

**The Role of
Nicotine and Nicotinic Agents in the Treatment of
Levodopa Induced Dyskinesia in a Rat Model of
Parkinson's Disease**

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Abstract

Background: Levodopa remains the most effective therapy for Parkinson's disease, but long-term use leads to many complications including L-DOPA induced dyskinesia, which affects approximately 80% all patients within a decade and limits treatment. Current antidyskinetic drugs offer only modest and temporary benefit. Nicotine and nicotinic agents are attractive potential treatments because they are safe, brain penetrable and have undergone numerous clinical trials for a variety of indications. Nicotine and nicotinic agents regulate striatal dopamine and glutamate release, and preclinical studies show that nicotinic stimulation can reduce dyskinesia by about half without weakening levodopa's therapeutic effect. The strongest evidence implicates $\beta 2$ containing receptors, especially $\alpha 4\beta 2$ and $\alpha 6\beta 2$ on dopaminergic terminals, and $\alpha 7$ receptors on interneurons and cortical inputs.

Methods: In this thesis the pharmacological profile of nicotine and nicotinic agents were investigated in a rat model unilaterally lesioned with 6-hydroxydopamine, primed with levodopa plus benserazide, until such time that rats displayed abnormal involuntary movements (AIMs) – a model of levodopa-induced dyskinesia. AIMs was scored across axial, limb, orolingual, and locomotor subtypes. The acute doses of the following were tested on AIMs: nicotine, varenicline, selective $\alpha 4\beta 2$ agonists (A-85380), and $\alpha 4\beta 2$ desensitiser (Sazetidine-A). The effects of the antagonist and positive allosteric modulator was also investigated. The effects of $\alpha 7$ agonists, an $\alpha 7$ positive allosteric modulator, and an $\alpha 7$ antagonist were also studied. Postmortem tissue was examined for dopaminergic loss and glial activation using tyrosine hydroxylase, IBA1, and GFAP using immunohistochemistry.

Results: Acute stimulation of nicotinic receptors reduced established AIMs in a dose dependent manner. Nicotine and varenicline both produced clear reductions in abnormal movements. When given throughout the priming period, these drugs also prevented levodopa

priming for AIMs. Rats treated with nicotine or varenicline from the start of levodopa dosing for priming showed very little AIMs even 3 weeks after cessation nicotine or varenicline administration. Their levodopa responsiveness remained intact.

Subtype selective compounds confirmed a dual mechanism. Activation and subsequent desensitization of $\alpha 4\beta 2$ receptors strongly reduced AIMs, consistent with a reduction of dopamine release. At the same time, enhancing $\alpha 7$ signalling with either an agonist or a positive allosteric modulator lowered AIMs severity, while $\alpha 7$ blockade worsened symptoms. Nicotinic treatments also produced signs of reduced neuroinflammation. Rats receiving varenicline or nicotine had lower microglial and astrocytic activation in the lesioned hemisphere. These effects align with known anti-inflammatory actions of $\alpha 7$ receptor stimulation. This suggested that onset of AIMs may be directly linked to the onset of inflammation.

Conclusion: Taken together, the work shows that targeting nicotinic receptors can reduce dyskinesia through multiple mechanism involving modulation of dopamine neurotransmission, modulation of the glutamatergic system and reduction of neuroinflammation which may become activated by chronic levodopa administration. These findings support advancing varenicline and $\alpha 4\beta 2$ or $\alpha 7$ selective compounds toward clinical testing as adjuncts that could improve long-term motor outcomes in Parkinson's disease.

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Acronyms

3-OMD 3-O-methyldopa.

5-HT 5-hydroxytryptamine (serotonin).

5-HT1A 5-hydroxytryptamine receptor 1A.

5-HT1B 5-hydroxytryptamine receptor 1B.

5-HT2A 5-hydroxytryptamine receptor 2A.

5-HT2C 5-hydroxytryptamine receptor 2C.

6-OHDA 6-hydroxydopamine.

8-OH-DPAT 8-hydroxy-2-dipropylaminotetralin (5-HT1A agonist).

A2A Adenosine A2A receptor.

A30P Alpha-synuclein A30P mutation.

A53T Alpha-synuclein A53T mutation.

A85380 Alpha4 beta2 nicotinic acetylcholine receptor agonist.

ABT-107 A selective alpha7 nicotinic acetylcholine receptor agonist.

ABT-894 Beta-2 nicotinic receptor agonist.

AADC Aromatic L-amino acid decarboxylase.

ABC Avidin Biotin Complex.

ABC-HRP ABC horseradish peroxidase detection complex.

AD Alzheimer's disease.

AFQ056 Mavoglurant (mGluR5 antagonist).

AG Antigen.

AIMs / AIMS Abnormal involuntary movements.

ALO Axial, Limb, Orolingual

AMPA Alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor.

AMPK AMP activated protein kinase.

ANOVA Analysis of variance.

AQW051 Selective alpha-7 nicotinic acetylcholine receptor agonist.

ARRIVE Animal Research: Reporting of In Vivo Experiments.

ASN Alpha synuclein.

ATP Adenosine triphosphate.

ATP13A2 Lysosomal P-type ATPase linked to PD.

AUC Area under the curve.

BBB Blood brain barrier.

BDNF Brain derived neurotrophic factor.

BG Basal ganglia.

BSA Bovine serum albumin.

CaMKII Calcium/calmodulin-dependent protein kinase II.

cAMP Cyclic adenosine monophosphate.

CEP-1347 Mixed lineage kinase inhibitor.

CNS Central nervous system.

COMT Catechol-O-methyltransferase.

CSF Cerebrospinal fluid.

DA Dopamine.

DAT Dopamine transporter.

DBS Deep brain stimulation.

DOPAC 3,4-dihydroxyphenylacetic acid (dopamine metabolite).

DRD2 Dopamine receptor D2.

DRT Dopamine replacement therapy.

DV Dorso-ventral.

E46K Alpha synuclein E46K mutation.

ERK Extracellular signal regulated kinase.

ERK1 Extracellular signal regulated kinase 1.

FDA Food and Drug Administration.

G2019S LRRK2 G2019S mutation.

GABA Gamma-aminobutyric acid.

GBA Glucocerebrosidase gene.

GFAP Glial fibrillary acidic protein.

GI Gastrointestinal.

HCl Hydrochloric acid.

D1 receptors Dopamine D1 subtype receptors.

D2 receptors Dopamine D2 subtype receptors.

DARPP-32 Dopamine and cAMP regulated phosphoprotein of 32 kDa.

DJ-1 Parkinson linked protein encoded by PARK7.

DMNV Dorsal Motor Nucleus of the Vagus

DRT Dopamine Replacement Therapy

GBA Glucocerebrosidase gene.

Globus Pallidus externus (GPe) External part of the globus pallidus.

GluA1 AMPA receptor subunit 1.

GluR1 Another name for the AMPA receptor subunit GluA1.

GPe/SNr Internal globus pallidus and substantia nigra pars reticulata.

HRP Horseradish peroxidase.

IBA-1 / IBA1 Ionised calcium binding adaptor molecule 1.

ICDs Impulse control disorders.

ILBD Incidental Lewy body disease.

IL-1 β Interleukin-1 beta.

IL-6 Interleukin-6.

IL-10 Interleukin-10.

IP Intraperitoneal.

JAK2/STAT3 Janus kinase 2 and signal transducer and activator of transcription 3.

LBs Lewy bodies

L-DOPA Levodopa.

LCIG Levodopa carbidopa intestinal gel.

LD Levodopa induced.

LID Levodopa induced dyskinesia.

LNAA Large neutral amino acid.

LPS Lipopolysaccharide.

LRRK2 Leucine-rich repeat kinase 2.

LTD Long term depression.

LTP Long term potentiation.

mAChRs Muscarinic Acetylcholine Receptors.

MAO Monoamine oxidase.

MAO-B Monoamine oxidase B.

MFB Medial forebrain bundle.

MIBG Metaiodobenzylguanidine.

ML Medio lateral.

MLA Methyllaconitine (alpha-7 nicotinic antagonist).

MPP+ 1-methyl-4-phenylpyridinium.

MPTP 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine.

MRI Magnetic resonance imaging.

MSN Medium spiny neuron.

nAChRs Nicotinic Acetylcholine Receptors

NBQX AMPA receptor antagonist.

NHS Nurses' Health Study.

NMDA N-methyl-D-aspartate receptor.

NSAIDs Non-Steroidal Anti-Inflammatory Drugs

NO Nitric oxide..

OD Optical density.

PAM Positive allosteric modulator.

PARK5 UCH-L1 gene.

PARK7 DJ-1 gene.

PARK8 LRRK2 gene.

PBS Phosphate buffered saline.

PD Parkinson's disease.

PDQ Parkinson's Disease Questionnaire.

PFA Paraformaldehyde.

PHA-543613 Alpha7 nicotinic receptor agonist.

PKA Protein kinase A

PNU-120596 Alpha7 nicotinic receptor PAM.

PTP Protein tyrosine phosphatase.

QBP Quasi-beta protein binder.

QOL Quality of life.

REM Rapid eye movement.

RNA Ribonucleic acid.

ROS Reactive oxygen species.

RPM Revolutions per minute.

RT Room temperature / reverse transcription (context-dependent).

SD Standard deviation.

SEM Standard error of the mean.

SN Substantia nigra.

SNc Substantia nigra pars compacta.

SNCA Alpha-synuclein gene.

SNr Substantia nigra pars reticulata.

SP Substance P.

SPN Spiny projection neuron

STN Subthalamic nucleus.

TBS Tris-buffered saline.

TH Tyrosine hydroxylase.

TH-IR Tyrosine hydroxylase immunoreactivity.

TNF- α Tumor necrosis factor alpha.

TRIS Tris(hydroxymethyl)aminomethane.

UCH-L1 (PARK5) UCH-L1 gene associated with familial Parkinson disease (PARK5).

UPS Ubiquitin proteasome system.

UPDRS Unified Parkinson's Disease Rating Scale.

VMAT2 Vesicular monoamine transporter 2.

VTa Ventral tegmental area.

WHO World Health Organization.

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Chapter 1

Introduction

1.1 Parkinson's Disease

Parkinson's disease (PD) is a progressive neurodegenerative movement disorder characterized by the selective degeneration of dopamine producing neurons in the substantia nigra pars compacta (SNc) of the basal ganglia (Björklund et al., 2008). It was first described by Dr. James Parkinson in 1817 in "An Essay on the Shaking Palsy", where he detailed six cases of what he called "shaking palsy" (Parkinson, 1817). The primary motor features of PD include bradykinesia (slowness of movement), muscle rigidity (stiffness), a resting tremor typically around 4-6 Hz, impaired gait, postural instability, and akinesia (absence or poverty of movement). These cardinal motor symptoms (resting tremor, bradykinesia, rigidity, and later postural instability), are often the most visible and debilitating aspects of PD, significantly impairing daily function and quality of life (Jankovic, 2008), (Figure.1.1).

In addition to its motor manifestations, PD is now recognised to have a broad array of non-motor symptoms, some of which can precede the motor signs by many years (Poewe et al., 2015). Common non-motor features include mood disorders such as depression and anxiety (affecting a large proportion of patients, with some studies suggesting up to 50% prevalence) (Aarsland et al., 2011), autonomic dysfunction (e.g. orthostatic hypotension, constipation, urinary incontinence, sexual dysfunction), cognitive decline (impacting memory, executive function, and visuospatial skills), and sleep disturbances (for example, REM sleep behaviour disorder, excessive daytime sleepiness, or insomnia), (Chaudhuri & Schapira, 2009; Chaudhuri et al., 2006), (Figure.1.1). Many of these non-motor symptoms can appear in the prodromal phase of PD, even years before diagnosis, for instance, chronic constipation, loss of smell (hyposmia), depression, or certain sleep problems in midlife are now understood as potential early indicators of PD (Kouli et al., 2018).

Symptoms of Parkinson's Disease

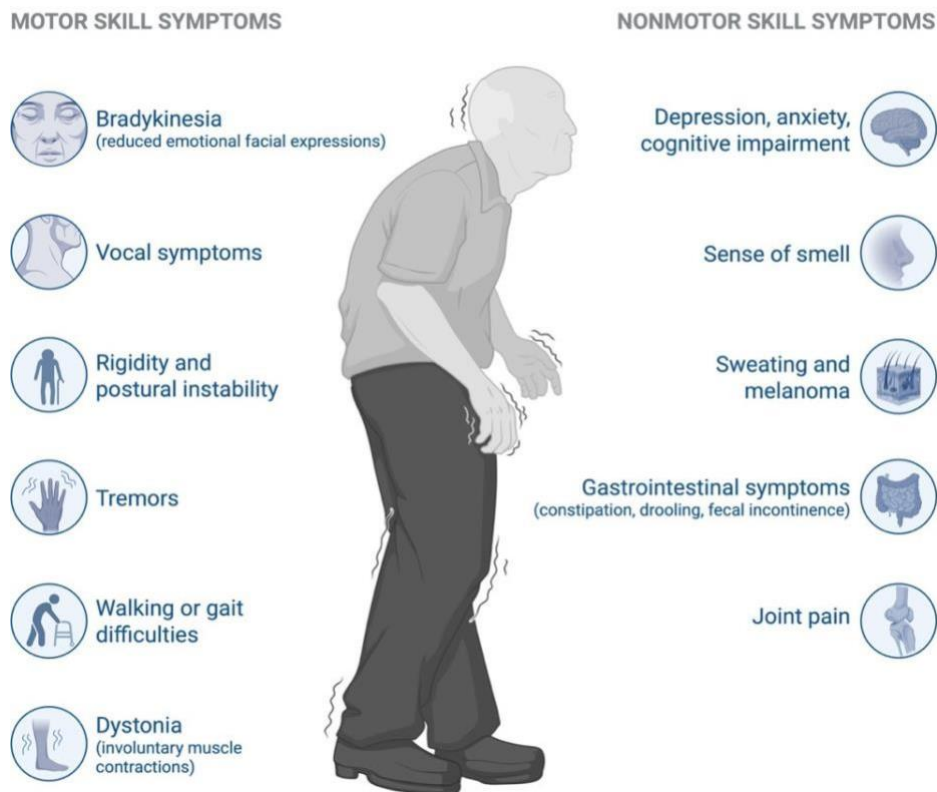


Figure 1. 1: Motor and non-motor symptoms. Overview of key motor and non-motor symptoms in Parkinson's disease. Motor symptoms include bradykinesia, rigidity, tremor, dystonia, gait disturbance, and vocal changes. Non-motor symptoms include cognitive and mood disorders, loss of smell, sweating abnormalities, gastrointestinal dysfunction, and joint pain. Made in Biorender.

1.2 History of Parkinson's Disease

Parkinson's disease has a long and rich history, with descriptions of PD like symptoms dating back to ancient times. Historical records indicate that symptoms resembling Parkinson's disease were noted in early Chinese and Indian medical texts (Goetz, 2011). In ancient India, for example, the Ayurvedic literature (circa 1000 BCE) described a condition called *kampavata*, characterised by tremors and loss of voluntary movement control. By around 300 BCE, the physician Charaka had outlined a coherent clinical picture of parkinsonism

including tremor, rigidity, bradykinesia, and gait disturbance and even recommended treatments such as *Mucuna pruriens* (a legume later found to contain levodopa), (Ovallath & Deepa, 2013). Similarly, in traditional Chinese medicine, documents as early as ~425 BCE described a syndrome of trembling and weakness known as “wind shaking”, which appears to correspond to Parkinsonian tremors (Ovallath & Deepa, 2013). These ancient accounts indicate that the symptoms of PD have been observed and pondered across civilisations for millennia, even if an integrated understanding of the disease was lacking.

1.2.1 James Parkinson’s Seminal Research

Despite these early references, PD was not formally delineated as a distinct medical entity until the early 19th century. James Parkinson, an English surgeon, provided the first comprehensive clinical description of the disease in 1817 (Parkinson, 1817). In his monograph “An Essay on the Shaking Palsy,” Parkinson described in detail six individuals in London who exhibited the hallmark signs of what he termed “paralysis agitans” (shaking palsy). He noted features such as a resting tremor (“involuntary tremulous motion, with lessened muscular power, in parts not in action”), abnormal posture and gait (a tendency to bend forward and festinate), and a marked slowness and hesitancy in movement initiation (DeMaagd & Phillip, 2015). Parkinson also observed that, despite the tremors and rigidity, sensory and cognitive functions appeared largely intact in these patients. His insightful clinical observations laid the groundwork for recognizing PD as a distinct disorder. Notably, James Parkinson’s essay drew attention to the progressive nature of the illness and the absence of effective treatments at the time. This seminal work remains a cornerstone in the history of neurology and earned Parkinson the posthumous honour of having the disease named after him.

1.2.2 The Contributions of Charcot

Following James Parkinson's publication, the medical community slowly began to take greater interest in the "shaking palsy". A major leap in understanding came from the French neurologist Jean-Martin Charcot in the mid 19th century (Charcot, 1872). Charcot built on Parkinson's work and is credited with renaming the illness "Parkinson's disease", firmly establishing it as a distinct neurological disorder in the medical literature. Charcot made several crucial contributions: he carefully differentiated Parkinson's disease from other tremor-dominant conditions (such as multiple sclerosis or essential tremor) and clarified its clinical presentation. For instance, Charcot emphasised that the rigidity in PD was not due to peripheral muscle problems but a central feature of the disease, and he distinguished true weakness from the bradykinesia and stiffness seen in PD (Charcot, 1872; Goetz, 2011). He also described "festinating gait" and rest tremor in detail and noted the asymmetrical onset of symptoms. Moreover, Charcot recognised that non-motor features (like sleep disturbances or sensory changes) could accompany the motor symptoms, an insight ahead of its time. Thanks to Charcot's influence, by the late 1800s the term "Parkinson's disease" was in common use, and the core clinical features, bradykinesia and rigidity especially were well established as diagnostic hallmarks. Charcot's work greatly refined the understanding of PD and can be seen as the foundation of modern Parkinsonology, bridging the gap between James Parkinson's initial observations and the more detailed neuropathological and clinical investigations that would follow in the 20th century.

1.3 Prevalence of PD

PD ranks as the second most prevalent neurodegenerative disorder, following Alzheimer's disease (Kalia & Lang, 2015), and the fastest growing neurodegenerative disease in the developed world (Chaudhuri et al., 2024). It affects 1% of individuals aged 60 and older,

with the incidence rising to 3% in those over 80 years old (Tanner & Goldman, 1996; Nussbaum & Ellis, 2003). In other words, roughly 1 in 100 seniors are living with PD, and this proportion triples in the oldest cohorts. Although PD is predominantly a disease of older adults, it is not exclusive to them; a significant minority of patients develop young onset PD in their 40s or even 30s (as noted in juvenile parkinsonism cases linked to specific genetic mutations). Nevertheless, age remains the single biggest risk factor for PD, with a median age of onset around 60 years and incidence rates climbing sharply in advanced age.

Global demographic trends have made PD an increasingly urgent public health issue. As lifespans lengthen and populations age, the number of people living with PD worldwide has been rising rapidly. According to the World Health Organization, the global prevalence of PD has doubled over the past 25 years in 2019, it was estimated that over 8.5 million individuals worldwide were living with PD (WHO, 2023). The increase could be reduced to the ageing population and improved diagnostic capabilities. This makes PD one of the fastest growing neurological disorders. Indeed, the Global Burden of Disease study reported that Parkinson's disease cases reached 1.02 million in 2017, with disability and mortality rates increasing faster than any other neurological disorder (Zevin, 2021). By 2030, the prevalence and incidence of PD are projected to rise by at least another 30% or more, potentially exceeding 11-12 million patients globally (Kouli et al., 2018). This burgeoning "Parkinson's pandemic" has profound implications, as it will result in escalating healthcare needs, costs, and caregiver burden across societies.

PD exhibits notable gender differences in its occurrence. Men are about 1.5 times more likely to develop Parkinson's disease than women (an incidence ratio of approximately 3:2) (DeMaagd & Philip, 2015). Moreover, women on average develop PD at a slightly later age. One hypothesis for this sex disparity is the neuroprotective effect of oestrogen on dopaminergic neurons. Oestrogen appears to support the nigrostriatal system through multiple mechanisms,

for example, by enhancing antioxidant enzyme activity, exerting anti-inflammatory effects, and promoting the expression of neurotrophic factors like brain-derived neurotrophic factor (BDNF), (Ascherio et al., 2003). Consistent with this idea, epidemiological studies have observed that post-menopausal women who undergo oestrogen replacement therapy tend to show a different relationship between caffeine intake and PD risk than those who do not, suggesting that high oestrogen levels can modulate the protective effects of other factors (like caffeine), (Ascherio et al., 2003). In any case, the lower risk in women might partly reflect hormonal protection and perhaps other gender related environmental exposures.

The societal and economic burden of Parkinson's disease is substantial and grows with disease progression. A recent multinational study (Chaudhuri et al., 2024) noted that annual healthcare costs for PD patients roughly triple from early to advanced stages. For example, in the United States, the yearly direct medical cost for a patient with intermediate stage PD was estimated to be around \$15,000, but this climbs to over \$35,000 per year in advanced PD due to increased medication needs, hospitalisations, and supportive care. European and Japanese data also show similar trends. In addition to direct costs, indirect costs (lost productivity, caregiver time) add significantly to the economic impact. The burden on caregivers intensifies as the disease advances: one survey found that around 40% of caregivers of late stage PD patients reported moderate to severe strain, compared to about 23% for those caring for patients in earlier stages. Thus, public health initiatives aimed at better PD management and potential neuroprotective therapies (once developed) will be crucial to mitigate the coming wave of Parkinson's cases around the world.

1.4 Aetiology of PD

PD is primarily considered a multifactorial disorder, influenced by a combination of genetic and environmental factors (Kouli et al., 2018). However, the precise cause in most individuals remains elusive the vast majority of PD cases are classified as idiopathic (Kouli et al., 2018), meaning no single identifiable trigger. It is likely that various contributing factors (ageing, genetic variants, toxins, lifestyle, etc.) converge to disrupt critical cellular processes in susceptible neurons. This complexity makes it difficult to pinpoint a universal cause of PD; rather, different patients may arrive at Parkinson's disease via different combinations of risk factors.

One of the strongest risk factors for PD is age itself (Lees et al., 2009). PD is predominantly a disease of older adults, and the risk of developing Parkinson's increases sharply with ageing (Lees et al., 2009). Normal ageing is associated with a slow attrition of dopaminergic neurons and a decline in mitochondrial and proteostasis function, which may lower the threshold for neurodegeneration (Lees et al., 2009). The incidence of PD rises from about 10-20 per 100,000 person-years in the sixth decade of life to around 90+ per 100,000 person years in those over 70–80 (Bower et al., 1999). In one population study, the incidence was measured at ~93 per 100,000 in the 70–79 age group (de Rijk et al., 1995). While increasing age is nearly a requisite risk factor, it is not sufficient by itself; many people live into very old age without Parkinson's. Thus, additional factors play a role in who among the elderly develops PD.

1.4.1 Cigarette smoking

An intriguing and highly replicated epidemiological finding is that cigarette smoking is associated with a lower risk of developing PD (Li et al., 2015). Most case control and cohort

studies over the past few decades have observed an inverse correlation between smoking and PD incidence (Li et al., 2015). A comprehensive meta-analysis (Hernán et al., 2002), which pooled data from 44 case control and 4 cohort studies across many countries, found that ever smokers had about a 40% reduced risk of PD compared to never smokers (pooled relative risk ~ 0.59). Moreover, current smokers at the time of the studies had an even lower risk, roughly 60% lower odds of PD than never smokers (RR ~ 0.39 for current smokers), (Hernan et al., 2002). The inverse relationship shows a dose dependent trend: heavy or long-term smokers are the least likely to develop PD. For example, risk decreases by an estimated $\sim 16\%$ for each additional 10 pack-years of smoking. These statistics are striking, especially given the broad consensus that smoking is harmful to health; it raises the question of what mechanism might underlie this unexpected protective effect.

Several explanations have been proposed. One possibility is that nicotine or other compounds in tobacco smoke exert a neuroprotective effect on dopaminergic neurons by stimulating nicotinic acetylcholine receptors or modulating dopamine release. Nicotine has been shown in animal models to have neurotrophic and anti-apoptotic effects on dopaminergic cells (covered in later chapters), (Gagne & Power, 2010). Another theory is the “self-selection” bias- i.e. people predisposed to PD might find it easier to quit smoking or never start (because incipient PD could cause behavioural changes or less reward from nicotine), (Gagne & Power, 2010). However, analyses that have looked at smoking cessation timing suggest the protective association holds even when accounting for early cessation in PD patients (Ritz et al., 2014). A third hypothesis involves monoamine oxidase-B (MAO-B), an enzyme that breaks down dopamine. Tobacco smoke contains compounds that are mild MAO-B inhibitors, which could raise dopamine levels or reduce oxidative stress in the brain over long periods of exposure. Despite decades of observational data, it remains unproven whether smoking truly confers a protective biological effect or whether some unmeasured confounding factors are at play (for

instance, smokers might have other lifestyle factors that reduce PD risk). Nonetheless, the association is robust: never-smoking is consistently associated with higher PD risk, and smoking (past or present) with lower risk (Li et al., 2015; Hernan et al., 2002). Of course, smoking has many deadly health consequences (cancer, cardiovascular disease, etc.), so it is emphatically not a recommended strategy to prevent PD. But understanding this epidemiological link could point scientists toward novel preventive avenues, for example, drugs that safely mimic the beneficial effects of nicotine or other components without the harms of smoking.

1.4.2 Caffeine and PD

Similar to smoking, caffeine consumption (particularly from coffee and tea) has been repeatedly linked to a reduced risk of Parkinson's disease PD (Ross et al., 2000; Chen et al., 2001). Caffeine, an adenosine A2A receptor antagonist, is thought to have neuroprotective properties, with mouse models suggesting its potential to protect against PD (Ross et al., 2000; Chen et al., 2001). The epidemiological evidence is quite strong: numerous large studies have found that people who regularly drink coffee have a lower incidence of PD than those who do not. For instance, a prospective analysis in male health professionals (Ross et al., 2000) and another in the HPFS/NHS cohorts (Ascherio et al., 2001) both showed a dose dependent inverse relationship between coffee intake and PD risk. In the meta-analysis by Hernán et al. (2002), (which looked at both smoking and coffee together), coffee drinkers had about a 30% lower risk of PD compared to non-drinkers (pooled relative risk ~0.69). Each additional 2–3 cups of coffee per day was associated with a further ~25% reduction in risk in case-control data. Similarly, black tea (which also contains caffeine) has been reported to be protective, although green tea evidence is less clear.

As with smoking, it is not definitively proven that caffeine causally prevents PD, but the association appears likely to reflect a real neuroprotective influence. Animal studies support that caffeine can protect dopaminergic neurons in toxin models of PD (Hernan et al., 2002). Furthermore, caffeine's effects seem to vary by sex in interesting ways. In men, the correlation between caffeine and lower PD risk is very robust and consistent (Kim et al., 2017). In women, the relationship has been more complex: some studies suggest that caffeine's protective benefit is evident mainly in women who are not taking postmenopausal oestrogen therapy, whereas women on oestrogen replacement might actually not experience the benefit or could even have a slight increase in PD risk with high caffeine intake (Ascherio et al., 2004). One analysis found that among women who never used hormone replacement, those who drank a lot of coffee had lower PD risk, but among women on oestrogen, high coffee intake did not reduce risk and in some cases these women showed a higher incidence of PD (Ascherio et al., 2004). The proposed explanation is that oestrogens inhibit the metabolism of caffeine (via hepatic enzymes), so oestrogen might raise caffeine levels and potentially negate the upregulation of certain neuroprotective pathways or otherwise interfere with the hormone/neurotransmitter interplay related to PD. This oestrogen caffeine interaction highlights how genetic and hormonal context can modulate an environmental factor's impact on PD risk (Ascherio et al., 2004).

1.4.3 Gut Microbiome

Emerging research over the last decade has pointed to the gut brain axis, in particular, the intestinal microbial community as a potential player in Parkinson's disease pathogenesis (Sampson et al., 2016). The gut microbiome in PD patients has been found to differ from that of healthy individuals, and scientists have hypothesised that misfolded α -synuclein (the protein that accumulates in PD brains) might originate in the gut in some cases and then spread to the

brain via the vagus nerve (the so called “gut first” PD hypothesis). Compelling evidence for a microbe PD connection came from a landmark study by Sampson et al. (2016). In this study, transgenic mice that overexpress human α -synuclein (and normally develop Parkinsonian features) were treated with antibiotics to drastically alter their gut microbiota. Strikingly, the antibiotic treated α -syn mice showed significantly reduced motor deficits and neuroinflammation compared to untreated mice. Furthermore, when the researchers transplanted gut microbiota from PD patients into these mice, the mice developed much worse motor impairments than mice colonized with microbiota from healthy human donors (Sampson et al., 2016). These findings demonstrate that gut bacteria can influence PD like symptoms in mice, presumably by producing metabolites or inflammatory signals that affect the brain. They also suggest that alterations in the human microbiome might be a risk factor or amplifier for PD.

Additional support comes from clinical observations: many PD patients experience chronic constipation decades before motor onset, and inflammatory changes in the enteric nervous system (and even α -synuclein aggregates in gut neurons) have been detected in early PD (Kelly et al., 2013). Some studies have identified specific microbial taxa that are over or underrepresented in PD patients’ intestines, for example, a decrease in beneficial short chain fatty acid producing bacteria and an increase in pro-inflammatory microbes (Scheperjans et al., 2015). While it’s not yet clear whether dysbiosis (an imbalance in gut microbes) is a cause or a consequence of PD, the gut brain connection is now firmly established as an important field of study. The concept of a “gut origin” of PD pathology was further bolstered by evidence that truncal vagotomy (surgical cutting of the vagus nerve, which connects gut to brain) is associated with lower subsequent risk of PD in epidemiological studies, implying a pathogenic agent might travel from gut to brain along that pathway (Svensson et al., 2015).

1.4.4 Well Water, Pesticides and MPTP

A longstanding question in PD aetiology is the role of environmental toxins. For many years, rural living and consuming private well water were thought to increase PD risk, possibly due to exposure to agricultural chemicals seeping into groundwater (Ascherio et al., 2006). However, the evidence for well water itself as a risk factor is mixed. A large population-based case control study in Michigan (Gorell et al., 1998) found no significant association between drinking well water and PD, despite earlier suspicions. Instead, what emerged from that and other studies was that the use of pesticides, particularly in occupational settings like farming correlates with higher PD risk (Hancock et al., 2008). Pesticides encompass insecticides, herbicides, fungicides, and other compounds; many are neurotoxic by design (to pests) and some can affect mammalian neurons.

Multiple epidemiological studies have reported a link between pesticide exposure and Parkinson's. For example, a family-based case control study (Hancock et al., 2008) found that individuals with PD were significantly more likely to have a history of direct pesticide application than their unaffected relatives (OR ~1.6) and that risk increased with cumulative exposure in a dose response manner (Hancock et al., 2008). Importantly, this study noted that living/working on a farm and consuming well water per se were not risk factors in their data, suggesting it is the direct handling or high-level exposure to chemicals that matters. Other investigations have identified specific pesticides of concern. Herbicides like paraquat (which structurally resembles MPP⁺) and insecticides such as rotenone, organochlorines (e.g. DDT), and organophosphates have all been implicated. One case control study in France reported that men with professional pesticide exposure had about double the odds of developing PD (OR ~2.2) compared to those without exposure (Elbaz et al., 2009). In another study, occupational use of herbicides was associated with roughly a 4-fold increased risk and insecticides with a ~3.5-fold increased risk of PD these high risk estimates likely reflect those with prolonged,

intensive exposures (Narayan et al., 2018). The consistency of findings across different populations (USA, Europe, Asia) strengthens the case that pesticide exposure is one environmental factor that can predispose to Parkinson's disease. Compounds like rotenone (a common pesticide) inhibit mitochondrial complex I, replicating a core cellular defect observed in PD. Administering rotenone chronically to rats causes selective degeneration of dopaminergic neurons and formation of Lewy body like protein aggregates, creating an animal model of PD (Betarbet et al., 2000).

Another infamous chemical, though not a pesticide, MPTP, provided dramatic proof of concept that environmental toxins can induce parkinsonism. MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) was identified in the early 1980s as a contaminant in illicit drug synthesis. In 1983, neurologist J. William Langston described a cluster of young drug users who developed acute, permanent parkinsonism after injecting a bad batch of synthetic heroin, the culprit was MPTP (Langston et al., 1983). MPTP itself is not toxic until it crosses into the brain and is metabolized by glial cells to MPP⁺ (1-methyl-4-phenylpyridinium), a molecule that selectively enters dopaminergic neurons via the dopamine transporter. Once inside, MPP⁺ poisons mitochondrial complex I, halting ATP production and generating oxidative stress, which causes rapid cell death in the substantia nigra (Mounsey & Teismann, 2010). The tragic cases of the “frozen addicts” (as chronicled by Langston) provided the first direct evidence that a chemical exposure can produce a Parkinsonian syndrome virtually indistinguishable from idiopathic PD (Langston et al, 1983). MPTP has since been used extensively in research to create primate and mouse models of PD for testing therapies.

The MPTP episode also led to the hypothesis that perhaps environmental complex I inhibitors (like certain pesticides or other pollutants) could be etiologic in typical PD. While no single toxin has been definitively singled out in common PD, epidemiological proxies for long-term exposure, such as having an agricultural occupation, living in rural areas, or well

water use in farming regions often correlate with increased PD risk (likely reflecting pesticide contact), (Hancock et al., 2008). By contrast, factors like metal exposures (e.g. heavy metals from welding or industry, such as iron, manganese, copper, lead) have been investigated for links to PD with mixed and generally inconclusive results. It's possible that chronic low level toxin exposures in the environment contribute small incremental risks that, over a lifetime and combined with genetic susceptibilities, tip the balance toward neurodegeneration. This area remains an active field of research, as identifying and mitigating environmental risk factors could be a path to PD prevention.

1.4.5 Genetics and PD

Although most Parkinson's disease is sporadic, a significant minority of patients (about 10–15%) have a family history of the disease, indicating that inherited genetic factors can play a role (Deng et al., 2018). Over the past 25 years, multiple PD related genes have been discovered through studies of familial Parkinson's and genome wide analyses. These discoveries have revolutionised understanding of PD pathogenesis, revealing that pathways involving protein misfolding, mitochondrial dysfunction, and defective cellular clearance can lead to dopaminergic neuron death.

One of the first major gene discoveries was SNCA, which encodes α -synuclein. In 1997, Polymeropoulos et al. (1997) identified a missense mutation (A53T) in SNCA in an Italian Greek family with autosomal dominant PD. Subsequently, additional SNCA mutations (A30P, E46K) were reported in other families, firmly establishing that α -synuclein mutations cause familial PD. This was a landmark discovery because soon after, α -synuclein was also found to be the major component of Lewy bodies, the pathological hallmark of PD. Spillantini et al. (1997, 1998) demonstrated that the filamentous aggregates in Lewy bodies and Lewy neurites are composed largely of misfolded α -synuclein. This linked familial and sporadic PD

at the molecular level via α -synuclein and suggested a unifying mechanism involving protein aggregation. Duplications and triplications of SNCA (causing excess α -synuclein) were later found to produce PD, confirming that α -synuclein overproduction or impaired clearance is toxic to neurons (Singleton et al., 2003).

Another group of PD genes involves intracellular protein degradation systems, namely the ubiquitin–proteasome system (UPS) and the autophagy lysosome pathway. Proper protein clearance is essential for neuronal survival, and several PD genes converge on these pathways. For example, parkin, encoded by PARK2, is an E3 ubiquitin ligase that tags defective proteins and mitochondria for degradation. Mutations in PARK2 were discovered in Japanese families with autosomal recessive juvenile parkinsonism (Kitada et al., 1998). Such mutations (often deletions or truncations) cause loss of function, impairing ubiquitination and the removal of damaged proteins. Patients with parkin type PD usually develop symptoms before 40 years of age, and their pathology often lacks Lewy bodies, possibly because α -synuclein does not accumulate when disease results primarily from proteasomal dysfunction. Parkin linked PD typically presents with classical motor features but may include dystonia and diurnal improvement.

At the cellular level, parkin mediates mitophagy, the autophagic turnover of damaged mitochondria in conjunction with another PD gene, PINK1. Mutations in PINK1 (a mitochondrial kinase) also cause recessive early onset PD (Valente et al., 2004). Together, PARK2 and PINK1 define a mitochondrial quality control pathway in which PINK1 identifies dysfunctional mitochondria and parkin labels them for autophagic degradation. When either gene is mutated, unhealthy mitochondria accumulate and may trigger neuronal death (Roosen and Cookson, 2016; Truban et al., 2017).

Other important PD genes include DJ-1 (PARK7), UCH-L1 (PARK5), LRRK2 (PARK8), and GBA. DJ-1 mutations (also recessive) lead to loss of a protein that normally

protects against oxidative stress; Bonifati et al. (2003) showed that DJ 1 acts as an antioxidant and molecular chaperone, and its absence increases vulnerability of dopaminergic neurons to reactive oxygen species. UCH-L1 was among the first genes linked to PD: a rare missense mutation (I93M) was identified in a German family (Leroy et al., 1998). UCH-L1 recycles ubiquitin, and although the I93M variant is extremely uncommon, its discovery supported the role of proteasomal dysfunction in PD. Interestingly, a common UCH-L1 polymorphism (S18Y) appears to protect against sporadic PD (Maraganore et al., 1999), suggesting that enhanced UPS function may confer neuroprotection.

LRRK2 (leucine rich repeat kinase 2) is the most common genetic cause of PD identified to date. Mutations, particularly G2019S, cause autosomal dominant PD often accompanied by typical Lewy body pathology (Paisán-Ruíz et al., 2004; Zimprich et al., 2004). LRRK2 is a large multidomain kinase that influences autophagy and endosomal trafficking; pathogenic mutations increase its kinase activity, leading to accumulation of toxic substrates and impaired clearance of α -synuclein. Because LRRK2 linked PD closely resembles idiopathic PD and occurs worldwide, accounting for up to 20% of PD cases in Ashkenazi Jewish and North-African Arab populations, it has become a major target for disease modifying therapy, with LRRK2 kinase inhibitors currently in clinical trials (Zabetian et al., 2006).

In summary, the genetic architecture of PD involves multiple interconnected pathways: α -synuclein aggregation (SNCA), proteasomal and lysosomal function (PARK2, PINK1, DJ-1, UCH-L1, LRRK2, ATP13A2), and mitochondrial maintenance. Many familial PD genes point to mechanisms that also operate in sporadic PD, including Lewy body formation, impaired UPS activity, and mitochondrial dysfunction in the substantia nigra pars compacta. These convergent findings indicate that PD is fundamentally a disorder of protein misfolding and defective cellular clearance. Not all genetic PD presents identically: *parkin* mutant patients

may lack Lewy pathology, whereas GBA mutation carriers (who exhibit impaired lysosomal glucocerebrosidase activity) tend to show greater cognitive decline (Sidransky et al., 2009). Collectively, these discoveries have transformed PD from a purely clinical syndrome into a set of molecularly defined subtypes, providing the foundation for targeted, gene specific therapeutic strategies.

1.5 Neuropathology of Parkinson's Disease

The two central pathological features of PD are: (1) the progressive loss of dopamine producing neurons in the brain, especially in the substantia nigra; and (2) the presence of intracellular protein aggregates known as Lewy bodies (LBs) in surviving neurons. These changes underlie the classic motor symptoms of PD and many of the non-motor aspects as well (Braak et al., 2003).

1.5.1 Loss of Dopaminergic neurons

The degeneration of dopaminergic neurons in the SNc and the resulting dopamine deficiency in the striatum are directly responsible for the classic motor symptoms of PD (Braak et al., 2003). In the healthy brain, dopamine in the striatum maintains a dynamic balance between two major basal ganglia pathways: the direct pathway, which facilitates movement, and the indirect pathway, which inhibits it (Albin et al., 1989). Dopamine activates D1 receptors on direct pathway neurons, thereby exciting the direct pathway, and inhibits D2 receptors on indirect pathway neurons, suppressing the indirect pathway. The net effect of normal dopaminergic tone is to enhance thalamo cortical activation and allow smooth, purposeful voluntary movement.

This classic circuit model, first formulated by Albin, DeLong, and colleagues in 1989, explains the core motor triad of PD: bradykinesia and akinesia (due to thalamic under activation and reduced cortical drive), rigidity (from excessive tonic output of the GPi/SNr affecting brainstem and spinal circuits), and tremor (arising from abnormal oscillatory activity in the interconnected thalamic and cerebellar networks). The pathophysiology of tremor remains complex, but it is likely modulated by the same basal ganglia thalamocortical dysrhythmia.

It is remarkable that by the time these motor symptoms manifest, the brain's compensatory reserves are already largely exhausted. Post-mortem and imaging studies indicate that roughly 50-60% of dopaminergic neurons in the SNc are lost and striatal dopamine levels are depleted by 70-80% at the onset of motor symptoms (Bernheimer et al., 1973; Kish et al., 1988). Before this point, compensatory mechanisms such as increased dopamine turnover, upregulation of post synaptic D2 receptors, and enhanced dopamine synthesis per surviving neuron help sustain near normal motor function (Zigmond & Stricker, 1984). As degeneration progresses, however, these adaptations fail, and the clinical phenotype of PD emerges.

In the later stages of disease, the effects of dopamine depletion extend beyond simple neurotransmitter loss to include abnormal synchronised oscillations within the basal ganglia thalamo cortical network. Neurons in the STN and GPi exhibit excessive oscillatory activity in the beta frequency range (~20 Hz), which correlates with bradykinesia and rigidity (Kouli et al., 2018). These aberrant rhythms further impair the transmission of excitatory signals through the thalamocortical pathway, compounding motor deficits. Therapeutically, this also explains why deep brain stimulation (DBS) of the STN or GPi can alleviate symptoms, it disrupts these pathological oscillations even though it does not restore dopamine levels (further in 1.7.2).

The pattern of degeneration within the SNc is regionally selective. Neurons in the ventrolateral tier are particularly vulnerable, while those in the dorsal tier are relatively spared until later in the disease (Damier et al., 1999). As a result, dopamine terminals in the posterior putamen are depleted early, correlating with motor symptoms, whereas the ventral striatum and caudate are affected later, contributing to the emergence of cognitive and psychiatric features (Kish et al., 1988). Reductions in TH expression and dopamine transporter (DAT) density within the striatum are well documented (Javoy-Agid et al., 1990; Miller et al., 1997), and other dopaminergic systems, such as those arising from the ventral tegmental area and hypothalamus, show relative preservation (Halliday et al., 1990).

Importantly, PD pathology is not confined to the dopaminergic system. It follows a topographic and progressive pattern, as proposed by Braak and colleagues (2003, 2004). According to this staging hypothesis (shown in Figure.2), α -synuclein pathology begins in the dorsal motor nucleus of the vagus (DMNV) and olfactory bulb (stage 1), progresses to the locus coeruleus and other pontine regions (stage 2), then involves the substantia nigra and midbrain (stage 3), before reaching limbic regions (stage 4) and finally the neocortex (stages 5-6). The appearance of motor symptoms generally corresponds to stages 3-4, when nigrostriatal neurons are substantially affected, while the earlier stages, involving the medulla, pons, and olfactory structures, are associated with prodromal non-motor symptoms such as constipation, REM sleep disturbance, and hyposmia.

As the disease advances, other neurotransmitter systems also degenerate, accounting for the broad range of non-motor symptoms seen in PD. Loss of noradrenergic neurons in the locus coeruleus leads to autonomic dysfunction and contributes to depression and fatigue; degeneration of serotonergic raphe nuclei is linked to anxiety, mood changes, and sleep disturbance; and loss of cholinergic neurons in the nucleus basalis of Meynert and pedunculopontine nucleus contributes to cognitive decline, gait freezing, and postural

instability (Zarow et al., 2003; Kouli et al., 2018). Degeneration of autonomic and enteric neurons, including those in the DMNV and sympathetic ganglia, underlies cardiac sympathetic denervation and gastrointestinal dysmotility, evidenced by reduced cardiac MIBG uptake and severe constipation in PD (Braak et al., 2003; Satoh et al., 1999; Orimo et al., 2005). In addition to the selective loss of neurons, PD is also characterised by abnormal protein aggregation. The most distinctive of these are Lewy bodies, which are discussed in the following section.

1.5.2 Lewy Bodies

Besides neuron loss, the other pathological signature of Parkinson's disease is the presence of Lewy bodies in the brain (Spillantini et al., 1998). Lewy bodies are eosinophilic, spherical inclusions within neurons, composed of aggregated proteins, primarily α -synuclein and ubiquitin, along with neurofilaments and other proteins. In PD, Lewy bodies are found in many of the remaining neurons of the substantia nigra and in various other regions (cortex, brainstem nuclei, etc.), while thread like Lewy neurites are seen in neurites and dendrites (Spillantini et al., 1998). The discovery that α -synuclein is the major constituent of Lewy bodies was a breakthrough that tied the sporadic disease to genetic forms. Under the microscope, a classic brainstem Lewy body appears as a dense protein core with a pale halo. Cortical Lewy bodies are often more irregular. Ubiquitin, a protein that tags other proteins for degradation, is present in LBs, indicating that these inclusions may form from accumulations of misfolded proteins that the cell's proteolytic machinery failed to dispose of (Beyer & Ariza, 2007).

The role of Lewy bodies in PD is complex and somewhat controversial. An inverse relationship is often observed between LB load and neuron survival in affected regions such as the SNc, suggesting toxicity (Power et al., 2016). Their formation has been associated with disruption of cellular homeostasis: LBs sequester various components of the proteasome and

autophagy systems (including ubiquitin, heat shock proteins, and proteasome subunits), potentially impairing protein degradation pathways (Olanow et al., 2004). Accumulation of α -synuclein in LBs can also lead to mitochondrial and lysosomal dysfunction and trigger inflammation (Erskine et al., 2021; Rocha et al., 2018).

On the other hand, a competing hypothesis proposes that Lewy bodies may represent a protective mechanism, the cell's attempt to isolate harmful protein aggregates into an enclosed structure (an "aggresome") and reduce their interaction with essential cellular components. In this view, neurons form LBs to sequester toxic oligomers of α -synuclein and other misfolded proteins, thereby delaying cellular damage.

Further insight comes from individuals with incidental Lewy body disease (ILBD), which are people without clinical PD symptoms but show LB pathology at autopsy. ILBD brains often exhibit reduced striatal dopamine markers and partial loss of nigral neurons compared to controls (DelleDonne et al., 2008), suggesting that Lewy pathology can precede overt neuronal loss and may reflect a preclinical stage. At the same time, not all neurons that die in PD contain LBs at the time of death, and in the SNc, many degenerating neurons are found without visible inclusions. This has led to the view that small, soluble α -synuclein oligomers (which accumulate before mature LBs form) are the most toxic species, and that Lewy bodies may be a downstream or compensatory response to their presence (Ingelsson, 2016; Cascella & Cecchi, 2022; Emin et al., 2022).

