a mouse model of Parkinson's disease. **Neuropharmacology**, v. 95, p. 367–376, ago. 2015.

CIRIC, J. et al. Alterations of Sleep and Sleep Oscillations in the Hemiparkinsonian Rat. **Frontiers in Neuroscience**, v. 13, p. 148, 25 fev. 2019.

CONG, S. et al. Prevalence and clinical aspects of depression in Parkinson's disease: A systematic review and meta-analysis of 129 studies. **Neuroscience & Biobehavioral Reviews**, v. 141, p. 104749, 1 out. 2022.

CZERNECKI, V. et al. Social cognitive impairment in early Parkinson's disease: A novel "mild impairment"? **Parkinsonism & Related Disorders**, v. 85, p. 117–121, abr. 2021.

DAN, R. et al. Separate neural representations of depression, anxiety and apathy in Parkinson's disease. **Scientific Reports**, v. 7, n. 1, p. 12164, 22 set. 2017.

DE WAELE, S.; CRAS, P.; CROSIERS, D. Apathy in Parkinson's Disease: Defining the Park Apathy Subtype. **Brain Sciences**, v. 12, n. 7, p. 923, 14 jul. 2022.

DER-AVAKIAN, A.; MARKOU, A. The neurobiology of anhedonia and other reward-related deficits. **Trends in Neurosciences**, v. 35, n. 1, p. 68–77, 1 jan. 2012.

DEUMENS, R.; BLOKLAND, A.; PRICKAERTS, J. Modeling Parkinson's Disease in Rats: An Evaluation of 6-OHDA Lesions of the Nigrostriatal Pathway. **Experimental Neurology**, v. 175, n. 2, p. 303–317, jun. 2002.

DI DOMENICO, D.; MAPELLI, L. Dopaminergic Modulation of Prefrontal Cortex Inhibition. **Biomedicines**, v. 11, n. 5, p. 1276, 25 abr. 2023.

DRUI, G. et al. Loss of dopaminergic nigrostriatal neurons accounts for the motivational and affective deficits in Parkinson's disease. **Molecular Psychiatry**, v. 19, n. 3, p. 358–367, mar. 2014.

DUJARDIN, K.; SGAMBATO, V. Neuropsychiatric Disorders in Parkinson's Disease: What Do We Know About the Role of Dopaminergic and Non-dopaminergic Systems? **Frontiers in Neuroscience**, v. 14, p. 25, 29 jan. 2020.

DUNCAN, W. C.; ZARATE, C. A. Ketamine, Sleep, and Depression: Current Status and New Questions. **Current Psychiatry Reports**, v. 15, n. 9, p. 394, set. 2013.

EDELMANN, E.; LESSMANN, V. Dopaminergic innervation and modulation of hippocampal networks. **Cell and Tissue Research**, v. 373, n. 3, p. 711–727, set. 2018.

EL-GAMAL, M. et al. Neurotoxin-Induced Rodent Models of Parkinson's Disease: Benefits and Drawbacks. **Neurotoxicity Research**, v. 39, n. 3, p. 897–923, jun. 2021.

ELMER, T.; STADTFELD, C. Depressive symptoms are associated with social isolation in face-to-face interaction networks. **Scientific Reports**, v. 10, n. 1, p. 1444, 29 jan. 2020.

FELIX-ORTIZ, A. C.; TYE, K. M. Amygdala inputs to the ventral hippocampus bidirectionally modulate social behavior. **The Journal of Neuroscience: The Official Journal of the Society for Neuroscience**, v. 34, n. 2, p. 586–595, 8 jan. 2014.

FERNAGUT, P. O. et al. A simple method to measure stride length as an index of nigrostriatal dysfunction in mice. **Journal of Neuroscience Methods**, v. 113, n. 2, p. 123–130, jan. 2002.

FETCHO, R. N. et al. Regulation of social interaction in mice by a frontostriatal circuit modulated by established hierarchical relationships. **Nature Communications**, v. 14, n. 1, p. 2487, 29 abr. 2023.

FINLAY, J. M. et al. Effects of prefrontal cortex and hippocampal NMDA-NR1 subunit deletion on complex cognitive and social behaviors. **Brain research**, v. 1600, p. 70–83, 10 mar. 2015.

FRIES, G. R. et al. Molecular pathways of major depressive disorder converge on the synapse. **Molecular Psychiatry**, v. 28, n. 1, p. 284–297, jan. 2023.

GRADOWSKI, R. W. et al. Antidepressant-like effect of curcumin in 6-hydroxydopamine model of Parkinson's disease. v. 7, 2013.

GYÖRFI, O. et al. Reduced CA2–CA3 Hippocampal Subfield Volume Is Related to Depression and Normalized by l-DOPA in Newly Diagnosed Parkinson's Disease. **Frontiers in Neurology**, v. 8, 2017.

HABER, S. N. The place of dopamine in the cortico-basal ganglia circuit. **Neuroscience**, v. 282, p. 248–257, 12 dez. 2014.

HABER, S. N. Corticostriatal circuitry. **Dialogues in Clinical Neuroscience**, v. 18, n. 1, p. 7–21, mar. 2016.

HASEGAWA, H. et al. The subcortical belly of sleep: New possibilities in neuromodulation of basal ganglia? **Sleep Medicine Reviews**, v. 52, p. 101317, ago. 2020.

HASSAMAL, S. Chronic stress, neuroinflammation, and depression: an overview of pathophysiological mechanisms and emerging anti-inflammatories. **Frontiers in Psychiatry**, v. 14, 2023.

HEINZEL, S. et al. Update of the MDS research criteria for prodromal Parkinson's disease. **Movement Disorders**, v. 34, n. 10, p. 1464–1470, out. 2019.

HENG, N. et al. Striatal Dopamine Loss in Early Parkinson's Disease: Systematic Review and Novel Analysis of Dopamine Transporter Imaging. **Movement Disorders Clinical Practice**, v. 10, n. 4, p. 539–546, abr. 2023.

HERNANDEZ-BALTAZAR, D.; ZAVALA-FLORES, L. M.; VILLANUEVA-OLIVO, A. The 6-hydroxydopamine model and parkinsonian pathophysiology: Novel findings in an older model. **Neurología (English Edition)**, v. 32, n. 8, p. 533–539, 1 out. 2017.

HERRERA, M. L. et al. Early Cognitive Impairment Behind Nigrostriatal Circuit Neurotoxicity: Are Astrocytes Involved? **ASN Neuro**, v. 12, p. 175909142092597, jan. 2020.