As discussed in 1.5.1, the relationship between LB and dopaminergic neuron survival is demonstrated by the work of Braak et al., (2003) who conducted an analysis of brains from PD patients and asymptomatic individuals with LB pathology (Figure.1.2). They proposed a neuropathological staging procedure based on the extent of α -synuclein immunopositive Lewy neurites and LBs as markers. This staging suggests that pathology begins in the dorsal motor nucleus of vagus (DMNV), (stage 1) and progressively ascends the neocortex at stages 5-6.

According to this model, the SNc is affected at stage 3, but the symptomatic phase is believed to commence at stages 4-5, marked by severe neuronal loss in the SN.

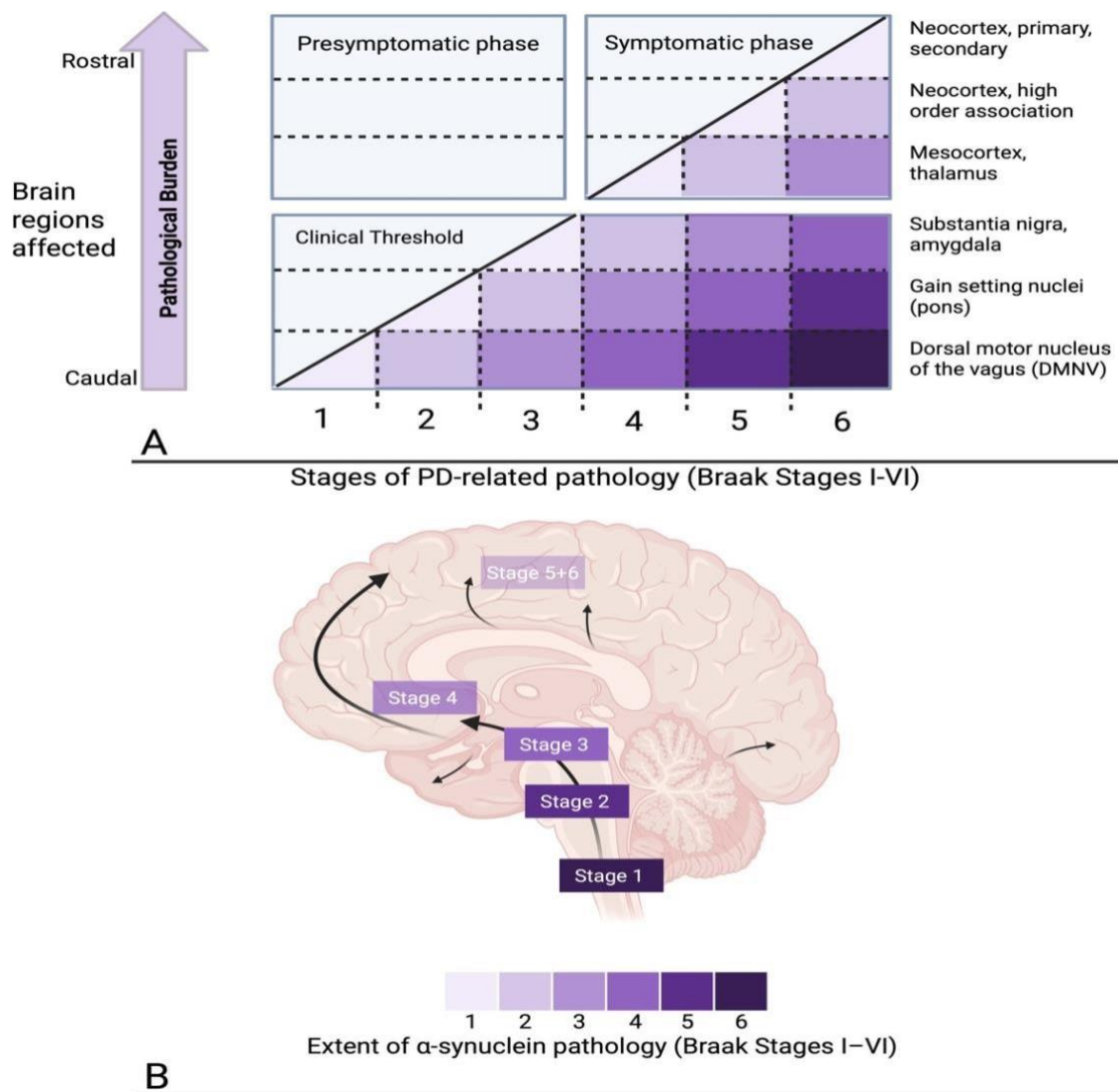


Figure 1. 2: Neuropathological stages of PD. Progressive spread of α -synuclein pathology in Parkinson's disease according to the Braak staging model. (A) Conceptual schematic showing the sequential involvement of brain regions and the clinical threshold marking the transition from presymptomatic to symptomatic phases. (B) Anatomical representation of the caudo rostral propagation of Lewy pathology from the dorsal motor nucleus of the vagus through the brainstem and midbrain to the neocortex (made with Biorender).

1.5.3 Neuroinflammation

Another critical aspect of PD pathology is the involvement of neuroinflammation, referring to the activation of the brain's immune cells and inflammatory responses. Over the past few decades, research has shown that inflammation is not merely a bystander in PD but may actively contribute to neurodegeneration. Post-mortem analyses of PD brains have consistently demonstrated activated glial cells (microglia and astrocytes) in affected regions such as the substantia nigra, along with elevated levels of pro-inflammatory cytokines. Microglia expressing interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α) have been identified in PD nigra (Boka et al., 1994; Hunot et al., 1999).

The connection between neuroinflammation and dopaminergic cell death was further illustrated through studies involving the neurotoxin MPTP. Following the discovery of MPTP's effects in humans, animal studies showed that even after the acute toxic insult, microglial cells remained activated for extended periods within the substantia nigra, indicating a chronic inflammatory response to the initial injury (McGeer et al., 1988; Langston et al., 1999). McGeer and colleagues first reported persistent microgliosis in MPTP exposed brains, suggesting that inflammation might perpetuate neurodegeneration.

Microglia, the resident macrophages of the central nervous system, can release a host of potentially neurotoxic substances upon activation, including pro-inflammatory cytokines (IL-1 β , TNF- α , IL-6), chemokines, reactive oxygen species (ROS), and nitric oxide (NO). Although microglia can produce neurotrophic factors and clearing debris, their pro-inflammatory output in PD appears to dominate, creating a self-propelling cycle of neuronal injury (Wojtera et al., 2005). Elevated cytokine levels have also been detected in the cerebrospinal fluid and blood of PD patients, correlating with disease severity (Halliday & Stevens, 2011).

The causal contribution of inflammation to neuronal death has been directly tested using the lipopolysaccharide (LPS) model, where injection of LPS into the substantia nigra of rats induces robust glial activation and progressive dopaminergic neuron loss (Castaño et al., 1998; Herrera et al., 2000; Iravani et al., 2002). Notably, LPS does not directly affect neurons but instead activates microglia, implicating inflammatory mechanisms as the driver of neuronal degeneration.

Astrocytes, the star-shaped glial cells supporting neurons, also contribute to the inflammatory milieu. In PD, reactive astrocytes can release glutamate, NO, and prostaglandins, which may exacerbate excitotoxic and oxidative stress on dopaminergic neurons (Ransohoff, 2016). While astrocytes can provide neuroprotection through the secretion of trophic factors and maintenance of ionic balance, their dysfunction can further increase neuronal vulnerability. The question of whether inflammation initiates or merely responds to neurodegeneration remains unresolved. Some evidence suggests that individuals with heightened innate immune activity, whether due to genetics or environmental factors, may be predisposed to neurodegeneration. Genetic associations between PD risk and immune-related loci, such as HLA-DR, support an immunological contribution (Hamza et al., 2010). However, neuronal death itself releases signals (e.g., extracellular α -synuclein, ATP, alarmins) that activate microglia, suggesting that neuroinflammation is also a reaction to ongoing neurodegeneration (Halliday & Stevens, 2011). This bidirectional interaction likely establishes a vicious cycle that amplifies neuronal injury.

Anti-inflammatory approaches have therefore gained attention as potential therapeutic strategies. In preclinical models, agents such as Minocycline, Dexamethasone, and Pioglitazone have shown neuroprotective effects by reducing microglial activation and cytokine release (Yrjänheikki et al., 1999; Sánchez-Pernaute et al., 2004; Dehmer et al., 2004). Epidemiological studies have also reported that regular use of non-steroidal anti-inflammatory

drugs (NSAIDs), particularly ibuprofen, is associated with a modestly lower risk of developing PD (Gagne & Power, 2010), although clinical trials with anti-inflammatory drugs like Pioglitazone and CEP-1347 have failed to demonstrate disease-modifying benefits (Iczkiewicz et al., 2006). Systemic inflammation can transiently worsen PD symptoms, and higher levels of peripheral inflammatory markers have been linked with faster disease progression. These observations suggest that anti-inflammatory interventions might be most effective during the prodromal or early disease stages.

1.6 The Normal and Parkinsonian projections and receptors in the basal ganglia

Following the loss of dopamine in the nigrostriatal projections in PD, extensive disorder in the activity of BG occurs. The BG are a group of nuclei located deep within the cerebral hemispheres of the brain that include the striatum (caudate nucleus and putamen), globus pallidus including internus (GPi) and globus pallidus externus (GPe), substantia nigra including substantia nigra pars compacta (SNc) and substantia nigra pars reticulata (SNr), and the subthalamic nucleus (STN), (Albin et al., 1989). The BG plays a critical role in regulating voluntary motor movements, procedural learning and routine behaviours.

In a normal brain, as seen in Figure.1.3A, the SNc projects dopaminergic neurons to the striatum, where dopamine (DA) binds to both D1 and D2 like receptors. Activation of D1 receptors, part of the direct pathway, leads to GABAergic projections to the GPi/SNr (Albin et al., 1989; DeLong, 1990). This increases GABA levels in the GPi/SNr, inhibiting these regions and reducing their inhibitory output to the thalamic relay nuclei. Consequently, the thalamus activates the motor cortex, facilitating movement. Conversely, DA binding to D2 receptors in the indirect pathway inhibits their GABAergic projections to the GPe, resulting in reduced

GABA levels in the GPe. This disinhibition allows the GPe to inhibit the STN. Consequently, the STN's excitatory glutamatergic projections to the GPi/SNr are inhibited, resulting in lower glutamate and higher GABA levels in the GPi/SNr. This inhibition reduces the GPi/SNr's inhibitory output to the thalamus, leading to the activation of the motor cortex and facilitating movement (Albin et al., 1989; Gerfen et al., 1990; DeLong, 1990).

In a Parkinsonian brain, as seen in Figure.1.3B the depletion of dopaminergic neurons in the SNc leads to an imbalance between the direct and indirect pathways. With reduced DA binding to D1 receptors, the GABAergic projections from the striatum to the GPi/SNr are underactive, leading to reduced GABA levels in these regions. Consequently, the GPi/SNr output centres have increased inhibitory output to the thalamus, suppressing thalamic activation and impairing motor cortex excitation, thus hindering movement initiation. Reduced DA binding to D2 receptors leads to overactivity of the indirect pathway. The striatum's GABAergic output to the GPe is increased, which inhibits the GPe. This inhibition decreases the GPe's suppression of the STN, resulting in increased glutamatergic output from the STN to the GPi/SNr. The heightened excitatory input to the GPi/SNr further increases their inhibitory output to the thalamus. This cumulative effect greatly suppresses the thalamus's activation of the motor cortex via glutamatergic projections, leading to significant motor deficits typical of Parkinson's disease (Albin et al., 1989; DeLong, 1990).

It has been found that lesions of the STN and pharmacological reduction of signalling through GABA receptor agonists can induce dyskinesia even in the absence of levodopa (L-DOPA), highlighting the importance of these structures in movement regulation (Burbaud et al., 1998; Hamada and DeLong, 1992). Conversely, the agonist action of apomorphine on D1 and D2 receptors modulates the activity of the STN and GPe (Marsden, 1982). Thus, lesions in the GPe tend to enhance dyskinesia, while lesions in the GPi can reduce it. These findings suggest that while the STN is crucial for regulating movement, the GPe and GPi have opposing

roles in the development of dyskinesia. Understanding these roles is essential for specifying targets (e.g., GPi) for deep brain stimulation (DBS) therapies to alleviate dyskinesia symptoms (Blanchet et al., 1994; Marsden and Obeso, 1994). Lesions of GPi and STN were the mainstay of pallidotomy and thalamotomy to treat the motor symptoms of PD.

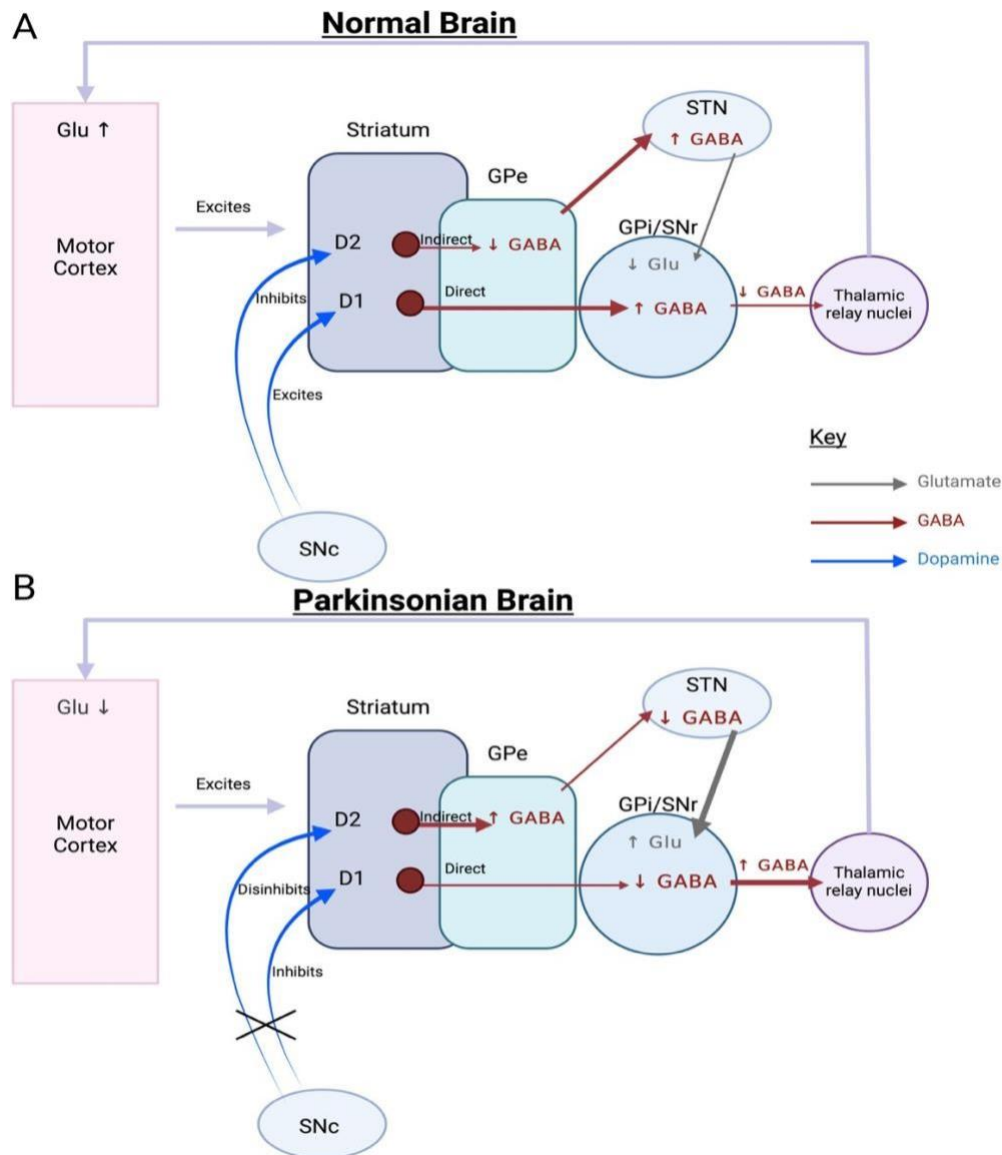


Figure 1. 3: Changes in Basal Ganglia Motor Pathways in Normal and Parkinsonian Brain. Comparison of basal ganglia circuitry in normal and Parkinsonian brains. In the normal brain (A), balanced excitation and inhibition within the striatum, GPe, STN, and GPi/SNr regulate motor control, allowing proper movement initiation via the thalamic relay nuclei. In Parkinson's disease (B), loss of dopaminergic neurons from the SNc disrupts this balance, leading to excessive inhibition of the thalamic relay nuclei, resulting in impaired movement control. Red lines indicate GABAergic projections, blue lines indicate dopaminergic projections, and grey lines indicate glutamatergic projections, with arrow thickness indicating pathway strength. Made with Biorender.

1.7 Current Treatment Strategies in PD

The management of PD relies on both pharmacological and surgical approaches that aim to restore dopaminergic tone and modulate abnormal basal ganglia activity. These

interventions provide significant symptomatic relief but, at present, none halt the underlying neurodegenerative process (Fox et al., 2018). Historically, surgical procedures targeting specific nuclei within the basal ganglia were among the first effective treatments for PD, preceding the development of modern pharmacotherapy (Pycroft et al., 2018). Over the past few decades, advances in neuroimaging, neurophysiology, and stereotactic techniques have allowed for more precise and safer interventions, including deep brain stimulation (DBS) and, more recently, non-invasive ablative methods such as MRI guided focused ultrasound (MRgFUS), (Chang et al., 2021). Alongside these developments, pharmacological strategies such as dopamine replacement therapy (DRT) remain the main means of management, either alone in early disease or in combination with surgical procedures in advanced stages.

1.7.1 Surgical Interventions

Surgical intervention in PD has evolved considerably since the mid 20th century. Early procedures, such as caudate nucleus resection, were attempted to alleviate tremor and rigidity but often resulted in variable outcomes and significant adverse effects (Meyers, 1942; Meyers, 1951). The introduction of stereotactic surgery in the 1950s marked a major advance, allowing neurosurgeons to target deep brain structures with millimetre precision. This paved the way for lesioning techniques such as thalamotomy, pallidotomy, and later, subthalamotomy.

Thalamotomy, targeting the ventral intermediate nucleus of the thalamus, proved highly effective for tremor dominant PD, particularly in reducing resting tremor and rigidity (Walker, 1952; Narabayashi et al., 1987). Similarly, pallidotomy, which involves lesioning the internal segment of GPi, demonstrated marked improvement in bradykinesia, rigidity, and especially levodopa induced dyskinesia in advanced PD (Laitinen et al., 1992). These benefits arise from disrupting the excessive inhibitory output of the GPi to the thalamus, thereby restoring thalamo cortical excitatory drive (Albin et al., 1989). Subthalamotomy, involving selective lesioning of

the STN, also produces powerful anti-parkinsonian effects, with unilateral procedures showing up to a 50% reduction in contralateral tremor, rigidity, and bradykinesia at six months post-operation (Martínez-Fernández et al., 2018). However, the potential for bilateral side effects such as dysarthria, postural instability, and cognitive changes limits the use of these lesioning procedures to unilateral applications (Zesiewicz & Hauser, 2001).

In recent years, MRgFUS has reintroduced lesion surgery in a minimally invasive form. This technique uses precisely targeted ultrasound beams to thermally ablate subcortical nuclei under real time MRI guidance. MRgFUS thalamotomy has received FDA approval for the treatment of PD related tremor (Elias et al., 2016), and clinical studies suggest that subthalamotomy using MRgFUS may also be effective for bradykinesia and rigidity (Martínez-Fernández et al., 2020). The key advantages of MRgFUS are its incisionless nature and the ability to monitor lesion size intraoperatively, providing safety and precision not possible with earlier ablative techniques. Nevertheless, its use remains limited to unilateral procedures, as bilateral ablation continues to carry significant risk.

Although these ablative surgeries offer reliable and often long-lasting symptomatic control, their irreversible nature and potential adverse effects have led to their replacement by DBS in most advanced cases. Today, lesion surgery is reserved for selected patients with focal or tremor predominant PD who are unsuitable candidates for DBS due to comorbidities, advanced age, or contraindications to implanted devices.

1.7.2 Deep brain Stimulation

DBS has become one of the most effective neurosurgical treatments for advanced PD, particularly in patients who no longer achieve adequate control from levodopa therapy or who experience disabling motor fluctuations and levodopa induced dyskinesia (Herrington et al., 2015; Hariz et al., 2022; Machado et al., 2006). The technique involves stereotactic

implantation of electrodes into deep nuclei of the basal ganglia, most commonly the STN or GPi, which are connected to an implanted pulse generator that delivers continuous high frequency electrical stimulation. This stimulation modulates abnormal oscillatory activity within the cortico basal ganglia thalamic circuitry, thereby restoring more physiological firing patterns and improving motor symptoms such as tremor, rigidity, and bradykinesia (Herrington et al., 2015).

The subthalamic nucleus is the principal DBS target for bradykinesia, postural instability, and tremor, and stimulation is typically applied bilaterally to the dorsolateral motor region of the nucleus (Machado et al., 2006). Long term improvement in tremor and rigidity has also been reported with DBS targeting the GPi and the ventral intermediate nucleus of the thalamus (Hariz et al., 2022). Comparative trials demonstrate that both STN and GPi stimulation yield substantial benefits, though they differ slightly in clinical emphasis: STN-DBS allows for greater reduction in dopaminergic medication, while GPi-DBS more directly suppresses dyskinesia (Follett et al., 2010).

Ideal candidates for DBS are those with idiopathic PD of at least four to five years' duration, a robust response to levodopa but disabling fluctuations or dyskinesia, and no significant cognitive impairment or psychiatric illness that might be worsened by surgery. Despite its benefits, DBS carries procedural and device related risks, including infection, lead fracture or migration, and intracranial haemorrhage, reported in 0.2–5% of cases (Benabid et al., 2009). Postoperative cognitive and mood changes can also occur, with GPi-DBS often associated with better mood outcomes than STN-DBS, which in some patients has been linked to transient depression or cognitive decline (Mikos et al., 2010; Zahodne et al., 2009).

1.7.3 Dopamine Replacement Therapy

DRT in PD aims to restore dopamine levels in the brain by using medications that either increase dopamine production, mimic its effects, or prevent its breakdown (Ferrazzoli et al., 2016). This therapy primarily targets the damaged nigrostriatal pathway, which is essential for motor control. By compensating for the loss of dopamine producing neurons, DRT alleviates movement related symptoms such as tremors, stiffness, and slowness. A prominent example of DRT is levodopa, a precursor converted into dopamine to improve motor function, which is further discussed in the following section.

Before the introduction of levodopa, PD therapy relied primarily on plant based compounds and anticholinergic agents. Belladonna alkaloids and related anticholinergics were commonly prescribed to counterbalance the relative excess of cholinergic activity resulting from dopaminergic loss (Kapp, 1992). Although these agents provided modest tremor control, their cognitive and peripheral side effects, including confusion, dry mouth, constipation, and blurred vision, led to their decline in routine use, especially in older adults (Perry et al., 2003).

The advent of selective dopamine agonists revolutionized early pharmacologic therapy. Drugs such as pramipexole, ropinirole, piribedil, and rotigotine act as direct dopamine receptor stimulants, predominantly targeting D2 like receptors. They offer symptomatic benefits comparable to those of levodopa in early disease and delay the need for levodopa initiation, thereby postponing the onset of levodopa induced motor complications (Hornykiewicz, 2010; Rascol et al., 2000). The CALM PD study demonstrated that pramipexole monotherapy effectively controlled early motor symptoms while reducing the risk of dyskinesia relative to initial levodopa therapy (Parkinson Study Group, 2000). These findings established dopamine agonists as an important option for early disease management, particularly in younger patients for whom long term motor complications are a concern.

However, the long-term tolerability of dopamine agonists is limited by adverse effects. Older ergot derivatives such as bromocriptine and cabergoline have largely fallen out of favour due to risks of cardiac valvopathy and fibrosis (Hornykiewicz, 2010). Non ergot agonists, though safer, can still cause nausea, orthostatic hypotension, somnolence, hallucinations, and impulse control disorders (ICDs), including pathological gambling and hypersexuality (Rascol et al., 2000; Weintraub et al., 2010). Cognitive changes involving executive function and impulse regulation have been reported with pramipexole and pergolide, likely reflecting overstimulation of mesolimbic dopaminergic circuits (Cools et al., 2003; Brusa et al., 2013). Such complications are especially problematic in older patients or those with pre-existing cognitive vulnerability (Redensek et al., 2020). Despite these challenges, dopamine agonists remain a valuable component of early PD management. Most patients, however, eventually require levodopa within 2–5 years of diagnosis as disease progression outpaces the efficacy of agonist monotherapy (Antonini et al., 2009).

The introduction of levodopa in the late 1960s transformed PD treatment. Cotzias and colleagues (1969) demonstrated that high dose oral administration of levodopa could dramatically reverse bradykinesia and rigidity, a discovery that reshaped both the prognosis and quality of life of PD patients. This pharmacological breakthrough rapidly supplanted earlier neurosurgical treatments, such as pallidotomy and thalamotomy, which had dominated the pre-levodopa era (Lang & Lozano, 1998). Nevertheless, with the recognition of long-term complications of levodopa therapy, including motor fluctuations and dyskinesia, research focus shifted toward refining pharmacologic selectivity and drug delivery.

Improvements in stereotactic neurosurgery have since redefined the role of surgical treatment in PD. Modern procedures such as DBS, developed through the pioneering work of Benabid et al. (1991), provide an adjustable and reversible alternative to ablative surgery. DBS offers sustained control of motor complications and remains an essential option when

pharmacotherapy, including optimized DRT, no longer achieves satisfactory control (Weaver et al., 2009).

1.8 Levodopa and its Role in PD Management

1.8.1 Levodopa (L-DOPA)

Arvid Carlsson's research in the late 1950s transformed the understanding of PD, by highlighting dopamine's role in motor control. His studies showed that dopamine was not only a precursor to noradrenaline, but also a critical neurotransmitter itself, especially in motor control (Carlsson et al., 1957). By inducing parkinsonian symptoms in animal models using reserpine and then administering L-DOPA to reverse these symptoms, Carlsson established dopamine's vital function in movement regulation, leading to the classification of PD as a dopamine deficiency disorder and laying the foundation for DRT (Duty et al., 2011).

Subsequent studies by George Cotzias and Oleh Hornykiewicz confirmed L-DOPA's efficacy in humans, demonstrating that it could cross the blood brain barrier and replenish dopamine levels in the brain, significantly improving motor symptoms (Cotzias et al., 1969; Hornykiewicz, 2002). Carlsson's work revolutionized Parkinson's treatment, turning L-DOPA into the gold standard therapy and illustrating the power of targeted neurotransmitter replacement. Subsequently, Hornykiewicz's research in the early 1960s demonstrated that patients had significantly reduced levels of DA in the basal ganglia (Ehringer & Hornykiewicz, 1963). Concurrently, Cotzias conducted clinical trials showing that administering large doses of levodopa could dramatically improve motor function in PD patients (Cotzias et al., 1969). Cotzias's method involved gradually increasing the doses to minimize side effects and optimize therapeutic outcomes. However, the use of levodopa introduced challenges, including development of motor fluctuations and dyskinesia with long term use. These complications

necessitated ongoing research into optimizing levodopa therapy; adjunct therapies such as the use of peripherally acting decarboxylase inhibitors (e.g. carbidopa) were developed to inhibit the peripheral metabolism of levodopa within the intestines, allowing more of the drug to reach the brain and reduce peripheral side effects. The introduction of levodopa revolutionized Parkinson's treatment, significantly improving the quality of life for many patients (Parkinson Study Group, 2004). Currently, L-DOPA remains the most widely used and is the gold standard for the treatment of PD.

L-DOPA, naturally occurring in *Mucuna pruriens* (velvet bean), serves as a precursor to dopamine and effectively restores its levels in the brain by crossing the blood brain barrier (BBB) (Lampariello et al., 2012). Once inside the brain, L-DOPA is converted into dopamine, enhancing synthesis and stimulating dopamine receptors, particularly D1 and D2 receptors, which are crucial for motor function regulation, as discussed in chapter 1.6. However, when taken orally, over 99% of L-DOPA is metabolized in the gut by amino acid decarboxylase (AADC) enzymes, significantly reducing its availability in the brain and leading to peripheral side effects (Contin & Martinelli., 2010).

As a result, early clinical trials of L-DOPA initially faced challenges due to its significant peripheral side effects and limited efficacy, as most of the administered dose was converted to dopamine outside the brain (van Gerpen et al., 2006). The introduction of AADC inhibitors, such as carbidopa, addressed this issue by preventing peripheral conversion, allowing more L-DOPA to cross the BBB. This combination significantly enhanced L-DOPA's therapeutic effect, expanding its 'therapeutic window' and increasing the duration of symptom relief ('on' time) (Nutt et al., 1987; Brooks and Sagar, 2003).

Despite its benefits, long-term L-DOPA therapy carries risks such as dyskinesia and motor fluctuations, which are common complications. Additionally, like dopamine agonists,

L-DOPA can lead to impulse control disorders. Discontinuing the drug may manage these issues, but withdrawal symptoms, including anxiety and pain, may arise (You et al., 2018).

1.8.2 Pharmacokinetics of Levodopa

Levodopa exhibits complex pharmacokinetic behaviour that is strongly influenced by its biochemical similarity to large neutral amino acids (LNAAs), including tyrosine, phenylalanine, tryptophan, leucine, and isoleucine (Contin & Martinelli, 2010). It is primarily absorbed in the proximal small intestine through saturable LNAA transporters, the same carriers used for dietary amino acids (Camargo et al., 2014). As a result, protein-rich meals compete with levodopa for uptake and can reduce its bioavailability. To improve consistency of absorption, patients are usually advised to take L-DOPA on an empty stomach, or at least 30 minutes before meals, and to maintain a regulated or redistributed daily protein intake (Tsui et al., 1989; Baruzzi et al., 1987; Contin & Martinelli, 2010).

After intestinal absorption, levodopa crosses the BBB using the same LNAA transport system operating in the intestinal mucosa. Competition with other circulating amino acids can hinder this process, explaining why high protein meals may worsen the therapeutic response even without lowering plasma levodopa concentrations (Nutt et al., 1984; Contin & Martinelli, 2010). Once within the CNS, levodopa is decarboxylated to dopamine by aromatic L-amino acid decarboxylase (AADC) within remaining dopaminergic neurons and other decarboxylating cells.

In the periphery, however, levodopa undergoes extensive presystemic metabolism by AADC and catechol-O-methyltransferase (COMT), meaning that without enzyme inhibition only about 30% of an oral dose reaches systemic circulation (Tambasco et al., 2018; Contin & Martinelli, 2010). Co-administration of peripherally acting AADC inhibitors such as carbidopa or benserazide markedly enhances levodopa bioavailability (nearly tripling systemic levels)

while reducing gastrointestinal and cardiovascular side effects (Contin & Martinelli, 2010). Subsequent metabolism shifts toward COMT activity, producing the long-lived metabolite 3-O-methyldopa (3-OMD). Although 3-OMD can compete with levodopa for LNAA transporters, its effect on brain uptake is modest at therapeutic concentrations (Nissinen et al., 2009; Contin & Martinelli, 2010).

Levodopa has a short plasma half-life of approximately 60-90 minutes when administered alone (Djaldetti et al., 1996), and even with AADC inhibition this extends only to 0.7-1.4 hours, necessitating frequent dosing to maintain motor benefit (Contin & Martinelli, 2010). The addition of COMT inhibitors such as entacapone or tolcapone prolongs levodopa's half-life by up to 50% and increases the area under the plasma concentration time curve by around 40%, providing smoother motor responses and reduced end of dose "wearing-off" (Nissinen et al., 2009; Kaakkola, 2000; Contin & Martinelli, 2010).

Nevertheless, levodopa plasma levels remain variable due to delayed gastric emptying, erratic intestinal motility, and dietary competition, all of which are accentuated PD because of autonomic dysfunction (Pardridge, 1979; Schapira, 2010). Slow gastric emptying can prolong the time to peak plasma concentration two- to threefold and decrease the maximum concentration by about 30%, further contributing to inconsistent clinical effects (Baruzzi et al., 1987; Contin & Martinelli, 2010).

As Parkinson's disease progresses and the striatal capacity to store dopamine diminishes, the motor response becomes increasingly dependent on fluctuating plasma levodopa concentrations. This tight concentration effect coupling underlies the development of motor fluctuations and "wearing-off" phenomena. Efforts to stabilize levodopa pharmacokinetics (including controlled release formulations, liquid levodopa preparations, and metabolic enzyme inhibition) have achieved only partial success due to the complex alterations

in cerebral levodopa handling that accompany nigrostriatal degeneration (Contin & Martinelli, 2010).

1.8.3 Clinical Efficacy of Levodopa

The "honeymoon" phase is an early period in PD management following the initiation of L-DOPA therapy, characterized by a substantial improvement in motor symptoms such as tremors, rigidity, and bradykinesia. Patients often experience a near normal level of motor function and a significant enhancement in their quality of life, as the medication effectively restores mobility (Cotzias et al., 1969). This phase typically lasts between 2 to 5 years, during which the brain's remaining dopamine producing neurons efficiently convert L-DOPA into dopamine, providing stable and predictable symptom control with relatively low doses (Fahn, 2008).

The clinical efficacy of levodopa during this honeymoon period is well documented, with Early vs. Later Levodopa Therapy in PD (ELLDOPA) study providing further support. This multicentre, double blind, placebo-controlled trial was designed to evaluate the impact of early versus delayed levodopa therapy on the progression of PD symptoms (Fahn et al., 2004). The ELLDOPA study involved 361 patients with early PD who were randomised to receive either levodopa at doses of 150 mg, 300 mg, or 600mg per day or a placebo for 40 weeks. The participants had not previously been treated with levodopa, and the primary endpoint was the change in the Unified Parkinson's Disease Rating Scale (UPDRS) score, which assesses motor and non-motor symptoms of PD. The results of the ELLDOPA study demonstrated that patients receiving levodopa experienced significant improvements in UPDRS scores compared to those receiving the placebo. Specifically, the higher doses of levodopa (300 mg and 600 mg per day) were associated with greater symptomatic relief, indicating a dose response

relationship. This study provided strong evidence that early levodopa therapy could significantly improve motor function and overall quality of life in PD patients, highlighting the importance of levodopa in early PD management (Fahn et al., 2004). However, the benefits seen during the honeymoon phase are temporary as the disease progresses. Over time, the number of dopamine producing neurons continues to decline, reducing the brain's ability to convert L-DOPA into dopamine. This decline necessitates higher doses of L-DOPA to maintain symptom control, leading to motor complications such as "wearing-off" periods and the development of L-DOPA induced dyskinesia (LIDs), (Olanow et al., 2001). These complications mark the end of the honeymoon phase and often require adjustments in treatment, such as the introduction of dopamine agonists or advanced therapies like DBS to maintain symptom relief (Obeso et al., 2000).

1.8.4 Disadvantages of Levodopa Therapy

As mentioned, a major disadvantage of levodopa therapy is the development of L-DOPA induced dyskinesia, which become increasingly common as PD progresses. As the therapeutic window for levodopa narrows, patients experience motor complications such as LID, characterized by involuntary, erratic, and dance like movements. Additionally, motor fluctuations become more pronounced, with patients experiencing unpredictable changes in motor performance and extended off phase periods where symptoms like rigidity, immobility, and tremors occur before the next dose is effective. Studies estimate that 40% of PD patients encounter these complications within five years of starting levodopa treatment, with some experiencing them as early as two years (Ahlskog & Muenter, 2001; Koller & Hubble, 1990). Specifically, LID risk among PD patients was found to be around 30% by 5 years, and 59% by 10 years (van Gerpen et al., 2006).

Prolonged levodopa therapy, especially in advanced stages of PD, not only increases the risk of LID but also poses additional complications. For example, the metabolism of levodopa by the enzyme COMT can lead to elevated homocysteine levels, which are associated with an increased risk of cardiovascular diseases, such as coronary artery disease, stroke, and peripheral vascular disease (Lamberti et al., 2005; Valkovic et al., 2005). Elevated homocysteine promotes atherosclerosis and thrombosis, adding further complexity to the management of PD and the treatment of patients undergoing long term levodopa therapy.

To enhance the efficacy of levodopa and mitigate its complications, adjunctive medications are recommended. One includes entacapone, which prevents the methylation of levodopa, thereby increasing its availability and reducing the formation of homocysteine (Lamberti et al., 2005; Valkovic et al., 2005). MAO inhibitors are also recommended, such as selegiline and rasagiline, which reduce the breakdown of DA and levodopa, enhancing their therapeutic effect and reducing the required dose of levodopa (Rabey et al., 2000; Riederer & Laux, 2011). Amantidine, an NMDA receptor antagonist, has shown efficacy in 60-70% of PD patients in alleviating dyskinesia by modulating glutamate activity (Snow et al., 2000; Sawada et al., 2010).

However, addressing the side effects of levodopa with additional pharmacological agents can create a complex cycle of treatment, where each new drug may introduce its own set of side effects. For instance, COMT inhibitors like entacapone can cause diarrhoea and abdominal pain (Fu et al., 2022) whilst MAO-B inhibitors might lead to insomnia and hypertension. Therefore, the management of PD with levodopa and adjuncts require a tailored approach to balance therapeutic benefits with minimization of side effects.

Nevertheless, early use of levodopa is associated with a higher likelihood of motor complications, particularly in younger patients. In such cases, delaying treatment may be warranted to avoid the potential complications of prolonged therapy. This strategy aims to

preserve the patient's quality of life over the long term by minimizing the risk of developing severe motor side effects, such as LID discussed in the following section and other complications associated with chronic levodopa use.

1.9 Levodopa Induced Dyskinesia

LIDs are a prevalent and challenging complication in the treatment of PD affecting the effectiveness and tolerability of L-DOPA therapy. First described by Cotzias et al. (1967), LID emerge over time as a side effect of levodopa, manifesting in various forms and often limiting its therapeutic benefits. Initially, LID was associated primarily with peak plasma levels of levodopa, but clinical observations have shown a more complex pattern, including diphasic dyskinesia (occurring at the start and end of the dose cycle) and early morning dystonia when patients experience reduced mobility upon waking (Cotzias et al., 1967; Tronci & Francardo, 2018). Estimates indicate that roughly 40% of patients exhibit LID within 5 years of therapy initiation, rising to nearly 90% after a decade (Carta & Tronci, 2014). Some studies report that about 30% of patients show dyskinesia as early as 2–3 years into levodopa therapy, which further shows the urgency for improved strategies (Alvarez et al., 2024).

LID fall under the broader "wearing off phenomenon," which describes the gradual return of PD symptoms before the next dose due to levodopa's short half-life. While early in treatment, L-DOPA may provide symptom relief for up to ten hours; as PD progresses, this duration can decrease to as little as two hours (Schapira et al., 2009). LID are specific abnormal involuntary movements (AIMs) that occur during "on" or "off" phases, linked to fluctuations in levodopa levels (Encarnacion & Hauser, 2008). They typically develop 5-10 years after initiating L-DOPA therapy due to its intermittent, pulsatile dosing, which causes maladaptive changes in the basal ganglia circuitry (Stocchi et al., 2009).

The clinical presentation of LID is diverse, including chorea (rapid, jerky movements), choreoathetosis (writhing and twisting actions), myoclonus (sudden muscle jerks), akathisia (inner restlessness), and ballism (large, flinging movements). These movements often appear on the more severely affected side of the body, starting in the lower extremities and progressing to the upper limbs as the disease advances. This pattern may be linked to early dopaminergic loss in the dorsolateral striatum (Schapira et al., 2009; Stocchi, Jenner & Obeso, 2010).

1.9.1 Prevalence of LID

The incidence of LID varies significantly, ranging from 3% to 94%, influenced by factors such as the age at disease onset, Parkinson's severity, levodopa dosage and duration, and potentially other unidentified factors (Carta & Tronci, 2014). However, variations in research methodologies, like patient reported histories versus comprehensive neurological assessments and the lack of standardized assessment scales, could contribute to these discrepancies (Tran et al., 2018). Earlier studies reported higher rates of LID, such as 81% after 12 months of Levodopa therapy, compared to later studies showing rates as low as 17%. This difference may be due to the initial use of higher levodopa dosages during advanced stages of Parkinson's disease. Levodopa dosage is critical in LID development. For example, in one study, LID did not occur with a mean daily dose of 338 mg but appeared at 387 mg. Thus, it could be suggested that higher doses are associated with prolonged dyskinesia (Hong et al., 2020).

Interestingly, not all patients develop dyskinesia despite prolonged Levodopa therapy. This could be attributed to genetic factors that play a significant role in susceptibility to LID. Studies have shown that certain genetic polymorphisms, particularly in the DA receptor D2 gene (DRD2), are associated with a reduced risk of developing peak dose dyskinesia (Oliver et al., 1999).

1.9.2 Types and Signs of LID

1.9.2.1 Clinical Presentation of LID

The most common form of LID is chorea, which involves involuntary, rapid, irregular movements that can shift between different body parts. The severity varies, ranging from mild movements provoked during activities like walking or speaking (overflow chorea) to severe flinging motions known as ballism. Chorea often includes choreoathetoid movements, characterized by superimposed twisting motions, and can affect various body regions, including the trunk, head, limbs, and speech muscles (Marsden & Quinn, 1990; Jankovic & Stacy, 2007).

Dystonia, the second most common form, involves sustained muscle contractions leading to abnormal postures or repetitive movements, such as a leg twisting while walking or an arm being pulled behind the back. It can be more disabling than chorea, especially during "off" periods when it is painful and debilitating (Fahn, 1988; Jankovic, 2008).

Less frequent forms of LID include akathisia (motor restlessness), a high-stepped gait, rapid alternating leg movements, blepharospasm, and mixed movement patterns. These varied presentations contribute to the complexity of managing LID effectively (Lang & Lozano, 1998; Rascol et al., 2000).

1.9.2.2 Types of LID

LID can manifest in different ways, generally classified into three main types: peak dose dyskinesia, wearing-off or off-period dyskinesia, and diphasic dyskinesia. Among these, peak-dose dyskinesia is the most prevalent, while diphasic dyskinesia is the least common. Patients might experience a single type of dyskinesia or a combination of two or three types (Hansen et al., 2022).

Table 1. 1: different types of levodopa induced dyskinesia and its medical management.

Types	Clinical description	Management strategies
Peak dose Dyskinesia	Most common type of dyskinesia (80%), which occurs at the time of peak plasma levels of levodopa, characterized by stereotypic head movements, choreiform truncal movement, and ballistic limb movement, rarely myoclonus. Can be ocular, respiratory, or abdominal muscle.	Decrease individual levodopa dosages, discontinue or reduce COMT and MAO-B inhibitors, switch to immediate release preparations. Consider adding amantadine.
Off period Dystonia	Second most common type (30%) and typically occurs in early morning, before the first dose of levodopa, usually involves legs.	Adding long-acting formulations at bedtime for off period symptoms during night or early morning. For off time during the day. consider adding COMT inhibitors. MAO-B inhibitors, or dopamine agonist
Diphasic Dyskinesia	Least common (20%) and starts 10-15 min after levodopa ingestion with ipsilateral leg movement and then contralateral involvement. Followed by improvement of parkinsonian symptoms for several hours and then recurrence of dyskinesia, when levodopa levels decline	Most difficult to treat. CIG infusion or subcutaneous infusion of apomorphine or surgical intervention. e.g. DBS

Peak dose dyskinesia, first described by Cotzias et al., occurs when L-DOPA concentration in plasma peaks, typically causing choreiform movements. Other motor symptoms such as dystonia, myoclonus, and ballism may also occur, involving the upper limbs, trunk, and orofacial muscles. A "square wave response" can happen, where dyskinesia starts with medication onset and persists until it wears off. Stress and anxiety often exacerbate these symptoms (Jankovic, 2008). Rare manifestations include isolated ballism, ocular, abdominal ("belly dancer's"), and respiratory dyskinesia. Ocular dyskinesia, causing involuntary upward eye movements, was found in 16% of advanced PD patients (Micheli et al., 1991). Abdominal dyskinesia involves involuntary abdominal muscle movements, reported in rare cases (Illiceto et al., 1990; Kono et al., 1994). Myoclonus, a sudden muscle jerk, occurs in peak dose dyskinesia, particularly in PD patients with dementia (Jankovic, 2008).

Off period dyskinesia manifests as a result of long-term L-DOPA therapy, where the effects of L-DOPA wear off, leaving the patient in a ‘medication-off’ state. Types of movements include dystonia, characterised by sustained muscle contractions that cause twitching and repetitive movements or abnormal postures (Jankovic, 2008). Dystonic movements often affect the feet and lower limbs but can also involve other body parts as well. Off-period dystonia can be painful and is frequently described as causing cramping sensations, particularly in the early morning before the first dose of L-DOPA. Off-period dyskinesia often affects focal or segmental regions of the limb/side of the body and tends to affect one side of the body more than the other. It has been found that continuous delivery systems, such as levodopa carbidopa intestinal gel (LCIG) and continuous subcutaneous apomorphine infusion significantly reduce the ‘off’ periods in advanced PD patients (Poewe et al., 2010).

Unlike peak dose dyskinesia, which occurs at highest plasma levels of L-DOPA, diphasic dyskinesia manifests when the drug levels are rising or falling, resulting in two peaks of abnormal movements: one at the onset of drug effect and another at its end. Patients can exhibit repetitive movements of the lower limbs, or a combination of choreiform and dystonic movements. Diphasic dyskinesia can affect one or both sides of the body and often involves the lower limbs more frequently than the upper limbs (Kumar et al., 2005; Nutt, 2001). Patients with diphasic dyskinesia may experience improvement during the on state, described as "dystonia (dyskinesia) improvement dystonia (dyskinesia)". This describes the pattern of initial dyskinesia as L-DOPA levels, a period of relatively normal movement (improvement), followed by another phase of dyskinesia as the drug levels fall. Some patients might also exhibit autonomic dysfunction, such as cardiac arrhythmias, during dyskinesia periods (Marsden & Parkes, 1976; Nutt et al., 2000).

1.9.3 Mechanisms of LID development – Evidence from animal models

1.9.3.1 Peripheral and Central Mechanisms

In the early stages of PD, remaining dopaminergic terminals in the striatum retain the ability to take up exogenous levodopa, convert it to dopamine and release it in a regulated manner. This buffering capacity smooths the dopaminergic input to postsynaptic targets. As degeneration progresses, however, this mechanism weakens: with fewer terminals available to store and release dopamine, striatal dopamine levels increasingly mirror the pharmacokinetics of each levodopa dose (Tumpa et al., 2025). Plasma peaks translate directly into striatal peaks, and troughs into periods of insufficient dopamine, giving rise to the well-known “on” and “off” fluctuations.

Once receptor stimulation becomes intermittent, central mechanisms begin to shift. Medium spiny neurons adopt a sensitised state, and the rules governing corticostriatal plasticity become distorted. A consistent observation across dyskinetic rodents and primates is the emergence of abnormally persistent long-term potentiation at corticostriatal synapses. This potentiation becomes unusually stable and resistant to reversal. Under physiological conditions, these synapses can depotentiate, enabling the system to erase outdated motor patterns. In dyskinetic animals, this capability is markedly impaired. Picconi et al. (2003) first demonstrated that dyskinetic rodents lose the ability to depotentiate strengthened corticostriatal synapses, linking this failure directly to involuntary movements. Ghiglieri and colleagues later confirmed that chronic levodopa exposure produces exaggerated LTP that cannot be reversed even with strong depotentiation protocols (Ghiglieri et al., 2012). Importantly, similar deficits have been documented in humans: Prescott et al. (2014) reported that neurons in the basal ganglia output nuclei of patients with dyskinesia also fail to depotentiate, indicating that this maladaptive plasticity is a conserved feature across species.

These physiological changes reflect a broader shift in intracellular signalling. Pulsatile dopaminergic input leads to exaggerated activation of D1linked pathways involving cAMP, PKA and DARPP-32, and this is closely coupled with robust activation of the ERK pathway. Elevated phosphorylated ERK has been repeatedly identified as a molecular hallmark of dyskinesia, correlating with dyskinesia severity and appearing consistently across experimental models. Pavón et al. (2006) showed that ERK activation tracks with the expression of Δ FosB, a stable transcription factor linked to sensitised motor output. Westin et al. (2007) demonstrated that ERK activation depends on D1receptor stimulation and arises early as dyskinesia develops. Santini et al. (2010) further confirmed that excessive recruitment of the cAMP–PKA–DARPP-32–ERK cascade plays a central role in the aberrant plasticity underlying dyskinesia, and that attenuation of ERK activity can reduce dyskinetic movements.

Together, these peripheral and central mechanisms create a state in which dopamine fluctuations are translated into exaggerated downstream responses, priming the circuit for the emergence of dyskinesia as levodopa therapy continues.

1.9.3.2 Presynaptic and Postsynaptic Mechanisms

From a mechanistic viewpoint, L-DOPA induced dyskinesia can be considered the result of both presynaptic changes (how dopamine is produced, released and regulated) and postsynaptic changes (how striatal neurons and basal ganglia networks respond to dopamine).

In the early stages of PD, some dopaminergic axon terminals remain in the striatum and can convert exogenous L-DOPA to dopamine via L-amino acid decarboxylase, storing it in vesicles through VMAT2 and releasing it in a regulated manner. This helps buffer dopamine delivery (Cenci, 2014; Mosharov et al., 2014; Aradi & Hauser, 2020). As PD progresses, the loss of these terminals means that more of the L-DOPA dose is taken up by non-dopaminergic cells that lack dopaminergic autoregulatory machinery. The consequence is unregulated

dopamine release and greater fluctuations in synaptic dopamine with each dose of L-DOPA. In advanced PD, levodopa's effects become closely tied to its pharmacokinetics, with plasma levodopa peaks correlating with dyskinesia severity (Stocchi, 2006). Altered dopamine turnover may also occur with chronic therapy as remaining terminals adjust their biosynthetic and catabolic pathways.

Postsynaptic mechanisms refer to changes in striatal neurons and downstream circuitry. A central postsynaptic event in dyskinesia is the hyperactivation of D1 receptor signalling in direct pathway medium spiny neurons. When L-DOPA causes large dopamine surges, D1 receptors trigger intracellular cascades involving cAMP, PKA, DARPP-32 and ERK. In the dyskinetic striatum, both DARPP-32 and ERK exhibit increased phosphorylation during L-DOPA “on” periods, contributing to heightened excitability of the direct pathway (Beaulieu & Gainetdinov, 2011; Santini et al., 2007; Santini et al., 2010; Santini et al., 2008; Feyder & Cenci, 2011). Indirect pathway neurons undergo their own adaptive changes; dopamine loss makes them supersensitive, yet during levodopa peaks they are strongly inhibited, facilitating a transient release of thalamocortical activity.

This imbalance is reflected at the level of basal ganglia output. Recordings show reduced firing of internal globus pallidus neurons during dyskinesia, consistent with excessive direct pathway drive and thalamocortical disinhibition (Ghiglieri et al., 2012). Imaging studies similarly demonstrate that dyskinesia corresponds to periods of increased synaptic dopamine and greater D1 receptor occupancy during the “on” state.

Additional postsynaptic changes involve ion channel plasticity and local inhibitory circuits. Altered expression of calcium channels and other membrane conductances can shift firing properties of striatal neurons, while dysfunction in GABAergic interneurons may disrupt the normal timing and patterning of MSN output (Ghiglieri et al., 2012; Borgkvist et al., 2017). Structural modifications, including changes in dendritic spine density on striatal MSNs, may

also impair indirect pathway function while strengthening aberrant connections in direct pathway neurons.

Glial cells form part of this maladaptive postsynaptic environment. Microglia and astrocytes can become activated in dyskinesia and influence neuronal excitability and synaptic plasticity through inflammatory mediators and transmitter release. Inhibiting specific glial pathways can reduce dyskinesia in experimental models, indicating that neuron glia interactions contribute to this condition (Carta et al., 2017; Cardinale et al., 2024).

In summary, L-DOPA induced dyskinesia arises from the convergence of presynaptic loss of dopamine buffering and postsynaptic neuroadaptations in basal ganglia pathways. The presynaptic loss sets the stage for dopamine spikes, and the postsynaptic changes in receptors, signalling cascades and synaptic architecture transform those spikes into pathological activity. The next section focuses specifically on how glutamate and serotonin systems contribute to these processes, as they are major non-dopaminergic modulators of dyskinesia.

1.9.4 Role of Glutamatergic and Serotonergic systems in LID

1.9.4.1 Glutamatergic system in LID

Glutamate, the primary excitatory neurotransmitter in the CNS, is essential for synaptic transmission, memory, and learning (Meldrum, 2000). In PD, glutamatergic dysfunction significantly contributes to motor and non-motor symptoms, particularly through alterations in glutamate receptor activity in the basal ganglia, which are implicated in dyskinesia development (Ambrosi et al., 2014; Picconi & Calabresi, 2014). Dyskinesia in PD is driven by both dopaminergic and glutamatergic dysfunction, with maladaptive plastic changes in the cortico-striatal pathway contributing to abnormal motor responses.

AMPA receptors play a key role in synaptic plasticity and fast excitatory neurotransmission. They are primarily localized on the postsynaptic membrane of medium spiny neurons (MSNs) in the striatum and are crucial for modulating the excitatory inputs that regulate motor function (Huganir & Nicoll, 2013). In the context of PD, AMPA receptor dysregulation, characterized by changes in receptor density and sensitivity, leads to enhanced excitatory neurotransmission and excitotoxicity, which contributes to the neurodegenerative processes and motor dysfunctions seen in dyskinesia. AMPA receptor subunits, particularly GluA1 through GluA4, determine receptor function, with calcium permeable AMPA receptors being especially implicated in promoting excitotoxic damage (Kwok et al., 2005). Efforts to modulate AMPA receptor activity through antagonists like NBQX have produced mixed results, reflecting the complexity of their role in PD pathophysiology (Klockgether et al., 1991; Konitsiotis et al., 2000).

NMDA receptors also play a critical role in synaptic plasticity and excitatory neurotransmission. Like AMPA receptors, NMDA receptors mediate glutamatergic inputs to the MSNs and are essential for regulating synaptic strength and motor control (Paoletti et al., 2013). In dyskinesia, NMDA receptor overactivation exacerbates the maladaptive plasticity associated with L-DOPA induced dyskinesia. The activation of NMDA receptors, which depends on both glutamate binding and cell depolarization (removing the Mg^{2+} block), allows excessive calcium influx, further amplifying excitotoxicity and the development of abnormal motor responses (Traynelis et al., 2010). Studies have shown that NMDA receptor antagonists targeting the NR2B subunit, in particular, hold promise in reducing dyskinesia by modulating this hyperactivity (Gardoni et al., 2006).

Dopamine receptor denervation in PD also alters the phosphorylation and sensitivity of NMDA and AMPA receptors, driving abnormal plasticity in the corticostriatal pathway. This contributes to the heightened sensitivity and maladaptive motor responses characteristic of LID

(Surmeier et al., 2007). Moreover, the interaction between D1 dopamine receptors and NMDA receptors in striatal postsynaptic densities further emphasizes their combined role in driving dyskinetic symptoms (Fiorentini et al., 2003).

Therapeutic strategies targeting both AMPA and NMDA receptors have been explored to mitigate dyskinesia by dampening the overactive glutamatergic transmission that fuels LID. While NMDA antagonists such as amantadine have shown clinical benefits, the complexity of glutamate receptor modulation continues to challenge the development of consistent and effective therapies (Blanchet et al., 1999). Recent research also suggests that focusing on more selective NMDA receptor subunits (such as NR2B) may hold greater potential in reducing dyskinesia while minimizing adverse effects (Alt et al., 2007).

1.9.4.2 Serotonergic System in LID

Research indicates that modulating serotonergic pathways using receptor agonists can lead to improvements in LIDs (Cenci, 2014; Politis et al., 2014). These neurons remain functional early in Parkinson's disease and are involved in the conversion of L-DOPA to dopamine (DA), its storage, and release (Carta et al., 2007; Tronci and Carta, 2013). This DA is stored together with serotonin in serotonergic terminals and released together, a process termed compensatory co-transmission (Mahmoudi et al., 2013). Unlike dopaminergic neurons, serotonergic neurons lack certain autoregulatory mechanisms such as the DAT and D2 inhibitory receptors. This absence leads to the unregulated release of DA in the striatum, contributing to the development of LIDs (Figure.1.4) (Selnow et al., 2019). The unregulated DA release results in excessive stimulation of postsynaptic receptors, leading to the dyskinetic movements observed in LIDs.

Long-term L-DOPA treatment exacerbates this problem by reducing the density of 5-HT nerve fibres in the striatum, especially when dopaminergic neurons are absent (Chen et al.,

2024). Chronic L-DOPA exposure can also be toxic to serotonergic cells, potentially generating harmful compounds like 6-OHDA, which decrease 5-HT levels in both striatal and extrastriatal regions, worsening dyskinesia in patients (Jaunarajas et al., 2013; Eskow et al., 2012). Additionally, long-term L-DOPA treatment can increase homocysteine levels, causing oxidative stress and excitotoxicity, which further damages serotonergic neurons (Lundblad et al., 2009; Müller, 2002; Navailles et al., 2011). Damage to serotonergic neurons results in lower 5-HT levels and increased sensitivity of 5-HT_{1A} receptors following L-DOPA treatment. This reduction in serotonergic activity leads to decreased dopamine levels in various brain regions that serotonergic neurons innervate. Ultimately, this decline in serotonergic function exacerbates LIDs, underscoring the critical role of the serotonergic system in dyskinesia pathogenesis (Lundblad et al., 2009; Müller, 2002; Navailles et al., 2011).

Modulation of serotonergic neurons influences LIDs through several mechanisms, including regulating DA release from serotonergic neurons (as a false transmitter see Figure 1.4). This may alter the arrangement of NR2B subunits of NMDA receptors between intra and extra synaptic locations, and modulating the activity of cortical glutamatergic neurons that project to the striatum (Iderberg et al., 2013; Munoz et al., 2009, 2008). These intricate interactions between serotonergic and dopaminergic systems, as well as the involvement of glutamatergic signalling, highlight the complex role of serotonergic neurons in both the development and potential treatment of LIDs in Parkinson's disease.

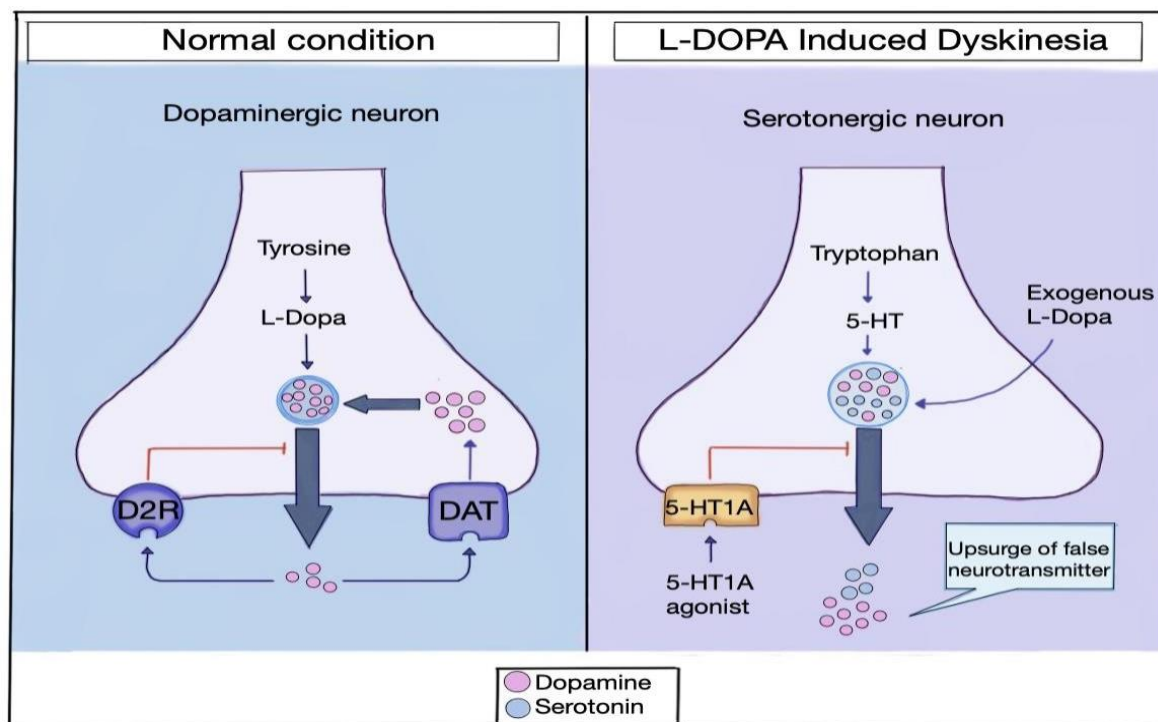


Figure 1. 4: Normal Condition Vs. L-DOPA Induced Dyskinesia. In normal conditions, dopamine (DA) is synthesized from tyrosine through the intermediate L-DOPA. DA is stored in synaptic vesicles and released into the synaptic cleft. The released DA can bind to D2 receptors (D2R) to exert its effects. Dopamine transporter (DAT) reuptakes DA back into the presynaptic neuron for reuse or degradation, providing autoregulatory control. In Parkinson's disease treated with exogenous L-DOPA, serotonergic neurons, which remain relatively intact early in the disease, convert L-DOPA to DA. Tryptophan is also converted to serotonin (5-HT) within these neurons. Both DA and 5-HT are co-stored in synaptic vesicles and co-released. Due to the lack of autoregulatory mechanisms such as DAT and D2R in serotonergic neurons, there is an unregulated release of DA. This release leads to an "upsurge of false neurotransmitter" activity, contributing to the development of LIDs. The administration of 5-HT1A receptor agonists modulates this activity by reducing the firing rate of serotonergic neurons, thereby decreasing the aberrant release of DA and mitigating LIDs. Adapted from Farajdokht et al. (2020).

1.10 Managing LID

Managing L-DOPA induced dyskinesia requires strategies that reduce dyskinetic movements without compromising the symptomatic benefits of dopaminergic therapy. Approaches typically include optimising levodopa delivery, using adjunctive medications to stabilise dopaminergic tone and employing treatments that target non-dopaminergic systems such as serotonin, glutamate, noradrenaline and acetylcholine.

Dopaminergic neuron of the substantia nigra

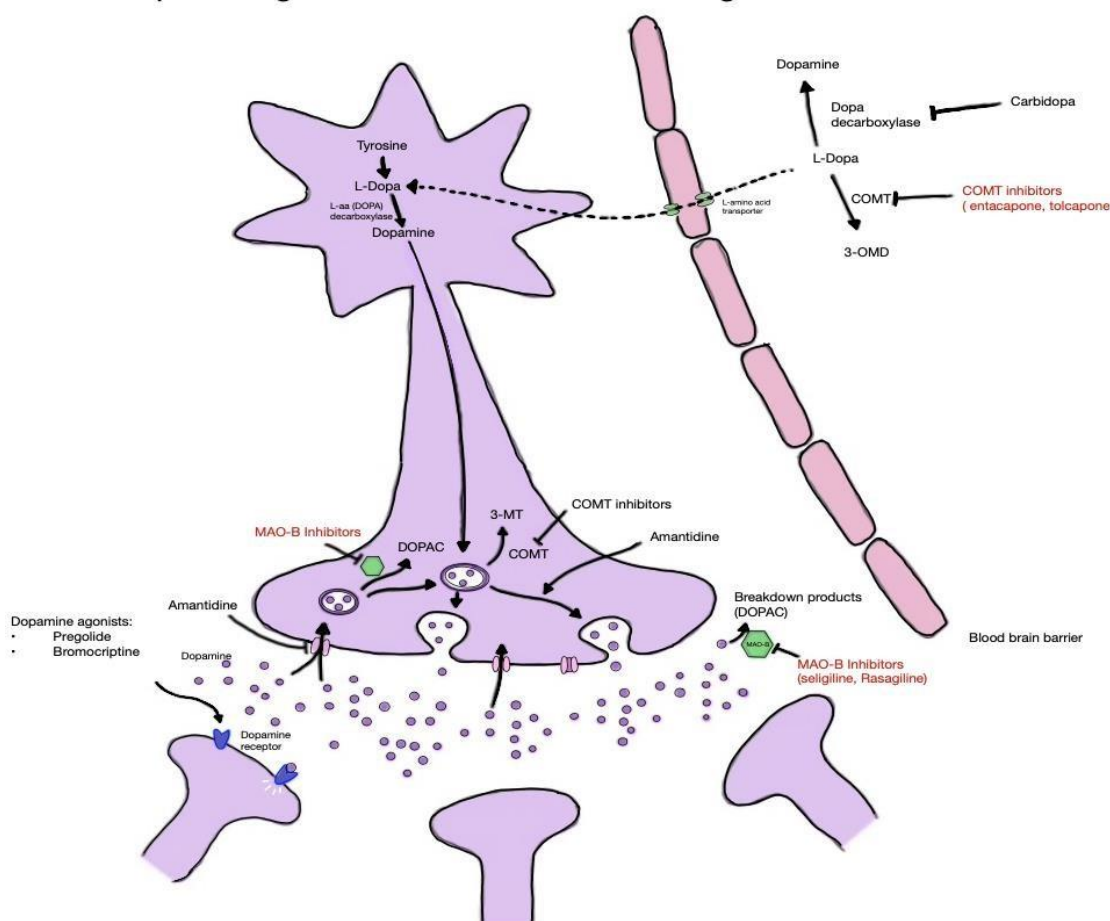


Figure 1. 5: Dopaminergic therapeutic targets in Parkinson’s disease. Schematic showing dopamine synthesis, vesicular storage, synaptic release and reuptake in a nigrostriatal dopaminergic neuron. Key enzymatic pathways (AADC, COMT, MAO-B) and their breakdown products are shown, together with the sites of action of major antiparkinsonian therapies. Peripheral and central COMT inhibitors (entacapone, tolcapone), MAO-B inhibitors (selegiline, rasagiline), dopamine agonists, and amantadine are indicated. Carbidopa blocks peripheral conversion of levodopa, enhancing central availability. Adapted from Rang et al. (2007).

1.10.1 LID: An Unmet Medical Need

L-DOPA induced dyskinesia is a major unmet clinical need in Parkinson’s disease. Long term studies show that a substantial proportion of patients develop dyskinesia, with roughly 30–40% affected within the first 3–5 years of levodopa therapy and up to 80% over the disease course (Ahlskog & Muenter, 2001; Fabbrini et al., 2007; Schrag & Quinn, 2000). As patients live longer on levodopa, dyskinesia becomes a key determinant of quality of life

because severe movements interfere with walking, feeding, speech, and daily functioning.

The pathophysiology is complex. Pulsatile levodopa dosing produces intermittent, non-physiological activation of dopamine receptors, which reshapes intracellular signalling and alters corticostriatal synaptic plasticity (Calabresi et al., 2010; Hong et al., 2014). This intermittent stimulation drives long lasting changes in gene expression, dendritic spine structure and firing patterns in direct pathway medium spiny neurons. Several neurotransmitter systems are involved in these adaptations. Glutamatergic transmission becomes dysregulated, with altered NMDA and AMPA receptor function supporting the persistence of maladaptive plasticity (Sgambato Faure & Cenci, 2012). Serotonergic neurons, which convert exogenous levodopa to dopamine but lack dopaminergic autoregulation, also contribute by releasing dopamine in a non-controlled manner, creating pathological synaptic surges (Sellnow et al., 2019).

Patient variability adds another layer of difficulty. Genetic differences, variation in the rate of nigrostriatal degeneration, and the degree of pathology in non-dopaminergic systems all influence dyskinesia susceptibility and therapeutic response (Sgambato Faure & Cenci, 2012). This heterogeneity complicates individualised treatment, especially as many available interventions have narrow therapeutic windows.

Current available treatments offer partial benefit but often come with trade-offs. Amantadine, the most widely used antidyskinetic drug, reduces dyskinesia in many patients but commonly causes confusion and hallucinations, particularly in older individuals (Rascol et al., 2021). Agents targeting glutamatergic or serotonergic receptors can reduce abnormal involuntary movements but show inconsistent efficacy and frequent side effects (Calabresi et al., 2010). These limitations highlight the need for approaches that address the underlying circuit pathology while maintaining levodopa's therapeutic benefit.

1.10.2 Continuous Dopaminergic Stimulation

Continuous dopaminergic stimulation aims to maintain a stable level of dopamine receptor activation and avoid the pulsatile stimulation associated with oral levodopa. Intermittent levodopa dosing drives rapid swings in synaptic dopamine, contributing to receptor sensitisation and the plastic changes that underlie dyskinesia (Cenci, 2007). Continuous approaches attempt to circumvent these fluctuations.

Levodopa carbidopa intestinal gel (LCIG) delivers levodopa directly into the jejunum, providing a steady plasma concentration independent of gastric emptying. This method significantly reduces motor fluctuations and dyskinesia. In a randomised controlled trial, LCIG decreased daily “off” time by more than four hours and improved dyskinesia severity without increasing levodopa burden (Olanow et al., 2013). By bypassing gastrointestinal variability, LCIG produces more predictable absorption and more physiological dopaminergic stimulation.

Continuous apomorphine infusion offers another means of stabilising dopaminergic signalling. As a potent dopamine agonist with rapid onset, apomorphine delivered subcutaneously reduces the peaks and troughs associated with oral levodopa. Clinical studies demonstrate significant reductions in dyskinesia and improved motor control in patients receiving continuous apomorphine therapy (Katzenschlager et al., 2005; Stocchi et al., 2009). Practical constraints, including injection site reactions and device maintenance, limit its broader use.

Rotigotine transdermal patches provide a non-invasive means of continuous dopaminergic stimulation. The patch releases rotigotine steadily over 24 hours, helping maintain stable dopaminergic tone and reducing motor complications in individuals who struggle with gastrointestinal absorption or oral formulations. In the RECOVER trial, rotigotine improved the total Non-Motor Symptoms Scale score compared with placebo, with the strongest effects in fatigue, apathy and mood related items (Chaudhuri et al., 2013). Patients

reported less tiredness, greater interest in their surroundings and activities, and fewer depressive symptoms. Although these findings were from *post hoc* analyses, they support the idea that steady dopaminergic delivery can stabilise non-motor domains that often fluctuate with levodopa.

Despite their benefits, continuous dopaminergic strategies can be limited by cost, invasiveness and the need for specialised care teams (Antonini & Odin, 2009). Nevertheless, they remain an important option in patients with troublesome dyskinesia who do not respond adequately to optimisation of oral therapy.

1.10.3 Adjunctive medications

Adjunctive therapies support levodopa by stabilising dopaminergic transmission and prolonging its therapeutic effect. COMT inhibitors such as entacapone and opicapone prevent peripheral levodopa breakdown, increasing levodopa's half-life and improving motor fluctuations (Kwon et al., 2022; Jenner et al., 2021). Experimental studies suggest that stabilising levodopa delivery may attenuate some forms of dyskinesia, though clinical responses vary (Tambasco et al., 2012). In practice, COMT inhibition can sometimes exacerbate dyskinesia due to increased synaptic dopamine availability, and careful dose adjustments of levodopa are required (Aradi et al., 2020). Stalevo, combining levodopa, carbidopa and entacapone, offers a simplified method of achieving this stabilisation and has shown benefits in reducing “off” time in PD (Koller et al 2005).

MAO-B inhibitors such as rasagiline and selegiline reduce the breakdown of dopamine in the brain and contribute to a more stable dopaminergic environment (Tan et al., 2022). Rasagiline has shown promise in reducing dyskinesia severity when added to levodopa; a double blind, placebo controlled trial demonstrated significant improvements in dyskinesia scores compared to placebo (Rascol et al., 2005). MAO-B inhibitors may also enhance

dopaminergic signalling by sparing endogenous dopamine and phenylethylamine, although elevated phenylethylamine levels may contribute to dyskinesia in some patients (Tan et al., 2022).

Overall, adjunctive medications can improve control of motor fluctuations and, in some cases, lessen dyskinesia. However, their effects vary between individuals, and careful titration is essential to avoid excessive dopaminergic stimulation.

1.10.4 Treatments based on Glutamatergic theory

Glutamatergic transmission plays a central role in the development and expression of LID. Dopamine and glutamate interact closely at corticostriatal synapses, and intermittent D1 receptor activation enhances NMDA receptor phosphorylation, trafficking and downstream signalling (Ahmed et al., 2011). These changes bias the system toward abnormal LTP, which supports the persistence of dyskinetic movements.

A consistent mechanistic feature of dyskinetic striatum is altered expression and subcellular localisation of the NR2B containing NMDA receptor. NR2B is redistributed to synaptic sites during dyskinesia, a change that amplifies calcium-dependent signalling and supports pathological potentiation (Gardoni et al., 2006). Pharmacological modulation of NR2B has produced mixed results across species. Several studies reported reduced abnormal involuntary movements with NR2B selective antagonists (Nash et al., 1999; Nutt et al., 2012), whereas others found that the NR2B antagonist CP-101,606 exacerbated dyskinesia in MPTP lesioned marmosets (Nash et al., 2004). These discrepancies suggest the complexity of NR2B involvement and suggest that its contribution varies with disease stage, receptor distribution, and species.

Broader NMDA receptor antagonism has shown more consistent antidyskinetic effects in animals. Amantadine, a low-affinity open channel NMDA antagonist with additional actions on cholinergic, dopaminergic and voltage gated channels, remains the clinical proof of concept (Fischer et al., 1997; Kornhuber et al., 1991). In practice, its pharmacology is highly non-selective: amantadine inhibits NMDA receptors only weakly and interacts with multiple targets including nicotinic acetylcholine receptors, sigma sites, and presynaptic dopamine handling. This broad activity contributes to its modest therapeutic window. Clinically effective doses often need to be pushed toward the upper recommended range, and tolerability becomes a major limiting factor. Cognitive disturbance, hallucinations, insomnia and peripheral edema are common at higher doses, which leads many patients, especially older individuals, to discontinue treatment or be unable to reach doses required for stable benefit (Metman et al., 1999; Sawada et al., 2010). Despite these limitations, controlled trials consistently show that amantadine can reduce dyskinesia without worsening motor symptoms (Metman et al., 1998; Oertel et al., 2017), but the effect is partial and sustained benefit is observed only in a subset of patients.

On the other hand, AMPA receptors mediate most fast glutamatergic transmission in the striatum and are strongly involved in shaping corticostriatal drive. In MPTP lesioned primates, AMPA receptor blockade consistently reduces dyskinesia (Konitsiotis et al., 2000; Bibbiani et al., 2005), whereas AMPA potentiation worsens it. Reports on AMPA receptor density and subunit changes in dyskinetic tissue remain mixed across rodent and primate studies (Bernard et al., 1996; Betarbet et al., 2000; Bagetta et al., 2012), likely reflecting methodological differences in lesion severity, chronicity and levodopa exposure. No AMPA specific therapy has yet translated successfully into clinical testing, and available antagonists often have broad activity profiles that complicate interpretation.

A recurring issue in glutamate targeting approaches is the limited pharmacological specificity of many commonly used compounds. Drugs such as amantadine, MK-801 and dextromethorphan interact with multiple transmitter systems, including serotonergic pathways (Baptista et al., 1997; Iravani et al., 1999; Callado et al., 2000; Gaikwad et al., 2005). This crosstalk is therapeutically relevant, as serotonin glutamate interactions contribute substantially to dyskinesia expression. It also complicates mechanistic interpretation of preclinical findings and helps explain why promising laboratory results do not always translate directly into human studies.

Metabotropic glutamate receptors add an additional layer of regulatory control. Group I mGluRs, particularly mGluR5, modulate NMDA receptor function, intracellular calcium signalling and the induction of LTP and LTD at corticostriatal synapses. Antagonists or negative allosteric modulators of mGluR5 produce robust antidyskinetic effects across 6-OHDA and MPTP models (Rylander et al., 2010; Mela et al., 2007). These findings generated substantial interest in mGluR5 antagonists as potential therapeutics.

Clinical translation, however, has been challenging. Two Phase II randomised trials of mavoglurant (AFQ056) failed to meet their primary antidyskinetic endpoints despite strong preclinical support (Trenkwalder et al., 2016). Dipraglurant, another mGluR5 negative modulator, produced positive signals in a small Phase IIa study but lacks larger confirmatory data (Tison et al., 2016). Across trials, central nervous system side effects (including dizziness, sedation and mood changes) have limited dose escalation. Together these outcomes illustrate the translational difficulty of targeting glutamate: although glutamatergic mechanisms are central to dyskinesia, the widespread distribution of glutamate receptors makes it difficult to modulate these pathways selectively without affecting cognition and mood.

Amantadine remains the only glutamate targeting drug with established benefit, and even this effect is partial and often transient. Circuit targeted NR2B modulation, biased

mGluR5 ligands or combined serotonin–glutamate interventions, may hold promise, but further development is needed.

1.10.5 Treatments based on Serotonergic theory

Serotonergic neurons play a central role in the expression of LID because they retain the capacity to convert exogenous L-DOPA into dopamine but lack the autoregulatory mechanisms of dopaminergic terminals. Without dopamine transporters or D2 autoreceptors, these neurons release dopamine in a non-physiological, unregulated manner (Carta et al., 2007; Politis et al., 2014). Each levodopa dose therefore produces sharp and poorly buffered dopamine surges, which exaggerate direct pathway drive and reinforce the maladaptive plasticity already established at corticostriatal synapses. Therapeutic strategies have focused on dampening this aberrant dopamine release or modifying downstream serotonin-dependent signalling.

Activation of somatodendritic 5-HT_{1A} autoreceptors in the raphe nuclei reduces the firing of serotonin neurons and correspondingly limits the L-DOPA driven dopamine surges that promote dyskinesia. This mechanism is well supported experimentally. The prototypical agonist $\pm$ 8-OH-DPAT consistently suppresses LID in both 6-OHDA lesioned rats and MPTP treated primates (Dupre et al., 2007; Bishop et al., 2009). Its effects include reduced raphe activity, normalisation of GluR1 phosphorylation and attenuation of dynorphin overexpression in the direct pathway (Lindenbach et al., 2015).

Buspirone, a partial 5-HT_{1A} agonist approved for clinical use, has shown similar results. In preclinical models it reduces both the expression and development of LID (Eskow et al., 2007). In patients, low-dose buspirone lessens dyskinesia without worsening parkinsonism, and PET imaging demonstrates that its antidyskinetic effect corresponds to blunting of L-DOPA evoked dopamine spikes from serotonergic terminals (Bara-Jimenez et

al., 2005; Politis et al., 2014). The development of more selective 5-HT_{1A} agonists such as NLX-112 (F15599) has strengthened the translational trajectory, showing consistent efficacy across rodent and primate models with improved tolerability (Fisher et al., 2020).

5-HT_{1B} receptors are located on serotonin neurons and modulate presynaptic transmitter release. Agonists such as CP-94253 reduce dyskinesia in 6-OHDA rats and influence downstream D1 linked signalling pathways (Jaunarajs et al., 2009). Benefits are more reliable with chronic rather than acute administration, aligning with the idea that sustained suppression of serotonergic output is necessary to reshape aberrant dopamine dynamics (Munoz et al., 2008; Carta et al., 2014).

Co-activation of 5-HT_{1A} and 5-HT_{1B} receptors appears particularly effective. Eltoprazine, a mixed 5-HT_{1A/1B} agonist, produces strong antidyskinetic effects in MPTP lesioned macaques without eliminating levodopa's therapeutic benefit (Bezard et al., 2013). A subsequent Phase I/IIa study in PD patients confirmed a dose-dependent reduction in dyskinesia severity, although mild attenuation of levodopa benefit was noted at higher doses (Svenningsson et al., 2015). These results support dual 5-HT_{1A/1B} targeting as one of the most promising serotonergic strategies currently in development.

At the cortical striatal level, 5-HT_{2A} receptors modulate glutamate output and influence the gain of cortico striatal transmission. Their blockade dampens the excessive excitatory drive that contributes to dyskinesia. Clozapine, a potent 5-HT_{2A} antagonist with minimal D₂ receptor blockade at low doses, remains the strongest clinical example. In a 10 week randomised, double-blind trial, low dose clozapine significantly reduced "on" time with troublesome dyskinesia and improved levodopa challenge responses without worsening parkinsonism (Durif et al., 2004). Its routine use is constrained by the need for haematological monitoring and risks of sedation or metabolic effects (Meltzer, 2012), but the mechanistic principle is clear.

Pimavanserin, a selective 5-HT_{2A} inverse agonist licensed for PD psychosis, has also shown modest antidyskinetic signals in exploratory studies (McFarland et al., 2011). Although not yet a dyskinesia therapy, it strengthens the case that 5-HT_{2A} modulation influences LID. Activation of 5-HT_{2C} receptors reduces glutamate release from corticostriatal terminals and offers a complementary route to attenuating dyskinesia. Lorcaserin, a 5-HT_{2C} agonist originally developed for obesity, reduces LID in both toxin lesioned rodents and MPTP lesioned macaques (Johnston et al., 2013). In primates the antidyskinetic effect was substantial, though accompanied by a modest worsening in baseline parkinsonism, illustrating the need for careful dose selection and receptor specific optimisation.

These observations show the dual influence of 5-HT_{2C} receptors on motor circuits: inhibiting cortico-striatal glutamate release can strongly suppress dyskinesia but may also reduce physiological excitatory drive needed for normal motor performance. In conclusion, among available strategies, 5-HT_{1A} and 5-HT_{1B} agonists, particularly when used in combination, have the strongest and most consistent preclinical support and have begun to show encouraging results in early clinical trials. 5-HT_{2A} antagonism and 5-HT_{2C} agonism offer additional avenues, though tolerability and motor side effects remain limiting factors.

1.10.6 Role of Acetylcholine in managing dyskinesia

The cholinergic system provides another route into the striatal mechanisms that sustain LID. Dopamine and acetylcholine normally operate in tight opposition in the striatum, and degeneration of nigrostriatal dopamine neurons shifts this balance toward relative cholinergic hyperactivity (Pisani et al., 2005). Striatal cholinergic interneurons regulate both glutamatergic and GABAergic transmission, and their activity feeds directly into the corticostriatal adaptations discussed earlier. Targeting muscarinic acetylcholine receptors (mAChRs),

particularly the M1 and M4 subtypes, has therefore been of particular interest for modulating dyskinesia.

The M4 receptor is of particular interest because it provides inhibitory control within direct pathway medium spiny neurons and on cholinergic interneurons. Through these actions, M4 serves as a brake on the dopamine dependent overactivation that characterises LID. Enhancing M4 receptor signalling with PAMs reduces abnormal involuntary movements in preclinical models by amplifying the response to endogenous acetylcholine without driving the receptor independently (Moehle & Conn, 2019). VU0152100, a selective M4 PAM, attenuates dyskinesia in 6-OHDA lesioned mice and normalises markers of striatal output associated with exaggerated direct-pathway drive (Brugnoli et al., 2020). These findings support M4 PAMs as a physiologically aligned and potentially well tolerated approach.

M1 receptors, in contrast, are excitatory and widely expressed on striatal projection neurons. They modulate corticostriatal glutamatergic input and intracellular calcium signalling, and their activation favours the same potentiation biased state seen in dyskinesia (Shen et al., 2016). Selective M1 receptor antagonists, such as pirenzepine analogues, reduce dyskinesia in rodent models by limiting excess excitatory drive while sparing other muscarinic subtypes important for cognition and autonomic function (Brugnoli et al., 2020). This specificity differentiates them from older, non-selective antimuscarinic agents, which have long been known to impair learning, memory and attention (Scatton et al., 1983). Non-selective muscarinic antagonists can suppress striatal cholinergic overactivity, but their poor tolerability in older adults has limited clinical use. In PD, anticholinergics improve motor function but are consistently associated with higher rates of neuropsychiatric and cognitive adverse events than placebo, and these effects are a common reason for treatment withdrawal (Katzenschlager et al., 2002, 2003). Large cohort studies in older adults similarly show that cumulative exposure to anticholinergic drugs increases the risk of cognitive decline, dementia and mortality

(Carrière et al., 2009; Fox et al., 2011), and in people with PD, greater anticholinergic burden is linked to a higher incidence of adverse outcomes (Crispo et al., 2016). These findings reinforce concerns about broad muscarinic blockade in an ageing PD population.

Subtype selective approaches offer a way forward. Experimental work indicates that M1 and M4 receptors are the key muscarinic subtypes involved in LID modulation: M1 receptors facilitate corticostriatal excitatory drive, whereas M4 receptors constrain direct pathway output (Moehle & Conn, 2019; Brugnoli et al., 2020). PAMs of M4, and selective M1 antagonists, reduce dyskinesia and normalise downstream signalling in rodent models without producing the global cognitive toxicity typical of non-selective antimuscarinics (Brugnoli et al., 2020; Nielsen et al., 2024). Together, these data support the view that targeting M1 and M4 receptors provides a more rational way to recalibrate striatal microcircuits, complementing glutamatergic and serotonergic strategies while minimising the risk of cognitive harm.

1.11 Nicotinic Acetylcholine Receptor Agonists; A new approach for managing LID

Nicotinic acetylcholine receptors (nAChRs) are pentameric ligand gated ion channels assembled from combinations of α ($\alpha 2$ – $\alpha 10$) and β ($\beta 2$ – $\beta 4$) subunits. In the nigrostriatal system, the predominant high-affinity subtypes are $\alpha 4\beta 2$ and $\alpha 6\beta 2$ receptors on dopaminergic terminals, together with homomeric $\alpha 7$ receptors on presynaptic and postsynaptic elements; the asterisk indicates that additional subunits may be present in the pentamer (for example, $\alpha 6\alpha 4\beta 2\beta 3$). These receptors regulate dopamine, glutamate and GABA release, and influence neuronal excitability on a millisecond timescale (Albuquerque et al., 2009; Gotti et al., 2009; Millar & Gotti, 2009; Quik et al., 2011).

Epidemiological studies have consistently reported an inverse association between cigarette smoking and PD risk, raising the possibility that chronic nicotine exposure may exert neuroprotective effects on nigrostriatal neurons (Li et al., 2015; Gallo et al., 2019). Experimental work supports this idea: nicotine and other nAChR ligands can attenuate dopaminergic cell loss, modulate neuroinflammation and normalise basal ganglia firing patterns in toxin-based PD models (Quik et al., 2014). In the context of L-DOPA induced dyskinesia, a series of preclinical studies has shown that nicotine and subtype selective nAChR agonists reduce abnormal involuntary movements in both 6-OHDA lesioned rats and MPTP lesioned monkeys, without diminishing the antiparkinsonian benefit of levodopa (Quik et al., 2007; Bordia et al., 2008; Bordia et al., 2010; Zhang et al., 2014).

Together these findings suggest that nAChR targeting drugs could represent a new class of antidyskinetic agents, acting by reshaping striatal neurotransmission rather than simply suppressing dopamine signalling. The following sections summarise how nAChR expression changes after nigrostriatal damage, and how $\alpha 4\beta 2$, $\alpha 6\beta 2$ and $\alpha 7$ subtypes contribute to LID expression and its pharmacological control.

1.11.1 Changes in nAChR Expression with Nigrostriatal Damage

nAChRs play key roles in motor loop regulation and in local immune and inflammatory processes, both of which are relevant for PD and LID (Quik et al., 2011; Perez et al., 2018). Experimental models have been especially informative in defining how individual receptor populations respond to nigrostriatal injury.

In 6-OHDA lesioned rodents, degeneration of nigrostriatal dopaminergic neurons leads to marked reductions in high affinity nicotine binding sites that correspond to $\alpha 4\beta 2$ and $\alpha 6\beta 2$ nAChRs on striatal dopamine terminals, while $\alpha 7$ binding is largely preserved (Kulak et al.,

2002; Quik et al., 2007). A similar pattern is seen in MPTP lesioned monkeys and in post-mortem PD striatum, where $\alpha 6\beta 2$ receptors decline early and disproportionately relative to $\alpha 4\beta 2$ sites (Quik et al., 2001; Bordia et al., 2015). Binding of ^{125}I - α -conotoxin MII, a selective ligand for $\alpha 6\beta 2$ containing receptors, falls in parallel with the dopamine transporter, indicating that $\alpha 6\beta 2$ nAChRs are mainly localised on dopaminergic terminals and closely track their integrity (Quik et al., 2007; Perez et al., 2010).

Immunohistochemical studies using subunit specific antibodies confirm this profile. After nigrostriatal lesion, there is a pronounced loss of $\alpha 6$ and $\beta 3$ subunit protein in striatum, with more modest decreases in $\alpha 4$ and $\beta 2$; $\alpha 7$ protein is relatively unchanged (Perez et al., 2010; Bordia et al., 2015). Subunits $\alpha 2$, $\alpha 3$ and $\beta 4$ are either absent or expressed at very low levels in rodent and primate dorsal striatum, consistent with the limited contribution of $\alpha 3\beta 4$ receptors at these synapses (Gotti et al., 2009).

These quantitative changes are summarised in Table 1.2, adapted from MPTP lesioned monkey studies. They show that $\alpha 6\beta 2$ nAChRs are particularly vulnerable, with substantial loss even after moderate nigrostriatal damage, whereas $\alpha 4\beta 2$ receptors are relatively spared until lesions become more severe. $\alpha 7$ receptors, which are not enriched on dopamine terminals, are much less affected by dopaminergic denervation.

Table 1.2: Declines in different nAChR subtypes in monkey striatum with nigrostriatal damage. The $\alpha 3\beta 2$ nAChR subtype is also expressed in monkey striatum. The $\alpha 4\beta 2$ subtype may contain either $\alpha 4\beta 2$ or $\alpha 4\alpha 2\beta 2$ subunits. Moderate lesion, $\zeta 50\%$ decline in the dopamine transporter; severe lesion $\zeta 90\%$ decline in dopamine transporter; Y indicates a decline; - indicates no change.

nAChR subtype	Present on striatal dopamine terminals	Severity of lesion	
		Moderate	Severe
$\alpha 6\alpha 4\beta 2\beta 3$	Yes	↓↓↓	↓↓↓
$\alpha 6\beta 2\beta 3$	Yes	-	↓
$\alpha 4\beta 2$	Yes	-	↓
$\alpha 4\beta 2^*$	No	-	-
$\alpha 7$	No	-	-

In brief, $\alpha 6\beta 2$ nAChRs are highly sensitive to nigrostriatal damage and decline early, while $\alpha 4\beta 2$ populations are more resistant and $\alpha 7$ receptors are largely spared. This differential vulnerability has implications for how nicotinic drugs will act at different disease stages and helps explain the subtype-specific contributions to LID modulation.

1.11.2 Role of $\alpha 4\beta 2$ and $\alpha 6\beta 2$ nAChRs in LID

The importance of $\beta 2$ nAChRs (receptors containing the $\beta 2$ subunit, including $\alpha 4\beta 2$ and $\alpha 6\beta 2$ assemblies) in LID has been demonstrated using genetic and pharmacological tools. In $\beta 2$ knockout mice, which lack both $\alpha 4\beta 2$ and $\alpha 6\beta 2$ receptors, L-DOPA induced abnormal involuntary movements are markedly reduced, and nicotine no longer exerts an antidyskinetic effect (Quirk et al., 2013). Selective deletion of $\alpha 6$ or $\alpha 4$ subunits produces a similar attenuation of LIDs, pointing to a direct contribution of both $\alpha 6\beta 2$ and $\alpha 4\beta 2$ populations to dyskinesia generation and its nicotinic modulation (Quirk et al., 2013; Perez et al., 2018).

Preclinical studies with subtype preferring agonists provide converging evidence. In 6-OHDA lesioned rats and MPTP lesioned monkeys, repeated nicotine administration reduces established LIDs by about 50-60%, whether given continuously in the drinking water or intermittently by injection (Quik et al., 2007; Bordia et al., 2008; Quik et al., 2013). More selective $\beta 2$ nAChR agonists such as A-85380, ABT-089 and ABT-894 produce comparable reductions in dyskinesia, again without worsening parkinsonism (Bordia et al., 2010; Zhang et al., 2014a). In a series of MPTP macaque studies, the $\alpha 4\beta 2/\alpha 6\beta 2$ preferring agonist ABT-894 reduced LIDs by roughly 60% and showed sustained benefit over weeks of dosing (Zhang et al., 2014).

TC-8831, another $\beta 2$ selective agonist, has been tested in dyskinetic MPTP macaques. It produced robust antidyskinetic effects while having minimal interaction with peripheral nAChRs, suggesting a favourable tolerability profile (Johnston et al., 2013). These results, together with those from ABT-089 and ABT-894, support the idea that carefully tuned activation or desensitisation of $\beta 2$ nAChRs on dopaminergic terminals and local interneurons can dampen the exaggerated dopamine release and downstream plasticity that sustain LID.

Varenicline, a partial agonist at $\alpha 4\beta 2$ and an agonist at $\alpha 7$ nAChRs developed for smoking cessation, also reduces LIDs in 6-OHDA rats and MPTP monkeys, with effects similar in magnitude to nicotine (Huang et al., 2011; Zhang et al., 2015). Its established clinical safety profile in humans makes it an attractive candidate for repurposing, although concerns about dependence and neuropsychiatric side effects mean that careful patient selection and dosing strategies would be essential (Rollema et al., 2007; Cahill et al., 2013).

Epidemiological observations of lower PD risk in smokers, combined with these animal data, have prompted speculation that chronic activation and desensitisation of $\beta 2$ nAChRs may induce long-lasting changes in dopamine release and striatal circuitry that reduce both neurodegeneration and dyskinetic sensitivity (Li et al., 2015; Quik et al., 2014). At the same

time, addiction liability and systemic side effects remain major barriers to using non-selective agonists such as nicotine itself for long term LID management. As a result, current efforts focus on subtype selective $\beta 2$ ligands that preserve antidyskinetic efficacy while minimising off target actions.