HORSAGER, J. et al. Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. **Brain**, v. 143, n. 10, p. 3077–3088, 1 out. 2020.

HSIEH, T.-H. et al. Time-course gait analysis of hemiparkinsonian rats following 6-hydroxydopamine lesion. **Behavioural Brain Research**, v. 222, n. 1, p. 1–9, set. 2011.

HUNT, J. et al. Sleep and circadian rhythms in Parkinson's disease and preclinical models. **Molecular Neurodegeneration**, v. 17, n. 1, p. 2, dez. 2022.

HUSTAD, E.; AASLY, J. O. Clinical and Imaging Markers of Prodromal Parkinson's Disease. **Frontiers in Neurology**, v. 11, p. 395, 8 maio 2020.

IKE, K. G. O. et al. Social withdrawal: An initially adaptive behavior that becomes maladaptive when expressed excessively. **Neuroscience & Biobehavioral Reviews**, v. 116, p. 251–267, 1 set. 2020.

IKEDA, K. et al. Dopamine Transporter Imaging in Parkinson Disease: Progressive Changes and Therapeutic Modification after Anti-parkinsonian Medications. **Internal Medicine**, v. 58, n. 12, p. 1665–1672, 15 jun. 2019.

ILKIW, J. L. et al. Dopaminergic Lesion in the Olfactory Bulb Restores Olfaction and Induces Depressive-Like Behaviors in a 6-OHDA Model of Parkinson's Disease. **Molecular Neurobiology**, v. 56, n. 2, p. 1082–1095, fev. 2019.

JELLINGER, K. A. The pathobiological basis of depression in Parkinson disease: challenges and outlooks. **Journal of Neural Transmission**, v. 129, n. 12, p. 1397–1418, 1 dez. 2022.

JI, N. et al. Efficacy and safety of pramipexole in Parkinson's disease with anxiety or depression: a meta-analysis of randomized clinical trials. **American Journal of Translational Research**, v. 14, n. 3, p. 1757–1764, 15 mar. 2022.

JONES, J. D. et al. Depressive symptoms precede cognitive impairment in de novo Parkinson's disease patients: Analysis of the PPMI cohort. **Neuropsychology**, v. 33, n. 8, p. 1111–1120, nov. 2019.

KACZYŃSKA, K.; ANDRZEJEWSKI, K. Chapter 40 - 6-hydroxydopamine-induced model of Parkinson's disease. Em: MARTIN, C. R.; PREEDY, V. R. (Eds.). **Genetics, Neurology, Behavior, and Diet in Parkinson's Disease**. [s.l.] Academic Press, 2020. p. 627–642.

KAISER, R. H. et al. Large-Scale Network Dysfunction in Major Depressive Disorder: A Meta-analysis of Resting-State Functional Connectivity. **JAMA Psychiatry**, v. 72, n. 6, p. 603–611, 1 jun. 2015.

KINGSBURY, L. et al. Correlated Neural Activity and Encoding of Behavior Across Brains of Socially Interacting Individuals. **Cell**, v. 178, n. 2, p. 429- 446.e16, 11 jul. 2019.

KIRIK, D.; ROSENBLAD, C.; BJÖRKLUND, A. Characterization of Behavioral and Neurodegenerative Changes Following Partial Lesions of the Nigrostriatal Dopamine System Induced by Intrastriatal 6-Hydroxydopamine in the Rat. **Experimental Neurology**, v. 152, n. 2, p. 259–277, ago. 1998.

KO, J. Neuroanatomical Substrates of Rodent Social Behavior: The Medial Prefrontal Cortex and Its Projection Patterns. **Frontiers in Neural Circuits**, v. 11, 2017.

KRACH, S. The rewarding nature of social interactions. **Frontiers in Behavioral Neuroscience**, 2010.

LAMA, J. et al. Animal models of Parkinson's disease: a guide to selecting the optimal model for your research. **Neuronal Signaling**, v. 5, n. 4, p. NS20210026, 8 dez. 2021.

LANCIEGO, J. L.; LUQUIN, N.; OBESO, J. A. Functional Neuroanatomy of the Basal Ganglia. **Cold Spring Harbor Perspectives in Medicine**, v. 2, n. 12, p. a009621–a009621, 1 dez. 2012.

LATREILLE, V. et al. Sleep spindles in Parkinson's disease may predict the development of dementia. **Neurobiology of Aging**, v. 36, n. 2, p. 1083–1090, fev. 2015.

LEE, E. et al. Enhanced Neuronal Activity in the Medial Prefrontal Cortex during Social Approach Behavior. **The Journal of Neuroscience**, v. 36, n. 26, p. 6926–6936, 29 jun. 2016.

LHOST, J. et al. Interplay Between Inhibitory Control and Behavioural Flexibility: Impact of Dorsomedial Striatal Dopamine Denervation in Mice. **Neuroscience**, v. 477, p. 25–39, nov. 2021.

LIAO, Y. et al. Is depression a disconnection syndrome? Meta-analysis of diffusion tensor imaging studies in patients with MDD. **Journal of Psychiatry & Neuroscience : JPN**, v. 38, n. 1, p. 49–56, jan. 2013.

LIM, J.; BANG, Y.; CHOI, H. J. Abnormal hippocampal neurogenesis in Parkinson's disease: relevance to a new therapeutic target for depression with Parkinson's disease. **Archives of Pharmacal Research**, v. 41, n. 10, p. 943–954, out. 2018.

LIU, Q. et al. Resting-state brain network in Parkinson's disease with different degrees of depression. **Frontiers in Neuroscience**, v. 16, 2022.

LOAS, G. et al. Hedonic Deficits in Parkinson's Disease: Is Consummatory Anhedonia Specific? **Frontiers in Neurology**, v. 5, 2014.

MA, J. et al. Associations of Sleep Disorders With Depressive Symptoms in Early and Prodromal Parkinson's Disease. **Frontiers in Aging Neuroscience**, v. 14, p. 898149, 10 jun. 2022.

MACPHERSON, T.; HIKIDA, T. Role of basal ganglia neurocircuitry in the pathology of psychiatric disorders. **Psychiatry and Clinical Neurosciences**, v. 73, n. 6, p. 289–301, jun. 2019.

MADIHA, S. et al. Assessment of gait dynamics in rotenone-induced rat model of Parkinson's disease by footprint method. **Pak. J. Pharm. Sci.**, 2017.