1.11.3 $\alpha 7$ nAChRs

The $\alpha 7$ nAChR subtype forms homomeric channels with high calcium permeability and rapid desensitisation kinetics, properties that place it at the intersection of synaptic plasticity, neurotransmitter release and cell survival pathways (Gotti & Clementi, 2004; Albuquerque et al., 2009; Papke, 2014). In basal ganglia circuits, $\alpha 7$ receptors are expressed on glutamatergic terminals, interneurons and some projection neurons, where they modulate glutamate and GABA release and shape local network activity (Perez et al., 2018).

Genetic studies suggest that $\alpha 7$ receptors exert a restraining influence on dyskinesia. Mice lacking $\alpha 7$ nAChRs show higher baseline AIM scores after L-DOPA treatment than wild type controls, indicating that the endogenous $\alpha 7$ system may normally dampen the development or expression of LID (Quik et al., 2013). This fits with the idea that $\alpha 7$ mediated control of glutamate release and calcium entry helps stabilise cortico-striatal signalling in the face of intermittent dopaminergic stimulation.

Pharmacological activation of $\alpha 7$ nAChRs has yielded promising antidyskinetic results in primate models. In MPTP lesioned squirrel monkeys with stable LIDs, the selective $\alpha 7$ agonist ABT-107 reduced dyskinesia scores by 40–60%, with no worsening of parkinsonism or cognition, and the effect persisted over several weeks without obvious tolerance (Zhang et al., 2014b). A related compound, ABT-126, showed dose dependent reductions of about 60% in LIDs in monkeys with both moderate and severe nigrostriatal damage, again without altering motor benefit from levodopa (Zhang et al., 2015). The $\alpha 7$ partial agonist AQW051 produced

similar dyskinesia reductions and extended the duration of levodopa benefit in MPTP monkeys (Di Paolo et al., 2014).

Interestingly, in monkeys treated with both an $\alpha 7$ agonist (ABT-107 or ABT-126) and a $\beta 2$ agonist (ABT-894), combined therapy did not further reduce LIDs beyond the effect of either agent alone, suggesting convergence on a shared downstream mechanism (Zhang et al., 2014b; Perez et al., 2018). This convergence may involve normalisation of abnormal corticostriatal glutamatergic drive and changes in striatal gene expression linked to plasticity.

Beyond motor effects, $\alpha 7$ receptors are key components of the so called cholinergic anti-inflammatory pathway. Activation of $\alpha 7$ nAChRs on immune cells suppresses pro-inflammatory cytokine production via JAK2/STAT3 and related cascades (Wang et al., 2003; Tracey, 2007). In brain, $\alpha 7$ stimulation can reduce microglial activation and oxidative stress, processes implicated in both PD progression and the amplification of dyskinesia (Gotti & Clementi, 2004; ElNebrisi et al., 2025). Thus, $\alpha 7$ -directed therapies may offer a dual benefit by modulating both synaptic transmission and inflammatory tone.

Not all $\alpha 7$ agonists have translated successfully to the clinic. In a Phase II trial in PD patients with LID, the $\alpha 7$ partial agonist AQW051 did not significantly reduce dyskinesia severity or parkinsonian disability, highlighting the gap between robust primate data and human outcomes (Foucault-Fruchard & Antier, 2017). Nonetheless, the consistent antidyskinetic effects of $\alpha 7$ activation in non-human primates, together with the broader neuroprotective and anti-inflammatory profile of this receptor, support ongoing efforts to refine $\alpha 7$ agonists and positive allosteric modulators for use in PD.

Overall, $\alpha 7$ nAChRs appear to play a critical regulatory role in LID. Loss of $\alpha 7$ function exacerbates dyskinesia, while selective activation can produce sustained reductions in abnormal movements without compromising dopaminergic benefit. Enhancing $\alpha 7$ signalling

therefore, represents a complementary strategy to $\beta 2$ targeted approaches, with the potential to stabilise both motor circuits and the inflammatory milieu in PD.

1.12 Significance and Potential Impact

The use of nicotinic medicines presents a promising treatment direction for managing LID in PD. nAChRs are considered potential therapeutic targets due to their role in regulating neurotransmitter secretion and neuronal excitability. Various studies have demonstrated that nAChR agonists can reduce dyskinetic movements in experimental models. Consequently, it has been postulated that these medications could significantly decrease the motor side effects associated with long-term L-DOPA therapy. Investigating the impact of nicotinic drugs on LID provides crucial insights into dyskinesia and offers a valuable tool for developing comprehensive treatments. nAChR agonists are believed to influence several aspects of LID pathophysiology, including the normalization of dopamine turnover, reduction of excitotoxic effects mediated by glutamate receptors, and modulation of serotonergic signalling pathways. Enhancing these mechanisms may not only open up additional therapeutic possibilities beyond nicotinic drugs but also provide a clearer understanding of CNS interactions in PD and associated treatment challenges.

Effective management of LID is essential for improving the quality of life in PD patients. While L-DOPA is highly effective for managing motor symptoms, its therapeutic window is limited by the significant impact of LID on daily functionality. Integrating nicotinic medications into the treatment regimen could enhance overall disease control, reduce motor complications, and improve the benefits of L-DOPA. This approach aims to achieve better long-term symptom control, thereby enhancing patients' overall functionality, stability, and engagement in daily activities. Beyond the therapeutic benefits for LID, developing potent

nicotinic based treatments also promotes further research into neuroprotective strategies PD. nAChRs have been reported to possess neuroprotective properties, suggesting that drugs activating these receptors could slow disease progression in neurodegenerative models. This represents a paradigm shift in PD management, moving from merely suppressing symptoms to potentially influencing the disease's natural course.

1.13 Study Rationale, Aims and Objectives

The aim of the study in this thesis is to extend investigations using in vivo models of Parkinson's disease to determine the effects of nicotinic medications on LID, building on promising trends observed in other preclinical studies. The hypothesis posits that nicotinic medications will reduce LID by modulating neurotransmitter activity. The null hypothesis states that nicotine and nicotinic agents have no effect on LID, suggesting that any observed changes are not due to the intervention of these agents.

Therefore, in a series of investigations a model of dyskinesia will be set up following levodopa priming in rats with a unilaterally 6-OHDA lesion of the median forebrain bundle. Specifically, the work will examine:

- How different nicotinic medications alter the development and expression of levodopa induced dyskinesia, with attention to nicotine, nicotinic agonists, antagonists and subtype specific modulators.
- Which dosing regimens and drug classes show the strongest antidyskinetic effect, and whether $\alpha 4\beta 2$ or $\alpha 7$ directed compounds offer advantages over nicotinic stimulation.
- Whether dyskinesia severity correlates with the loss of dopaminergic neurons in the substantia nigra and the extent of glial activation, and whether nicotinic treatment reduces these inflammatory changes.

- Whether early nicotinic co-treatment can interrupt or prevent levodopa priming, limiting the sensitization that normally leads to dyskinesia.

1.14 Gaps in the Literature and Novelty of the Present Work

Despite a substantial and growing body of preclinical evidence supporting nicotinic receptor modulation as a therapeutic strategy for levodopa induced dyskinesia, several critical gaps in the literature remain unaddressed. The majority of published work has focused on chronic or repeated nicotinic drug administration, leaving the anti-dyskinetic potential of acute nicotinic interventions largely unexplored. Similarly, while broad nicotinic receptor stimulation using non-selective agents has been extensively studied, the relative contributions of individual receptor subtypes in particular $\alpha 4\beta 2$ and $\alpha 7$ nAChRs have not been systematically characterised within a single, directly comparable experimental framework. The question of whether nicotinic co-treatment initiated during levodopa priming can prevent sensitisation rather than merely suppress established dyskinesia has received limited attention using subtype-selective agents. Furthermore, the mechanistic potential of allosteric modulation as a physiologically distinct alternative to direct receptor agonism has not been rigorously evaluated in the understanding of levodopa induced dyskinesia. Finally, the relationship between nicotinic receptor mediated anti-inflammatory effects and dyskinesia severity has not been directly characterised within the same experimental cohorts. The present thesis addresses each of these gaps through a series of systematic in vivo investigations in the unilateral 6-OHDA lesioned rat model, and the principal novelties are described below in the order in which they are presented.

Chapter 3 addresses the gap in acute nicotinic pharmacology. In contrast to previous studies that have predominantly focused on chronic nicotine exposure or repeated administration of $\alpha 7$ nicotinic receptor agonists to attenuate levodopa induced dyskinesia, this chapter examined the effects of acute nicotinic receptor modulation. The data demonstrate that a single, low-dose

administration of nicotinic agents including nicotine and varenicline produces a rapid and dose-dependent reduction in abnormal involuntary movements in the 6-OHDA model. This finding extends the current mechanistic understanding of nicotinic involvement in dyskinesia by establishing that anti-dyskinetic effects are not limited to chronic treatment paradigms but can be achieved acutely, highlighting a more immediate therapeutic potential for nicotinic receptor modulation than has previously been recognised.

Chapter 4 shifts from symptomatic suppression to disease modifying potential, addressing a gap that is both mechanistically and clinically significant. Unlike previous studies that primarily evaluated nicotine and varenicline after levodopa induced dyskinesia had already developed, this chapter examined their role as a pre-dyskinetic intervention specifically, their capacity to prevent or reduce the onset of abnormal involuntary movements when co-administered during the levodopa priming phase. By targeting early neurochemical events that precede the manifestation of dyskinesia, these agents may exert effects on maladaptive striatal plasticity rather than merely suppressing symptoms after they emerge. Evaluating nicotine and varenicline in parallel additionally enabled direct comparison of full versus partial receptor agonism and differences in receptor subtype selectivity, providing insight into how pharmacological profile influences dyskinesia vulnerability during the critical priming window.

Chapter 5 advances the mechanistic framework further by moving from broad nicotinic receptor involvement to a more defined, subtype-specific analysis. Building on the findings of Chapters 3 and 4, this chapter addresses a key gap by isolating the contribution of $\alpha 4\beta 2$ nicotinic acetylcholine receptors and critically by distinguishing between different modes of receptor engagement; agonism, antagonism and desensitisation rather than treating receptor activity as a uniform process. This approach provides the first systematic examination of whether specific

receptor states, rather than receptor presence alone, underlie the nicotinic modulation of levodopa induced dyskinesia, establishing a more precise mechanistic basis for evaluating $\alpha 4\beta 2$ nAChRs as targets for future nicotinic based therapies in Parkinson's disease.

Chapter 6 presents the principal and most translational novelty of the thesis. A central objective was to move beyond receptor agonism and evaluate $\alpha 7$ positive allosteric modulation as a mechanistically distinct therapeutic strategy. This chapter investigated whether enhancing endogenous cholinergic signalling using the $\alpha 7$ PAM PNU-120596 could attenuate established dyskinesia while preserving normal physiological function, an approach that has not previously been examined in the understanding of Parkinson's disease. In parallel, receptor blockade was used to define the contribution of endogenous $\alpha 7$ activity to dyskinesia regulation. This work presents the first demonstration that selective $\alpha 7$ nAChR positive allosteric modulation using PNU-120596 significantly reduces levodopa induced abnormal involuntary movements in the 6-OHDA rat model without attenuating levodopa's therapeutic efficacy. Together, the data establish $\alpha 7$ allosteric modulation as a more physiologically aligned and therapeutically relevant strategy for the treatment of levodopa induced dyskinesia than direct agonism.

Across Chapters, a further and understudied dimension of nicotinic pharmacology is characterised: the relationship between nicotinic receptor modulation, neuroinflammation and dyskinesia severity. Using immunohistochemistry within the same experimental cohorts, this thesis directly characterises the effects of nicotinic treatment on microglial activation (IBA1) and astrocytic responses (GFAP) across the lesioned hemisphere and correlates these inflammatory changes with the severity of abnormal involuntary movements. The data provide evidence that nicotinic stimulation reduces glial activation in the dopamine depleted striatum and substantia nigra and that the magnitude of neuroinflammatory change is associated with anti-dyskinetic outcomes.

This represents the first integrated characterisation of nicotinic anti-inflammatory effects and dyskinesia endpoints within a single cohort-level analysis, implicating neuroinflammation as a contributory mechanism in the onset and maintenance of levodopa induced dyskinesia and identifying it as a secondary therapeutic target.

Together, these findings advance the mechanistic understanding of nicotinic receptor involvement in levodopa induced dyskinesia across acute, preventive, subtype specific and allosteric dimensions. They provide translational evidence supporting the clinical investigation of subtype-selective nicotinic agents in particular varenicline and $\alpha 7$ PAMs as adjunctive treatments with the potential to improve long-term motor outcomes in Parkinson's disease.

Chapter 2

General Material & Methods

2.1 6-OHDA Lesion Model of Parkinson's Disease

In the following studies, Parkinsonian symptoms were modelled using the 6-hydroxydopamine lesion in rats. The 6-OHDA model is a long established and widely used rodent model of PD that selectively destroys catecholaminergic neurons, especially dopaminergic neurons in the nigrostriatal pathway (Torres & Dunnett, 2011). Mechanistically, 6-OHDA is a dopamine analog taken up by the dopamine transporter (DAT) into neurons, where it accumulates and causes toxicity. It generates reactive oxygen species and inhibits mitochondrial complex I, triggering oxidative stress and ultimately apoptotic cell death of dopamine neurons (Kim et al., 2024). When stereotactically injected into appropriate brain regions (such as the medial forebrain bundle or substantia nigra), 6-OHDA produces a lesion of the nigrostriatal pathway that closely mimics the dopamine depletion seen in PD. Unilateral infusion of 6-OHDA leads to pronounced motor asymmetry (hemiparkinsonian behaviour), whereas bilateral infusions can induce severe akinetic deficits similar to advanced PD.

A key strength of the 6-OHDA model is that it reproduces many cardinal features of PD, including bradykinesia, rigidity, and postural instability, due to the extensive loss of dopaminergic neurons. Importantly for our purposes, 6-OHDA lesioned rodents respond to dopamine replacement therapy (e.g. L-DOPA) and can develop abnormal involuntary movements analogous to the levodopa induced dyskinesia seen in PD patients (Wang & Shao, 2020). In rats with extensive unilateral 6-OHDA lesions, chronic L-DOPA administration reliably triggers dyskinetic behaviours, making this model highly suitable for studying L-DOPA induced dyskinesia and potential antidyskinetic treatments (such as nicotinic agents). Previous studies have demonstrated that targeting nicotinic acetylcholine receptors can modulate dyskinesia in the 6-OHDA model. For example, nicotine treatment has been shown to reduce L-DOPA induced AIMs in parkinsonian rodents (Quik et al., 2012). Such findings suggest that nicotinic receptor modulation may be a promising strategy for reducing LID (a

hypothesis central to this thesis). Overall, the 6-OHDA lesion model provides a robust and well characterized platform to investigate both the pathophysiology of dyskinesia and the efficacy of novel therapeutic interventions.

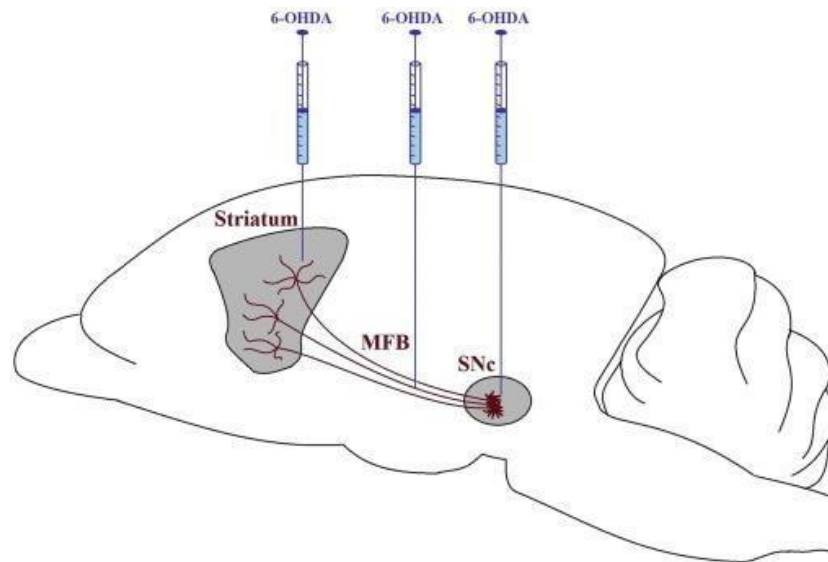


Figure 2. 1: 6-Hydroxydopamine lesion sites commonly used to model Parkinson's disease. Schematic of a rodent brain showing three standard targets for 6-OHDA delivery: the striatum, the medial forebrain bundle (MFB), and the substantia nigra pars compacta (SNc). Injection into the striatum produces a gradual, partial loss of nigrostriatal terminals, while MFB or SNc injections cause a rapid and more extensive degeneration of dopaminergic neurons. Adapted from Kaczynska and Andrzejewski, 2020.

2.2 Experimental Model Selection and Rationale

For the present research, the unilateral 6-OHDA lesion rat model was selected to induce Parkinsonian dyskinesia. This choice was guided by the model's strong validity and reproducibility in capturing the specific loss of nigrostriatal dopamine neurons that characterizes PD. The 6-OHDA model, first developed by Ungerstedt in the early 1970s (Breese et al., 1974), produces reliable Parkinsonian motor deficits in rats and upon chronic L-DOPA administration, robust dyskinetic symptoms. Unilateral lesions in particular allow the use of the contralateral (intact) side as an internal control for behavioural and biochemical comparisons. Compared to other PD models, 6-OHDA offered distinct advantages for the

experimental goals. The MPTP toxin model, for instance, is excellent in primates and mice but is less consistent in rats due to species differences in MPTP metabolism and neuroanatomy. By contrast, 6-OHDA allows precise stereotaxic targeting of the nigrostriatal pathway in rats, enabling near complete dopamine neuron degeneration in specific regions (medial forebrain bundle, substantia nigra pars compacta, or striatum) as needed. This precision is critical for dyskinesia studies, as it ensures a well-defined lesion in circuits known to underlie LID. 6-OHDA was chosen over MPTP for its reliability in rats and its ability to produce consistent, severe dopamine denervation.

The rotenone model was also considered, as it induces PD like neurodegeneration via systemic mitochondrial inhibition and oxidative stress (Betarbet et al. 2000). Rotenone can replicate certain PD features (including α -synuclein aggregation), but its use is confounded by high variability in lesion extent and significant systemic toxicity. These factors make rotenone lesioned animals less suitable for quantitative motor assays like dyskinesia scoring. In contrast, the 6-OHDA lesion can be titrated in magnitude and precisely placed, causing a more uniform cohort of Parkinsonian rats (Ungerstedt 1971). This reproducibility supports robust assessments of dyskinetic behaviours and therapeutic effects. Indeed, the unilateral 6-OHDA model has become a foundation of preclinical LID research: it consistently mirrors the motor complications arising from long-term L-DOPA therapy in PD (Cenci and Lundblad 2006).

The value of the 6-OHDA model for dyskinesia research is underscored by extensive prior work. For example, Cenci and colleagues have shown that unilateral 6-OHDA lesions produce not only the expected motor impairments but also the underlying molecular changes associated with LID (Cenci & Konradi, 2010). Dopamine denervation leads to supersensitivity of striatal dopamine D_1 receptors, and repeated L-DOPA dosing in lesioned rats triggers pathological signalling cascades in striatal neurons. Notably, dyskinetic rats exhibit excessive phosphorylation of intracellular kinases (such as ERK1/2) and other proteins (e.g.

ribosomal S6) in striatal neurons, reflecting aberrant plasticity caused by pulsatile dopamine stimulation (Cenci & Konradi, 2010). These molecular adaptations closely parallel those observed in dyskinetic PD patients and highlight the model's strong construct validity. As summarized by Cenci and collaborators, an inability to appropriately down regulate D₁ receptor mediated signalling in the dopamine depleted striatum is a key driver of LID (Cenci & Konradi, 2010). Thus, the 6-OHDA model not only reproduces dyskinetic behaviours but also provides insight into their neurobiological underpinnings (e.g., maladaptive striatal plasticity; Cenci & Konradi, 2010). This dual relevance reinforced the rationale for adopting the 6-OHDA lesioned rat as the primary experimental platform.

In designing the experiments, special attention was paid to the laterality and extent of 6-OHDA lesions. Unilateral lesions were implemented (targeting the left hemisphere) because they offer practical and ethical advantages. For histochemical studies, each rat effectively served as its own control, when the intact side of the brain remains undamaged (Ungerstedt and Arbuthnott 1970). This internal control greatly facilitates behavioural tests based on asymmetry (such as rotation or forelimb use) and reduces inter animal variability in outcome measures. Unilateral lesions also reflect the often-asymmetric onset of motor symptoms in early PD (Kempster et al. 1989), while avoiding the severe debilitation that bilateral lesions cause. It is worth noting that unilateral models only partially mimic human PD, which eventually affects both hemispheres.

By contrast, bilateral 6-OHDA lesions produce a more global parkinsonian state but were considered impractical for the study. Near complete bilateral dopamine loss induces profound akinesia as rats become nearly unable to initiate movement, often requiring intensive care (hand feeding, hydration) for survival (Sachs and Jonsson 1975). While bilateral lesioned rats can display extremely severe dyskinesia when given L-DOPA (often exceeding those in unilateral models), (Lundblad et al. 2004), the welfare concerns and technical challenges are

considerable. Moreover, bilateral lesions eliminate the internal control, requiring different behavioural tests (e.g. general locomotion or eating behaviour) and larger group sizes to achieve statistical power. Given these issues, a bilateral lesion approach was not pursued. Instead, near complete unilateral lesions (sometimes termed a full unilateral lesion, aiming for >90% dopamine neuron loss on one side) was carried out, which caused robust Parkinsonian deficits while allowing the animal to maintain basic functions (Ungerstedt 1971). Unilaterally lesioned rats typically continue to eat and drink independently (although with mild weight loss), have high postoperative survival, and exhibit clear, quantifiable motor asymmetries (such as biased rotations and impaired use of the affected limbs), (Ungerstedt and Arbuthnott 1970). These features made the unilateral 6-OHDA model the most appropriate and highly translational choice for in vivo studies of L-DOPA induced dyskinesia and its modulation by nicotinic drugs.

2.3 Animals

All experiments were conducted on female Sprague Dawley rats (Charles River, UK). At the start of the study, rats were ~8–10 weeks old, weighing approximately 250 g. Animals were housed in pairs in standard polycarbonate cages under controlled laboratory conditions (12:12 h light/dark cycle, lights on at 07:00; ambient temperature 22–23 °C; relative humidity 50– 60%). Food and water were available *ad libitum*. After arrival in the facility, rats were allowed to acclimatize for at least one week before any procedures. During this period, they were handled daily and monitored for general health and body weight, to reduce stress and familiarize them with gentle human contact. All experiments complied with the ARRIVE guidelines and were carried out in accordance with the UK Animals (Scientific Procedures Act 1986) and associated guidelines, EU Directive 2010/63/EU for animal experiments. All

procedures and protocols were approved by the Animal Welfare and Ethical Review Board (AWERB) at the University of Hertfordshire, which were carried out under the UK Home Office approved Project License PD72555B4.

Female rats were chosen exclusively in order to reduce biological variability in our outcome measures. Female Sprague Dawley rats tend to have a more stable body weight track over the months of behavioural testing, whereas age matched males gain substantially more weight, which can affect drug dosing (on a mg/kg basis), pharmacokinetics, and motor performance (Dorfman et al., 2017; Yu et al., 2016). Using females thus minimized complications related to body size changes. Additionally, restricting to a single sex avoided potential sex specific differences in the dopamine system or nicotinic receptor pharmacology that might complicate data interpretation (Betz & Sibley, 2023). Essentially, a homogeneous group of female rats improved our statistical power to detect treatment effects by limiting between subject variability. All procedures involving animals were carried out in accordance with the UK Animals (Scientific Procedures) Act 1986 and institutional ethical guidelines, under project and personal licenses approved by the UK Home Office. Every effort was made to ensure animal welfare and to use the minimum number of animals necessary to achieve scientific objectives.

2.4 Stereotaxic Surgery for 6-OHDA Lesioning

Rats received unilateral lesions of the left nigrostriatal pathway via stereotaxic infusion of 6-hydroxydopamine into the medial forebrain bundle (MFB). Surgeries were performed under deep isoflurane (Isoflo, Abbot Laboratories, Maidenhead, UK) anesthesia (4% for induction, ~2.5% for maintenance in oxygen enriched air). Each rat was placed in a stereotaxic

frame (Kopf Instruments), with the incisor bar adjusted to achieve a flat skull position (bregma and lambda at the same dorsoventral plane). The scalp was shaved and disinfected (chlorhexidine scrub), (hibiscrub Medical; NVS; UK), and a midline incision was made using a sterile disposable scalpel (No. 15, Swan-Morton, UK) to expose the skull. Using the Paxinos and Watson rat brain atlas coordinates (Paxinos & Watson, 1986), two injection sites along the MFB were targeted (a protocol modified from Cenci et al., 1998). The stereotaxic coordinates relative to bregma were as follows:

- Site 1: AP -4.4 mm, ML $+1.2$ mm, DV -7.8 mm (with the tooth bar set at -3.3 mm). This site lies in the posterior lateral hypothalamic area, along the ascending dopaminergic fiber bundle of the MFB.
- Site 2: AP -4.0 mm, ML -0.8 mm, DV -8.0 mm (tooth bar at $+3.4$ mm). This is a slightly more rostral and medial point on the MFB trajectory, nearer to the level of the substantia nigra.

At each site, a small hole was drilled in the skull. A $10\text{-}\mu\text{L}$ Hamilton syringe (701 ASN, Fisher Scientific, UK) with a 34 gauge blunt needle (Hamilton, Fisher Scientific, UK) was lowered to the target depth. 6-OHDA hydrochloride (Sigma-Aldrich) was prepared fresh on the day of surgery at $4\text{ }\mu\text{g}/\mu\text{L}$ (free base weight) in sterile 0.9% saline (Saline; Aquapharm; NVS; UK), containing 0.1% (w/v) ascorbic acid (to prevent oxidation of 6-OHDA). Each rat in the lesion group received a total of $16\text{ }\mu\text{g}$ of 6-OHDA, divided as $8\text{ }\mu\text{g}$ at each of the two sites (i.e. $2\text{ }\mu\text{L}$ of the $4\text{ }\mu\text{g}/\mu\text{L}$ solution per site). The neurotoxin was injected slowly at a rate of $1\text{ }\mu\text{L}/\text{min}$ using an automated micro infusion pump. After each infusion, the needle was left in place for an additional 2 minutes to allow diffusion into the tissue and to minimize reflux of the solution up the injection tract. The needle was then withdrawn slowly. Sham lesioned control rats (if any) received identical stereotaxic procedures except that vehicle (ascorbate

saline) was injected instead of 6-OHDA. The skull was then swabbed with saline and the wound closed using sutures (Ethicon-coated vicryl, NVS, UK).

Throughout surgery, body temperature was maintained at ~37 °C with a heating pad to prevent hypothermia. Aseptic technique was strictly observed to prevent infection (sterile instruments, gloves, and drapes; betadine/ethanol for skin preparation). To provide preventative analgesia, each rat received buprenorphine (0.03 mg/kg subcutaneous) during the surgery (approximately 15–20 minutes prior to the skin incision). Immediately following wound closure, 5 mL of warm sterile saline with 5% glucose solution (Saline +5% glucose; Aquapharm; NVS; UK) was administered subcutaneously to rehydrate the animal and aid recovery. A second smaller dose of buprenorphine (0.01 mg/kg s.c.) was given within a few hours post-surgery to ensure continued pain relief. Additionally, a non-steroidal anti-inflammatory analgesic, meloxicam (1 mg/kg p.o., Metacam), (Metacam Oral Susp (dog); NVS, UK), was provided once daily for 3–4 days post-op to control any inflammation or discomfort.

Rats were closely monitored during recovery from anesthesia. They were placed in clean cages partially on a warming pad and observed until fully conscious. Soaked food pellets and palatable hydrogel (nutrition enriched gelatin), (ElectroGel, Strawberry; Electrolyte Replenisher; LBS Biotechnology, US) were provided in the cage to encourage eating and drinking, since lesioned rats can have mild neglect of food on the affected side. Body weight was recorded daily for at least one-week post-surgery. If any rat lost more than ~10% of its pre-surgery weight, nutritional supplementation (high calorie diet paste) was given until weight stabilized. Under this comprehensive post-surgical care regimen, all 6-OHDA lesioned rats recovered uneventfully from surgery. Typical signs of a successful lesion (e.g. spontaneous ipsiversive circling, contralateral forelimb use difficulty) were noted in the days following the lesion, but rats remained generally healthy and active.

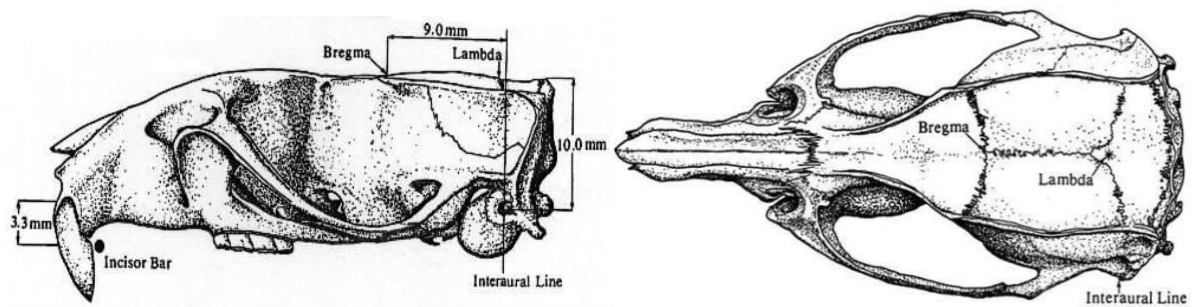


Figure 2. 2: Stereotaxic positioning of the rat skull. (A) Horizontal placement of the rat head in the stereotaxic frame, defined by lowering the incisor bar 3.3 mm below the interaural line. This alignment brings bregma and lambda into the correct horizontal plane. (B) Top down view of the rat skull showing the location of bregma and lambda, which serve as reference points for stereotaxic coordinates. Adapted from Paxinos & Watson atlas, 1986.

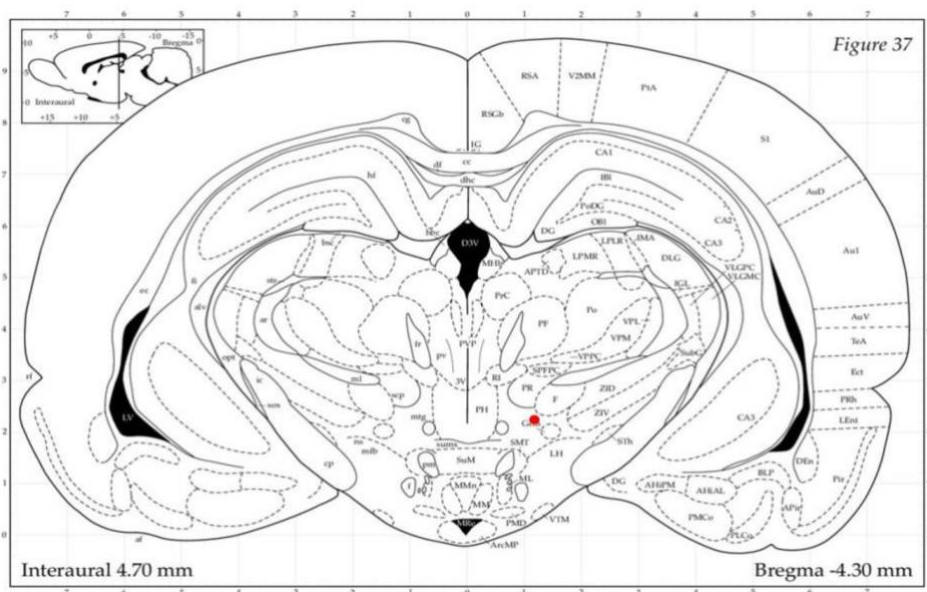


Figure 2. 3: Diagram of the medial forebrain bundle lesion site. Coronal section showing the position of the unilateral 6-OHDA lesion in the left medial forebrain bundle. The diagram is oriented as if the observer is facing the rat, with the left hemisphere on the right side of the image. Adapted from Paxinos and Watson, 1986.

2.5 Stepping Test (Forelimb Akinesia)

Two weeks after 6-OHDA lesion surgery, rats were behaviourally screened for forelimb akinesia using the adjusting step (stepping) test, as originally described by Olsson et al. (1995). This test assesses the animal's ability to initiate and sustain stepping movements with the forepaws, and it is highly sensitive to unilateral dopamine depletion (Olsson et al., 1995; Montoya et al., 1991). Briefly, the experimenter held the rat such that only one forelimb was free to move while the other three limbs were gently restrained. For example, to test the left forelimb, the right forelimb was held along with the hindlimbs, leaving the left forepaw resting on a tabletop. The rat was then moved sideways along the table in the direction of the free paw (forehand direction) for a distance of 90 cm over ~5 seconds. As the rat was moved, it reflexively attempted to adjust its body position by taking small steps with the free forepaw. The number of adjusting steps taken by the free forelimb during the 90 cm traversal was counted manually by the observer. The procedure was then repeated (in the opposite direction) to measure steps with the other forelimb. Each forepaw was tested twice, and the results were averaged.

In an intact rat, both forelimbs make a comparable number of steps, but a rat with a unilateral 6-OHDA lesion shows a dramatic impairment in the paw contralateral to the lesion (Schallert et al., 1979). The lesioned side forepaw typically fails to step normally, reflecting akinesia due to the loss of striatal dopamine (Schallert et al., 1979). In the experiments carried out, a successful left side lesion was expected to show markedly fewer adjusting steps with the right forepaw (contralateral to the lesion) compared to the left (ipsilateral) forepaw. This asymmetry provides a convenient behavioural indicator of lesion severity. Therefore, the stepping test was used as a secondary inclusion criterion: rats that did not show obvious forelimb akinesia on the contralateral side (indicating a possible incomplete lesion) were earmarked for exclusion.

2.6 Rotational Behaviour Test

Three weeks post lesion, the extent of dopamine denervation was assessed quantitatively using drug induced rotational behaviour, a classic outcome measure in unilateral PD models (Ungerstedt, 1971). Each rat was tested on two separate occasions: once following administration of D-amphetamine (Tocris; Biotechnique, UK) and once following administration of apomorphine (Tocris; Biotechnique, UK), to elicit rotational responses dependent on the lesion status of the nigrostriatal system. Prior to each test session, rats were placed in a cylindrical open field arena (40 cm diameter, with 40 cm high walls) for 10–15 minutes to acclimate to the environment.

For the amphetamine rotation test, rats received an intraperitoneal injection of D-amphetamine sulphate (5 mg/kg i.p.). Amphetamine induced ipsiversive rotation in unilateral 6-OHDA rats was originally described by Ungerstedt & Arbuthnott (1970). Immediately after D-amphetamine administration, the rat was placed in the centre of the circular arena. D-amphetamine causes endogenous dopamine release from intact dopamine terminals; in a unilaterally lesioned rat, this leads to asymmetrical activation of the striatum. Specifically, D-amphetamine releases more dopamine on the intact side (since the lesioned side has far fewer dopamine terminals), causing the animal to rotate toward the lesion (ipsiversive turning) (Ungerstedt & Arbuthnott, 1970). The rotational activity (asymmetry) was recorded for 90 minutes following D-amphetamine administration. Typically, within minutes, lesioned rats began to turn continuously in the ipsilateral direction, especially during the peak drug effect (20–60 minutes post-injection).

On a separate day (at least 48 hours later), the same rats were tested with apomorphine. Apomorphine HCl (0.5 mg/kg, s.c.) was administered, and rotations were monitored for 40–60 minutes following apomorphine administration. The foundational description of apomorphine induced contraversive turning following 6-OHDA denervation comes from Ungerstedt (1971). Apomorphine is a dopamine receptor agonist that directly stimulates dopamine receptors. In unilateral 6-OHDA rats, dopamine receptor density is greatly upregulated (supersensitive) on the lesioned side due to denervation. A low dose of apomorphine predominantly activates these supersensitive receptors, inducing the rat to rotate away from the lesion (contraversive turning) (Ungerstedt, 1971). Notably, the dose of 0.5 mg/kg apomorphine induces rotations in rats with mild or partial lesions, with contraversive circling occurring only if >90% of striatal dopamine is depleted. Thus, a strong contralateral rotational response to 0.5 mg/kg apomorphine is a stringent indicator of a severe (>90%) lesion in the nigrostriatal pathway (Ungerstedt, 1971).

Rotational behaviour was quantified using an automated video tracking system (Noldus EthoVision XT 15). A ceiling mounted camera tracked each rat's movement in the arena. The software computed rotations by comparing the orientation of the rat's body over time. A full 360° revolution (in either direction) was counted when the rat's nose to tail axis rotated through a complete circle. The total number of full rotations made in each direction was classified as ipsiversive (toward the lesioned hemisphere) or contraversive (away from the lesioned hemisphere), recorded in consecutive 10-minute time bins. Rats with successful left side lesions showed a predominance of clockwise (ipsiversive) rotations following D-amphetamine administration, whereas following apomorphine administration they showed predominantly counterclockwise (contraversive) rotations. The net rotation was expressed as the difference between turns in the two directions (e.g. net ipsiversive or contraversive turns per minute).

To quantify lesion success, a standard threshold was used: rats that exhibited an average of 6 or more full body rotations per minute (≥ 6 rpm net turning, in the expected direction) during the peak response period were defined as having a severe hemiparkinsonian lesion (corresponding to $>90\%$ dopamine depletion in the striatum), (Ungerstedt, 1971; Carman et al., 1991; Wang & Shao, 2020). This criterion is based on established correlations between rotation rates and nigrostriatal neuron loss. In this study, only rats meeting or exceeding this rotation threshold (for either amphetamine or apomorphine tests, as appropriate) were included for subsequent experimentation. Rats with subthreshold rotation behaviour (indicating incomplete lesions) were excluded from the dyskinesia induction phase and were humanely terminated. In practice, most of operated rats (approximately 80–90%) achieved the criteria.

2.7 Induction and Rating of Dyskinesia

After verifying successful lesions, induction of stable L-DOPA induced AIMs in the hemiparkinsonian rats commenced. Following a one week rest period after rotation testing, rats began a priming regimen of daily L-DOPA to show abnormal involuntary movements. Each rat received Levodopa (L-DOPA methyl ester; 8 mg/kg; Tocris; Biotechnique, UK), combined with benserazide (15 mg/kg) (Benserazide - HCL; Tocris; Biotechnique, UK) once daily. Benserazide, a peripheral aromatic amino acid decarboxylase inhibitor, was included to prevent peripheral conversion of L-DOPA to dopamine, thereby increasing L-DOPA bioavailability to the brain (Bartholini & Pletscher, 1962). The drugs were administered via oral gavage: L-DOPA and benserazide were dissolved in a small volume of a palatable vehicle (5% sucrose in water) and delivered directly into the stomach using a feeding needle. Dosing was done at a consistent time each morning to minimize variability due to circadian rhythms.

This daily L-DOPA/benserazide treatment was continued for at least 21 days. Over this period, the previously akinetic 6-OHDA lesioned rats gradually developed abnormal involuntary movements on the side of the body contralateral to the lesion, typically emerging within the first 1–2 weeks of treatment and increasing in severity thereafter. This is known as priming for dyskinesia that caused by repeated dopaminergic stimulation leads to progressive sensitization of downstream signalling pathways, culminating in overt dyskinetic motor outputs (Cenci et al., 1998). In this protocol, most rats showed observable AIMs (e.g. rapid jerking of the forelimb, twisting of the neck, or hyperkinetic orolingual movements) by around 10–14 days of L-DOPA treatment. Stable dyskinesia was operationally defined as the point at which an individual rat exhibited a reproducible level of AIM severity in response to the L-DOPA dose on at least two consecutive days. This typically occurred by 3–4 weeks of daily treatment, indicating that the dyskinetic response had plateaued (no further sensitization). At that stage, the rats were considered primed, and their dyskinesia were robust and reliable.

Once repeated daily doses of levodopa plus benserazide produced stable maximum AIMs, the rats were deemed dyskinetic. Quantitative AIM ratings were then performed to document each rat's baseline dyskinesia severity. These baseline ratings served as the reference point for evaluating the effects of investigational drugs (e.g. nicotinic agents) on dyskinesia. On assessment days, rats were given their usual oral dose of L-DOPA/benserazide (8 mg/kg L-DOPA + 15 mg/kg benserazide). Immediately after dosing, they were placed individually in clear observation chambers (40 × 40 × 40 cm). Dyskinetic behaviour was scored using the established Abnormal Involuntary Movement (AIM) scale for rodents (Cenci et al., 1998; Lundblad et al., 2002). Observers who were blinded to treatment condition performed the ratings to eliminate bias. Starting 20 minutes after L-DOPA administration (when AIMs typically begin), rats were observed at 20 minute intervals up to 120 minutes post dose (i.e. at approximately 20, 40, 60, 80, 100, and 120 min). During each observation period, lasting 1 minute, the rater recorded the presence and severity of dyskinetic

movements. In total, 6–7 observation time points per session were scored for each rat, capturing the time course of dyskinesia from onset to offset. All animals were evaluated with four abnormal involuntary movement scales using two established AIM systems, referred to here as Scale A and Scale B. Each animal was observed directly for one minute every 20 minutes over a 120 minute period following levodopa administration. All ratings were performed concurrently by three trained and blinded observers.

Abnormal involuntary movements were classified into four subtypes; axial, limb, orolingual, and locomotive and scored following the framework introduced by Cenci et al. (1998). Axial AIMs were defined as torsion/twisting of the head and neck (and upper trunk) toward the side contralateral to the lesion. Forelimb (limb) AIMs were repetitive, purposeless tapping, jerking or dystonic postures of the contralateral forelimb. Orolingual AIMs were vacuous jaw movements (opening/closing) often with repetitive tongue protrusion (usually directed contralaterally). Locomotive dyskinesia was excessive, non-goal directed locomotion (a strong contralateral turning or circling bias). Each subtype was rated independently on two complementary 0–4 scales and on a combined severity scale.

Importantly, different AIM metrics were used across the experimental chapters. In Chapters 3, 4 and 6, analyses were based on the frequency of AIM subtypes (Scale A) only. In contrast, Chapter 5 incorporated both frequency (Scale A) and amplitude/severity (Scale B), as well as their combined global scores, to provide a more comprehensive assessment of dyskinetic expression.

- Scale A (Time Based AIM Frequency): Each subtype's presence during a 1-minute observation was scored 0–4. 0 = no dyskinesia; 1 = infrequent (<50% of the time); 2 = frequent (>50%); 3 = continuous but briefly interruptible by a strong sensory stimulus; 4 = continuous, uninterrupted. For example, an axial dystonic twist lasting most of the minute but stopping briefly

when the cage is opened would score a “3” on this scale. The three sub-scores (axial, limb, orolingual) were summed (maximum 12 per observation). Across nine observations, this resulted a possible session total of 108.

- Scale B (Amplitude/Severity): Each dyskinesia subtype was also given an amplitude (or severity) score 0–4 based on movement magnitude (Winkler et al. 2002). This scale reflects how pronounced or forceful the movements are:

- Axial: 0 = normal; 1 = mild head/neck tilt ($\sim 30^\circ$); 2 = moderate tilt ($\sim 60^\circ$); 3 = severe torsion ($\sim 90^\circ$); 4 = extreme torsion ($>90^\circ$) often causing a fall.
- Limb: 1 = tiny forepaw taps; 2 = larger limb displacement (paw to snout, etc.); 3 = large forelimb sweep engaging shoulder; 4 = vigorous swing crossing midline.
- Orolingual: 1 = small orofacial twitching/jaw movement; 2 = clear jaw opening (tongue may briefly protrude); 3 = obvious jaw opening with occasional tongue excursions; 4 = forceful, sustained jaw opening with frequent vigorous tongue protrusions.
- Locomotive Dyskinesia: This subtype (contralateral circling/pacing) was scored with a single 0–4 scale, analogous to Scale A above. A score of 0 = normal locomotion; 1–4 reflect progressively more pronounced circling and hyperactivity, with 4 indicating continuous, exaggerated turning that disrupts normal movement patterns.

Each subtype’s Scale A and B scores were then multiplied for each observation and summed across subtypes/timepoints to give a “global” AIM score (higher values indicate more severe dyskinesia) (Elabi et al., 2023).

Once the three observers completed their independent ratings, the scores were entered into a standardized scoring sheet and transferred to Excel for processing (figure 2.4). For each time point, individual observer scores were summed to obtain a total AIMs value for each rat. The mean of the summed scores across AIM subtypes was then calculated and used to generate the final graphical summaries in GraphPad Prism as described in section 2.11.

For each time point, scores for the axial, limb and orolingual categories also were summed to get an overall ALO dyskinesia score (range 0–12). Locomotive dyskinesia was scored separately and not included in the ALO sum, since abnormal locomotion represents a distinct phenomenon. Using the ALO scores collected across the session, the time course of dyskinesia frequency and severity was plotted for each animal. In addition, the area under the curve (AUC) of the ALO score versus time plot was calculated for each rat as an integrated measure of overall dyskinetic severity. These baseline measures, including peak ALO score and AUC, were then compared across all lesioned rats to ensure that dyskinesia severity and pattern were consistent.

	cage	id	chip	weight	20 min				40 min				60 min				80 min				100 min				120 min			
					Lo	Li	Ax	OI	Lo	Li	Ax	OI	Lo	Li	Ax	OI	Lo	Li	Ax	OI	Lo	Li	Ax	OI	Lo	Li	Ax	OI
basic ampl	1																											
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Figure 2. 4: Data collection sheet for dyskinesia ratings. Template used to record abnormal involuntary movements over a 120 minute session. Gray cells represent the basic AIMs score, and white cells capture the amplitude ratings for each subtype (locomotive, limb, axial, and orolingual) across time points.

2.8 Tissue Collection

At the conclusion of the behavioural experiments, rats were sacrificed for neurochemical and histological analyses to validate lesion extent and to examine any treatment related neurobiological changes. To avoid acute drug effects on tissue markers, a washout period of approximately two weeks was implemented after the final L-DOPA or experimental drug injection before euthanizing the animals.

Rats were deeply anesthetized with an overdose of isoflurane and transcardially perfused. The transcardial perfusion method originates from the classical technique described by Rosene and Rhodes (1990). The perfusion was performed in two stages: first with ice cold phosphate buffered saline (PBS, 0.01 M, pH 7.4) (Gibco PBS; pH 7.4; Thermo Fisher Scientific, UK) for ~2–3 minutes to flush blood from the vasculature, and then with freshly prepared 4% paraformaldehyde (PFA) (Sigma Aldrich, Dorset, UK), in 0.1 M phosphate buffer (pH 7.4) for ~10 minutes to fix the tissues. Approximately 250 mL of PFA fixative was perfused per rat. Successful perfusion was confirmed by observing liver blanching and rigid limb musculature (from PFA cross linking), as described in standard perfusion protocols (Rosene & Rhodes, 1990). After perfusion, the brain was carefully removed from the skull and post fixed by immersion in 4% PFA at 4 °C for 24 hours.

Following fixation, brains were transferred to a cryoprotectant solution of 30% sucrose in 0.1 M PBS. The sucrose cryoprotection step follows the original method introduced by Cryer (1980). The brains were stored at 4 °C for 2–3 days until they sank in the sucrose solution (indicating complete infiltration). Cryoprotection prevents ice crystal artifact during subsequent freezing. Once equilibrated, brains were rapidly frozen by placing them on a metal plate over dry ice. Frozen brains were then stored at –80 °C until sectioning.

For histology, serial coronal sections were cut on a cryostat microtome (Leica CM1900) at a thickness of 30 μm . Cryostat sectioning for fixed brain tissue follows the principles established by Konigsmark and Murphy (1970). Sections were collected throughout the striatum and midbrain (including substantia nigra) in a series (usually 1 in 6 or 1 in 3 sampling, see below). Sections were kept free floating in a tissue storage solution (PBS with ethylene glycol and glycerol, i.e. antifreeze solution) at $-20\text{ }^{\circ}\text{C}$ until processing. To systematically sample the lesion and relevant structures, a fractionator approach was used: for the striatum, every 6th section was reserved for immunohistochemistry (yielding $\sim 5\text{--}6$ sections per striatum across its rostrocaudal extent). For the substantia nigra pars compacta (SNpc), which is smaller in anteroposterior range, every 3rd section through the SNpc was collected for staining (providing $\sim 4\text{--}5$ sections covering the entire SNpc). Additional sections were saved in parallel sets for potential future analyses or alternative stains. This sampling approach is based on the systematic random sampling principles introduced by Gundersen (1986). This approach ensured that representative cross sections of the key regions (striatum and SNpc) were examined for each brain, allowing quantitative comparisons of the lesioned versus intact hemispheres.

2.9 Histological Characterization of the Lesion

2.9.1 Tyrosine Hydroxylase Immunohistochemistry

To evaluate the degree of dopaminergic neuron loss produced by the 6-OHDA lesions, the immunohistochemical staining for tyrosine hydroxylase (TH) was performed. TH is the rate limiting enzyme in dopamine (and noradrenaline) synthesis, and it serves as a specific marker for catecholaminergic neurons and it was first described by Pickel et al. (1975). In the nigrostriatal system, TH labelling visualizes the cell bodies of dopaminergic neurons in the

SNpc and their axonal projections and terminals in the striatum. By comparing TH immunoreactivity in the lesioned vs. unlesioned hemisphere, the extent of dopamine neuron degeneration could be quantified.

For each brain, TH staining was examined in two main regions: (1) the SNpc, to count the number of surviving dopamine cell bodies, and (2) the striatum, to assess the density of dopaminergic fibers and terminals. In the SNpc, care was taken to distinguish this nucleus from the adjacent ventral tegmental area (VTA), since both contain TH-positive neurons. Anatomical markers and established criteria as defined by Carman et al. (1991) were used to define the SNpc (which lies more laterally and contains larger, more densely packed cells) versus the VTA (more medial and diffuse). Only TH-positive cells within the defined SNpc boundaries were counted as nigral dopamine neurons. In the striatum, TH staining appears as a diffuse plexus of fibers on the intact side, whereas on the lesioned side a dramatic loss of TH-positive innervation is expected. The pattern of striatal TH loss was qualitatively noted, and optical density of TH staining was quantitatively measured to estimate the percentage depletion of striatal dopamine terminals.

2.9.2 GFAP and IBA1 Immunohistochemistry

Next, the neuroinflammatory response associated with the 6-OHDA lesion and chronic L-DOPA treatment was investigated, focusing on astrocytes and microglia, the two key glial cell types involved in inflammation in the brain. For this purpose, immunostaining for glial fibrillary acidic protein (GFAP) and ionized calcium binding adapter molecule 1 (IBA1) was performed.

GFAP is an intermediate filament protein expressed predominantly by astrocytes in the central nervous system which it was first identified and characterized by Eng et al. (1971). In

normal conditions, astrocytes have relatively low basal GFAP expression. However, following neural injury or neurodegeneration, astrocytes become reactive (a process called astrogliosis) and dramatically upregulate GFAP synthesis (Sofroniew & Vinters, 2010). Thus, elevated GFAP immunoreactivity is a hallmark of astrocyte activation. In PD models like 6-OHDA lesions, the loss of dopamine neurons and ensuing tissue stress typically provoke a strong astrocytic response. Reactive astrocytes often undergo hypertrophy or proliferation, forming a glial “scar” around areas of damage. They also release a variety of signalling molecules, including proinflammatory cytokines (e.g. interleukin-1 β , tumor necrosis factor- α), chemokines, and reactive oxygen species which can influence neuronal survival and plasticity (Sofroniew & Vinters, 2010). By staining for GFAP in the lesioned rats, the aim was to measure the extent of astrogliosis in the striatum and SNpc and to determine whether the treatments (e.g. nicotinic drugs) altered the astrocytic response. An attenuation of GFAP upregulation in a treatment group, for instance, might suggest an anti-inflammatory or neuroprotective effect of that treatment.

IBA1 is a calcium binding protein expressed in microglia, the resident immune cells of the CNS. IBA1 was originally discovered and described by Imai et al. (1996) as a microglia/macrophage specific marker. Under basal conditions, microglia have a ramified morphology, constantly surveilling the brain parenchyma. When they detect injury, infection, or abnormal protein accumulation, microglia become activated, they retract their processes, enlarge the cell body (amoeboid shape), and often proliferate. Activated microglia highly express IBA1 (among other markers) and can release neurotoxic mediators such as nitric oxide, superoxide, and inflammatory cytokines (Hirsch & Hunot, 2009). In PD, microglial activation has been widely observed in the substantia nigra of patients and in toxin based models, where it may contribute to the progression of neurodegeneration (Hirsch & Hunot, 2009). IBA1 immunohistochemistry was used to label microglia in the samples. On the lesioned side, an

increase in both the number of IBA1-positive cells and a transformation of their morphology indicative of activation (thicker processes or amoeboid shapes) was expected. By comparing IBA1 staining between groups, it was possible to assess whether certain treatments modulated the microglial response to the lesion. For example, a reduction in IBA1-positive cell activation in a drug treated group might correlate with neuroprotective effects or reduced dyskinesia, whereas an exacerbation of microglial activation might indicate pro-inflammatory consequences.

In summary, GFAP and IBA1 staining allowed the glial environment in the dopamine depleted striatum and SNpc to be characterized. These data provided background for the neuronal findings: persistent inflammation (gliosis) in the brain can influence dyskinesia and neurodegeneration. Monitoring astrocytes and microglia thus helped interpret the results in terms of potential neuroinflammatory changes associated with L-DOPA dyskinesia and its modulation by nicotinic agents.

2.9.3 Immunohistochemistry Procedure and Image Analysis

The standard avidin biotin peroxidase immunohistochemistry protocol (ABC method), (Hsu et al., 1981) for all the above markers (TH, GFAP and IBA1) was used. Free floating sections were processed in parallel sets to minimize variability. Unless stated otherwise, all steps were carried out at room temperature with gentle agitation. The procedure was as follows:

1. Tissue Preparation: Sections were first rinsed thoroughly in 0.01 M PBS (pH 7.4) with 0.3% Triton X-100 (PBS-T) to remove cryoprotectant and rehydrate the tissue. Endogenous peroxidase activity was quenched by incubating the sections in 0.3% H₂O₂ in methanol for 15 minutes (to prevent nonspecific background from endogenous peroxidases). After several PBS washes, sections were blocked for 1 hour in 20%

normal goat serum serum (Animal Free Blocker; 2B Scientific, UK). This blocking step reduces nonspecific antibody binding by saturating potential sticky sites in the tissue.

2. **Primary Antibody Incubation:** Sections were incubated overnight (≈ 18 h) with the appropriate primary antibody diluted in PBS-T + 1% NGS. The primary antibodies used were: rabbit anti-TH (1:1000 dilution, Anti-Tyrosine Hydroxylase; Abcam; UK), rabbit anti-GFAP (1:5000, Anti-GFAP; Abcam; UK), and rabbit anti-IBA1 (1:5000, Anti-IBA- 1; Abcam; UK). All primary antibodies were raised in rabbit to allow use of a common secondary. The overnight incubation at 4 °C (or room temp) allowed ample time for antibody penetration and binding to target antigens within the 30 μ m sections.
3. **Secondary Antibody Incubation:** The next day, sections were rinsed in PBS and then incubated for 1 hour at dilution of 1:200 with a biotinylated goat anti-rabbit IgG secondary antibody (Horse Anti-Rabbit IgG Antibody (H+L), Biotinylated; Abcam, UK). This secondary antibody binds to the primary antibodies attached in the tissue. After this step, the target protein sites in the tissue are “tagged” with biotin molecules (via the secondary).
4. **ABC Detection and Chromogen Development:** After washes, sections were incubated with the avidin biotin peroxidase complex (VECTASTAIN[R] ABC-HRP Kit, Peroxidase; 2B Scientific, UK) for 1 hour. The ABC prepared by mixing two drops of Reagent A and two drops of Reagent B with 10 ml of 0.1M PBS and it was prepared 1 hour in advance according to the manufacturer’s instructions. Avidin has a very high affinity for biotin and each avidin can bind multiple biotin molecules; by using a biotinylated horseradish peroxidase (HRP) complex, this step effectively loads many HRP enzymes onto each secondary antibody site (amplifying the signal; Key, 2009). After ABC, the sections were washed and then incubated them in a solution of the

chromogen 3,3'-diaminobenzidine (DAB), with hydrogen peroxide to visualize the antibody binding (DAB Substrate Kit, Peroxidase (HRP), with Nickel; 2B Scientific, UK). Specifically, sections were placed in 0.05% DAB with 0.015% H₂O₂ in Tris buffer (pH ~7.6). The colour development was monitored under a microscope and stopped by transferring sections to PBS once the desired intensity was achieved (usually 5–10 minutes, varying by antibody). The result of this step was a permanent visible stain (brown or black precipitate) at the sites of antigen antibody binding.

5. Section Mounting: After a final series of washes, sections were mounted onto glass SuperFrost Plus adhesion slides (Thermo Fisher Scientific, Massachusetts, US and VWR, Pennsylvania, US). Mounted sections were air dried overnight. The next day, slides were dehydrated through graded ethanol solutions (70% → 95% → 100%, 2 minutes each), cleared in xylene substitute (Histoclear, 2 × 5 min), and cover slipped with DPX mounting medium (Sigma Aldrich, Dorset, UK).

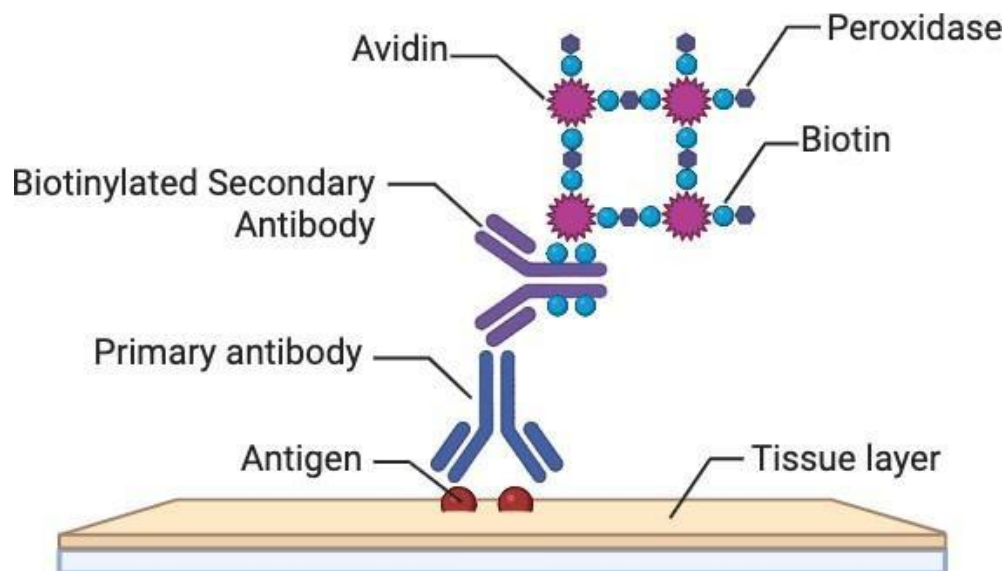


Figure 2. 5: Avidin biotin complex peroxidase method for immunohistochemistry. Diagram showing the sequence of antibody binding and signal amplification. The primary antibody binds the tissue antigen, followed by a biotinylated secondary antibody. Avidin, which has a high affinity for biotin, forms a complex that recruits multiple peroxidase molecules. This amplification step enhances the final chromogenic signal used to visualize the antigen, made with Biorender.

After immunostaining, quantitative image analysis was carried out following the optical fractionator approach (Gundersen et al., 1988; West et al., 1991) to assess the extent of the lesion and any treatment effects. Sections were imaged with a Nikon microscope fitted with a digital camera. For each rat, low magnification images of the SNpc were acquired that captured the full medial-lateral extent of the region, along with striatal images taken at a consistent rostrocaudal level. Both hemispheres were imaged using the same settings. Illumination and camera parameters were kept constant across all animals to allow direct comparison between the lesioned and intact sides. Images were saved in an uncompressed format and analysed in ImageJ (NIH, USA). Before analysis, ImageJ was calibrated using a greyscale reference to confirm that optical density values fell within a linear range. All images were converted to 8-bit greyscale to standardise the dynamic range. The same magnification, exposure, and processing settings were applied throughout the dataset.

Dopaminergic neuron counts in the SNpc were obtained using an optical fractionator approach to reduce bias from section thickness and cell size and to avoid overcounting (Gundersen et al., 1988; West et al., 1991). For each brain, a systematic series of coronal sections spanning the SNpc was selected (e.g. every third 30 μ m section from rostral to caudal SNpc). Only sections with well-preserved morphology and clear TH labelling were included. The anatomical boundaries of the SNpc were defined for each section using a rat brain atlas and established criteria, and the ventral tegmental area (VTA) was excluded from analysis. Within the SNpc, a sampling grid and counting frame were overlaid using ImageJ. A grid-based sampling strategy with a 200 $\times$ 200 μ m step size and a 50 $\times$ 50 μ m counting frame was used. At each sampling site, the counting frame was positioned, and TH-positive cell bodies were counted. The counting followed standard stereological rules. TH-positive neurons were counted if their cell body came into focus within the defined optical dissector height and did not touch the exclusion borders of the counting frame. Cells touching inclusion borders were

counted, whereas those touching exclusion borders were not. Only clearly labelled TH-positive neurons with a visible soma and nucleolus were included. Counts were performed manually in ImageJ, and separate estimates were obtained for each hemisphere.

For each animal and hemisphere, the total number of TH-positive neurons in the SNpc was estimated from the sampled sections according to the optical fractionator principle, taking into account the section sampling fraction (e.g. every third section) and the area sampling fraction defined by the grid and counting frame. These estimates were used to derive the percentage loss of TH-positive neurons in the lesioned hemisphere relative to the contralateral, intact side.

Striatal dopaminergic terminal loss was quantified by optical densitometry of TH staining in the dorsal striatum. For each animal, images of TH-stained sections at the level of the dorsal striatum were analysed in ImageJ. A region of interest (ROI) of fixed size and shape was placed within the dorsal striatum of each hemisphere, avoiding borders and areas with artefacts. The mean grey value (optical density) within each striatal ROI was recorded. To correct for non-specific background staining, a second ROI was placed in an adjacent TH poor region such as the somatosensory cortex or corpus callosum on the same section. This background value was subtracted from the striatal measurement to yield a corrected optical density for each hemisphere. For each animal, the corrected TH optical density in the lesioned striatum was expressed as a percentage of the corrected value in the intact striatum. Lower percentages indicated greater loss of TH-positive fibres. In animals with extensive lesions, TH optical density in the lesioned striatum was typically reduced to a small fraction of the contralateral side, in line with marked dopamine terminal depletion



Figure 2. 6: Grayscale stepladder. A continuous 8 bit grayscale gradient displaying increments from white to black. The scale illustrates the full range of luminance values used for calibration and visual inspection of contrast levels during image analysis (Schneider et al., 2012).

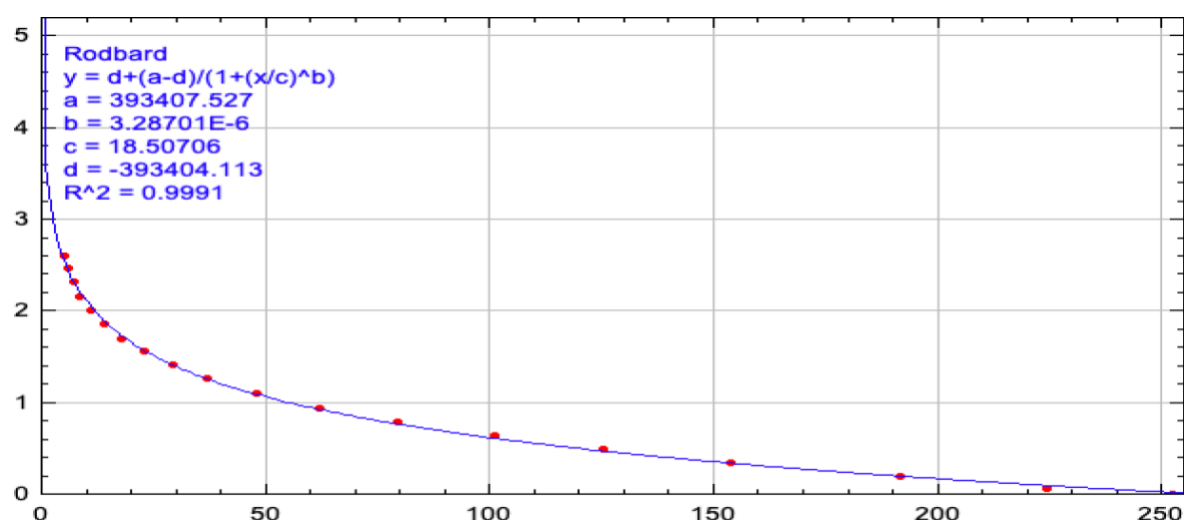


Figure 2. 7: Optical density calibration curve of the grayscale stepladder. Red points represent measured optical density values for each grayscale step. The blue line shows the fitted Rodbard function used to model the nonlinear relationship between pixel intensity and optical density. Fit parameters are shown in the panel, with an R^2 of 0.9991, indicating excellent agreement between measured and fitted values. This calibration was used to convert grayscale intensity into quantitative OD values for subsequent image analyses.

For glial markers, slightly different analyses were used given their distribution. In the striatum, GFAP and IBA1 staining tends to be diffuse. The area fraction of immunopositivity was measured by thresholding images and calculating the percentage of the striatal area occupied by positively stained processes or cells. Mean optical density of the stain in the striatum was also measured. These metrics were compared between lesioned and intact hemispheres. In the SNpc, where individual glial cells could be distinguished against a clearer background, the number of GFAP-positive astrocytes and IBA1-positive microglia was

counted. Using the ImageJ “Analyze Particles” function, immunoreactive cell profiles were counted after adjusting threshold and size filters to exclude artifactual noise. Care was taken to count only robustly stained cell bodies or processes indicative of reactive glia, sometimes also categorizing microglial morphology (ramified versus amoeboid). All counting and measurements were performed by an observer blinded to the treatment group of the animals to prevent bias.

These histological analyses allowed us to correlate the behavioural outcomes with the underlying biological effects of 6-OHDA and our interventions. A successful lesion was confirmed by >90% loss of TH-positive neurons in the SNpc and dense loss of TH-positive fibers in the striatum on the lesioned side (in line with our behavioural inclusion criteria). The glial markers provided insight into whether chronic L-DOPA and/or nicotinic treatments modulated the injury response, for instance, a reduction in IBA1 activation could imply an anti-inflammatory, neuroprotective effect of a drug. By integrating these data, the aim was to draw connections between neurochemical changes (dopamine loss, neuronal activity, gliosis) and the behavioural changes (rotational asymmetry, dyskinesia severity) in the

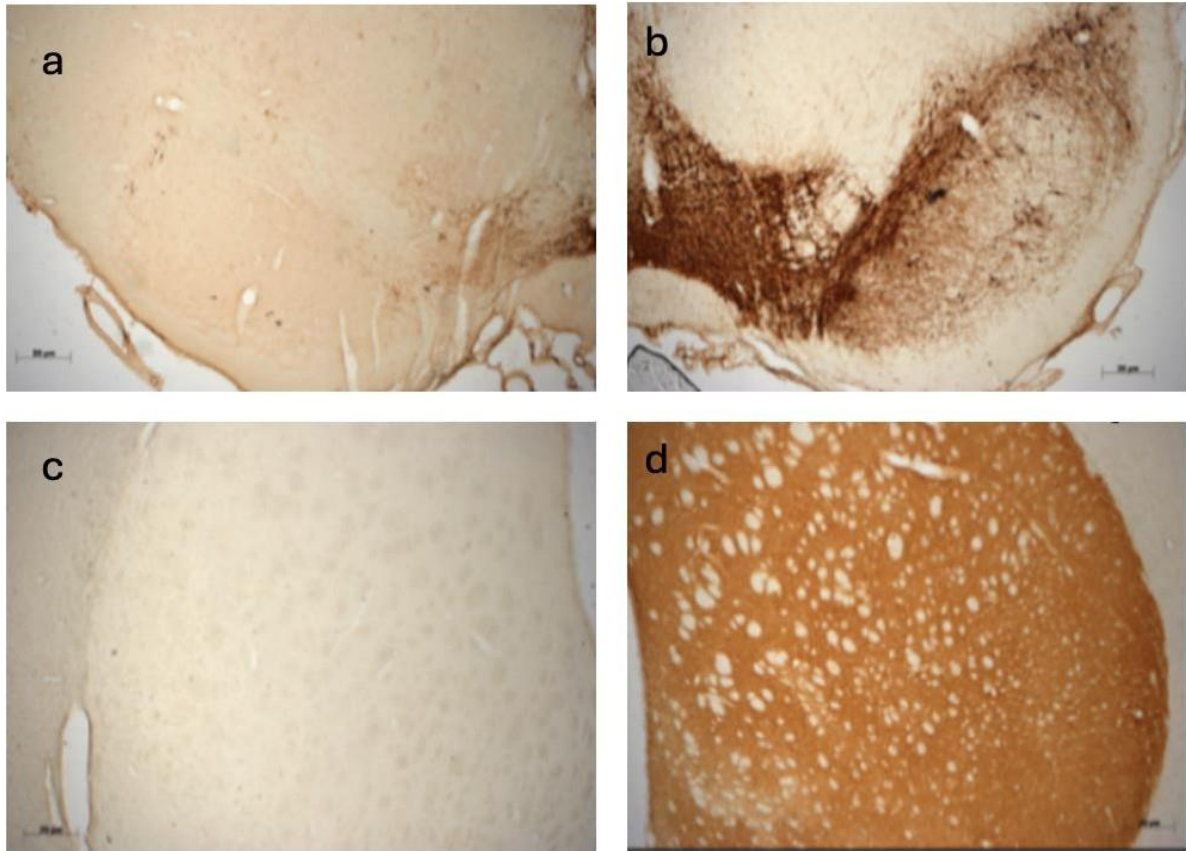


Figure 2.8. Representative TH immunostaining in the substantia nigra pars compacta (SNpc) and striatum of unilaterally 6-OHDA-lesioned rats. (a–b) TH-positive neurons in the SNpc of the lesioned (a) and non-lesioned (b) hemispheres. (c–d) Striatal TH immunoreactivity in the lesioned (c) and non-lesioned (d) hemispheres. Marked reduction in TH staining is observed in the lesioned hemisphere, confirming successful dopaminergic denervation. Images were captured at 2.5 $\times$ magnification. Scale bar = 20 μ m.

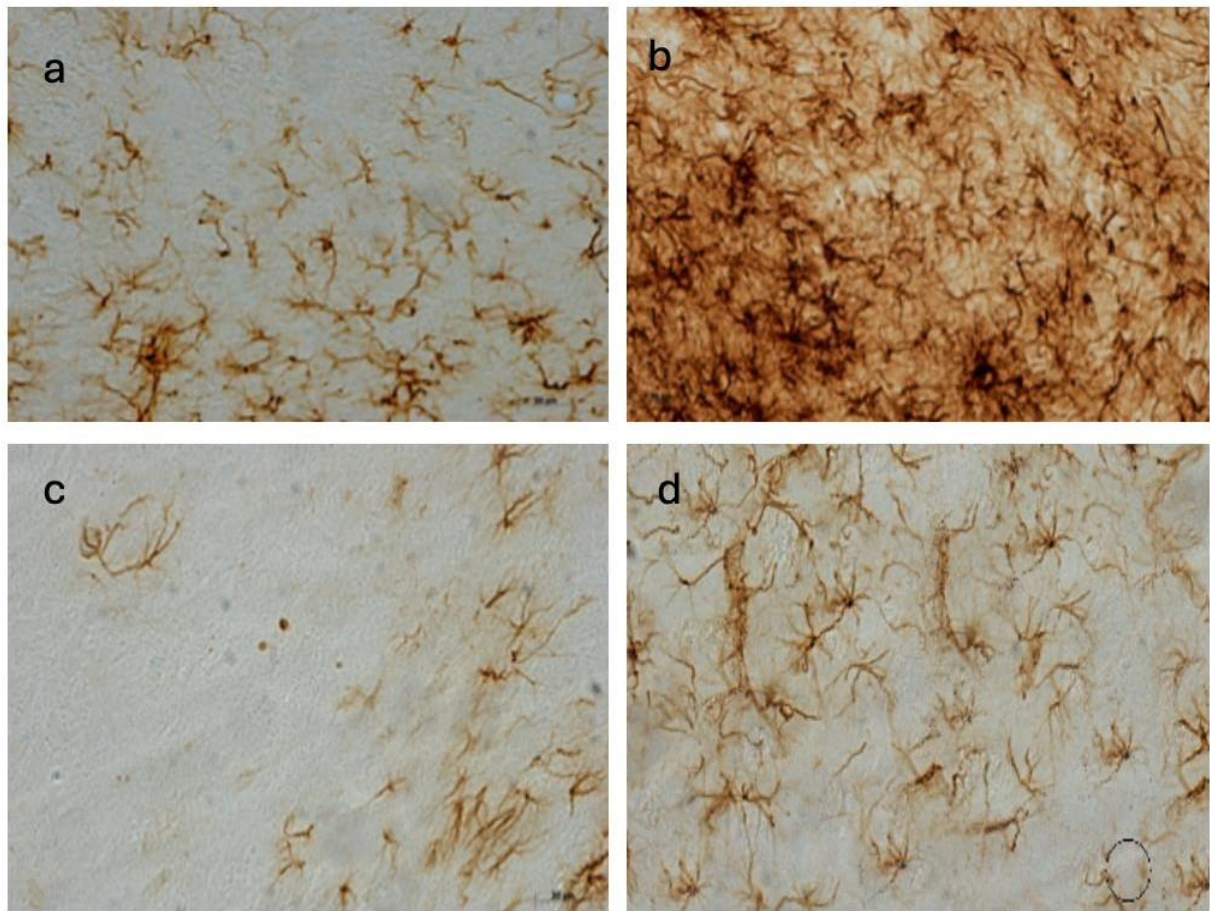


Figure 2.9. Representative GFAP immunostaining in the substantia nigra pars compacta (SNpc) and striatum of unilaterally 6-OHDA-lesioned rats. (a–b) GFAP immunoreactivity in the non-lesioned (a) and lesioned (b) hemispheres. (c–d) striatal sections showing non-lesioned (c) and lesioned (d) hemispheres. Increased GFAP staining is observed in the lesioned hemisphere, consistent with reactive astrogliosis following dopaminergic denervation. Images were captured at 20× magnification. Scale bar = 20 μ m.

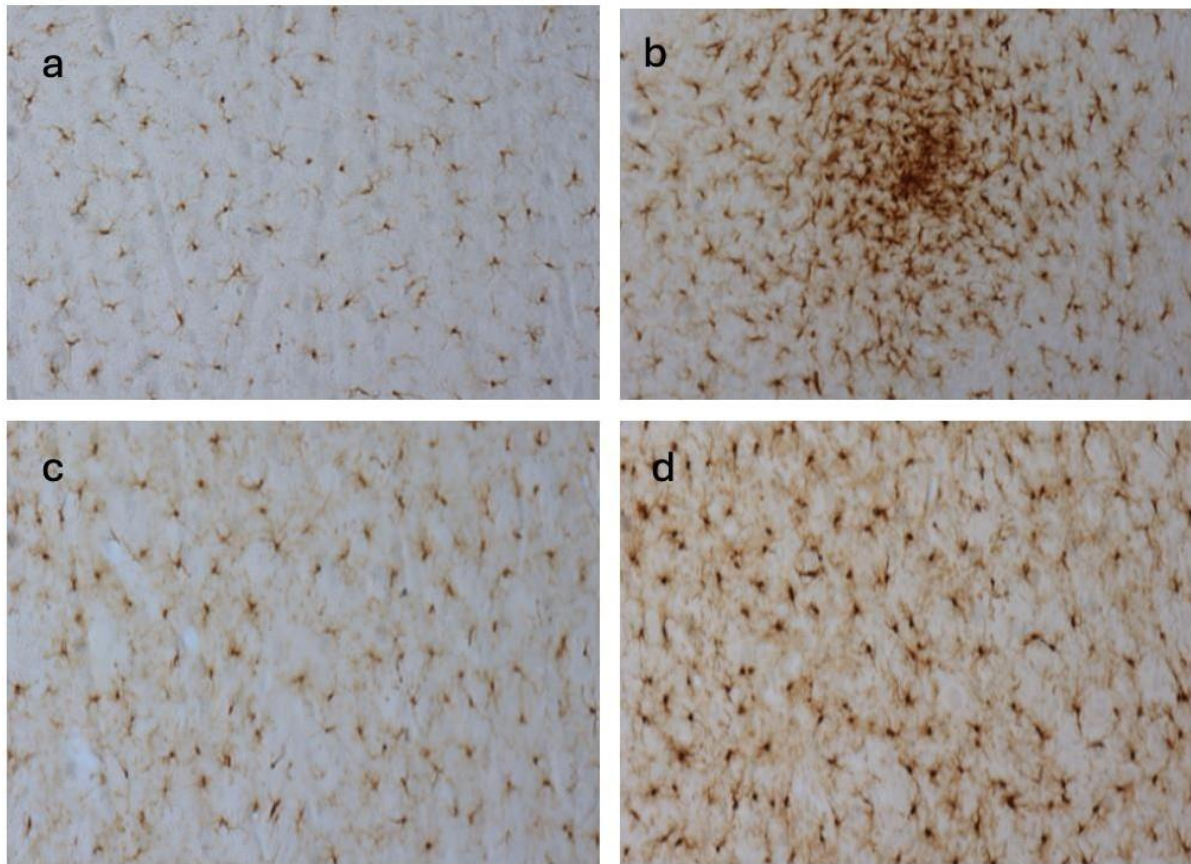


Figure 2.10. Representative Iba1 immunostaining in the substantia nigra pars compacta (SNpc) and striatum of unilaterally 6-OHDA lesioned rats. (a–b) Iba1 immunoreactivity in the non-lesioned (a) and lesioned (b) hemispheres. (c–d) Striatal sections showing non-lesioned (c) and lesioned (d) hemispheres. Increased Iba1 staining and morphological changes consistent with microglial activation are observed in the lesioned hemisphere. Images were captured at 40× magnification. Scale bar = 20 μ m.

2.10 Statistical Analysis

Data are presented as mean $\pm$ standard error of the mean (SEM) for each experimental group, unless stated otherwise. All statistical analyses were performed using GraphPad Prism (Version 10.0). Prior to hypothesis testing, datasets were examined for normality using the D'Agostino–Pearson omnibus test. In all cases reported, the data met the assumptions of normal distribution and homogeneity of variances, allowing the use of parametric tests. (If any dataset had significantly violated normality or homoscedasticity, appropriate non-parametric tests would have been used and noted, but this was not necessary for the results presented.

For experiments involving comparisons among multiple groups or conditions, an analysis of variance (ANOVA) techniques were used. Specifically, one-way ANOVA was used when comparing three or more independent groups for a single outcome measure (e.g. effect of different drug doses on dyskinesia score, measured in separate groups of rats). When the experimental design had two factors (for example, treatment group and time, or lesion status and drug), a two-way ANOVA was used. In cases where measurements were repeated on the same subjects over time or under multiple conditions (e.g. AIMs scores at multiple time points in the same animals, or rotational behaviour measured before and after a treatment in the same animal), Repeated measures ANOVA was applied to account for within subject correlations. The specific type of ANOVA (one-way, two-way, repeated measures, mixed model, etc.) for each result is detailed in the respective Results section corresponding to that experiment.

When ANOVA indicated a significant overall effect ($p < 0.05$), a *post hoc* test was performed to probe pairwise comparisons while controlling for multiple comparisons. In most cases, Dunnett's *post hoc* test was used when comparing several treatment groups against a single control group (e.g. multiple dose groups vs. a vehicle group), since Dunnett's test is

designed for multiple comparisons against a common control. In other instances, where all pairwise comparisons were of interest (not just vs. control), Tukey's honestly significant difference (HSD) test was applied, as it controls the family wise error rate for all pairwise contrasts. The choice of *post hoc* test is indicated alongside the results as needed. For the stepping test (which involved a within subject comparison of contralateral vs. ipsilateral forelimb steps in the same animals), a paired two-tailed Student's t-test was used. For comparisons between two independent groups (e.g. lesion vs. sham in a certain assay), an unpaired two-tailed t-test was used.

The criterion for statistical significance was set at $p < 0.05$ for all analyses. Exact p-values are reported where significant, and results are often annotated with significance levels such as $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****) in figures and text for brevity. All hypothesis tests were two-tailed unless otherwise specified, reflecting the possibility of differences in either direction. Data were managed and plotted in Prism, and all values were double checked for accuracy. By using these statistical methods, the conclusions drawn (for example, a reduction in dyskinesia by a nicotinic drug, or a difference in TH fiber density between groups) were supported by appropriate analysis and not likely due to chance. Each analysis method used is described alongside the results in the thesis for clarity, and assumptions (such as normality) were verified as noted above.

Chapter 3

The effects of Nicotine and Varenicline
on Levodopa Induced Abnormal
Involuntary Movements in a Levodopa
Primed, Unilaterally 6-OHDA Lesioned
Rats

3.1 Introduction

Levodopa remains the gold standard for managing motor symptoms in Parkinson's disease, offering reliable relief across disease stages. However, its long-term use is frequently accompanied by debilitating motor complications, particularly levodopa induced dyskinesia (LIDs), which substantially impair quality of life (Ahlskog & Muentner, 2001; Cenci & Lundblad, 2006). Given the progressive nature of PD and the limitations of current dopaminergic therapies, there is an urgent need for adjunctive treatments that can reduce LIDs without compromising the therapeutic efficacy of levodopa.

Nicotinic acetylcholine receptors (nAChRs), particularly the $\alpha 4\beta 2$ and $\alpha 6\beta 2$ subtypes, have emerged as promising therapeutic targets for addressing levodopa induced motor complications. These receptors are critical modulators of striatal dopamine release and are expressed on dopaminergic terminals in the nigrostriatal pathway. The $\alpha 6\beta 2$ subtype is of particular interest due to its selective localization to dopaminergic axons and its high vulnerability to nigrostriatal damage, an observation consistent across rodent, primate, and human studies (Quik et al., 2008). In contrast, $\alpha 4\beta 2$ receptors are relatively more resistant, suggesting expression on both dopaminergic and non-dopaminergic striatal neurons. Postmortem findings in PD patients confirm significant declines in both $\alpha 6\beta 2$ and $\alpha 4\beta 2$ receptor populations, with losses correlating closely with dopaminergic degeneration as indicated by reduced dopamine and transporter levels (Quik et al., 2008; Gotti et al., 2006). These findings provide a mechanistic rationale for targeting these receptors in therapeutic strategies aimed at preserving striatal dopaminergic function.

Nicotine, a well-characterized nAChR agonist, has shown considerable promise as a potential adjunct therapy in PD. A compelling body of epidemiological evidence has consistently demonstrated an inverse association between tobacco use and PD incidence, with

current smokers exhibiting a lower risk compared to non-smokers or former smokers (Thacker et al., 2007; Hernán et al., 2002). In vivo, nicotine pretreatment consistently protected against dopaminergic damage in 6-hydroxydopamine lesioned rats and MPTP treated mice, although the latter model has shown variable results due to specific differences in nicotine metabolism. More robust effects have been observed in non-human primates, where chronic nicotine exposure preserved striatal dopamine markers, maintained synaptic plasticity, and reduced overactive dopaminergic signaling following MPTP lesioning (Quik et al., 2008). In addition to neuroprotection, nicotine has demonstrated efficacy in reducing levodopa induced dyskinesia in primates (Quik et al., 2013) and abnormal involuntary movements in rodents (LID), (Bordia et al., 2013), further supporting its utility as an adjunctive therapy in PD. In rats in particular, several studies have shown that nicotine significantly decreases abnormal involuntary movements when co-administered with levodopa in previously levodopa primed animals. For example, Quik et al. (2007) reported that nicotine delivered either intermittently through drinking water or continuously via minipump led to approximately a 50% reduction in LIDs. Notably, nicotine was effective both in animals undergoing levodopa priming by reducing the development of dyskinesia and in animals with established levodopa induced dyskinesia, suggesting both preventative and therapeutic potential (Bordia et al., 2013; Quik et al., 2013). In non-human primates another study by Quik et al. (2013), showed that when nicotine was added to the drinking water at 300 µg/mL either before or after the initiation of levodopa/carbidopa administration (10/2.5 mg/kg twice daily) there was a sustained 60–70% reduction in dyskinetic movements, with effects persisting for up to 23 weeks. Importantly, nicotine did not compromise the antiparkinsonian action of levodopa and did not worsen parkinsonian symptoms. These findings show that nicotine can effectively reduce dyskinetic symptoms during both the development and maintenance phases of levodopa treatment, supporting its utility in preclinical studies of Parkinson's disease.

Although nicotine has demonstrated consistent antidyskinetic effects in both rodent and primate models, its chronic administration presents several challenges that limit its translational potential. In previous studies, nicotine was typically administered over extended periods, which raises concerns about the contribution of nicotine dependence and associated neuroadaptations to the observed reductions in dyskinesia (Quik et al., 2007; Quik et al., 2013). Long term nicotine exposure is known to affect synaptic plasticity through mechanisms such as altered AMPA/NMDA receptor ratios, thereby limiting the clarity with which its therapeutic effects can be interpreted (Calabresi et al., 2006). Moreover, nicotine has a short half-life, similar to levodopa, and long term use can lead to receptor desensitization and tolerance, thereby limiting its efficacy in prolonged therapeutic applications. These factors emphasize the importance of investigating the acute effects of nicotine administration on dyskinesia, independent of long-term receptor adaptation or addiction liability.

Nicotine's addictive properties further complicate its clinical application. Its interaction with $\beta 2$ containing nicotinic receptors shows both its therapeutic and addictive effects, making it a difficult candidate for long term use in PD patients. This has led to interest in alternative compounds that retain the therapeutic potential of nicotine while avoiding its adverse effects.

Varenicline tartrate is one such candidate. Clinically approved for smoking cessation, varenicline functions as a partial agonist at $\alpha 4\beta 2$ and a full agonist at $\alpha 7$ nicotinic acetylcholine receptors. These receptor subtypes are implicated in both dopaminergic and glutamatergic signalling within the striatum, which are relevant to the pathophysiology of levodopa induced dyskinesia. By partially stimulating $\alpha 4\beta 2$ receptors, varenicline may reduce excessive dopamine release without overstimulating the system, while $\alpha 7$ receptor activation may offer additional neuroprotective and anti-inflammatory benefits. Importantly, varenicline is not associated with the same risk of dependence as nicotine and offers a more favorable safety and pharmacokinetic profile. Preclinical studies in MPTP lesioned primates support

varenicline's potential, demonstrating significant reductions in LID severity and frequency without impairing the antiparkinsonian efficacy of levodopa (Bordia et al., 2012). These findings provide a strong rationale for further exploration of varenicline as a safer, non-addictive nicotinic receptor modulator for the treatment of LIDs.

Given these advantages, the current study aims to systematically evaluate and compare the acute antidyskinetic effects of nicotine and varenicline in a 6-OHDA rat model of Parkinson's disease. By investigating their efficacy across a range of doses in levodopa primed animals, this study seeks to clarify their potential as adjunctive treatments for LID and to provide further insight into nicotinic receptor targeted strategies for managing motor complications in PD.

3.1.1 Aims and objectives

The primary aim of this chapter was to systematically evaluate the acute effects of nicotine (0.01, 0.03, 0.1, and 0.3 mg/kg) administered following levodopa and benserazide priming as well as the effects of varenicline (0, 0.1, 0.3, and 1 mg/kg) administered following levodopa and benserazide priming phase.

Experiments were conducted using a 6-OHDA lesioned hemiparkinsonian rat model in which animals were primed through chronic daily administration of levodopa together with the peripheral dopa decarboxylase inhibitor benserazide to induce abnormal involuntary movements (AIMs). This model was selected due to its reproducibility, reliability and ethical advantages over primate models as detailed previously (see Chapter 2). Following unilateral injection of 6-OHDA, rats developed dopaminergic neuron depletion, mimicking the unilateral dopamine deficiency observed in Parkinson's disease. Chronic administration of levodopa and benserazide resulted in the predictable emergence of AIMs within approximately 3–4 weeks.

Once AIMs were reliably established, the study assessed the acute co-administration of levodopa with nicotine, varenicline, or vehicle. AIMs were quantified by trained observers using a well-established behavioural rating scale (Cenci and Lundblad), evaluating axial, limb and orolingual components (see Chapter 2).

The working hypothesis underpinning this study was that acute modulation of nicotinic acetylcholine receptors via nicotine and varenicline would result in a dose dependent reduction in AIM severity in levodopa treated hemiparkinsonian rats. This hypothesis is based on evidence implicating specific nAChR subtypes in the modulation of basal ganglia circuitry and motor complications associated with Parkinson's disease. Dose selection was guided by previous studies demonstrating effective therapeutic ranges for these compounds (Bordia et al., 2008).

The inclusion of both compounds was hypothesis driven. Nicotine acts as a non-selective full agonist at multiple nAChR subtypes, whereas varenicline is a partial agonist with high selectivity for the $\alpha 4\beta 2$ subtype and partial activity at $\alpha 6\beta 2$ containing receptors, both highly expressed in striatal circuits implicated in dyskinesia. Assessing both agents allowed comparison between full and partial receptor activation, providing insight into the receptor mechanisms underlying anti-dyskinetic effects. In addition, varenicline has a longer half-life, improved bioavailability and reduced toxicity compared to nicotine, making it a promising candidate for clinical translation.

In contrast to previous studies, which have predominantly focused on chronic nicotine exposure or repeated administration of $\alpha 7$ nicotinic receptor agonists to attenuate L-DOPA induced dyskinesia, the present study examined the effects of acute nicotinic receptor modulation. Here, we demonstrate that a single low dose administration of nicotinic agents, including nicotine and varenicline, produces a rapid and dose dependent reduction in abnormal involuntary movements in the 6-OHDA model. This finding extends the current understanding of nicotinic mechanisms in dyskinesia by showing that anti-dyskinetic effects are not limited to chronic treatment paradigms but

can also be achieved acutely, highlighting a more immediate therapeutic potential for nicotinic receptor modulation.

3.2 Material and Methods

3.2.1 *Animals*

Two cohorts of female Sprague Dawley rats ($n = 30$ per group; Charles River, UK, Harlow, Essex) were used. The nicotine cohort (250–275 g at the start of the experiment) was taken from a larger study presented in Chapter 6 from which the relevant animals and data are included here. The varenicline cohort (250–300 g at the start of the experiment) was taken from the study described in Chapter 4, with only the data relevant to the present experiments included in this chapter. Animals were housed in pairs in standard plastic cages and maintained under a 12:12 h light/dark cycle (lights on at 07:00, off at 19:00) in a temperature-controlled room (20 ± 1 °C). Food and water were available *ad libitum*.

Female rats were used to reduce variability associated with sex differences. In particular, females show more stable body weight over prolonged experimental periods, supporting more consistent behavioural and pharmacokinetic measures. In addition, sex dependent differences in pharmacokinetics and behavioural responses may emerge with age, which can complicate interpretation in long-term studies. Restricting the study to females therefore allowed more consistent assessment of treatment effects.

To clarify the relationship between datasets across chapters, the nicotine cohort described here forms part of a larger dataset presented in Chapter 6 and the same levodopa primed control animals are used for comparison across both chapters. Specifically, data obtained from the primed control group in this chapter are also used as the reference condition for the nicotine experiments presented in Chapter 6, allowing direct comparison of drug effects without introducing additional inter cohort variability. In contrast, the varenicline cohort was derived from the study described in

Chapter 4, and only the subset of animals and data relevant to the present analysis are included here. No drugs were administered during the priming period beyond levodopa and benserazide, ensuring consistency of the primed baseline across experimental conditions.

3.2.2 Unilateral 6-OHDA Lesions

All surgical procedures, including acclimatisation, anesthesia, preparation of 6-OHDA, stereotaxic injections, post-operative care and analgesia, followed the protocol described in Chapter 2 (see Section 2.4 for full methodological details). In the Nicotine experiment, 24 rats received unilateral 6-OHDA lesions, and 6 rats received vehicle injections. In the Varenicline experiment, all animals (n = 30) received unilateral 6-OHDA lesions. The same stereotaxic coordinates, injection parameters and lesioning procedures described in Chapter 2 were used throughout.

3.2.3 Assessment of 6-OHDA induced Motor Asymmetry (rotational behaviour)

Assessment of lesion severity followed the procedures detailed in Chapter 2.6, using the stepping test in combination with amphetamine and apomorphine induced rotational behaviour. Successful lesioning was defined based on established behavioural criteria and corresponding reduction in tyrosine hydroxyl immunoreactivity, indicative of substantial dopaminergic depletion.

In the nicotine cohort, 12 out of 30 animals met the lesion criteria. In the varenicline cohort, 16 out of 30 animals were confirmed as fully lesioned, of which 10 were assigned to varenicline treatment and the remaining animals were allocated to a separate study. Animals that did not meet the predefined lesion threshold were excluded from further experiments.

Following lesion verification, fully lesioned animals underwent levodopa priming. In the nicotine group, rats received daily oral doses of 8.0 mg/kg levodopa methyl ester plus 15.0 mg/kg benserazide for 21 days. In the varenicline group, animals underwent the same levodopa and benserazide priming regimen for 34 days.

Animals were considered successfully lesioned based on behavioural criteria supported by a corresponding reduction in Tyrosine Hydroxylase (TH) immunoreactivity in the lesioned hemisphere relative to the contralateral side, indicative of substantial dopaminergic depletion.

It should be noted that not all animals developed lesions of sufficient severity to meet these predefined criteria, reflecting the inherent variability of the unilateral 6-OHDA lesion model. This variability was confirmed histologically by differences in TH immunoreactivity between animals. As a result, group sizes were reduced following lesion verification, and only animals meeting both behavioural and histological thresholds were included in subsequent analyses.