MAGGI, G. et al. Sleep and wakefulness disturbances in Parkinson's disease: A meta-analysis on prevalence and clinical aspects of REM sleep behavior disorder, excessive daytime sleepiness and insomnia. **Sleep Medicine Reviews**, v. 68, p. 101759, abr. 2023.

MAGNARD, R. et al. What can rodent models tell us about apathy and associated neuropsychiatric symptoms in Parkinson's disease? **Translational Psychiatry**, v. 6, n. 3, p. e753–e753, 8 mar. 2016.

MANFRÉ, G. et al. BACHD rats expressing full-length mutant huntingtin exhibit differences in social behavior compared to wild-type littermates. **PLOS ONE**, v. 13, n. 2, p. e0192289, 7 fev. 2018.

MARQUES, N. F. et al. Guanosine prevents depressive-like behaviors in rats following bilateral dorsolateral striatum lesion induced by 6-hydroxydopamine. **Behavioural Brain Research**, v. 372, p. 112014, out. 2019.

MARSH, L. Depression and Parkinson's Disease: Current Knowledge. **Current neurology and neuroscience reports**, v. 13, n. 12, p. 409, dez. 2013.

MARSHALL, C. A.; KING, K. M.; KORTAGERE, S. Limitations of the rat medial forebrain lesion model to study prefrontal cortex mediated cognitive tasks in Parkinson's disease. **Brain Research**, v. 1702, p. 105–113, jan. 2019.

MASINI, D. et al. The histamine H3 receptor antagonist thioperamide rescues circadian rhythm and memory function in experimental parkinsonism. **Translational Psychiatry**, v. 7, n. 4, p. e1088–e1088, abr. 2017.

MASINI, D. et al. A Guide to the Generation of a 6-Hydroxydopamine Mouse Model of Parkinson's Disease for the Study of Non-Motor Symptoms. **Biomedicines**, v. 9, n. 6, p. 598, 25 maio 2021.

MATHEUS, F. C. et al. Decreased synaptic plasticity in the medial prefrontal cortex underlies short-term memory deficits in 6-OHDA-lesioned rats. **Behavioural Brain Research**, v. 301, p. 43–54, mar. 2016a.

MATHEUS, F. C. et al. Temporal Dissociation of Striatum and Prefrontal Cortex Uncouples Anhedonia and Defense Behaviors Relevant to Depression in 6-OHDA-Lesioned Rats. **Molecular Neurobiology**, v. 53, n. 6, p. 3891–3899, ago. 2016b.

MCDONALD, C. et al. 200 Years of Parkinson's disease: what have we learnt from James Parkinson? **Age and Ageing**, v. 47, n. 2, p. 209–214, 1 mar. 2018.

MEDEIROS, D. DE C. et al. Sleep Disorders in Rodent Models of Parkinson's Disease. **Frontiers in Pharmacology**, v. 10, p. 1414, 27 nov. 2019.

MENG, D. et al. Longitudinal cognitive changes in patients with early Parkinson's disease and neuropsychiatric symptoms. **CNS Neuroscience & Therapeutics**, v. 29, n. 8, p. 2259–2266, ago. 2023.

NAGAHARA, A. H.; TUSZYNSKI, M. H. Potential therapeutic uses of BDNF in neurological and psychiatric disorders. **Nature Reviews Drug Discovery**, v. 10, n. 3, p. 209–219, mar. 2011.

OBESO, J. A. et al. The expanding universe of disorders of the basal ganglia. **The Lancet**, v. 384, n. 9942, p. 523–531, ago. 2014.

OU, Z. et al. Global Trends in the Incidence, Prevalence, and Years Lived With Disability of Parkinson's Disease in 204 Countries/Territories From 1990 to 2019. **Frontiers in Public Health**, v. 9, 2021.

PALASZ, E. et al. BDNF as a Promising Therapeutic Agent in Parkinson's Disease. **International Journal of Molecular Sciences**, v. 21, n. 3, p. 1170, 10 fev. 2020.

PANDI-PERUMAL, S. R. et al. Clarifying the role of sleep in depression: A narrative review. **Psychiatry Research**, v. 291, p. 113239, set. 2020.

PAREDES-RODRIGUEZ, E. et al. The Noradrenergic System in Parkinson's Disease. **Frontiers in Pharmacology**, v. 11, p. 435, 8 abr. 2020.

PAUL, E. R. et al. Towards a multilevel model of major depression: genes, immuno-metabolic function, and cortico-striatal signaling. **Translational Psychiatry**, v. 13, n. 1, p. 171, 19 maio 2023.

PAXINOS, G.; WATSON, C. **The rat brain in stereotaxic coordinates**. 6. ed ed. Amsterdam Heidelberg: Academic Press; Elsevier, 2007.

PENTTINEN, A.-M. et al. Characterization of a new low-dose 6-hydroxydopamine model of Parkinson's disease in rat. **Journal of Neuroscience Research**, v. 94, n. 4, p. 318–328, 2016.

PETERS, S. M. et al. Novel approach to automatically classify rat social behavior using a video tracking system. **Journal of Neuroscience Methods**, v. 268, p. 163–170, ago. 2016.

PHARMATHER HOLDINGS LTD. **PharmaTher Announces FDA Approval of Ketamine IND In The Treatment of Parkinson's Disease**. Disponível em: <https://www.globenewswire.com/news-release/2021/05/17/2230622/0/en/PharmaTher-Announces-FDA-Approval-of-Ketamine-IND-In-The-Treatment-of-Parkinson-s-Disease.html>. Acesso em: 5 nov. 2023.

POEWE, W. et al. Parkinson disease. **Nature Reviews Disease Primers**, v. 3, n. 1, p. 17013, 21 dez. 2017.

POLITIS, M. et al. Depressive symptoms in PD correlate with higher 5-HTT binding in raphe and limbic structures. **Neurology**, v. 75, n. 21, p. 1920–1927, 23 nov. 2010.

PONTONE, G. M. et al. The longitudinal impact of depression on disability in Parkinson disease. **International Journal of Geriatric Psychiatry**, v. 31, n. 5, p. 458–465, maio 2016.

PORCELLI, S. et al. Social brain, social dysfunction and social withdrawal. **Neuroscience & Biobehavioral Reviews**, v. 97, p. 10–33, fev. 2019.

POSTUMA, R. B. et al. MDS clinical diagnostic criteria for Parkinson's disease: MDS-PD Clinical Diagnostic Criteria. **Movement Disorders**, v. 30, n. 12, p. 1591–1601, out. 2015.