3.2.4 Assessment of 6-OHDA induced Motor Asymmetry (stepping test)

Following priming, all lesioned animals (n = 30 in the nicotine group; n = 29 in the varenicline group) were assessed for forelimb akinesia using the stepping test, as described in Chapter 2, Section 2.5. The procedure was repeated twice, and the average number of steps was calculated for each forelimb.

3.2.5 Experimental Protocol

All experiments were performed between 10.00h and 16.00h. For the assessment of drug effects on levodopa induced dyskinesia experiments, the animals were randomised into groups of 4 rats where the effects of different doses of drugs were assessed. The observations and data collection were conducted in Latin Square double blinded. The observer was blinded to the drug treatment of the animals in all instances. The scores for each AIMs subtype were

combined and taken as an overall index of dyskinesia (Lundblad et al., 2002) or assessed individually to assess the effect of the drugs on each component differentially. When overall AIMs were compared over the experimental period of 120 minutes, area under the curve (AUC) was used as a measure to total AIMs in response to levodopa.

For all experiments, levodopa primed animals were acclimatised to the AIMs observation boxes for 10 min and baseline AIMs measurements obtained prior to acute drug administration. A washout period of 48-72 hours was considered between each dose within an experiment and between different drug treatments.

3.2.6 Drugs

Levodopa (L-DOPA methyl ester; 8 mg/kg; Tocris; Biotechne, UK) and benserazide (Benserazide - HCL; Tocris; Biotechne, UK) dissolved in 5% w/v sucrose in deionised water and they were administered orally at the time of which was considered 0 mins.

Nicotine [(-)-nicotine ditartrate] obtained from (Biotechne; UK), was dissolved in sterile saline and the pH of the final concentration was adjusted to ~pH 7.0 by dropwise addition of NaOH. Nicotine was administered subcutaneously at doses of 0.01, 0.03, 0.1 and 0.3 mg/kg 20 minutes following levodopa and benserazide administration.

Varenicline [Varenicline Tartrate] obtained from (Merck; UK), was dissolved in saline it was adjusted to ~pH 7.0 and administered at doses of 0.1, 0.3 and 1 mg/kg subcutaneously 20 minutes following levodopa/benserazide administration

3.2.7 *Histological Analysis*

Upon conclusion of the behavioural experiments, animals were transcardially perfused, and brains were collected for immunohistochemical evaluation. Brain sections were processed to visualise dopaminergic neurons and fiber via tyrosine hydroxylase (TH) immunolabelling. A primary anti-TH antibody (1:1000, Abcam, UK; cat. no. ab112) was used, followed by incubation with a biotinylated goat anti-rabbit secondary antibody (1:200, Abcam, UK; cat. no. ab6720). This staining protocol was used to evaluate TH positive cell bodies in the substantia nigra pars compacta (SNpc) and TH-positive terminals in the striatum, consistent with protocols previously described (see Chapter 2).

Quantification of TH immunoreactivity was performed using ImageJ software (NIH, USA). Calibration was first conducted using a greyscale standard curve and images were then converted to 8-bit. For the striatum, optical density (OD) values were extracted from manually defined regions of interest (ROIs) and corrected using the somatosensory cortex as a background reference. In the SNpc, dopaminergic neuron counts were estimated using an optical fractionator approach to account for section depth and avoid overcounting. A grid based sampling strategy ($200 \times 200 \mu\text{m}$) with a $50 \times 50 \mu\text{m}$ counting frame was employed, and cells were counted manually in ImageJ following stereological conventions. TH⁺ cell counts and striatal OD values were recorded for each hemisphere. Representative TH immunohistochemical images illustrating dopaminergic depletion in the lesioned hemisphere are shown in Figure 3.16 to 3.19.

All quantitative outcomes (including TH cell counts) and striatal OD values were tabulated in Microsoft Excel and subjected to statistical analysis using GraphPad Prism version 10.0. Data are presented as mean $\pm$ SEM, and group comparisons were analysed using two-way ANOVA followed by Dunnett's post-hoc test, in line with the statistical procedures outlined in chapter 2.

3.2.8 *Statistical Analysis*

All data were assessed for normality of distribution using D'Agostino-Pearson test and parametric statistical analysis was mandated on the results of this test being nonsignificant test. To measure the effect of each drug on rotation behaviour or AIMs subtype scores or AUCs, repeated measure one-way ANOVA followed by a Dunnett's post-hoc test was used. In the data where the interactions of drug dose and time was compared, i.e. AIMs scores at the different doses at different time, a two-way ANOVA followed by a Dunnett's post-hoc test was used. Data from stepping test were analysed using two tailed, paired student t-test. Data were expressed as means $\pm$ standard error of the mean and a level of $p \leq 0.05$ was considered statistically significant. Statistical analyses were performed using the GraphPad Prism version 10 software.

3.3 Results

3.3.1 *Amphetamine induced ipsiversive rotation test (nicotine group)*

The assessment of drug induced rotational behavior was conducted 2 weeks subsequent to the administration of a 6-OHDA lesion to evaluate the extent of dopamine neuronal damage. A dose of 5 mg/kg (+) amphetamine was administered to induce rotational movements, specifically causing pronounced ipsilateral (towards the lesioned side) rotations in the subjects. The results showed marked and significant ipsilateral rotation to 5 mg/kg (+) amphetamine [$F(1,23) = 7.77$; $P = 0.0105$] in the nicotine group and [$F(2.940, 79.37) = 19.07$; $P < 0.0001$] in the varenicline group (figure 3.2 and 3.3).

The onset of this rotational behavior was observed approximately 10 minutes following the amphetamine in injection both groups and persisted for a duration exceeding 90 minutes. Notably, the frequency of rotations increased progressively over the 90-minute observation period, indicating a time dependent intensification of the rotational response.

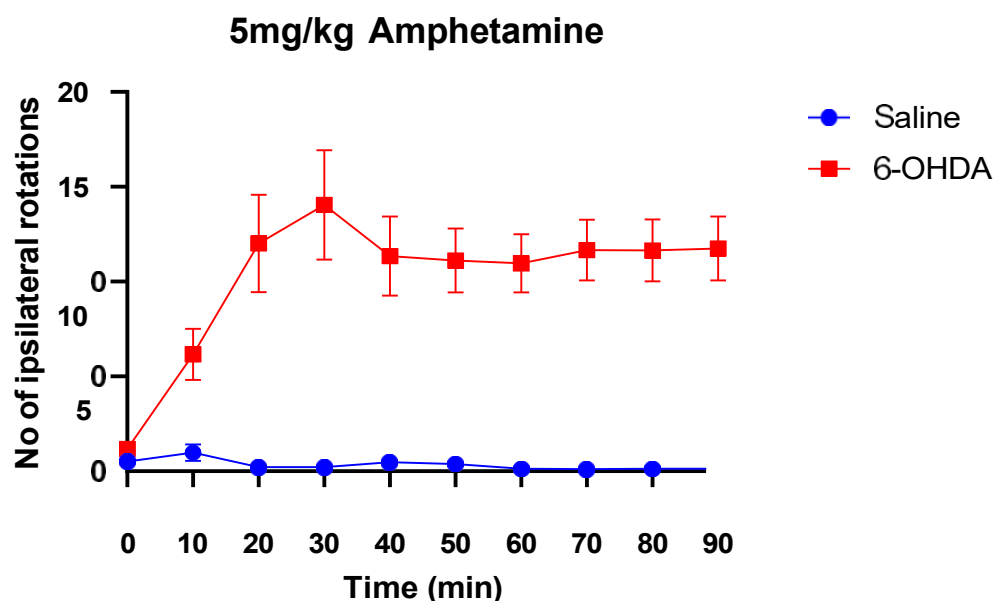


Figure 3. 1: Amphetamine induced rotational behaviour over 90 minutes (nicotine group). Ipsilateral rotational behaviour in unilaterally lesioned rats treated with 8 μ l 6-OHDA compared to saline treated controls. Rats were assessed for lesion integrity prior to levodopa priming by measuring the number of ipsiversive rotations in response to 5 mg/kg (+)-amphetamine. 6-OHDA treated rats (n=24) showed a significant increase in rotational behaviour compared to saline controls (n=6) over a 90 minute period, starting at 10 minutes post amphetamine administration. Statistical analysis was performed using a two-way ANOVA.

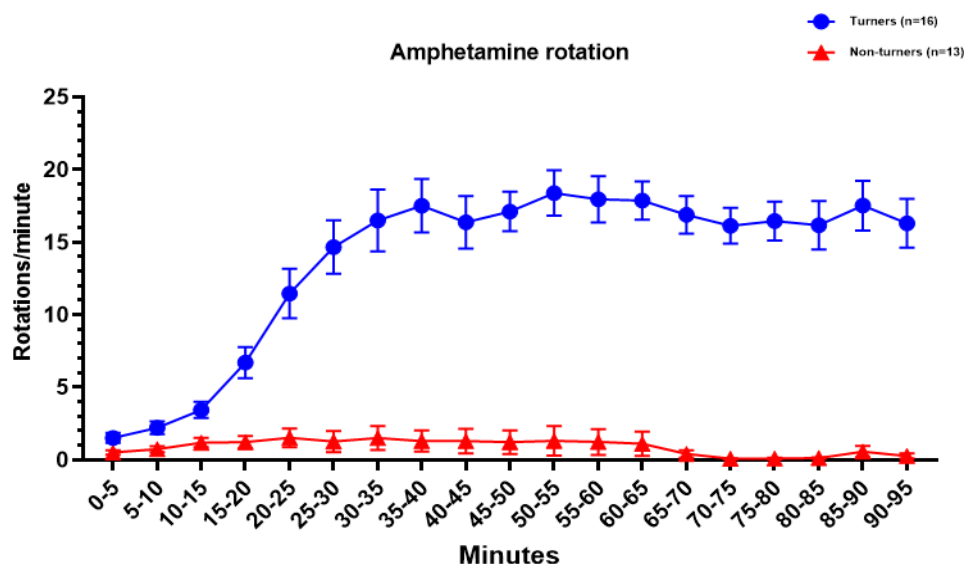


Figure 3. 2: Amphetamine induced rotational behaviour over 90 minutes (Varenicline group). Ipsilateral rotational behaviour in unilaterally lesioned rats treated with 8 μ l 6-OHDA comparing number of turners (n=16) and non-tuners (n=13). Statistical analysis was performed using a two-way ANOVA.

3.3.2 Apomorphine induced contravasive rotation test

The evaluation of apomorphine induced rotational behavior was conducted 18 days following the administration of 6-OHDA lesion to assess the integrity of dopaminergic pathways. Apomorphine, administered at a dosage of 0.5 mg/kg, produced a pronounced contralateral (away from the lesioned side) rotational response in the nicotine group [$F(1,17) = 5.19$; $P = 0.0359$] and [$F(1.381, 41.42) = 2.261$; $P = 0.1320$] for varenicline group. This rotational behavior displayed shortly after the apomorphine injection and persisted for an approximate duration of 90 minutes for nicotine group and about for 60 minutes (figure 3.4 and 3.5).

A peak in the rotational activity was observed around 20 minutes post injection, indicating the most intense period of rotational behavior induced by apomorphine. Throughout the observation period, from 10 to 90 minutes post injection, a statistically significant difference in the number of rotations was noted between the saline treated control group and the 6-OHDA lesioned group in the nicotine group, with the lesioned group exhibiting more pronounced rotational behavior ($P < 0.05$).

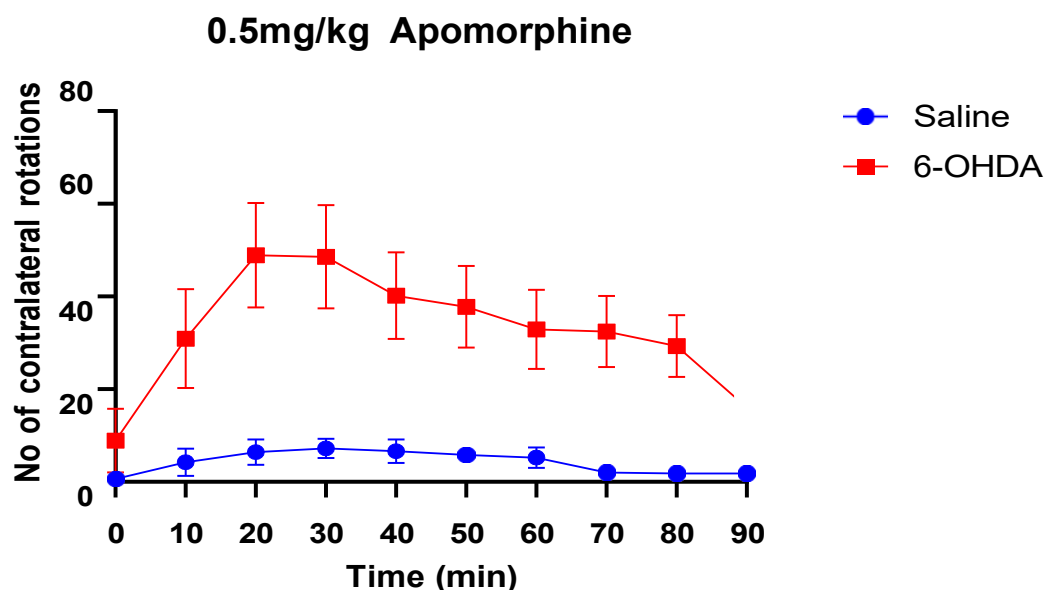


Figure 3. 3: Rotational behaviour induced by apomorphine post 6-OHDA lesion (nicotine group). Contralateral rotational behaviour induced by apomorphine (0.5 mg/kg) in saline treated controls and 6-OHDA lesioned rats (n=24). A significant increase in rotational behaviour was observed in the 6-OHDA group compared to the saline group (n=6), with rotations peaking at 20 minutes post-injection and persisting for the 90 minute observation period. Lesion integrity was assessed prior to levodopa priming. Statistical analysis was conducted using a two-way ANOVA.

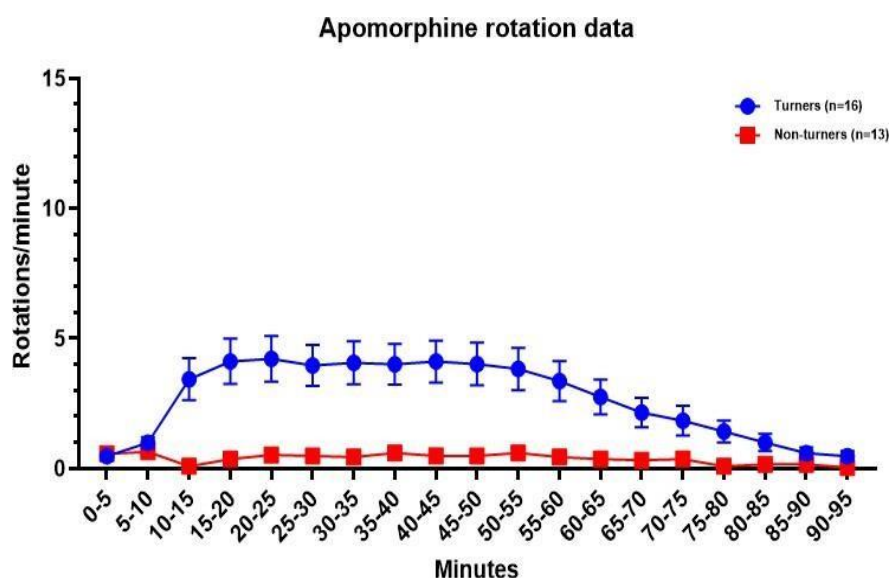


Figure 3. 4: Apomorphine induced rotational behaviour over 90 minutes (Varenicline group). Ipsilateral rotational behaviour in unilaterally lesioned rats treated with 8 μ l 6-OHDA comparing number of turners (n=16) and non-tuners (n=13). Statistical analysis was performed using a two-way ANOVA. Error bars represent mean $\pm$ SEM.

3.3.3 Adjusted step test

All lesioned and primed animals (n=30; nicotine group) and (n=29; varenicline group) were further assessed for lesion integrity using the stepping test (Olsson et al., 1995) as fully described in chapter 2. During the session, it was shown that the impairment was pronounced, with a highly significant p-value of less than 0.001 (n=16; ****p ≤ 0.0001) for nicotine group and (n=16; p= ****p ≤ 0.0001) for varenicline group, as indicated by the Tukey's post-hoc test results.

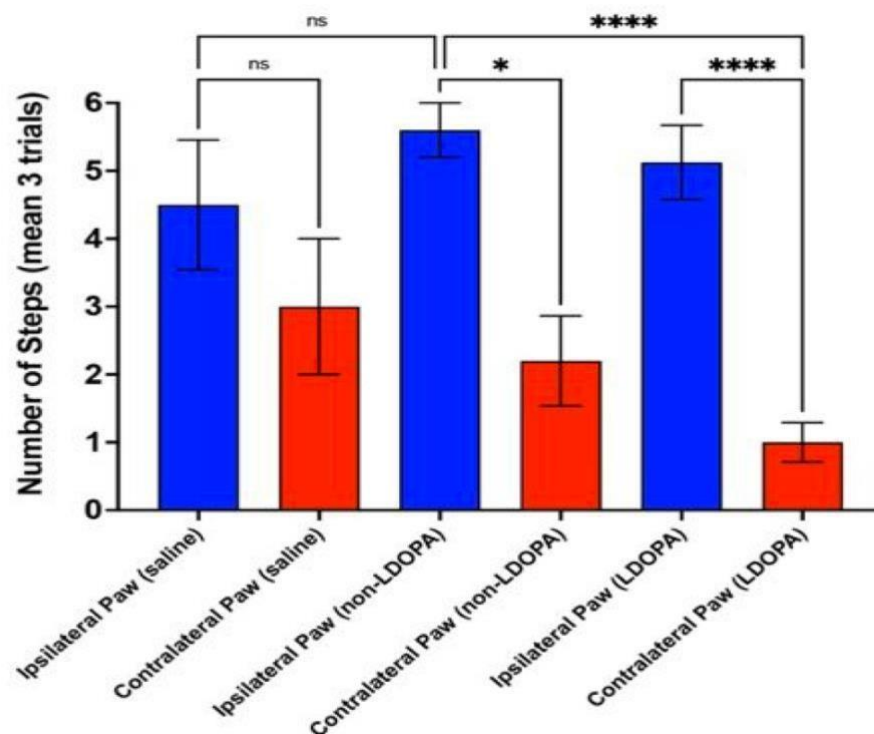


Figure 3. 5: Analysis of stepping test performance in unilaterally 6-OHDA lesioned rats (nicotine group). After 21 days of treatment with levodopa + benserazide rats were further tested using forelimb akinesia stepping test in absence of acute levodopa treatment. Saline (n=6) or primed 6-OHDA treated (n=24) rats produced greater level of limb asymmetry respectively. *P<0.05, ****P<0.0001, mean ± SEM.

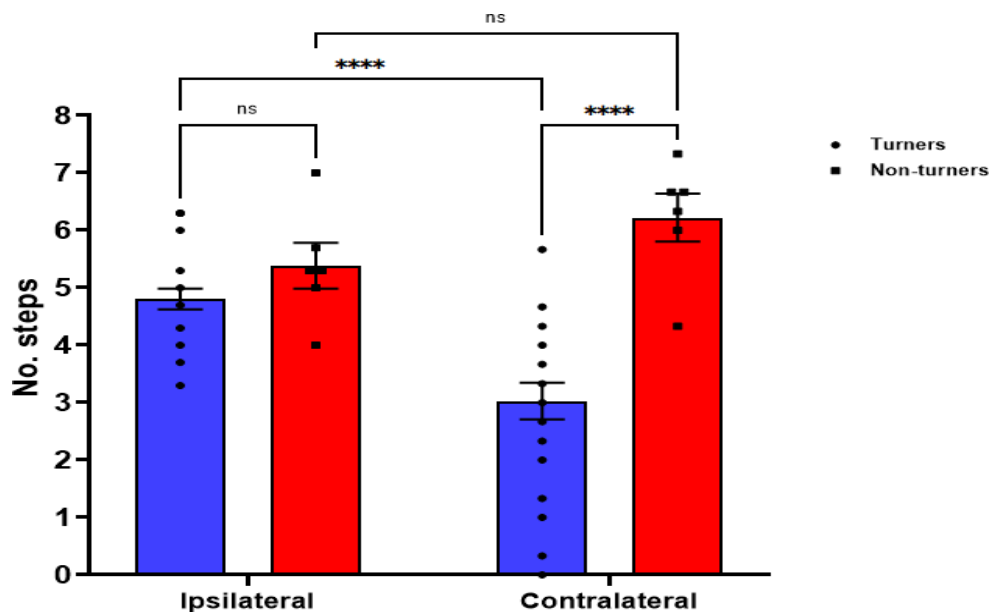


Figure 3. 6: Analysis of stepping test performance in unilaterally 6-OHDA lesioned rats (Varenicline group). Following 34 day of treatment with levodopa + benserazide along with levodopa rats were further tested using forelimb akinesia stepping test in absence of levodopa and saline treatment. Turner group rats produced greater level of limb asymmetry. ****P<0.0001, mean ± SEM.

3.3.4 Development of abnormal involuntary movements across 7 sessions

Rats that exhibited more than 6 rotations per minute post lesion were selected for chronic treatment over the course 21 days for nicotine group and 34 days for the varenicline group. The treatment consisted of acute daily administration of L-DOPA (8 mg/kg) in conjunction with benserazide (15mg/kg) for the nicotine group and of L-DOPA (8 mg/kg) with benserazide (15mg/kg) in combination of saline. Dyskinetic movements induced by this treatment regimen were then assessed in 7 sessions (twice a week) in nicotine group and once a week in varenicline group to monitor the progression of LID using a standardized rating scale, with observations made by three experimentally blinded observers to eliminate bias.

Figure 3.8 shows that chronic levodopa and benserazide treatment consistently induced AIMs across all sessions in the nicotine group, with peak dyskinesia occurring approximately

between 40 and 80 minutes after administration, as indicated by the sharp increase in AIMS scores. Analysis revealed a significant effect of time ($F(3.803, 295.0) = 45.49, p < 0.001$). AIMS scores then gradually declined but did not return to baseline within the 120 minute observation window, suggesting a sustained dyskinetic effect of the treatment.

In the varenicline group, levodopa induced AIMS peaked between 60 and 100 minutes. Similarly, analysis showed a significant effect of time ($F(4.371, 134.3) = 73.76, p < 0.001$).

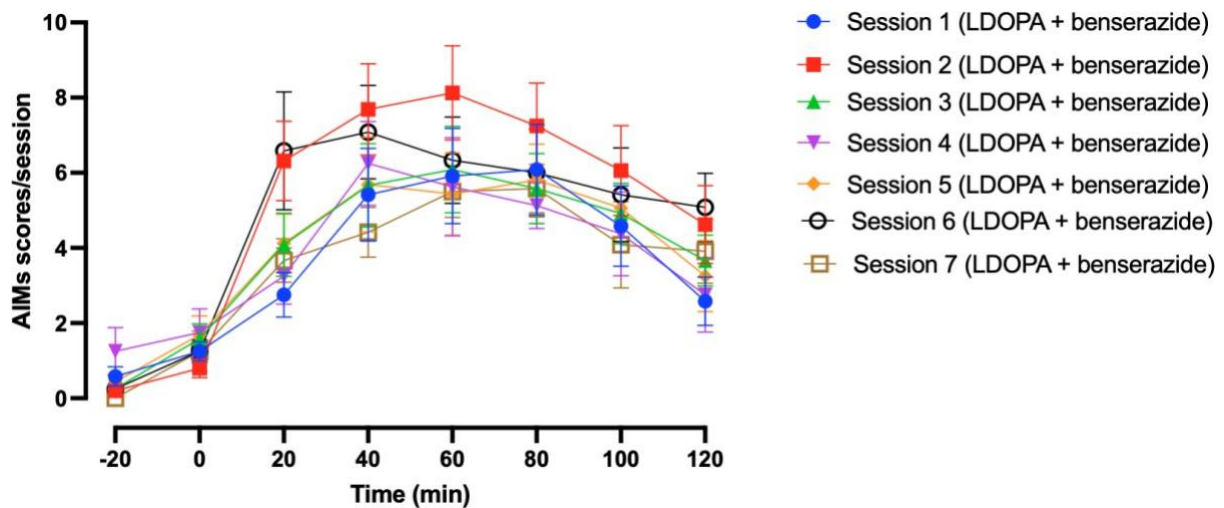


Figure 3. 7: Progression of levodopa induced AIMS over time in 6-OHDA lesioned rats (nicotine group). After establishing a baseline of rotational behavior exceeding six rotations per minute post 6-OHDA lesion, rats were subjected to chronic treatment with levodopa (8 mg/kg) in combination with benserazide (15 mg/kg) daily. Over three weeks, this regimen induced stable dyskinetic movements. The AIMS scores per session charted here reveal the onset and development of LID (n=12). The error bars represent the standard error of the mean (SEM).

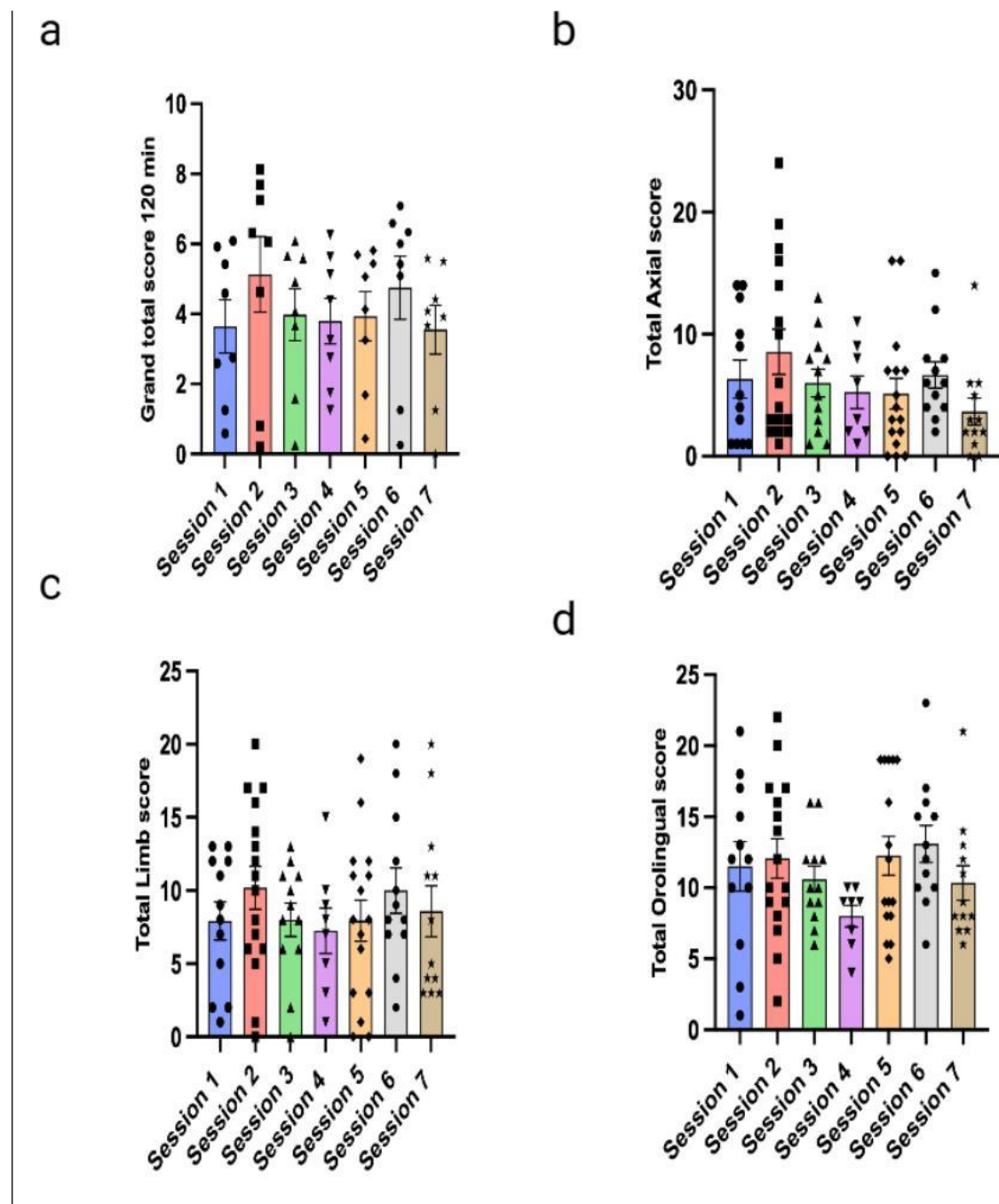


Figure 3. 8: Development of LID over chronic treatment with levodopa and benserazide across 7 sessions. The bar graph represents the progression of LID in rats receiving a daily dose of 8 mg/kg L-DOPA in combination with 15 mg/kg benserazide across seven sessions, conducted twice weekly over three weeks. Graph (a) shows the grand total score across 7 sessions, The effects of oral administration of levodopa and benserazide on axial, limb and orolingual components of AIMs over 120 min are summarized in panels b, c and d. The error bars represent the standard error of the mean (SEM).

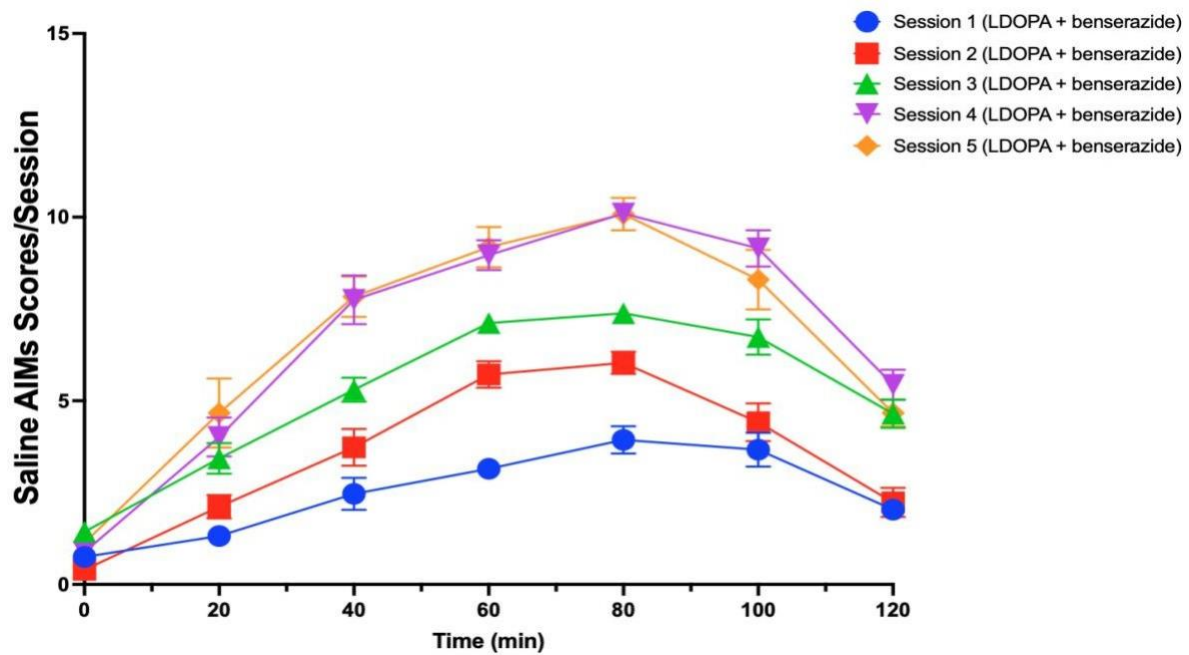


Figure 3. 9: Time course of AIMs development in 6-OHDA lesioned rats following levodopa and benserazide treatment. Levodopa and Benserazide administration in combination with saline induced AIMs over five sessions in lesioned rats ($n = 10$). Peak dyskinetic responses were consistently observed between 60 and 100 minutes post treatment. Statistical comparisons were performed using two-way ANOVA with Tukey's *post-hoc* test. Data are presented as mean $\pm$ SEM.

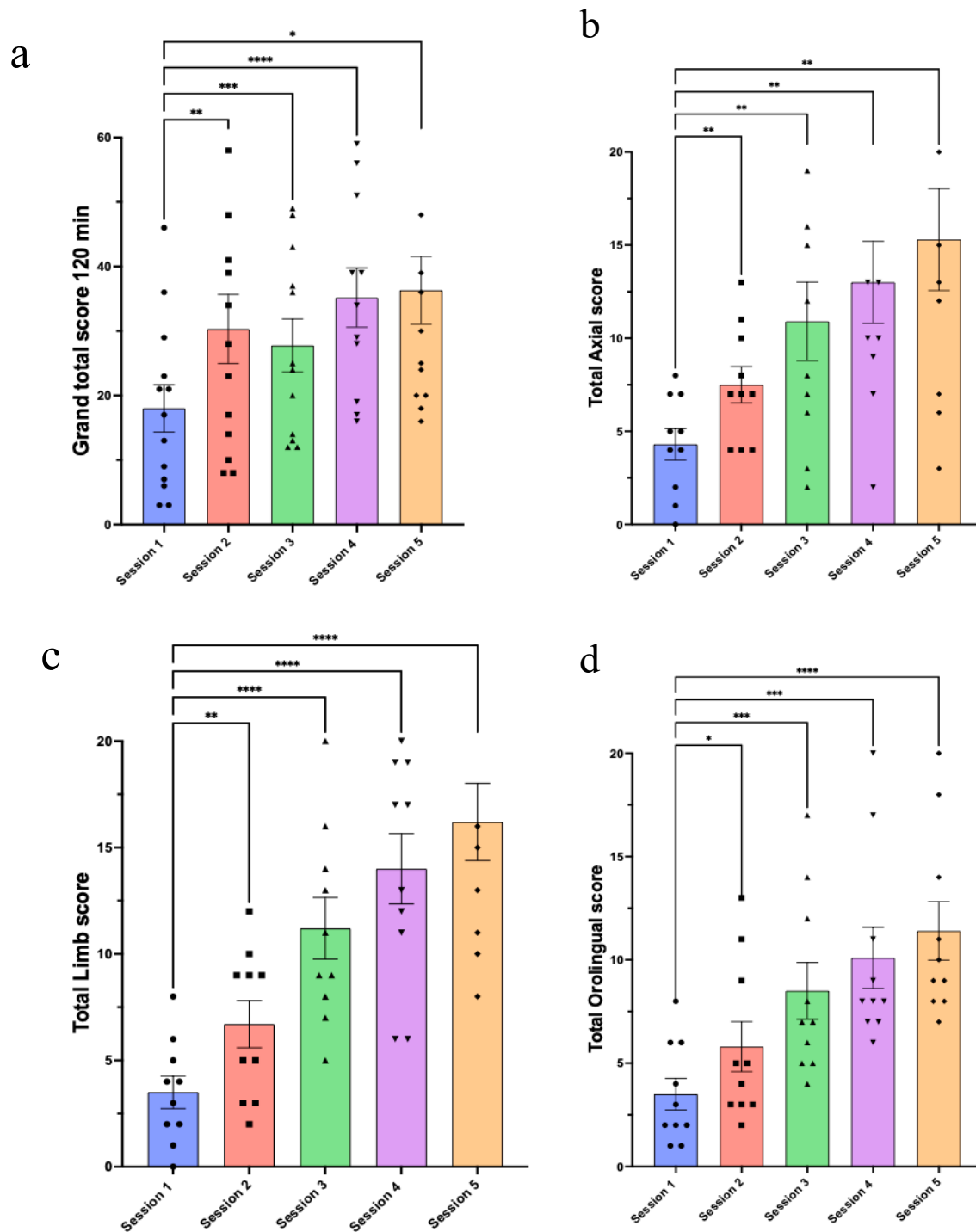


Figure 3.10: ALO AIMs progression in the varenicline treated group across five sessions.

Rats with unilateral 6-OHDA lesions received daily oral levodopa (8 mg/kg) and benserazide (15 mg/kg) alongside subcutaneous saline and were assessed weekly over a five week period. Panel (a) shows the cumulative AIMs scores across all sessions, while panels (b), (c), and (d) present axial, limb, and orolingual AIMs over the 120 minute post-treatment timeframe. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0010$. Results are shown as mean $\pm$ SEM.

3.3.5 Nicotine dose dependently reduces AIMS

The effects of nicotine bitartrate in doses ranging from 0.01 to 0.3 mg/kg were investigated on AIMS. The levodopa AIMS AUC was significantly reduced by all doses of nicotine bitartrate treatment [$F(2.975, 32.73) = 7.792$; $P=0.0005$] and time [$F(11,44) = 2.622$; $P=0.0115$]. Nicotine bitartrate dose dependently reduced levodopa induced ALO AIMS [nicotine dose x time interaction, $F(28,497)=1.512$, $P=0.0465$] (figure 3.7).

The AUC for levodopa induced AIMS over 120 min showed statistically significant AIMS (0.01 mg/kg, $P=0.0074$; 0.03 mg/kg, $P=0.0006$; 1.0 mg/kg, $P=0.0069$; 3.0 mg/kg $P=0.0120$; one-way ANOVA Dunnett's *post hoc* test). However, these doses also significantly reduced axial, limb and orolingual components by similar level (figure 3.8).

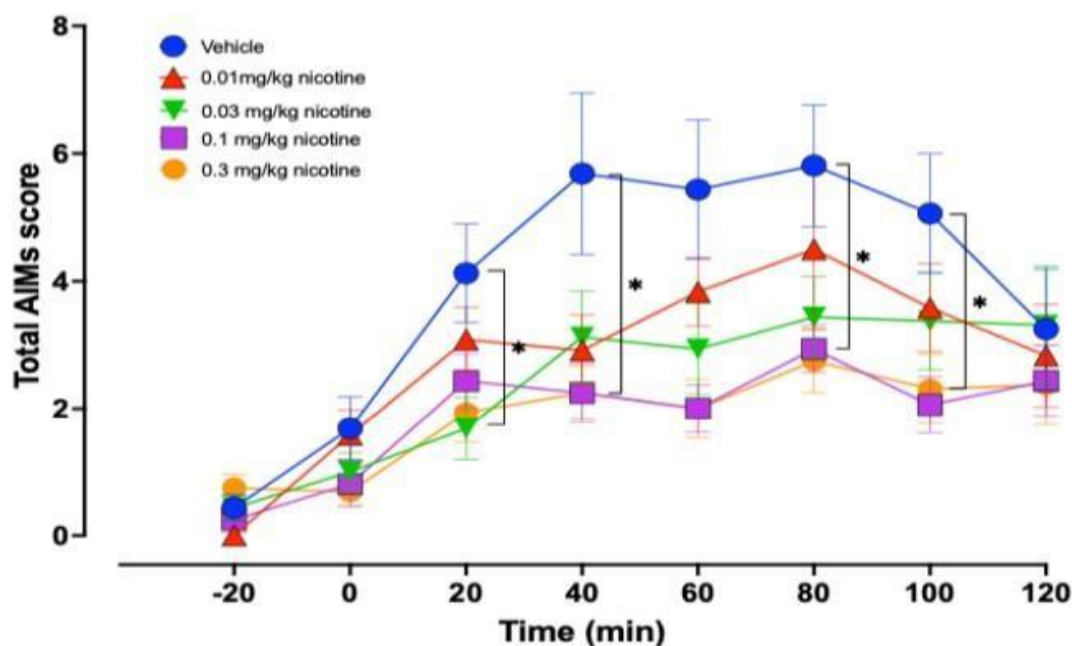


Figure 3. 11: Reduction in AIMS scores per session following nicotine treatment. The effects of subcutaneous administration of nicotine on unilaterally 6-OHDA lesioned AIMS in levodopa primed rats (n=12). Following 20 min post subcutaneous administration, nicotine dose dependently reduced peak total ALO AIMS between 20-80 min. Data are presented as mean $\pm$ standard error, and significance is considered when $p < 0.05$.

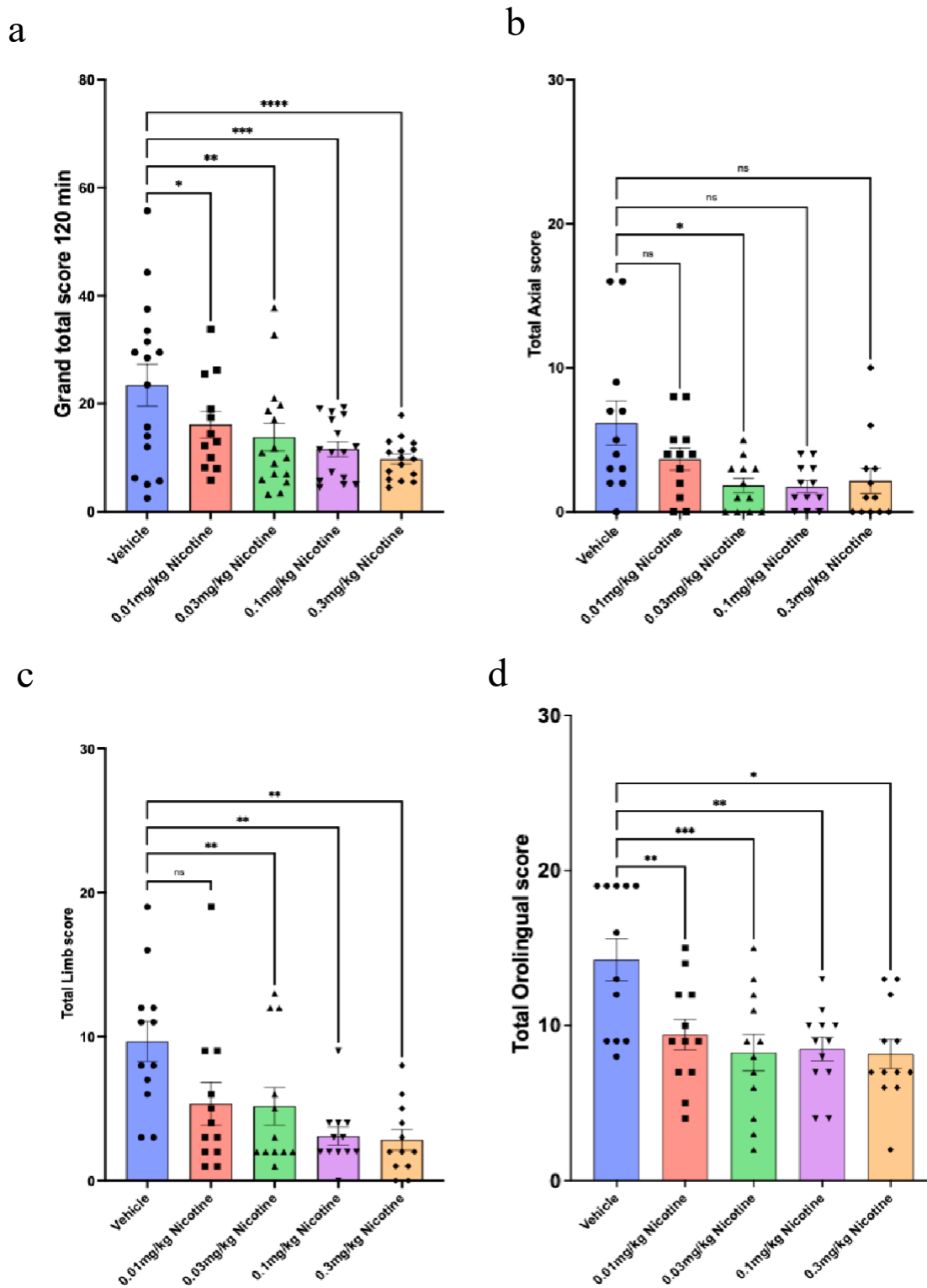


Figure 3. 12: The effects of administration of nicotine on 6-OHDA lesioned AIMs in levodopa primed rats. Following 20 min post subcutaneous administration, nicotine dose dependently reduced the grand total of ALO AIMs over the course of 120 min (a). The effects of nicotine on axial, limb and orolingual components of AIMs over 120 min are summarized in panels b, c and d. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, mean $\pm$ SEM, (n=12).

3.3.6 Varenicline dose dependently reduced AIMS

The effects of Varenicline in doses ranging from 0.1, 0.3 and 1 mg/kg were investigated on AIMS. The levodopa AIMS AUC was significantly reduced by all doses of varenicline treatment [$F(3, 60) = 7.726$; $P=0.0002$] and time [$F(3.965, 237.9) = 25.26$; $p < 0.0001$]. However, the dose $\times$ time interaction was not statistically significant [$F(21, 420) = 1.378$, $p = 0.1236$] (figure 3.9).

The AUC for levodopa induced AIMS over 120 min showed statistically significant AIMS (0.01 mg/kg, $P=0.0074$; 0.03 mg/kg, $P=0.0006$; 1.0 mg/kg, $P=0.0069$; 3.0 mg/kg $P=0.0120$; ` Moreover, these doses also significantly reduced axial, limb and orolingual components by similar level (figure 3.10)

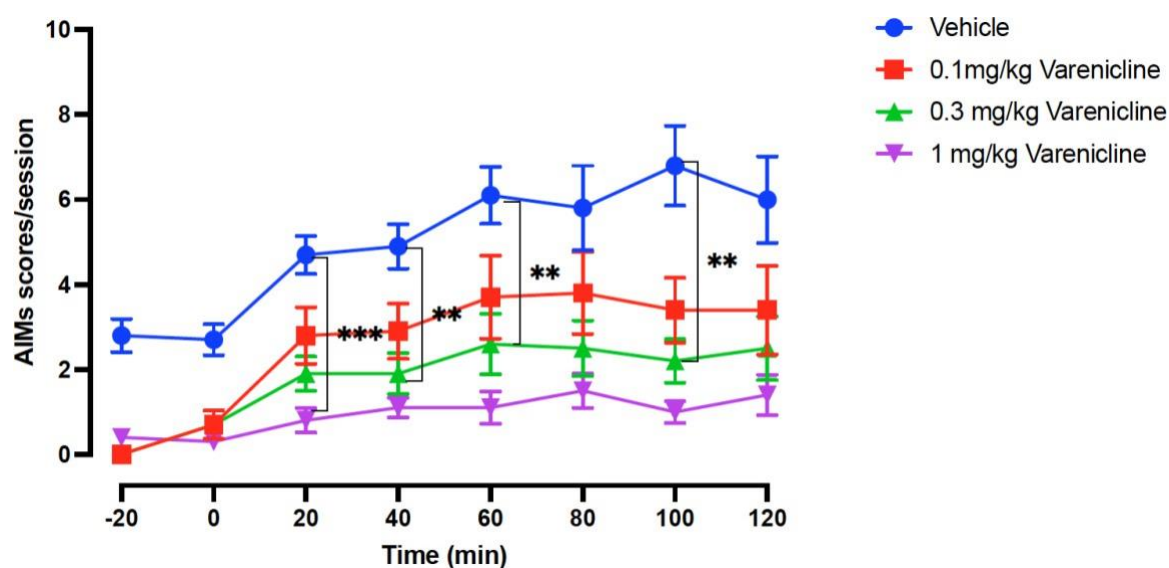


Figure 3. 13: Reduction in AIMS scores per session following varenicline treatment. The effects of subcutaneous administration of varenicline on unilaterally 6-OHDA lesioned AIMS in levodopa primed rats ($n=10$). Following 20 min post subcutaneous administration, varenicline dose dependently reduced peak total ALO AIMS between 20-100 min. Data are presented as mean $\pm$ standard deviation, and significance is considered when $p < 0.05$.