PRANGE, S. et al. Depression in Patients with Parkinson's Disease: Current Understanding of its Neurobiology and Implications for Treatment. **Drugs & Aging**, v. 39, n. 6, p. 417–439, jun. 2022.

PRENGER, M. T. M. et al. Social Symptoms of Parkinson's Disease. **Parkinson's Disease**, v. 2020, p. 1–10, 31 dez. 2020.

QAMHAWI, Z. et al. Clinical correlates of raphe serotonergic dysfunction in early Parkinson's disease. **Brain**, v. 138, n. 10, p. 2964–2973, 1 out. 2015.

REAL, C. C. et al. BDNF receptor blockade hinders the beneficial effects of exercise in a rat model of Parkinson's disease. **Neuroscience**, v. 237, p. 118–129, maio 2013.

REDCAY, E.; SCHILBACH, L. Using second-person neuroscience to elucidate the mechanisms of social interaction. **Nature Reviews Neuroscience**, v. 20, n. 8, p. 495–505, ago. 2019.

REQUEJO, C. et al. Changes in Day/Night Activity in the 6-OHDA-Induced Experimental Model of Parkinson's Disease: Exploring Prodromal Biomarkers. **Frontiers in Neuroscience**, v. 14, p. 590029, 14 out. 2020.

RIZVI, S. J. et al. Assessing anhedonia in depression: Potentials and pitfalls. **Neuroscience and biobehavioral reviews**, v. 65, p. 21–35, jun. 2016.

SANTANGELO, G. et al. Neuropsychological correlates of theory of mind in patients with early Parkinson's disease. **Movement Disorders**, v. 27, n. 1, p. 98–105, jan. 2012.

SANTANGELO, G. et al. Relationship between apathy and cognitive dysfunctions in *de novo* untreated P arkinson's disease: a prospective longitudinal study. **European Journal of Neurology**, v. 22, n. 2, p. 253–260, fev. 2015.

SANTIAGO, R. M. et al. Depressive-like behaviors alterations induced by intranigral MPTP, 6-OHDA, LPS and rotenone models of Parkinson's disease are predominantly associated with serotonin and dopamine. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, v. 34, n. 6, p. 1104–1114, ago. 2010.

SANTIAGO, R. M. et al. Induction of depressive-like behavior by intranigral 6-OHDA is directly correlated with deficits in striatal dopamine and hippocampal serotonin. **Behavioural Brain Research**, v. 259, p. 70–77, fev. 2014.

SEPPI, K. et al. Update on treatments for nonmotor symptoms of Parkinson's disease—an evidence-based medicine review. **Movement Disorders**, v. 34, n. 2, p. 180–198, fev. 2019.

SERDAR, C. C. et al. Sample size, power and effect size revisited: simplified and practical approaches in pre-clinical, clinical and laboratory studies. **Biochemia Medica**, v. 31, n. 1, p. 010502, 15 fev. 2021.

SILVA, T. P. D. et al. Time course study of microglial and behavioral alterations induced by 6-hydroxydopamine in rats. **Neuroscience Letters**, v. 622, p. 83–87, maio 2016.

SOMENSI, N. et al. Role of toll-like receptor 4 and sex in 6-hydroxydopamine–induced behavioral impairments and neurodegeneration in mice. **Neurochemistry International**, v. 151, p. 105215, dez. 2021.

SOTOYAMA, H. et al. The dual role of dopamine in the modulation of information processing in the prefrontal cortex underlying social behavior. **The FASEB Journal**, v. 36, n. 2, p. e22160, 2022.

SPANAGEL, R. Ten Points to Improve Reproducibility and Translation of Animal Research. **Frontiers in Behavioral Neuroscience**, v. 16, p. 869511, 21 abr. 2022.

Stereotaxic Administration. Disponível em: <https://encapsula.com/stereotaxic-administration/>. Acesso em: 2 nov. 2023.

SU, W. et al. Correlation between depression and quality of life in patients with Parkinson's disease. **Clinical Neurology and Neurosurgery**, v. 202, p. 106523, mar. 2021.

SUGIMOTO, H.; KAWAKAMI, K. Low-cost Protocol of Footprint Analysis and Hanging Box Test for Mice Applied the Chronic Restraint Stress. **Journal of Visualized Experiments**, n. 143, p. 59027, 23 jan. 2019.

TADAIESKY, M. T. et al. Emotional, cognitive and neurochemical alterations in a premotor stage model of Parkinson's disease. **Neuroscience**, v. 156, n. 4, p. 830–840, out. 2008.

TAGLIAFERRO, P.; BURKE, R. E. Retrograde Axonal Degeneration in Parkinson Disease. **Journal of Parkinson's Disease**, v. 6, n. 1, p. 1–15, 2016.

TESSITORE, A. et al. Dopamine Modulates the Response of the Human Amygdala: A Study in Parkinson's Disease. **The Journal of Neuroscience**, v. 22, n. 20, p. 9099–9103, 15 out. 2002.

UNGERSTEDT, U. 6-hydroxy-dopamine induced degeneration of central monoamine neurons. **European Journal of Pharmacology**, v. 5, n. 1, p. 107–110, 1 dez. 1968.

VAREŠLIJA, D. et al. 6-Hydroxydopamine: a far from simple neurotoxin. **Journal of Neural Transmission**, v. 127, n. 2, p. 213–230, fev. 2020.

VECCHIA, D. D. et al. Effects of ketamine on vocal impairment, gait changes, and anhedonia induced by bilateral 6-OHDA infusion into the substantia nigra pars compacta in rats: Therapeutic implications for Parkinson's disease. **Behavioural Brain Research**, v. 342, p. 1–10, abr. 2018.

VECCHIA, D. D. et al. Ketamine reversed short-term memory impairment and depressive-like behavior in animal model of Parkinson's disease. **Brain Research Bulletin**, v. 168, p. 63–73, mar. 2021.

VISANJI, N. P. et al. The prion hypothesis in Parkinson's disease: Braak to the future. **Acta Neuropathologica Communications**, v. 1, n. 1, p. 2, 8 maio 2013.

VO, Q. et al. Polysomnographic Features of Sleep Disturbances and REM Sleep Behavior Disorder in the Unilateral 6-OHDA Lesioned Hemiparkinsonian Rat. **Parkinson's Disease**, v. 2014, p. 1–8, 2014.

VON MÜCKE-HEIM, I.-A. et al. Introducing a depression-like syndrome for translational neuropsychiatry: a plea for taxonomical validity and improved comparability between humans and mice. **Molecular Psychiatry**, v. 28, n. 1, p. 329–340, jan. 2023.

WANG, Y. et al. Association of low serum BDNF with depression in patients with Parkinson's disease. **Parkinsonism & Related Disorders**, v. 41, p. 73–78, ago. 2017.

WILLIS, A. W. et al. Incidence of Parkinson disease in North America. **npj Parkinson's Disease**, v. 8, n. 1, p. 1–7, 15 dez. 2022.

YANG, T. et al. The Role of BDNF on Neural Plasticity in Depression. **Frontiers in Cellular Neuroscience**, v. 14, p. 82, 15 abr. 2020.

YANG, Z. et al. Molecular Mechanisms Underlying Reciprocal Interactions Between Sleep Disorders and Parkinson's Disease. **Frontiers in Neuroscience**, v. 14, p. 592989, 10 fev. 2021.

YOO, S.-W. et al. "Depressed" caudate and ventral striatum dopamine transporter availability in de novo Depressed Parkinson's disease. **Neurobiology of Disease**, v. 132, p. 104563, dez. 2019.

ZAHED, H. et al. The Neurophysiology of Sleep in Parkinson's Disease. **Movement Disorders**, v. 36, n. 7, p. 1526–1542, jul. 2021.

ZHOU, C. et al. Two distinct trajectories of clinical and neurodegeneration events in Parkinson's disease. **npj Parkinson's Disease**, v. 9, n. 1, p. 111, 13 jul. 2023.

ZHURAKOVSKAYA, E. et al. Sleep-State Dependent Alterations in Brain Functional Connectivity under Urethane Anesthesia in a Rat Model of Early-Stage Parkinson's Disease. **eneuro**, v. 6, n. 1, p. ENEURO.0456-18.2019, jan. 2019.

APPENDIX A – Systematic review

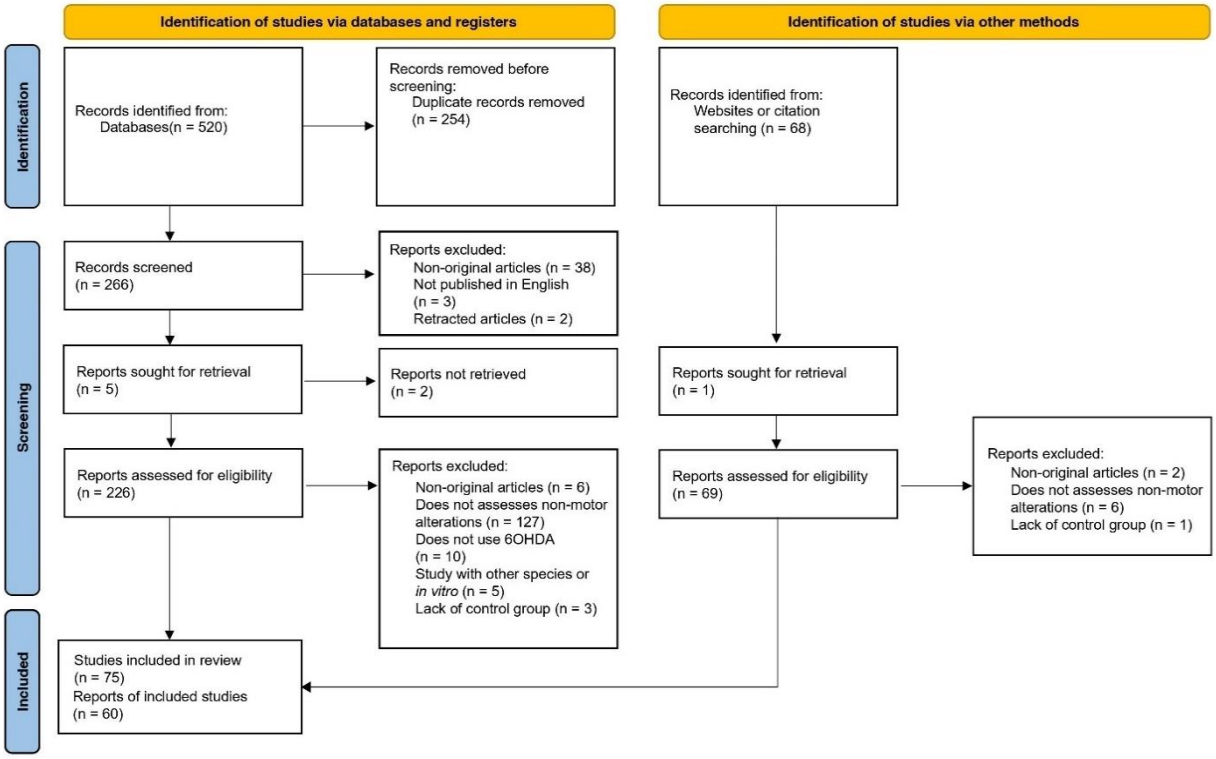


Figure A1. **PRISMA flow chart of search strategy.** Description of the phases of inclusion and exclusion in the articles in review.