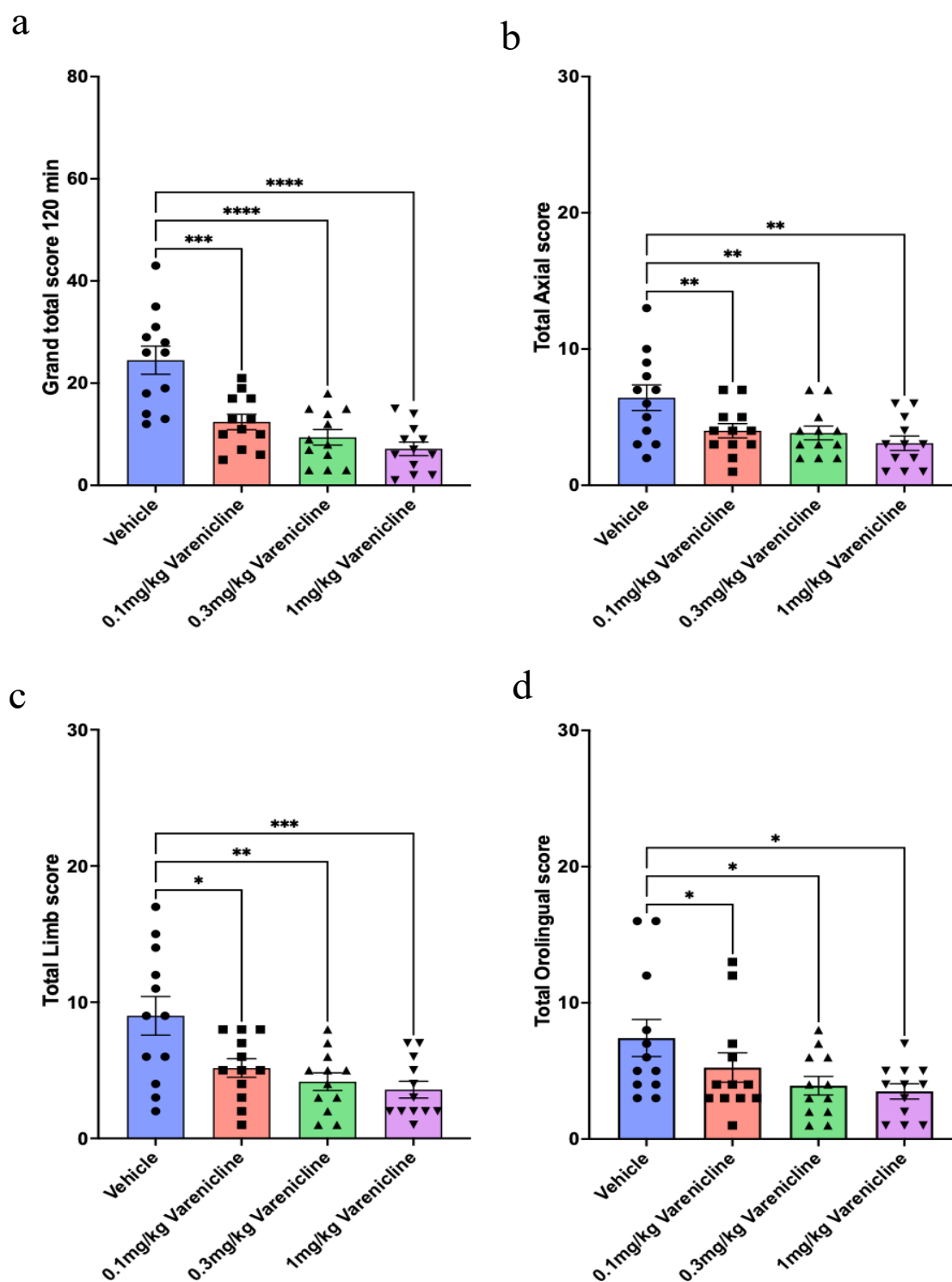


Figure 3. 14: The effects of administration of varenicline on 6-OHDA lesioned AIMs in levodopa primed rats. Following 20 min post-subcutaneous administration, varenicline dose dependently reduced the grand total of ALO AIMs over the course of 120 min (a). The effects of varenicline on axial, limb and orolingual components of AIMs over 120 min are summarized in panels b, c and d. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, mean $\pm$ SEM, (n=12).

3.3.7: 6-OHDA lesioning induces over 90% dopaminergic cell loss in the striatum and substantia nigra of nicotine group

3.3.7.1 Nicotine treated group

Immunohistochemical analysis of tyrosine hydroxylase expression was performed to assess dopaminergic neurodegeneration in the substantia nigra pars compacta and the loss of dopaminergic terminal density in the striatum following unilateral 6-hydroxydopamine lesioning. Unilateral 6-OHDA injection resulted in variable degrees of TH-positive neuronal loss in the SNpc as well as different levels of TH fiber density reduction in the striatum of nicotine group.

In animals that did not exhibit abnormal involuntary movements, TH immunoreactivity appeared largely preserved in both the SNpc and striatum, with minimal difference between hemispheres. In contrast, animals with moderate AIMs showed approximately 40–60% reduction in TH-positive neurons in the SNpc and a visible reduction in striatal TH fiber density. Animals that developed pronounced AIMs exhibited extensive loss of TH-positive neurons in the SNpc (greater than 90%) and a marked decrease in TH fiber density in the striatum. These observations are illustrated in the representative images below (figure 3.16 to 3.19).

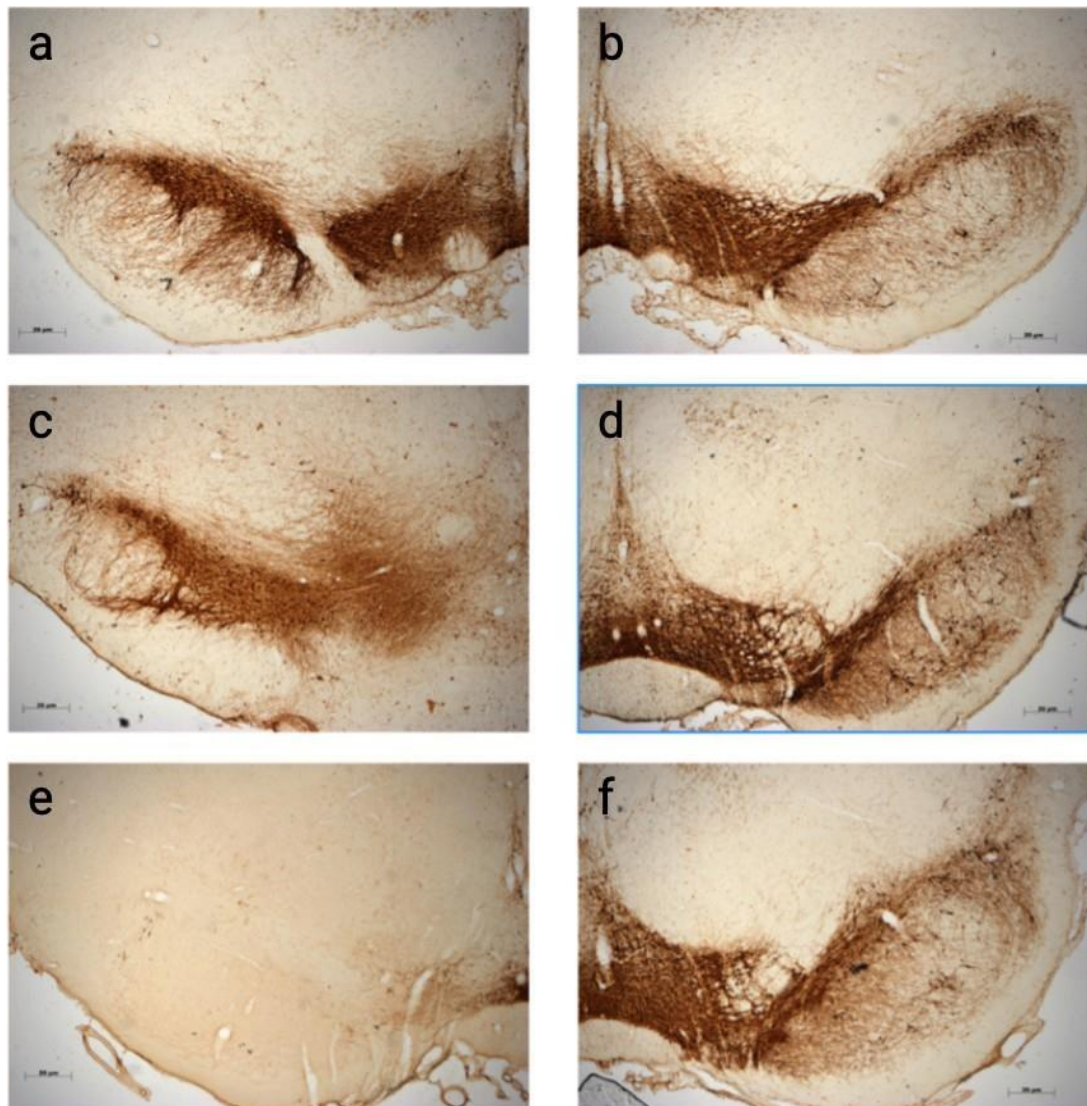


Figure 3. 15: TH immunostaining in the SNpc of animals treated with nicotine, comparing the non-lesioned and lesioned hemispheres. (a–b) TH-positive neurons in the lesioned (a) and non-lesioned (b) hemispheres of animals from the saline treated group (n = 6). (c–d) Lesioned (c) and non-lesioned (d) hemispheres from a 6-OHDA treated animal with partially developed AIMs. (e–f) Lesioned (e) and non-lesioned (f) hemispheres from a 6-OHDA treated animal with fully developed AIMs. Images were captured at 2.5× magnification. Scale bar = 20 μ m.

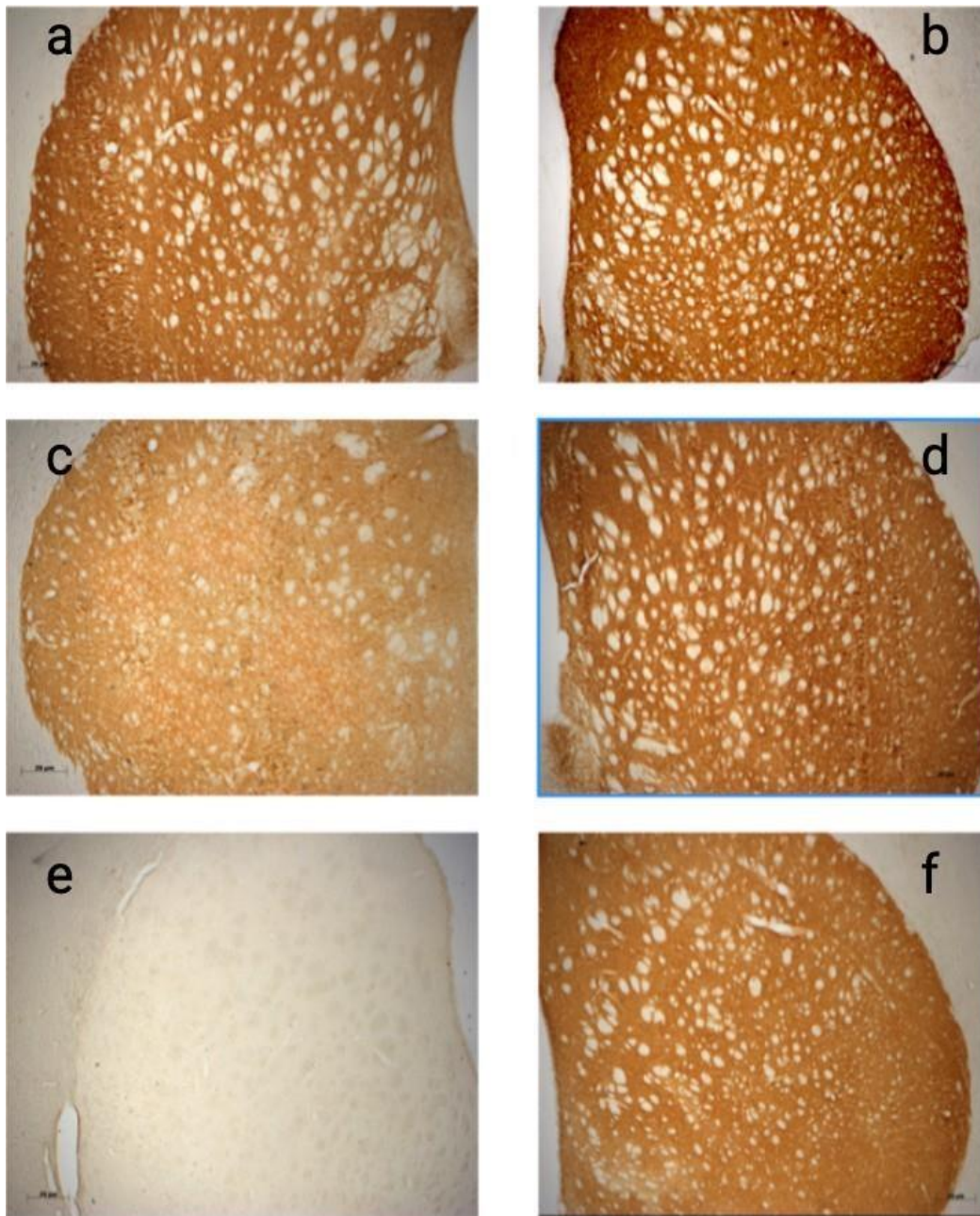


Figure 3. 16: TH immunostaining in the striatum of animals treated with nicotine, comparing the non-lesioned and lesioned hemispheres. (a–b) TH-positive fibers in the lesioned (a) and non-lesioned (b) striatum of an animal from the saline treated group (n = 6). (c–d) Lesioned (c) and non-lesioned (d) striatum from a 6-OHDA treated animal with partially developed AIMs. (e–f) Lesioned (e) and non-lesioned (f) striatum from a 6-OHDA treated animal with fully developed AIMs. Images were captured at 2.5× magnification. Scale bar = 20 μ m.

3.3.7.2 *Varenicline treated group*

In the varenicline treated group ($n = 10$), which received varenicline in combination with levodopa and benserazide following 6-OHDA lesioning, displayed a range of AIM severities. One rat showed no evidence of AIMs and retained TH immunoreactivity in the lesioned SNpc similar to the contralateral side (Figure 3.18a). One animal exhibited moderate AIMs, accompanied by an estimated 67% reduction in TH-positive neurons (Figure 3.18c). The remaining eight animals developed high AIM scores and showed marked dopaminergic cell loss in the lesioned SNpc, with over 90% reduction in TH immunoreactivity (Figure 3.18e). Striatal TH labelling followed a similar pattern. The rat without AIMs exhibited similar dopaminergic terminal density between hemispheres. In the moderately affected animal, TH fibre density was visibly reduced in the lesioned striatum. Animals with pronounced AIMs displayed near complete loss of striatal TH-positive fibers (Figure 3.19).

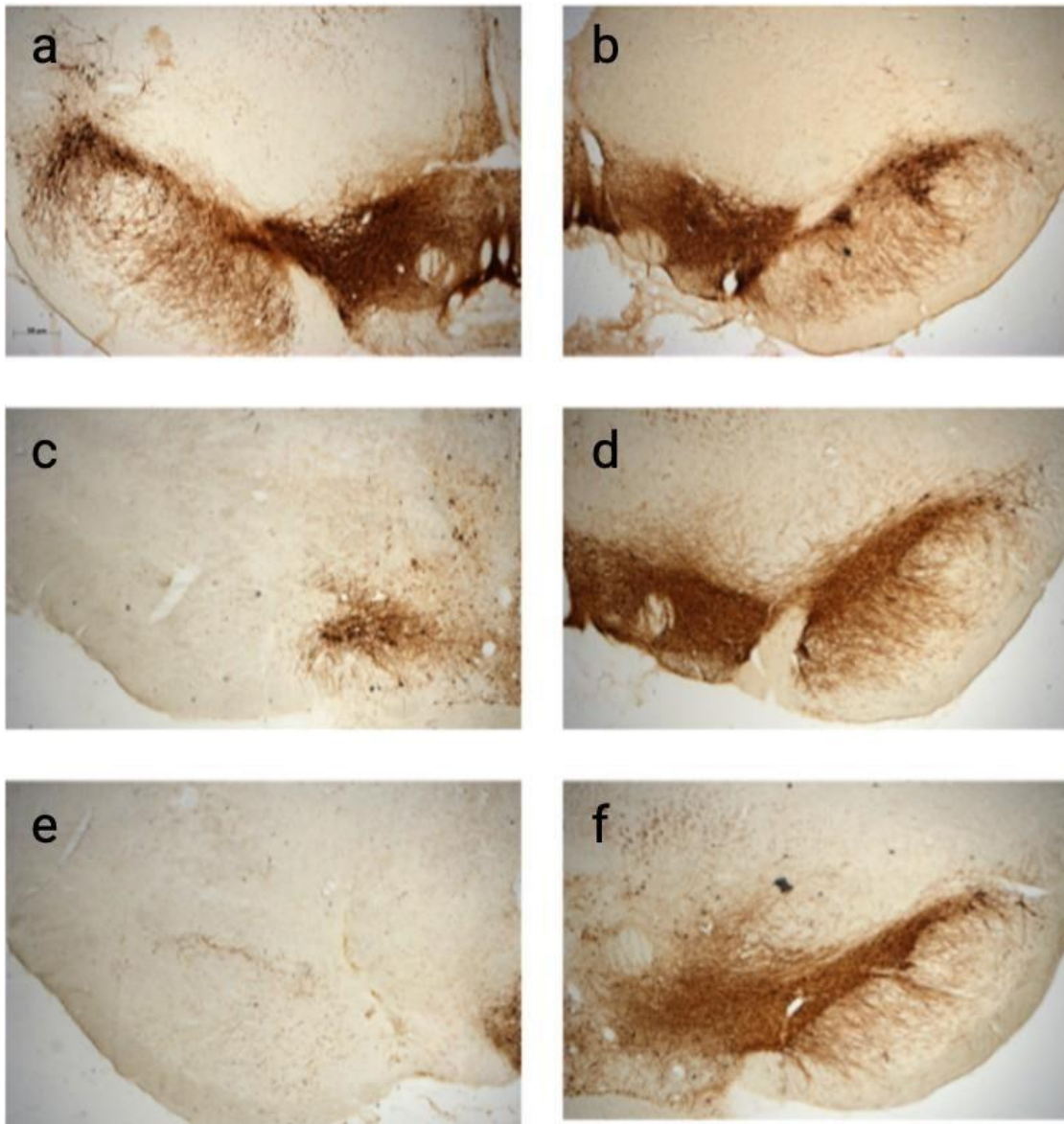


Figure 3. 17: TH immunostaining in the SNpc of the varenicline treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) TH-positive neurons in the lesioned (a) and non-lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) lesioned (c) and non-lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) lesioned (e) and non-lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 2.5× magnification. Scale bar = 20 μ m.

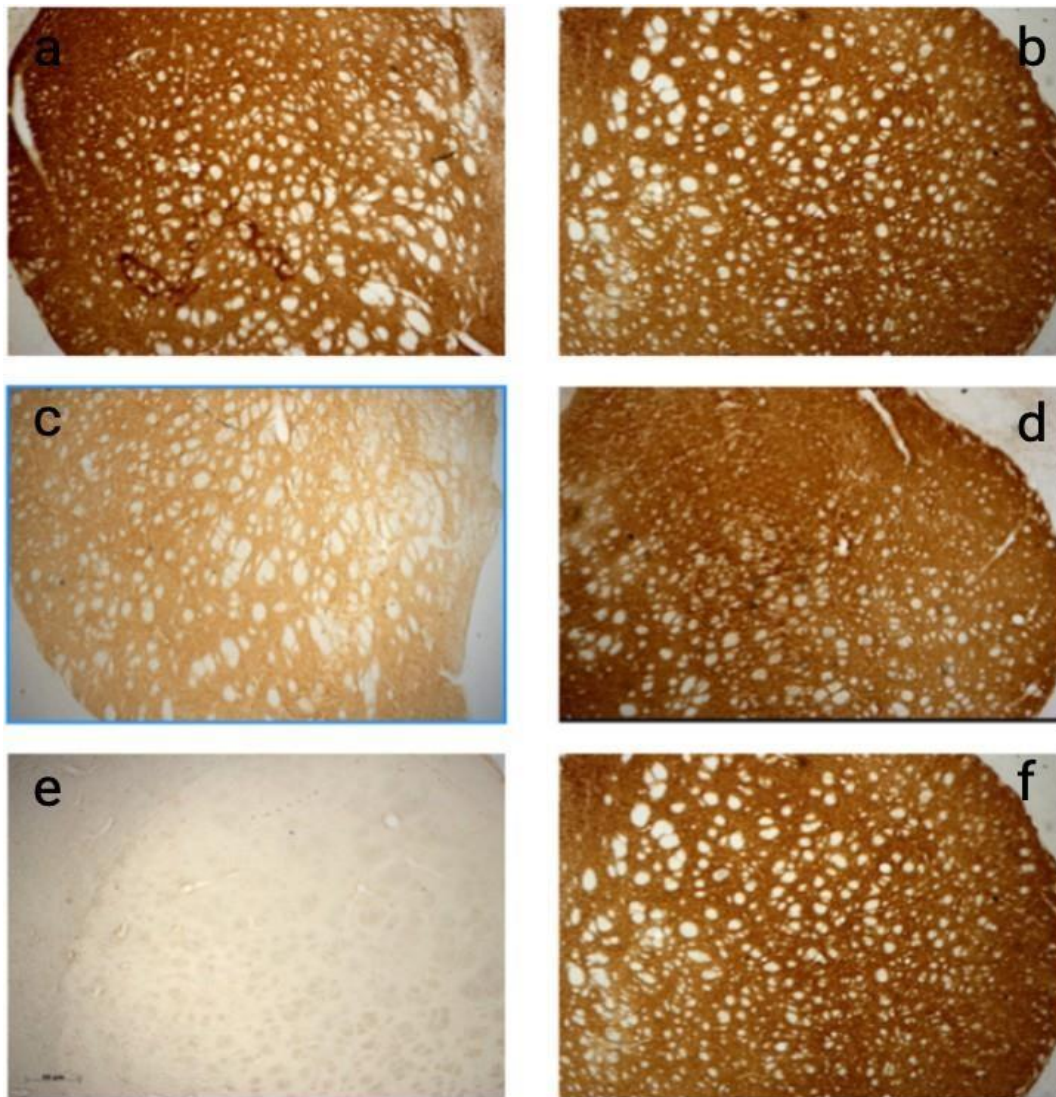


Figure 3.18: TH immunostaining in the striatum of the varenicline treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) TH-positive fibers in the lesioned (a) and non-lesioned (b) striatum of animals that did not develop AIMs. (c–d) Lesioned (c) and non-lesioned (d) striatum from animals with partially developed AIMs. (e–f) Lesioned (e) and non-lesioned (f) striatum from animals with fully developed AIMs. Images captured at 2.5× magnification. Scale bar = 20 μ m.

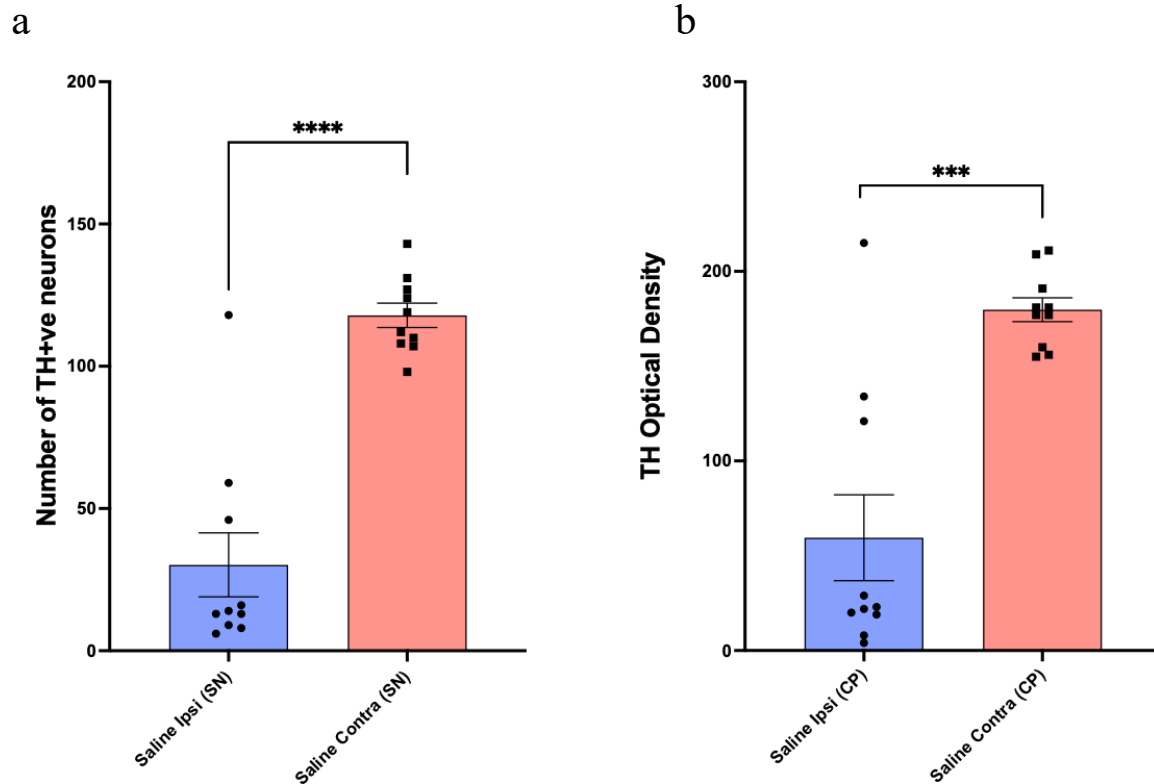


Figure 3.19: Quantification of TH positive neurons in the SNpc and TH optical density, expressed in arbitrary units, in the striatum of nicotine treated rats, comparing the control and 6-OHDA lesioned hemispheres in rats. (a) Significant reduction in the number of TH positive neurons in the lesioned substantia nigra of rats. (b) Corresponding decrease in TH optical density in the lesioned striatum compared to the control hemisphere. *** $P < 0.001$, **** $P < 0.0001$ (two-way ANOVA followed by Tukey's *post hoc* test).

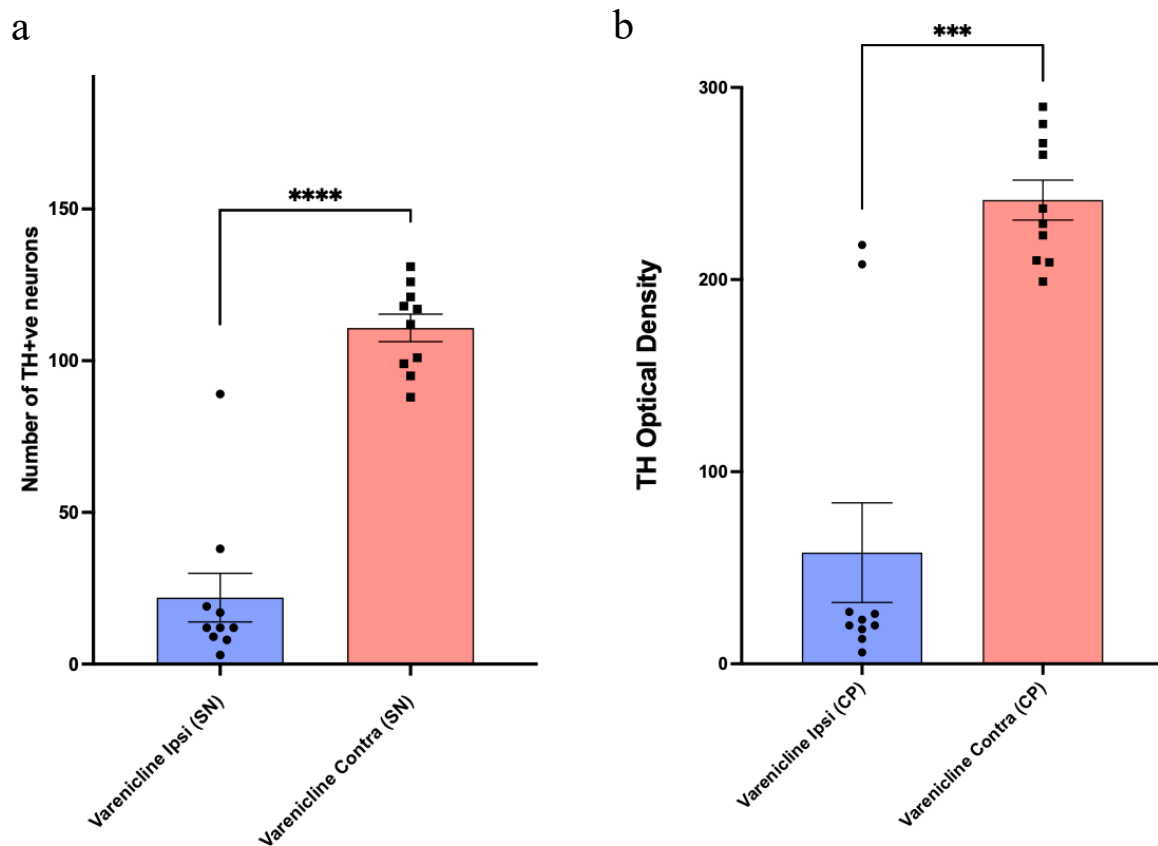


Figure 3.20: Quantification of TH positive neurons in the substantia nigra and TH optical density, expressed in arbitrary units, in the striatum of Varenicline treated rats, comparing the control and lesioned hemispheres in rats. (a) Significant reduction in the number of TH positive neurons in the lesioned substantia nigra of rats. (b) Corresponding decrease in TH optical density in the lesioned striatum compared to the control hemisphere. ** $P < 0.0001$ (two-way ANOVA followed by Tukey's *post hoc* test).**

3.4 Discussion

That nicotine may be beneficial in the treatment of Parkinson's disease was first suggested by early epidemiological studies demonstrating an inverse association between cigarette smoking and the incidence of PD in humans (Allam et al., 2004; Tanner, 2010). Subsequent preclinical studies reported that chronic nicotine administration, delivered via drinking water, significantly reduced levodopa induced dyskinesia and abnormal involuntary

movements in both rodent and primate models (Quik et al., 2007; Bordia et al., 2008). Importantly, similar effects have been demonstrated in mouse models. For example, Huang et al. (2011) showed that nicotine treatment markedly attenuated LID in parkinsonian mice, an effect mediated by $\beta 2$ containing nicotinic acetylcholine receptors, as confirmed using $\beta 2$ knockout mice. Consistent findings were also reported by Quik et al. (2013), who demonstrated that chronic nicotine exposure via drinking water significantly reduced LID severity in a mouse model. Together, these findings support the cross species efficacy of nicotine in reducing LID and suggest a key role of nAChRs in mediating its therapeutic effects.

Nicotine, the active constituent of tobacco and tobacco smoke, non-selectively targets a range of nicotinic acetylcholine receptor subtypes. Its action on receptors, particularly the $\alpha 4 \beta 2$ and $\alpha 7$ nAChR subtypes modulates a variety of neurochemical events. Nicotine, acting through these receptors, influences the activity of the nigrostriatal and corticostriatal pathways, enhancing dopaminergic and glutamatergic transmission while also exerting inhibitory effects on certain neural circuits (Lichtensteiger et al., 1982; Reavill, 1987; Janson & Fuxe, 1997; Meshul et al., 2002; Valjent et al., 2005; Quik et al., 2006; Xiao et al., 2009; Storey et al., 2016; Matityahu et al., 2022; Ye et al., 2024).

It has been shown that ACh through activation of either nAChR or blockade of muscarinic receptors reduces the expression of levodopa induced dyskinesia (Bordia et al., 2010; Shen et al., 2015; Conti et al., 2018; Bordia and Perez, 2019; Chambers et al., 2019; Brugnoli et al., 2020). However, blockade of muscarinic receptors is problematic due to side effects, such as confusion, hallucinations, dry mouth, memory disturbance, and urinary retention (Perry and Perry, 1995; Van Der Zee and Luiten, 1999; Hegde, 2006).

Previously, nicotine and varenicline administered chronically reduced levodopa induced dyskinesia (LID) in macaques and AIMs in rats (Bordia et al., 2012; Quik et al., 2013; Quik et al., 2014). In this chapter, it was examined the acute administration of various doses

of nicotine and varenicline on levodopa induced AIMs in two separate groups of rats that were unilaterally lesion and primed with daily doses of levodopa (8mg/kg + 15 mg/kg of benserazide). To do this, it was first ensured that the nigrostriatal tract in this model was extensively lesioned. Extensive lesioning is defined as at least more 70 % but typically 90-95% loss of dopaminergic neurons. This is set up and assessed according to the protocol of Ungerstedt (Ungerstedt and Arbuthnott, 1970; Ungerstedt, 1971) in which release of endogenous dopamine by (+) amphetamine from the intact hemisphere or super sensitivity of dopamine receptors by a dopamine agonist, apomorphine on the lesioned of a rat hemi parkinsonian cause the rotation of the animal towards or away from the lesioned hemisphere respectively. In this model, the number of rotations is directly proportional to the size of lesion. From the 24 rats used in this group, only 16 rats met the circling threshold of 6 turns/minute. To investigate the above results, lesion integrity was first confirmed behaviourally in 16 lesioned and 8 non-turners presumed to be partially lesioned animals. In the 16 turners, (+) amphetamine induced ipsilateral rotations and apomorphine induced contralateral turning robustly validated dopaminergic depletion, consistent with lesioning of the nigrostriatal pathway. The stepping test further evidenced significant motor asymmetry and forelimb akinesia, particularly in levodopa primed animals. Immunohistochemical analysis confirmed over 90% loss of tyrosine hydroxylase (TH) positive neurons in the substantia nigra and striatum of the fully lesioned hemisphere.

Subsequently, following levodopa priming, animals developed pronounced LID, with AIMs peaking 40–80 minutes post dose and persisting beyond 120 minutes. This AIMs was stable across 7 sessions which was required to complete the block randomisation, confirming a reliable and reproducible model for testing pharmacological treatments. The consistent induction of axial, limb, and orolingual AIMs provides further evidence of the model's reliability for evaluating dyskinetic behaviors.

Both nicotine and varenicline. Nicotine at doses of 0.01, 0.03, 0.1, and 0.3 mg/kg, dose dependently and significantly reduced axial, limb, and orolingual (ALO) AIMS, confirming a robust antidyskinetic activity when given acutely also particularly between 20–80 minutes post administration. Importantly, each dose significantly reduced axial, limb, and orolingual AIMS. These findings align with influential work by Quik et al. (2007), who showed that nicotine reduces dyskinesia in non-human primates and rodents. A similar finding was also observed with varenicline at doses of 0.1, 0.3, and 1 mg/kg. However, the extent AIMS reduction was less than nicotine in line with its partial agonistic property (Quik et al., 2007; Huang et al., 2011).

The action of nicotine is likely to be mediated through a whole host of nicotinic receptor subtypes. Mechanistically, the antidyskinetic effect of nicotine is thought to come from activation and subsequent desensitisation of $\beta 2$ containing nicotinic acetylcholine receptors particularly the $\alpha 4\beta 2$ and $\alpha 6\beta 2$ subtypes (Quik & Wonnacott, 2011; Millar & Gotti, 2009). The effects of varenicline is likely to point to activation of two main subtypes: $\alpha 4\beta 2$ as it has been designed to act as partial the $\alpha 4\beta 2$ agonist for smoking cessation and $\alpha 7$ receptors where it acts like full agonist. These receptors are heavily expressed in the striatum and on dopaminergic terminals. Additionally, studies in knockout models show that nicotine's effects are abolished in the absence of these subunits (Huang et al., 2011), supporting their critical involvement. The effects of chronic administration of varenicline through a partial agonist at $\alpha 4\beta 2$ and full agonist at $\alpha 7$ nAChRs in reducing dyskinesia has been demonstrated in rodents and primates (Zhang et al., 2013). In the current study varenicline dose-dependently and significantly decreased axial, limb, and orolingual AIMS.

The apparent differential effect between nicotine and varenicline may be also due to their distinct pharmacodynamics. While nicotine broadly activates and desensitises multiple

nAChR subtypes, varenicline's partial agonism allows for subtler receptor modulation. Notably, $\alpha 7$ nAChRs, abundantly expressed on glutamatergic cortical terminals, may contribute to its antidyskinetic effects by reducing excessive excitatory activity onto striatal neurons (Quik et al., 2012).

Considering potential clinical applications, varenicline's more favourable side effect profile compared to the peripheral and cognitive effects of muscarinic antagonists (Perry & Perry, 1995; Hegde, 2006) that positions it as a viable adjunct therapy for LID. Moreover, its efficacy at low doses and capacity to modulate multiple nAChR targets supports further clinical exploration.

It is also important to note that although the immunohistochemical analyses confirmed extensive dopaminergic cell loss in both nicotine and varenicline treated animals, behavioural improvements were still observed. This suggests that the antidyskinetic effects observed are not a result of structural neuroprotection but instead reflect functional neuromodulation. These observations indicate that the extent of dopaminergic degeneration alone does not fully account for AIMs severity, emphasising the importance of receptor plasticity and downstream basal ganglia mechanisms.

In summary, the findings of this study support the therapeutic potential of nicotine and varenicline in reducing levodopa induced AIMs and subtypes of AIMs in Parkinsonian rats. Despite profound nigrostriatal degeneration, both compounds were able to modulate motor output and reduce AIMs severity, likely through receptor neuromodulation involving $\alpha 4\beta 2$ and $\alpha 6\beta 2$ nAChRs. These results are in strong agreement with previous studies by Quik and provide additional support for the continued investigation of nicotinic agents as adjunctive treatments for managing motor complications in Parkinson's disease. Future studies may explore dose dependent effects, combination therapy with dopaminergic agents.

3.5 Conclusion

In conclusion, this study has provided two key sets of findings that contribute to the evolving understanding of LID and its management. Firstly, while the anti-dyskinetic effects of nicotinic agents are not novel, our findings reinforce and extend previous research by demonstrating that nicotine, when administered at very low doses and acutely, exhibits a dose dependent inhibition of abnormal involuntary movements. This contrasts with earlier studies where chronic administration of nicotine or $\alpha 7$ nAChR agonists was emphasized (Bordia et al., 2008; Zhang et al., 2015). The acute inhibitory effects observed in this study suggest that nicotine's action may be mediated through rapid modulation of nicotinic acetylcholine receptors, providing new insights into its potential for immediate symptom relief in AIMs. In the subsequent chapters the effects nicotine and varenicline on the AIMs priming and use of more selective nicotine agents will be investigated in AIMs reduction in order to better understand nicotine and more selective nicotinic may reduce AIMs.

Chapter 4

The effects of Nicotine and Varenicline
on Levodopa Priming of Involuntary
Movements in Unilaterally 6-OHDA
Lesioned Rats

4.1 Introduction

Building on the previous chapter's exploration of levodopa's therapeutic efficacy and its long-term complications, this chapter shifts focus to two pharmacological agents nicotine and varenicline as potential modulators of levodopa induced AIMs when administered during the critical period of AIMs development. While levodopa remains the gold standard in Parkinson's disease treatment, its chronic use often leads to LID. The emergence of LID is tightly linked to pulsatile dopaminergic stimulation and abnormal plasticity within the basal ganglia circuitry (Stocchi et al., 2005). Consequently, there is a pressing need for adjunctive therapies that not only maintain levodopa's motor benefits but also prevent or reduce the maladaptive neuroplasticity driving dyskinesia.

Findings across several experimental models now indicate nicotinic acetylcholine receptors as key modulators of striatal dopaminergic activity and synaptic plasticity. Among these, the $\alpha 4\beta 2$ and $\alpha 6\beta 2$ nAChR subtypes have attracted particular interest due to their expression on dopaminergic terminals and their involvement in dopamine release regulation within the nigrostriatal pathway. These receptor subtypes are known to undergo significant degeneration in PD, correlating with dopaminergic neuron loss and altered motor function (Gotti et al., 2006; Quik et al., 2008). Modulating these receptors through targeted agonists offers a promising therapeutic avenue for influencing both motor symptoms and levodopa related complications.

Nicotine, a non-selective full agonist at multiple nAChR subtypes, has consistently demonstrated antidyskinetic properties across various animal models of PD. Chronic nicotine exposure has been shown to reduce LID severity in both rodents and non-human primates (Quik et al., 2007; Bordia et al., 2008; Quik et al., 2013). Its therapeutic effects are thought to arise from both activation and subsequent desensitisation of $\beta 2$ containing nAChRs,

particularly $\alpha 4\beta 2$ and $\alpha 6\beta 2$ subtypes, leading to altered dopaminergic transmission and dampened maladaptive plasticity. Moreover, nicotine's ability to modulate glutamatergic and GABAergic inputs in the striatum may further contribute to its capacity to normalise basal ganglia output (Quirk & Wonnacott, 2011).

However, despite its efficacy, nicotine's short half-life, broad receptor profile, and addictive potential limit its translational utility. Chronic administration poses a risk of tolerance, receptor desensitisation, and off-target effects, making its clinical deployment in PD ethically and pharmacologically problematic (Calabresi et al., 2006; Millar & Gotti, 2009). These limitations have prompted investigation into safer, more selective alternatives that can engage nicotinic receptor targets without the drawbacks associated with nicotine.

Varenicline is one such candidate. Initially developed for smoking cessation, varenicline is a partial agonist at $\alpha 4\beta 2$ and a full agonist at $\alpha 7$ nAChRs, offering a more targeted approach with a reduced risk of dependency. By partially stimulating $\alpha 4\beta 2$ receptors, varenicline can maintain sufficient dopaminergic tone without overstimulation, potentially reducing the sensitisation of striatal circuits implicated in LID development. Concurrently, $\alpha 7$ receptor activation has been linked to anti-inflammatory and neuroprotective effects, which may support synaptic stability and resilience in the lesioned striatum (Bordia et al., 2012; Zhang et al., 2013). Varenicline's pharmacokinetic profile marked by a longer half-life, favourable absorption, and lower abuse liability, makes it a compelling candidate for adjunctive use during levodopa treatment.

Unlike previous studies that primarily evaluated the therapeutic effects of nicotine or varenicline after LID had already developed, this chapter focuses on their role as pre-dyskinetic intervention that is, their potential to prevent or reduce the onset of LID when co-administered during the levodopa priming phase. This is a critical distinction. By targeting early neurochemical events that precede the manifestation of dyskinesia, nicotine and varenicline

may exert disease modifying effects on maladaptive plasticity, rather than only suppressing symptoms after they emerge. Furthermore, evaluating these agents in parallel offers the opportunity to compare full versus partial receptor agonism, as well as differences in receptor subtype selectivity, and how these pharmacological properties influence dyskinesia vulnerability.

4.1.1 Aims and objectives

The chapter explores whether modulating nAChRs acutely during the sensitisation phase of levodopa treatment which is the priming phase for AIMs can effectively disrupt the cascade of molecular and circuit level changes that culminate in AIMs. This approach provides insight into the preventative potential of nicotinic agents in PD, extending beyond symptomatic relief and toward disease modifying strategies. Moreover, the differential effects observed between nicotine and varenicline may elucidate specific receptor mechanisms that contribute to the development or prevention of LID, thereby refining future pharmacological targets.

The investigation is grounded in the well-characterised 6-OHDA rat model of Parkinson's disease, which mimics the dopaminergic cell loss and striatal hypersensitivity associated with dyskinesia in human PD. Within this model, the chapter assesses whether early exposure to nicotinic agents alters the priming process itself, thereby mitigating or delaying the onset and severity of AIMs.

To evaluate this, AIMs is systematically measured using established behavioural criteria across axial, limb, and orolingual domains. The results are then compared to post priming data, enabling a direct assessment of whether the timing of nicotinic receptor modulation influences therapeutic outcome.

This approach also provides an opportunity to differentiate the pharmacological profiles of nicotine and varenicline. While both agents target $\alpha 4\beta 2$ and $\alpha 6\beta 2$ nicotinic acetylcholine

receptors subtypes implicated in modulating striatal dopaminergic release and corticostriatal transmission varenicline's additional activity as a full agonist at $\alpha 7$ receptors introduces the possibility of broader neuromodulatory effects. These may include anti-inflammatory signalling or neuroprotection, both of which could influence the development of dyskinesia beyond simple dopaminergic modulation.

In contrast to previous studies, which have largely focused on the treatment of established levodopa induced dyskinesia, this chapter adopts a preventative approach by investigating the effects of early nicotinic receptor modulation during the priming phase of levodopa administration. Nicotine and varenicline were co-administered from the onset of levodopa treatment to determine whether the development of dyskinesia could be attenuated before its full expression when is compared with saline treated group.

This approach allows evaluation of whether nicotinic interventions can modify the underlying processes driving dyskinesia, rather than simply reducing symptoms once they have developed. The findings demonstrate that early administration of nicotinic agents significantly attenuates the progression of dyskinesia without compromising the therapeutic efficacy of levodopa, suggesting a potential disease modifying strategy. These results further indicate that nicotinic receptor modulation may interfere with early neuroplastic and inflammatory changes associated with dyskinesia development.

4.2 Material and Methods

4.2.1 *Animals*

Female Sprague Dawley rats (n=30, Charles River UK, Harlow, Essex), each weighing between 250-300 grams, was grouped into three separate groups for the study. Animals were housed 3 per in standard plastic cages and maintained on a 12:12 hour light/dark cycle (lights on at 07:00, off at 19:00) in a temperature-controlled room (20 ± 1 °C). Food and water were available *ad libitum*.

4.2.2 *Unilateral 6-OHDA Lesions*

All surgical procedures, including acclimation, anesthesia, preparation of 6-OHDA, stereotaxic injections, post-operative care and analgesia, followed the protocol described in Chapter 2 (see Section 2.4 for full methodological details). After a one week acclimation period, rats received unilateral 6-OHDA lesions prepared in 0.1% L-ascorbic acid in 0.9% saline, as outlined in Chapter 2. To produce strong dopaminergic loss, two injections of 6-OHDA were delivered stereotaxically into the MFB using the same coordinates and injection parameters described previously. All animals recovered uneventfully, and no adverse effects were observed. The experimental timeline is shown in Figure 4.1.