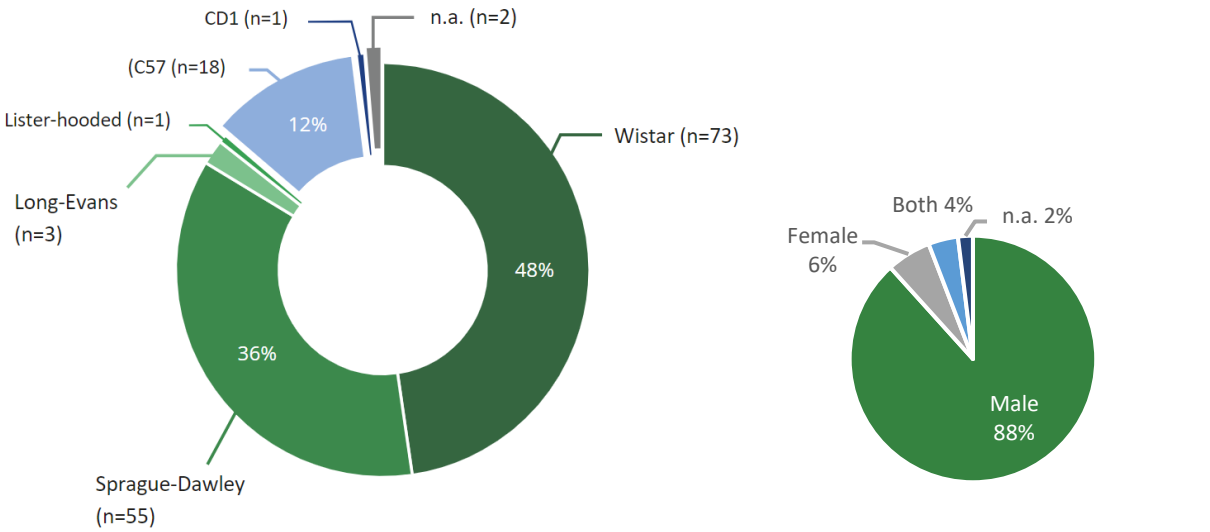
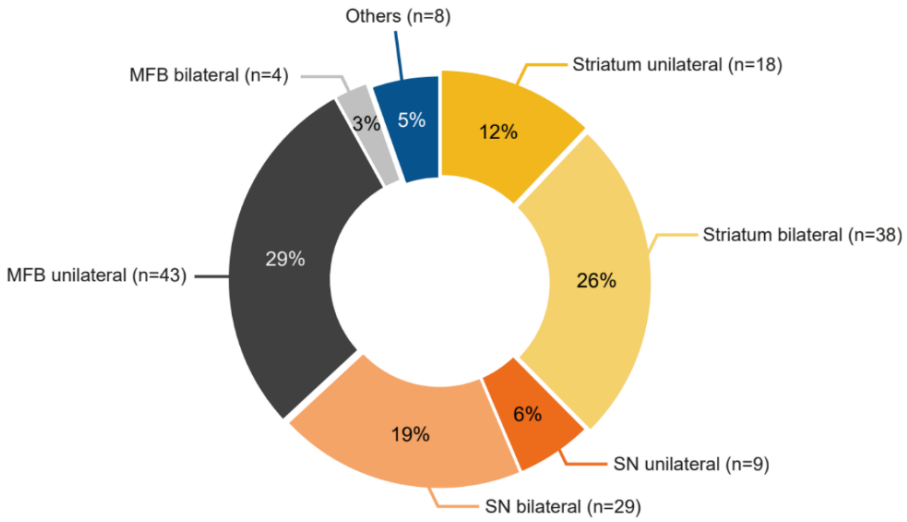


Figure A2. **Animals used in experimental research. (A)** Distribution of lineages of rats (green and grey) and mice (blue). **(B)** Proportion of studies that used male, female, or both. n.a: not informed

A



B

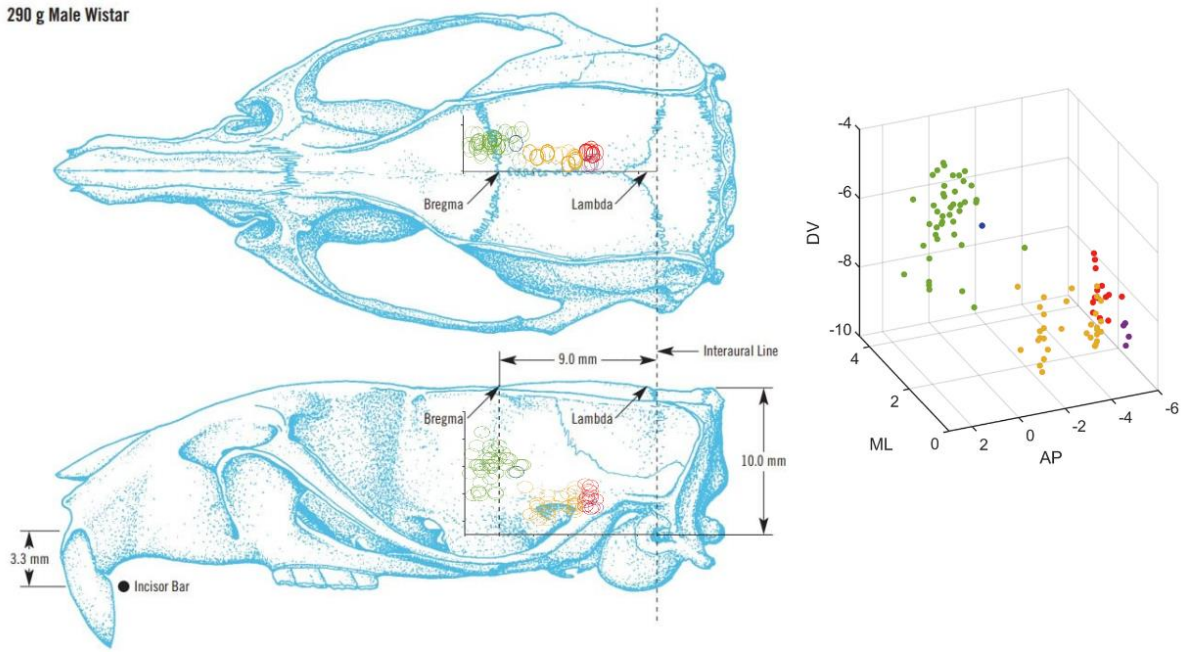


Figure A3. Site of infusion of 6-OHDA. (A) Proportion distribution of the areas injected in unilateral and bilateral infusions. In yellow the striatum site injection, orange SN, grey MFB pathway and in blue the other areas (amygdala basolateral, globus pallidus and VTA). **(B)** Schematic representation of the coordinates of injection indicated by the studies in the striatum (green), MFB (yellow), SN (red) and VTA (purple).

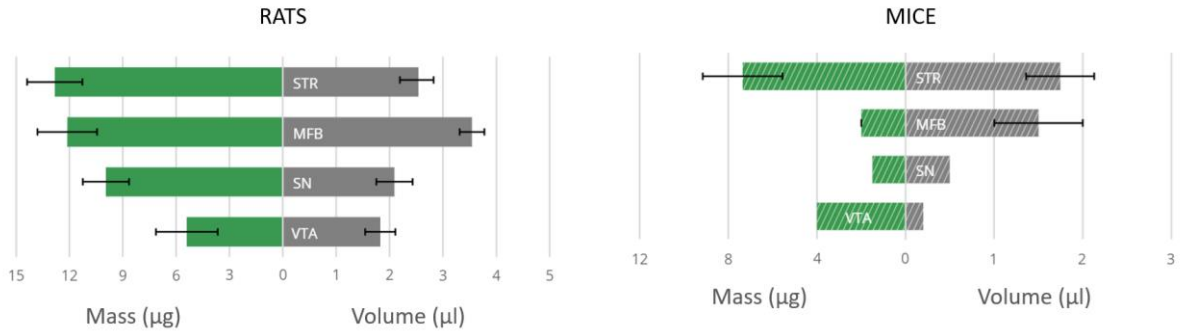


Figure A4. Averaged 6-OHDA concentrations by species and region of infusion. Mean amount of 6-OHDA mass and volume injected into each region from studies using rats and mice.

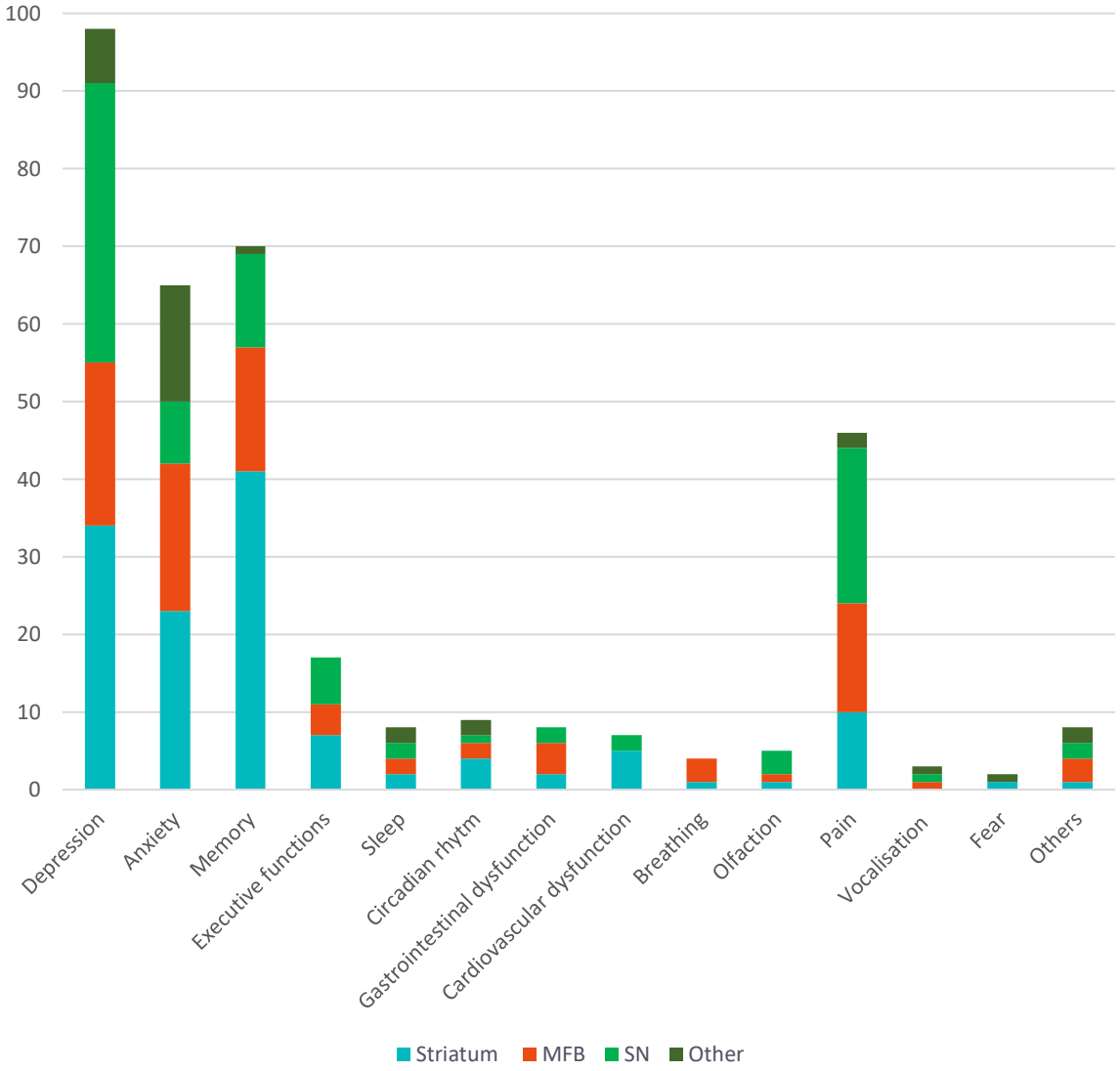


Figure A5. **Nonmotor symptoms assessed in studies with 6-OHDA models.** Number of studies that assessed each non-motor symptom, classified according to site of 6-OHDA infusion: striatum (blue); MFB (orange) and SN (green) and others (grey).

APPENDIX B – Protocol used for tissue sectioning

DATA: _____ **IDENTIFICAÇÃO ANIMAL: _____**

1. Instruções

- Coletar todas as secções com 50 µm de espessura em quatro tubos de 2 ml contendo solução anti-congelante. A coleta deve ser em blocos.
- Iniciar e finalizar a coleta de acordo com o bregma indicado para cada região (6ª edição do atlas Paxinos)

*Isto é uma aproximação e para cada animal é útil saber o bregma inicial e final de cada tubo
* Indicar secções que foram perdidas
* Coletar cada 4ª seção de que as secções em cada tudo tenham uma distância de 200 µm:

Coleta de secções no nível do córtex motor M1

Seccionamento:

- Inicio no bregma: **+3,72 mm**; Final no bregma: **+2,52** mm;
- Total da espessura do córtex: 1.20 mm
- Número total de secções (50 µm de espessura) em 1.2 mm: ~24 secções.

Tubo 1:	1	5	9	13	17	21
Tubo 2:	2	6	10	14	18	22
Tubo 3:	3	7	11	15	19	23
Tubo 4:	4	8	12	16	20	24

Início: _____ Final:_____

Observações:

_____

_____

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Coleta de secções no nível do corpo estriado

Seccionamento:

- Inicio no bregma: **+2.16 mm**; Final no bregma: **- 0.84 mm;** Total da espessura do CPu: 2.28 mm
- Número total de secções (50 µm de espessura) em 3.0 mm: ~60 secções.
- secções 1-32 nos tubos 1 a 4 e secções 33-60 nos tubos 5-8.

Tubo 1:	1	5	9	13	17	21	25	29	Tubo 5:	33	37	41	45	49	53	57
Tubo 2:	2	6	10	14	18	22	26	30	Tubo 6:	34	38	42	46	50	54	58
Tubo 3:	3	7	11	15	19	23	27	31	Tubo 7:	35	39	43	47	51	55	59
Tubo 4:	4	8	12	16	20	24	28	32	Tubo 8:	36	40	44	48	52	56	60

Início: _____ Final:_____

Observações:

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Coleta de seção no nível da substância negra compacta (SNc)

Data: _____ Identificação do animal: _____

Seccionamento:

- Inicio no bregma: **- 4.44 mm**; Final no bregma: **- 6.72 mm**
- Total da espessura da SNc: 2.28 mm
- Número total de secções (50 µm de espessura) em 2.2 mm: ~44 secções.

Tubo 1:	1	5	9	13	17	21	25	29	33	37	41
Tubo 2:	2	6	10	14	18	22	26	30	34	38	42
Tubo 3:	3	7	11	15	19	23	27	31	35	39	43
Tubo 4:	4	8	12	16	20	24	28	32	36	40	44

Início: _____ Final:_____

Observações:

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Appendix C – DESCRIPTION OF RESULTS

Table C1. Footprint test. Descriptive statistics of the control and 6-OHDA groups and results from the Student's t test or Mann-Whitney U test. *Min: minimum; Max: Maximum; SD: Standard deviation; SEM: Standard Error of Mean*

				N	Median	Min	Max	Mean	SD	SEM
Week 3	Forelimb	Stride length	Control	8	13.32	12.25	14.88	13.50	1.07	0.38
			6-OHDA	8	13.79	10.92	14.25	13.25	1.26	0.44
		Base width	Control	8	2.67	2.17	3.33	2.69	2.37	0.15
			6-OHDA	8	2.33	2.00	3.00	2.37	0.32	0.11
	Hindlimb	Stride length	Control	8	13.71	12.25	14.42	13.54	0.78	0.27
			6-OHDA	8	13.88	11.67	15.00	13.65	0.97	0.34
		Base width	Control	8	4.34	3.00	5.67	4.36	0.99	0.35
			6-OHDA	8	4.33	4.00	5.00	4.42	0.36	0.13
Week 4	Forelimb	Stride length	Control	6	14.80	13.50	15.50	14.68	0.67	0.27
			6-OHDA	10	14.15	12.80	16.50	14.40	1.24	0.39
		Base width	Control	6	2.50	2.00	3.50	2.67	0.68	0.28
			6-OHDA	10	2.50	2.00	5.00	2.71	0.88	0.28
	Hindlimb	Stride length	Control	6	14.65	12.80	16.00	14.57	1.05	0.43
			6-OHDA	10	13.90	12.30	16.60	14.24	1.45	0.46
		Base width	Control	6	5.00	3.00	5.50	4.60	1.05	0.43
			6-OHDA	10	5.00	2.50	5.50	4.50	1.03	0.32

Table C2. Open field test. Descriptive statistics of the control and 6-OHDA groups and results from the Student's t test.

			N	Median	Min	Max	Mean	SD	SEM
Week 3	Total distance (m)	Control	12	65.46	39.21	82.44	60.65	14.82	4.278
		6-OHDA	18	49.5	21.88	88,91	50.04	17	4.006
	Average speed (m/s)	Control	12	0.0727	0.0436	0.0916	0.0674	0.01648	0.00476
		6-OHDA	18	0.055	0.024	0.099	0.05561	0.01904	0.00449
Week 4	Total distance (m)	Control	6	41.88	25.78	72.83	44.75	18.93	7.73
		6-OHDA	9	53	27.99	79.64	49.5	18.2	6.068
	Average speed (m/s)	Control	6	0.0465	0.029	0.081	0.04983	0.02095	0.00855
		6-OHDA	9	0.059	0.031	0.088	0.05489	0.02016	0.00672

Table C3. Sucrose preference test. Descriptive statistics of the control and 6-OHDA groups and results from the Student's t test or Mann-Whitney U test.

			N	Median	Min	Max	Mean	SD	SEM
Week 1	Sucrose preference (%)	Control	8	91.44	64.67	96.64	86.20	11.34	4.01
		6-OHDA	8	64.10	2.99	88.34	55.77	32.41	11.46
	Water (ml)	Control	8	3.90	1.57	16.60	6.36	5.19	1.84
		6-OHDA	8	13.57	4.00	32.40	15.66	9.73	3.44
	Sucrose (ml)	Control	8	39.95	30.38	57.50	40.26	8.54	3.02
		6-OHDA	8	24.31	1.00	45.33	22.47	14.75	5.22
Week 3	Sucrose preference (%)	Control	8	93.31	86.17	97.71	92.90	4.08	1.44
		6-OHDA	8	87.67	1.96	97.31	72.95	35.51	12.56
	Water (ml)	Control	8	2.57	0.80	5.09	2.82	1.65	0.58
		6-OHDA	8	4.37	1.03	30.00	8.39	10.36	3.66
	Sucrose (ml)	Control	8	33.41	30.88	50.60	36.47	6.67	2.36
		6-OHDA	8	30.75	0.60	48.50	28.76	17.39	6.15

Table C4. Social interaction test. Descriptive statistics of the control and 6-OHDA groups and results from the Student's t test or Mann-Whitney U test.

			N	Median	Min	Max	Mean	SD	SEM
Week 3	Social interaction	Control	12	178.3	120.2	335.3	206.3	69.67	20.11
		6-OHDA	18	156	49.7	213	146.7	48.47	11.42
	Cage exploration	Control	12	329.6	196.7	408.6	324.3	58.23	16.81
		6-OHDA	18	364	56.13	469.4	324.3	113.7	26.8
	Social avoidance	Control	12	31.21	6.75	87.95	34.55	22.27	6.428
		6-OHDA	18	36.56	7.95	53.17	31.19	14.15	3.334
	Self-grooming	Control	12	23.89	7.45	59.35	28.87	18.38	5.306
		6-OHDA	18	27.53	8.45	88.87	34.56	23.05	5.432
	Quiet-awake	Control	12	6.31	0	155.1	21.41	43.9	12.67
		6-OHDA	18	9.85	0	140.8	35.47	45.19	10.65
	Submissive/passive	Control	12	8.85	0	43.2	12.12	14.38	4.152
		6-OHDA	18	8.035	0	70.25	18.86	23.38	5.511
	Aggressive/dominant	Control	12	0	0	17.77	2.828	5.903	1.704
		6-OHDA	18	0	0	55.1	6.889	14.78	3.484