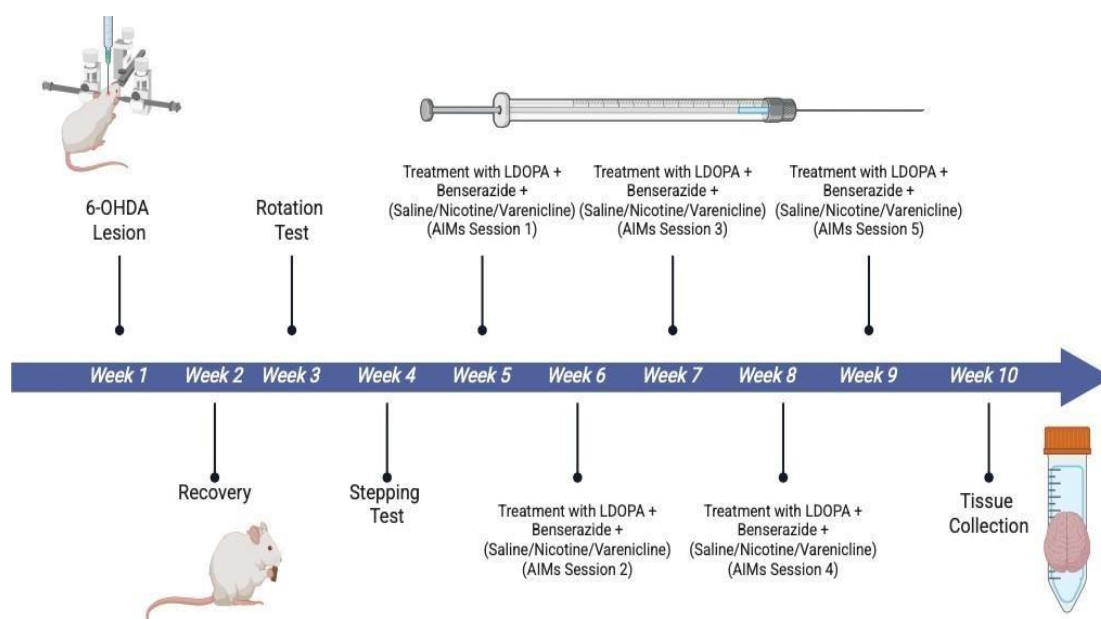


Figure 4. 1: Experimental timeline for the preparation of levodopa primed rats and the expression of abnormal involuntary movements. Rats were unilaterally lesioned with (6-OHDA; 8 μ g) and allowed to recover for two weeks. In week 3, lesion efficacy was confirmed using rotational behavior tests with amphetamine (5.0 mg/kg) and apomorphine (0.5 mg/kg). In week 4, all animals were further assessed using the stepping test. From week 5, all animals received daily injections of levodopa (8 mg/kg) and benserazide (15 mg/kg), in addition to their allocated group treatment (saline, nicotine or varenicline), for 34 days. AIMs scoring began after one week of levodopa treatment and continued once weekly until all animals were reliably primed. In week 10, animals were perfused, and brain tissue was collected for immunohistochemical analysis.

4.2.3 Assessment of 6-OHDA induced Motor Asymmetry (rotational behaviour)

Rotational behaviour was assessed following the method described in Chapter 2.6. Unilateral 6-OHDA lesioned rats were tested with amphetamine or apomorphine and animals displaying at least 6 ipsiversive or contraversive turns per minute were classified as fully lesioned. Based on these results, rats were allocated to the saline, nicotine or varenicline groups and subsequently underwent levodopa and benserazide priming.

4.2.4 Assessment of 6-OHDA induced Motor Asymmetry (stepping test)

Following priming, all lesioned animals (n = 29) were assessed for forelimb akinesia using the stepping test, as described in Chapter 2, Section 2.5.

4.2.5 Experimental Protocol (drug administration)

Following 6-OHDA lesioning, all rats (n = 29) were systematically allocated into three experimental groups by a non-blinded individual who also assigned treatment labels (Drug A, B, and C) but did not participate in the assessment of abnormal involuntary movements. Group A (control) received saline, Group B received nicotine (0.3 mg/kg, subcutaneously; Biotechne; UK), identified as the most effective dose based on previous study and Group C received varenicline (1 mg/kg, subcutaneously; Merck, UK), selected for its efficacy at a moderate dose. Both nicotine and varenicline were dissolved in saline with the pH adjusted between 7.0 and 7.4. In addition to their assigned treatments, all animals were administered 8 mg/kg L-DOPA (L-DOPA methyl ester; Tocris; Biotechne, UK) and 15 mg/kg benserazide (Benserazide-HCL; Tocris; Biotechne, UK), dissolved in 10 mL of distilled water containing 5% (w/v) sucrose, delivered orally for 34 consecutive days.

4.2.6 Levodopa Priming for Inducing AIMs

Abnormal involuntary movements were assessed once weekly for five weeks using the AIMs scoring procedures described in Chapter 2, section 2.7.

4.2.7 Experimental Protocol

Abnormal involuntary movements were assessed using a well-established behavioural rating scale, as described in detail in Chapter 2. Briefly, AIMs were scored across axial, limb, and

orolingual subtypes and individual subtype scores were either analysed separately or combined to generate an overall dyskinesia score (Lundblad et al., 2002). When comparing overall dyskinesia over the 120 minute observation period, the area under the curve (AUC) was calculated to quantify total AIMs response to levodopa.

The levodopa primed control group receiving saline during the priming phase was common to both this chapter and Chapter 3. Data from this group were used as the reference condition to enable direct comparison between nicotine and varenicline treated animals under equivalent primed conditions.

4.2.8 Drugs

Levodopa (L-DOPA methyl ester; 8 mg/kg; Tocris; Biotechnie, UK) and benserazide (Benserazide - HCL; Tocris; Biotechnie, UK) dissolved in 5% w/v sucrose in deionised water and they were administered orally at the time of which was considered 0 mins.

Nicotine [(-)-nicotine ditrate] obtained from (Biotechnie; UK), was dissolved in sterile saline and the pH of the final concentration was adjusted to ~pH 7.0 by dropwise addition of NaOH. Nicotine was administered subcutaneously at dose of 0.3 mg/kg 20 minutes following levodopa and benserazide administration.

Varenicline [Varenicline Tartrate] obtained from (Merck; UK), was dissolved in saline it was adjusted to ~pH 7.0 and administered at dose of 1 mg/kg subcutaneously 20 minutes following levodopa/benserazide administration.

4.2.9 Histological Analysis

Histological processing and immunohistochemistry followed the procedures detailed in Chapter 2. TH staining was performed using the same dilutions described in Section 3.2.8. GFAP and IBA1 staining were carried out using primary antibodies at 1:5000 (GFAP) and 1:5000 (IBA1), followed by standard secondary labelling and DAB development. Quantification of TH, GFAP, and IBA1 (optical density, area fraction and cell counts) was performed in ImageJ, and statistical analyses followed the methods outlined in Chapter 2.

4.2.10 Statistical Analysis

Statistical analyses followed the procedures outlined in Section 3.2.9.

4.3 Results

4.3.1 *Amphetamine induced ipsiversive rotation test*

As previously shown for the saline treated group in Chapter 3 (Section 3.2.3), administration of (+)-amphetamine (5 mg/kg, i.p.) produced a significant increase in ipsilateral rotations, reflecting the same pattern of dopaminergic lesioning observed in the present cohort. Consistent with these findings, a subset of animals ($n = 16$) were classified as turners (see Graph 3.3).

4.3.2 *Apomorphine induced contravasive rotation test*

As shown in the saline treated group in Chapter 3 (Section 3.3.2), apomorphine (0.5 mg/kg, s.c.) produced a robust contralateral rotational response. Consistent with this, a similar pattern was observed in the present cohort [$F(3.537, 95.50) = 18.13, p < 0.0001$], confirming successful lesioning (figure 3.5).

4.3.3 *Adjusted stepping test*

As previously demonstrated in the saline treated group in Chapter 3 (Section 3.3.3), the stepping test revealed a marked impairment in contralateral forelimb use following 6-OHDA lesioning. Consistent with these findings, all lesioned animals ($n = 29$) in the present cohort showed a significant reduction in contralateral stepping, with a highly significant difference between the turner ($n = 16$) and non-turner ($n = 13$) groups ($****p \leq 0.0001$); (figure 3.7).

4.3.4 Levodopa + Benserazide AIMS Sessions (ALO development)

All 6-OHDA treated rats ($n = 29$) were divided into three groups: a saline treated group ($n = 10$), a nicotine treated group ($n = 9$), and a varenicline treated group ($n = 10$). All animals received a daily oral dose of 8 mg/kg levodopa and 15 mg/kg benserazide. In addition, the saline group received 0.3 mL of saline (subcutaneously), the nicotine group received 0.3 mg/kg nicotine (subcutaneously), and the varenicline group received 1 mg/kg varenicline (subcutaneously) for 34 consecutive days. Dyskinetic movements induced by this treatment regimen were then assessed in 5 sessions (once a week) to monitor the progression of LID using a standardized rating scale as described in chapter 2, with observations made by three experimentally blinded observers.

4.3.4.1 Development of ALO Scores (Saline treated group)

As previously demonstrated in the saline treated group in Chapter 3 (Graph 3.10), acute administration of levodopa (8 mg/kg) and benserazide (15 mg/kg) with saline reliably induced abnormal involuntary movements (AIMs) across all assessment sessions. Consistent with these findings, peak dyskinesia in the present cohort ($n = 10$) was observed between 40 and 100 minutes post-treatment, with AIMs scores remaining elevated throughout the 120 minute observation period (Graph 3.10). Statistical analysis confirmed a significant main effect of time [$F(3.879, 174.6) = 168.7, p < 0.0001$].

The progression of dyskinesia across sessions was further reflected in the analysis of AIMs subtypes (Figure 3.11). A one-way ANOVA revealed a significant increase in global AIMs AUC over the 120-minute assessment period compared to session 1 [$F(1.204, 13.54) = 6.993$], with Dunnett's post hoc test confirming significant elevations in all subsequent sessions.

A similar pattern was observed across individual AIMs subtypes. Axial AIMs showed a significant effect of session [$F(1.529, 13.76) = 20.21$], with scores elevated in all sessions relative to session 1. Limb AIMs demonstrated a highly significant session effect [$F(2.379, 21.41) = 65.58$], with consistent increases across sessions. Orolingual AIMs also showed a robust increase over time [$F(1.727, 15.54) = 51.60$], with all subsequent sessions significantly elevated compared to baseline (figure 3.11).

4.3.4.2 Development of ALO Scores (Nicotine)

In the nicotine treated group ($n = 9$), co-administration of levodopa (8 mg/kg, oral) and benserazide (15 mg/kg, oral) with subcutaneous nicotine (0.3 mg/kg) led to the development of levodopa induced AIMs, but with significantly reduced abnormal involuntary movements compared to the saline treated group across all five assessment sessions. Although peak dyskinesia consistently occurred between 40 and 100 minutes post treatment, as reflected by elevated AIMs scores, the overall severity was notably reduced. Statistical analysis revealed a significant main effect of time [$F(6, 315) = 64.49, p < 0.0001$]. AIMs scores gradually declined following the peak but did not return to baseline within the 120 minute observation period (figure 4.2).

A one-way ANOVA was performed to assess the effects of nicotine (0.3 mg/kg) on the progression of AIMs across sessions. Although a main effect of session was observed for global AIMs, $F(2.374, 18.99) = 6.055$, *post hoc* Dunnett's comparisons did not reveal any statistically significant differences between sessions relative to Session 1 (all $P > 0.05$) (Figure 4.3 panel a). For axial AIMs, a main effect of session was detected, $F(2.894, 23.15) = 4.561$; however, *post hoc* analysis indicated that none of the sessions differed significantly from baseline (all $P > 0.05$), despite a general upward trend across sessions (Figure 4.3 panel b). Similarly, for limb AIMs, a main effect of session was observed, $F(2.266, 18.13) = 2.695$, but *post hoc* comparisons did not identify any statistically significant differences between sessions and Session 1 (all $P > 0.05$) (Figure 4.3 panel c). For orolingual AIMs, a main effect of session was observed, $F(2.996, 23.97) = 1.818$. However, *post hoc* Dunnett's comparisons did not reveal any statistically significant differences between sessions and Session 1 (all $P > 0.05$), despite a tendency towards increased scores at later time points (Figure 4.3 panel d).

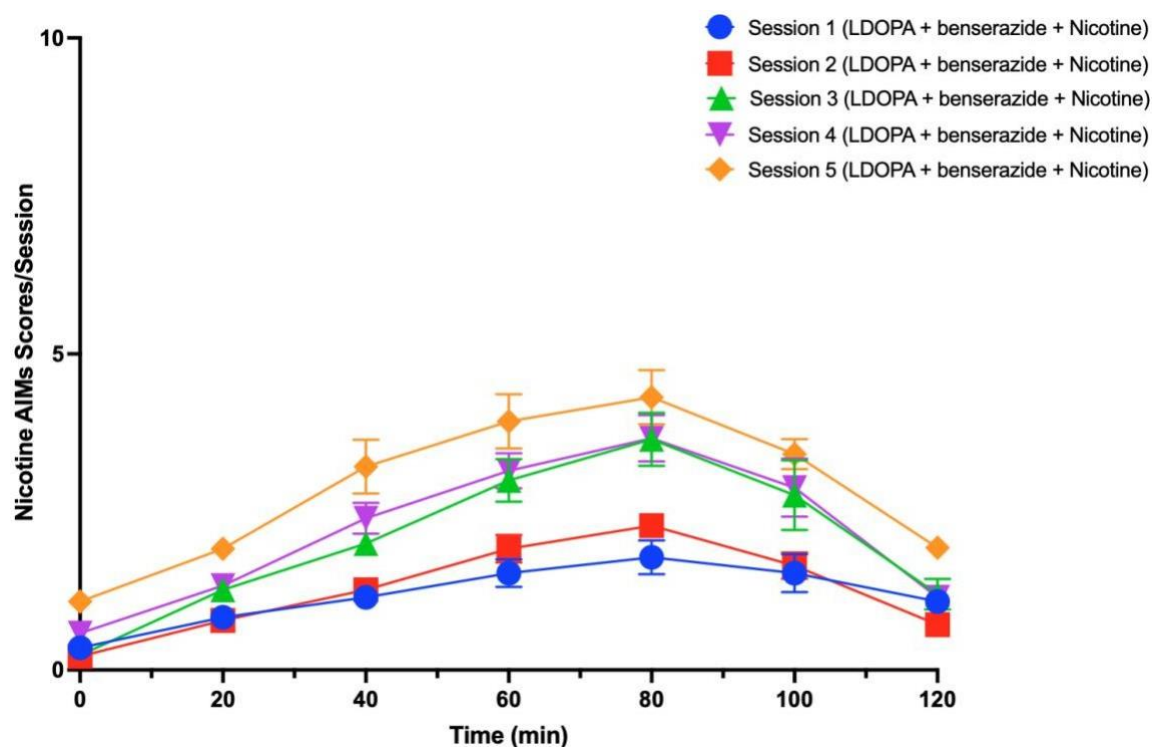


Figure 4. 2: Progression of AIMs expression following levodopa, benserazide and nicotine treatment in 6-OHDA lesioned rats. Across all five assessment sessions, co-administration of levodopa (8 mg/kg) and benserazide (15 mg/kg) with subcutaneous nicotine (0.3 mg/kg) induced AIMs in 6-OHDA lesioned rats ($n = 9$), with peak dyskinesia consistently occurring between 40 and 100 minutes post-injection. Data is analysed using two-way ANOVA followed by Tukey's post-hoc test. Error bars represent the standard error of the mean (SEM).

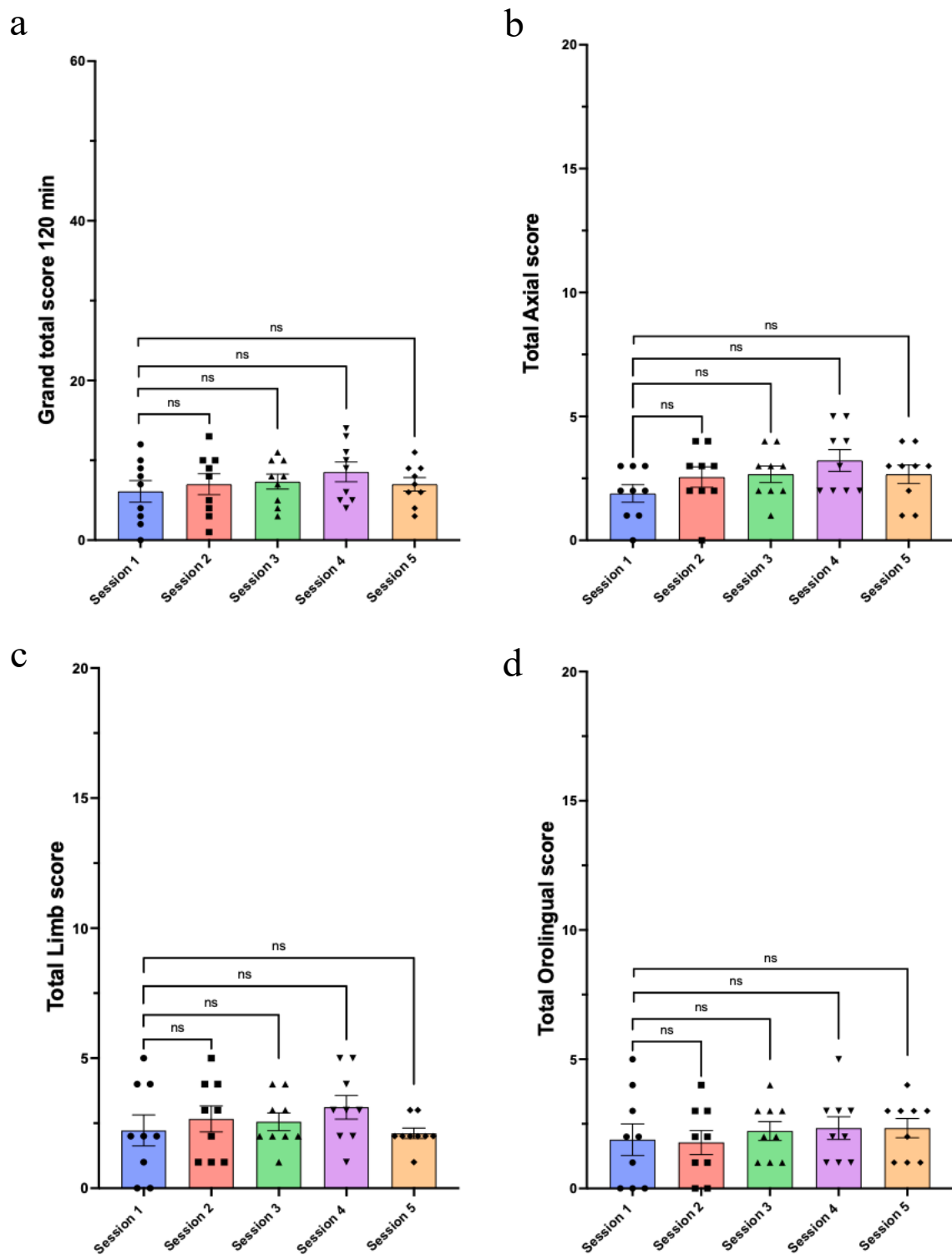


Figure 4. 3: Development of ALO in the nicotine treated group over five assessment sessions. 6-OHDA lesioned rats treated with daily oral levodopa (8 mg/kg) and benserazide (15 mg/kg), in combination with subcutaneous nicotine (0.3 mg/kg), were evaluated once per week for five weeks. Graph (a) illustrates the total ALOs scores summed across sessions, while graphs (b), (c), and (d) display axial, limb, and orolingual subtypes over the 120 minute post treatment period. Data are expressed as mean $\pm$ SEM.

4.3.4.3 Development of ALO Scores (Varenicline)

In rats treated with varenicline ($n = 10$), the combination of levodopa, benserazide, and subcutaneous varenicline (1 mg/kg) also resulted in the development of levodopa induced AIMs. However, similar to the nicotine group, the severity of AIMs was significantly decreased in comparison with the saline treated controls across all five sessions. Dyskinetic activity peaked between 60 and 100 minutes post-administration, and although scores declined thereafter, they remained elevated compared to baseline. A significant main effect of time was observed [$F(6, 315) = 88.03, p < 0.0001$], (figure 4.5).

To evaluate the impact of varenicline (1 mg/kg) on AIM development, a one-way ANOVA was conducted for each subtype. A main effect of session was observed for global AIMs, $F(1.911, 17.20) = 5.738$; however, *post hoc* Dunnett's comparisons did not reveal any statistically significant differences between sessions and Session 1 (all $P > 0.05$), although a general increase in scores across sessions was observed (Figure 4.6 panel a). The development of axial AIMs also showed a main effect of session, $F(2.550, 22.95) = 10.69$. Despite an apparent increase in scores over time, *post hoc* analysis indicated that none of the sessions differed significantly from Session 1 (all $P > 0.05$) (Figure 4.6 panel b). For limb AIMs, a main effect of session was detected, $F(2.074, 18.66) = 1.206$; however, *post hoc* comparisons did not identify any statistically significant differences between sessions and Session 1 (all $P > 0.05$), despite a trend towards increased scores across sessions (Figure 4.6 panel c). In contrast, orolingual AIMs showed a weaker effect of session, $F(2.554, 22.98) = 0.8928$. *Post hoc* Dunnett's comparisons did not reveal any statistically significant differences between sessions and Session 1 (all $P > 0.05$), although variability across sessions was observed (Figure 4.6 panel d).

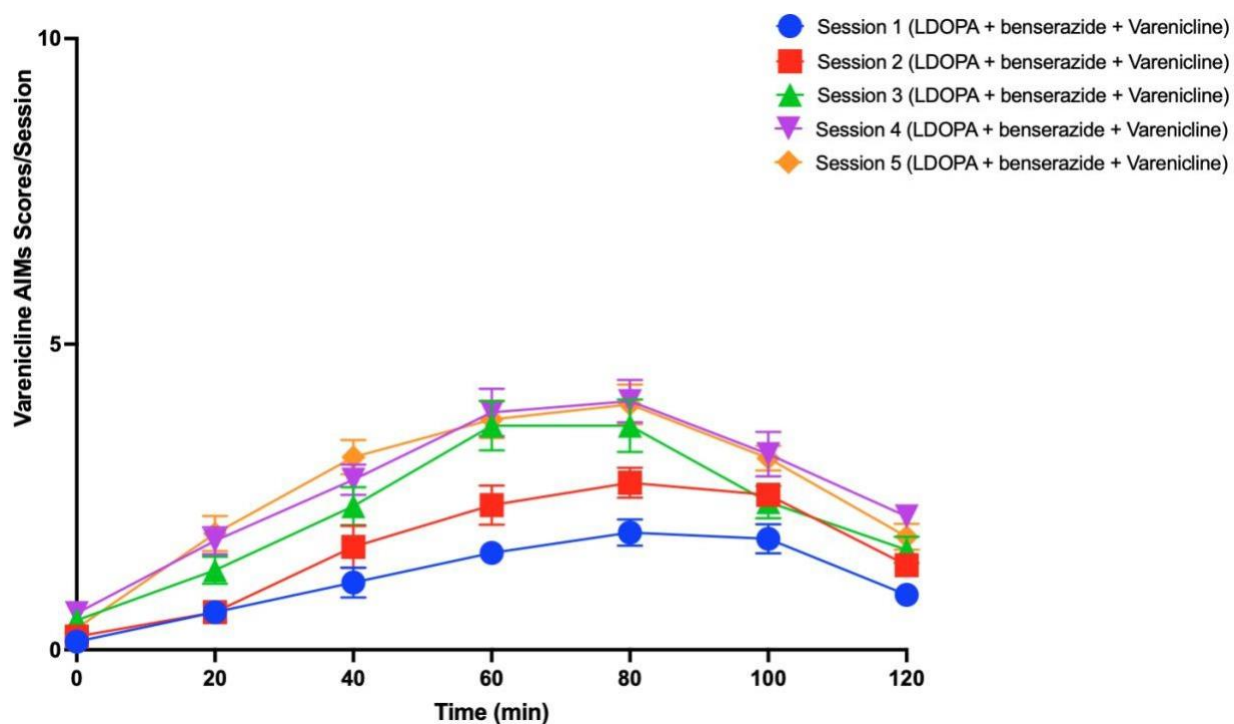


Figure 4. 4: Time course of AIMs development in 6-OHDA lesioned rats following varenicline treatment. Levodopa and benserazide administration in combination with varenicline (1 mg/kg, s.c.) induced AIMs over five sessions in lesioned rats ($n = 10$). Peak dyskinetic responses were consistently observed between 60 and 100 minutes post-treatment. Statistical comparisons were performed using two-way ANOVA with Tukey's post-hoc test. Data are presented as mean $\pm$ SEM.

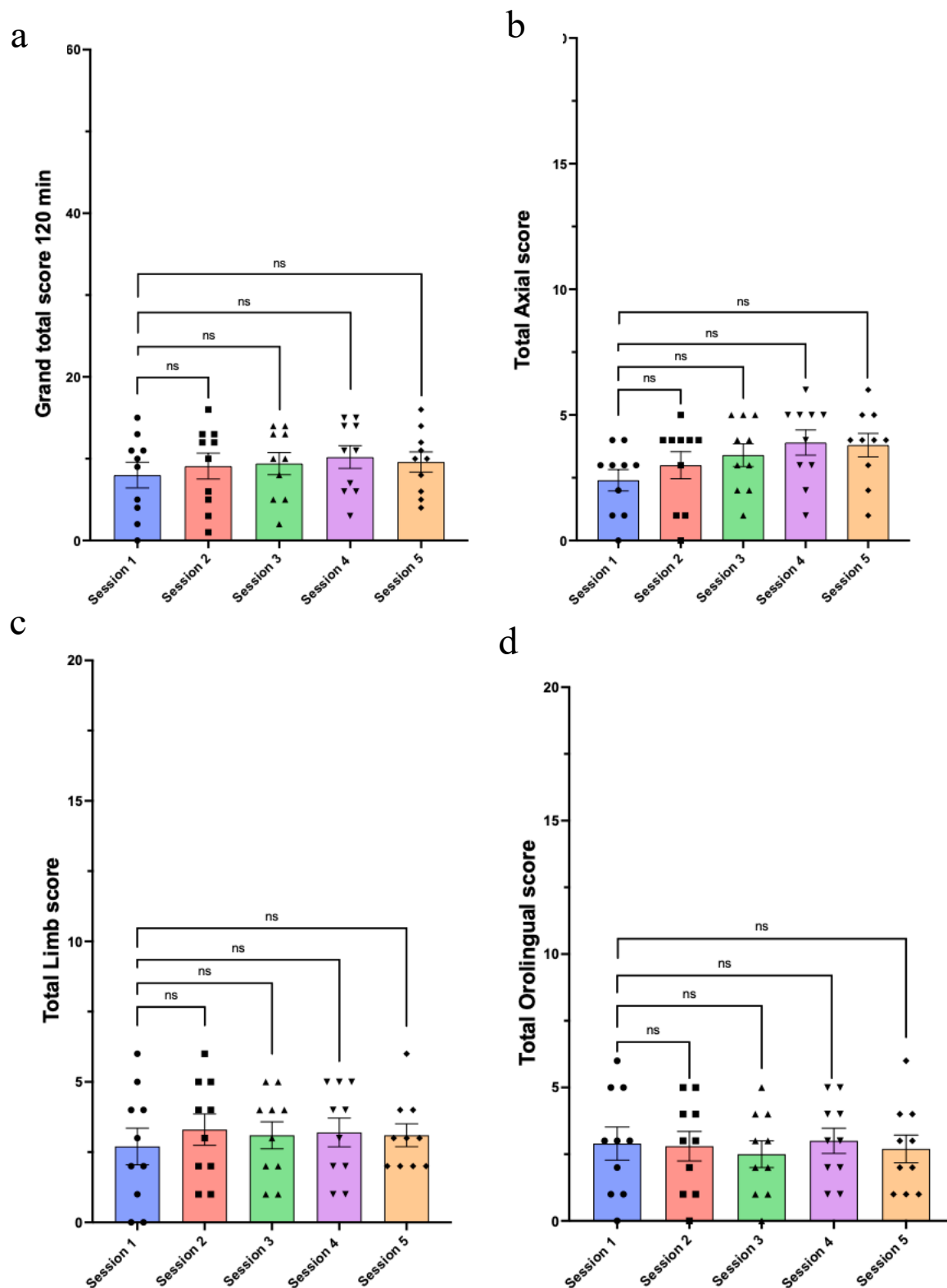


Figure 4. 5: ALO AIMs progression in the varenicline treated group across five sessions. Rats with unilateral 6-OHDA lesions received daily oral levodopa (8 mg/kg) and benserazide (15 mg/kg) alongside subcutaneous varenicline (1 mg/kg) and were assessed weekly over a five week period. Panel (a) shows the cumulative AIMs scores across all sessions, while panels (b), (c), and (d) present axial, limb, and orolingual AIMs over the 120 minute post-treatment timeframe. Results are shown as mean $\pm$ SEM.

4.3.5 : Development of ALO subtype comparison (by group)

To compare the effects of nicotine (0.3 mg/kg) and varenicline (1 mg/kg) with the saline control on the development of abnormal involuntary movements, a two-way ANOVA followed by Tukey's *post hoc* test was performed. The analysis assessed drug effects across global, axial, limb, and orolingual AIM subtypes, as illustrated in Figure 4.7.

A two-way ANOVA revealed a significant main effect of treatment, showing that nicotine and varenicline significantly reduced the development of global AIMs compared to the saline group. Dunnett's *post hoc* analysis confirmed that global AIM scores were significantly lower in the nicotine group compared to saline ($P = 0.0004$), and in the varenicline group compared to saline ($P = 0.0067$) as shown in (figure 4.7 panel a).

The analysis also showed a significant reduction in axial AIMs in both drug treated groups compared to saline. Axial AIM scores were significantly lower in the nicotine group ($P = 0.0007$) and the varenicline group ($P = 0.0004$), as shown in figure 4.7, panel b.

A similar effect was observed for limb AIMs, with both nicotine ($P < 0.0001$) and varenicline ($P = 0.0009$) producing significant reductions compared to the saline group, as shown in figure 4.7, panel c.

Orolingual AIMs were also significantly reduced by both nicotine ($P = 0.0003$) and varenicline ($P = 0.0009$) compared to the saline group, as illustrated in figure 4.7, panel d.

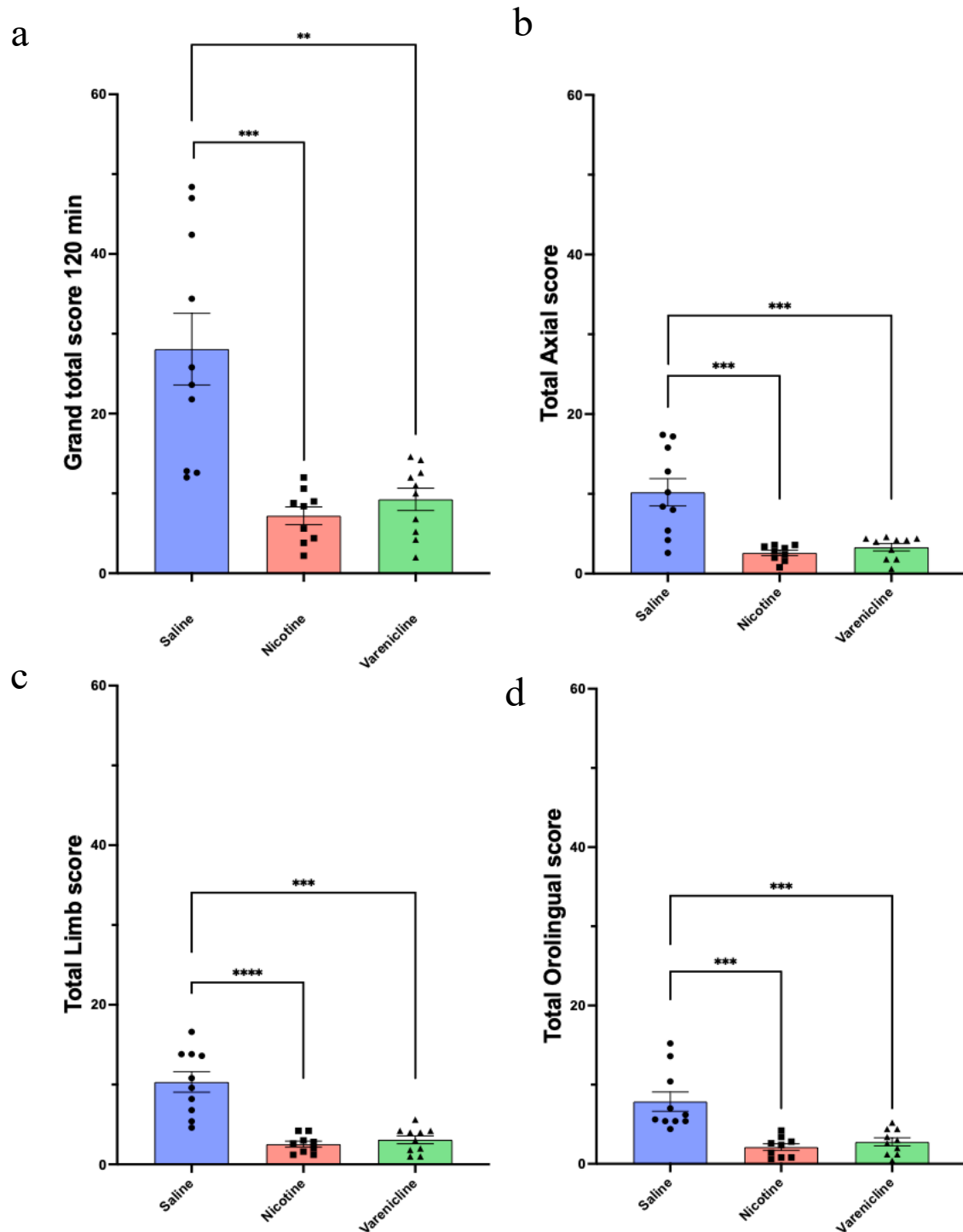


Figure 4. 6: Comparison of AIM scores across treatment groups over five sessions. Rats with unilateral 6-OHDA lesions received daily oral levodopa (8 mg/kg) and benserazide (15 mg/kg) in combination with either saline, nicotine, or varenicline. AIMS were assessed weekly over a five-week period. Panel (a) illustrates the development of total AIM scores (global AIMS), while panels (b), (c), and (d) show axial, limb, and orolingual AIM subtypes, respectively. Both nicotine and varenicline significantly reduced global and subtype specific AIMS compared to saline across the treatment period. Data are presented as mean ± SEM. *P < 0.05, **P < 0.01, ***P < 0.001.

4.3.6: Development of Locomotive scores by group

To evaluate the effects of nicotine (0.3 mg/kg) and varenicline (1 mg/kg) on the progression of AIMS, compared to saline control, statistical analyses were performed across five sessions. A one-way ANOVA followed by Tukey's *post hoc* test was conducted to determine differences in locomotor AIM scores, with results illustrated in Figure 4.8.

In the saline treated group ($n = 10$), co-administration of levodopa (8 mg/kg, oral) and benserazide (15 mg/kg, oral) with subcutaneous saline led to a robust development of dyskinesia over time. A significant main effect of session was observed ($F(2.506, 22.55) = 63.74, p < 0.0001$), with Dunnett's *post hoc* comparisons indicating significant increases in locomotor AIM scores from session 1 to session 2 ($p = 0.0012$), session 3 ($p = 0.0003$), and sessions 4 and 5 ($p < 0.0001$) (Figure 4.8, panel a).

In the nicotine treated group ($n=9$), a one-way ANOVA revealed a significant effect of session on locomotor AIMS ($F(2.217, 17.74) = 28.86, p < 0.0001$). *Post hoc* analysis indicated significantly elevated AIM scores at session 2 ($p = 0.0090$), session 3 ($p = 0.0022$), session 4 ($p = 0.0020$), and session 5 ($p = 0.0004$), relative to session 1 (Figure 4.8, panel b).

Similarly, varenicline treatment was associated with a significant session effect on locomotive AIM development ($F(2.387, 21.48) = 15.48, p < 0.0001$). Dunnett's *post hoc* tests demonstrated significant increases in locomotor AIMS at session 2 ($p = 0.0006$), session 3 ($p = 0.0004$), session 4 ($p = 0.0007$), and session 5 ($p = 0.0101$), when compared to session 1 (Figure 4.8, panel c).

To compare treatment effects directly, a two-way ANOVA was performed, assessing the interaction between treatment (saline, nicotine, varenicline) and session, followed by Tukey's *post hoc* test (Figure 4.8, panel d). A significant main effect of treatment was observed, indicating that both nicotine and varenicline significantly reduced the development of locomotor AIMS compared to saline. Dunnett's comparisons further confirmed that

locomotive AIM scores were significantly reduced in the nicotine group ($p < 0.0001$) and the varenicline group ($p < 0.0001$), relative to saline controls.

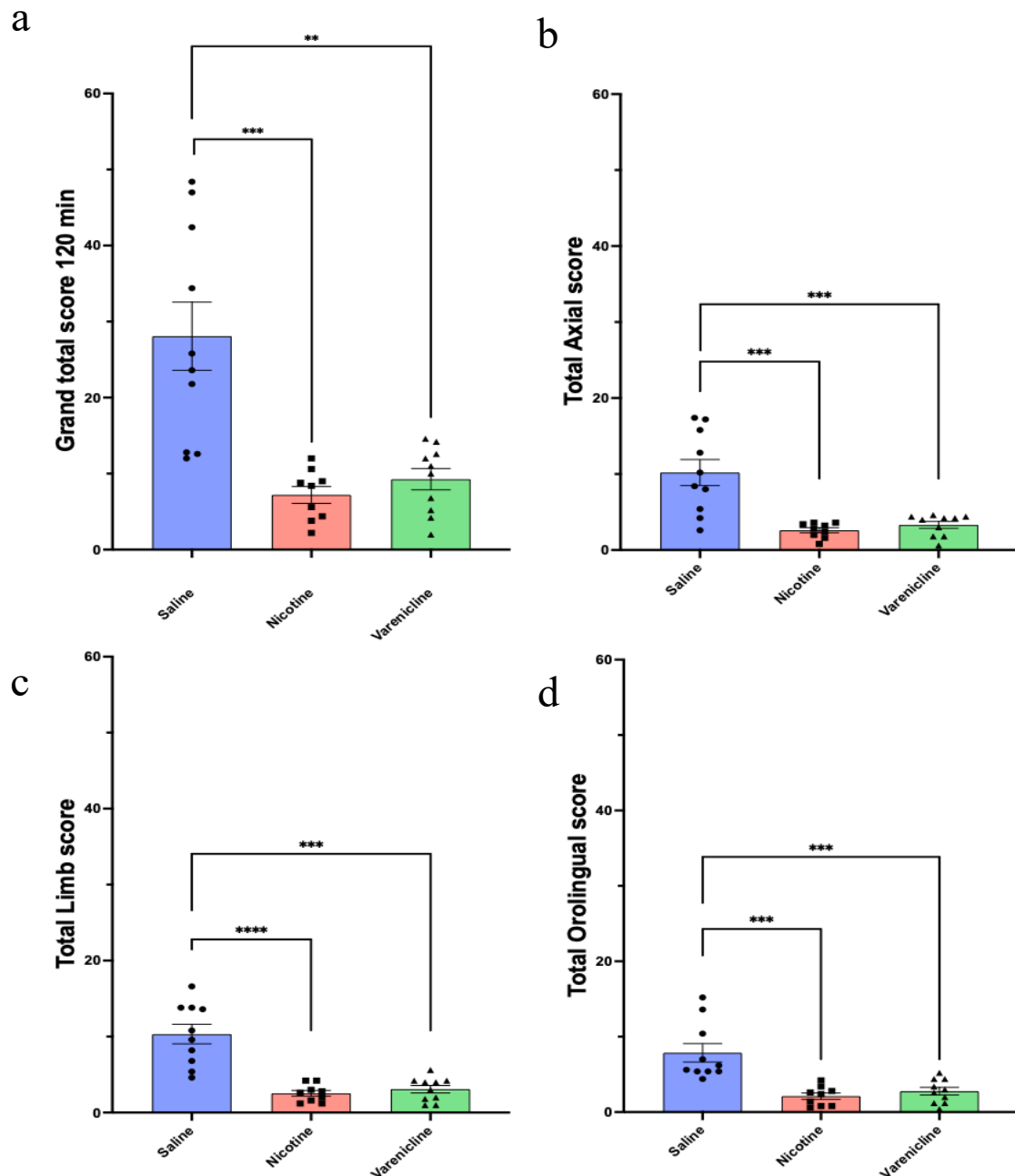


Figure 4. 7: Comparison of locomotive AIM scores across treatment groups over five sessions. Rats with unilateral 6-OHDA lesions received daily oral levodopa (8 mg/kg) and benserazide (15 mg/kg) in combination with either saline, nicotine, or varenicline. Locomotive AIMs were assessed weekly over a five-week period. Panel (a) illustrates the development of total locomotive AIM scores in saline group, panels (b) show the development of locomotive AIM score in nicotine group and panel (c) shows the locomotive AIM development in varenicline group. Panel (d) show the comparison of 3 groups in development of locomotive AIMs. Data are presented as mean $\pm$ SEM. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

4.3.7: 6-OHDA lesioning induces over 90% dopaminergic cell loss in the striatum and substantia nigra

Immunohistochemical analysis of tyrosine hydroxylase expression was performed to assess dopaminergic neurodegeneration in the substantia nigra pars compacta and the loss of dopaminergic terminal density in the striatum following unilateral 6-hydroxydopamine lesioning. Unilateral 6-OHDA injection resulted in variable degrees of TH-positive neuronal loss in the SNpc as well as different levels of TH fiber density reduction in the striatum.

In animals that did not exhibit abnormal involuntary movements, TH immunoreactivity appeared largely preserved in both the SNpc and striatum, with minimal difference between hemispheres. In contrast, animals with moderate AIMs showed approximately 40–60% reduction in TH-positive neurons in the SNpc and a visible reduction in striatal TH fibre density. Animals that developed pronounced AIMs exhibited extensive loss of TH-positive neurons in the SNpc (greater than 90%) and a marked decrease in TH fibre density in the striatum. These observations are illustrated in the representative images below (figure 4.9 to 4.14).

4.3.7.1 Saline treated group

In the saline treated group ($n = 10$), which received co-administration of levodopa and benserazide following 6-OHDA lesioning, animals exhibited varying degrees of abnormal involuntary movements. One animal did not develop AIMs and showed no apparent dopaminergic cell loss in the lesioned hemisphere, as evidenced by TH immunoreactivity in the substantia nigra pars compacta (Figure 4.9a). Two animals exhibited partial AIM expression and demonstrated approximately 47% reduction in TH positive neurons on the lesioned side (Figure 4.9c). The remaining seven animals developed robust AIMs and showed

extensive dopaminergic neuronal loss, with greater than 90% reduction in TH positive cells in the lesioned substantia nigra (Figure 4.9e).

Corresponding differences were observed in the striatum, where TH immunoreactivity reflects the density of dopaminergic terminals. In the animal with no AIMs, TH fibre density was intact across both hemispheres. Animals with partial AIM expression exhibited moderate reductions in TH positive terminal density in the lesioned striatum. In contrast, those with fully developed AIMs displayed a marked depletion of striatal TH staining, indicating extensive loss of dopaminergic innervation in the lesioned hemisphere (figure 4.10).

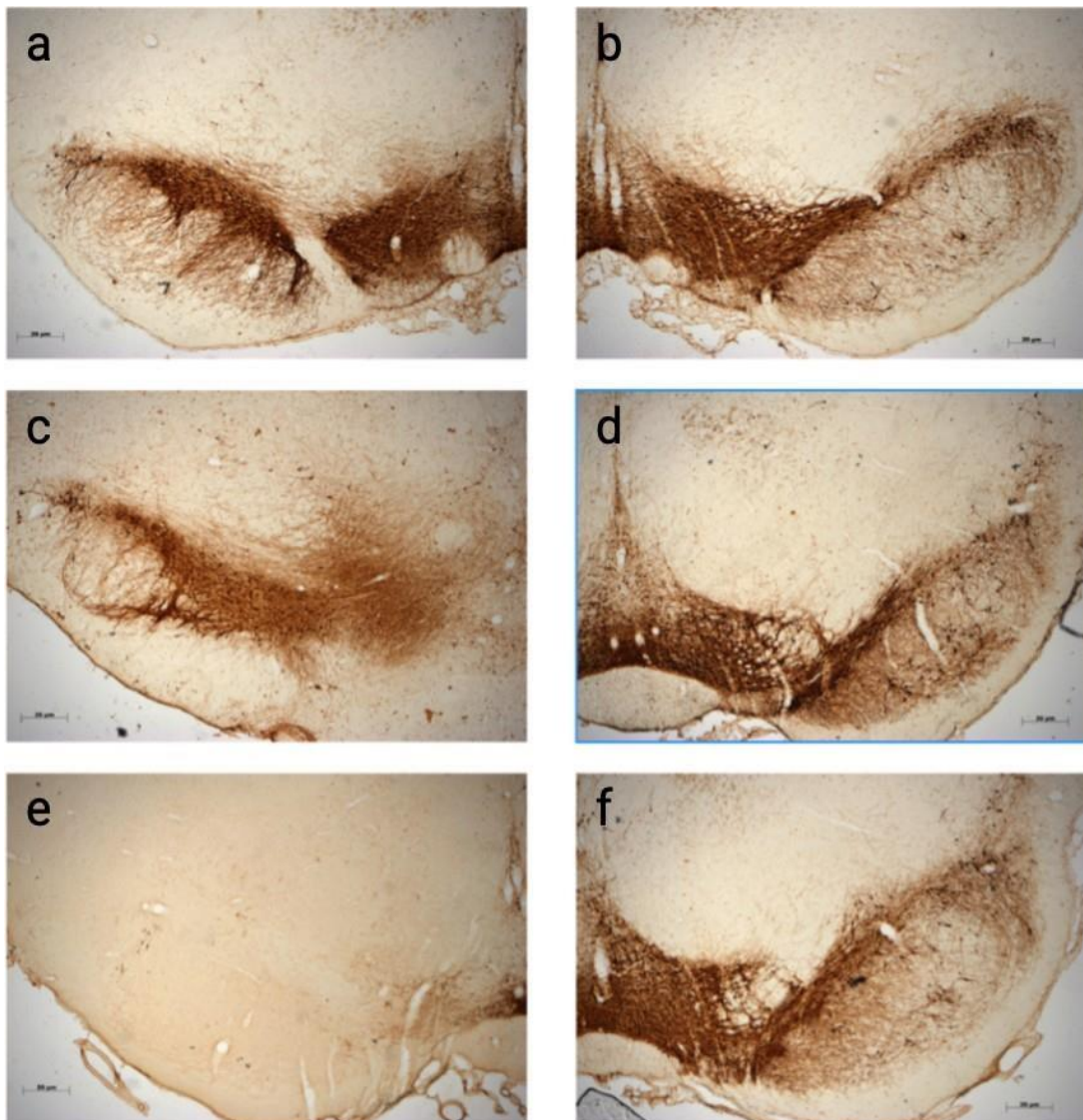


Figure 4. 8: TH immunostaining in the SNpc of the saline treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) TH-positive neurons in the lesioned (a) and non-lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) lesioned (c) and non-lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) lesioned (e) and non-lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 2.5× magnification. Scale bar = 20 µm.

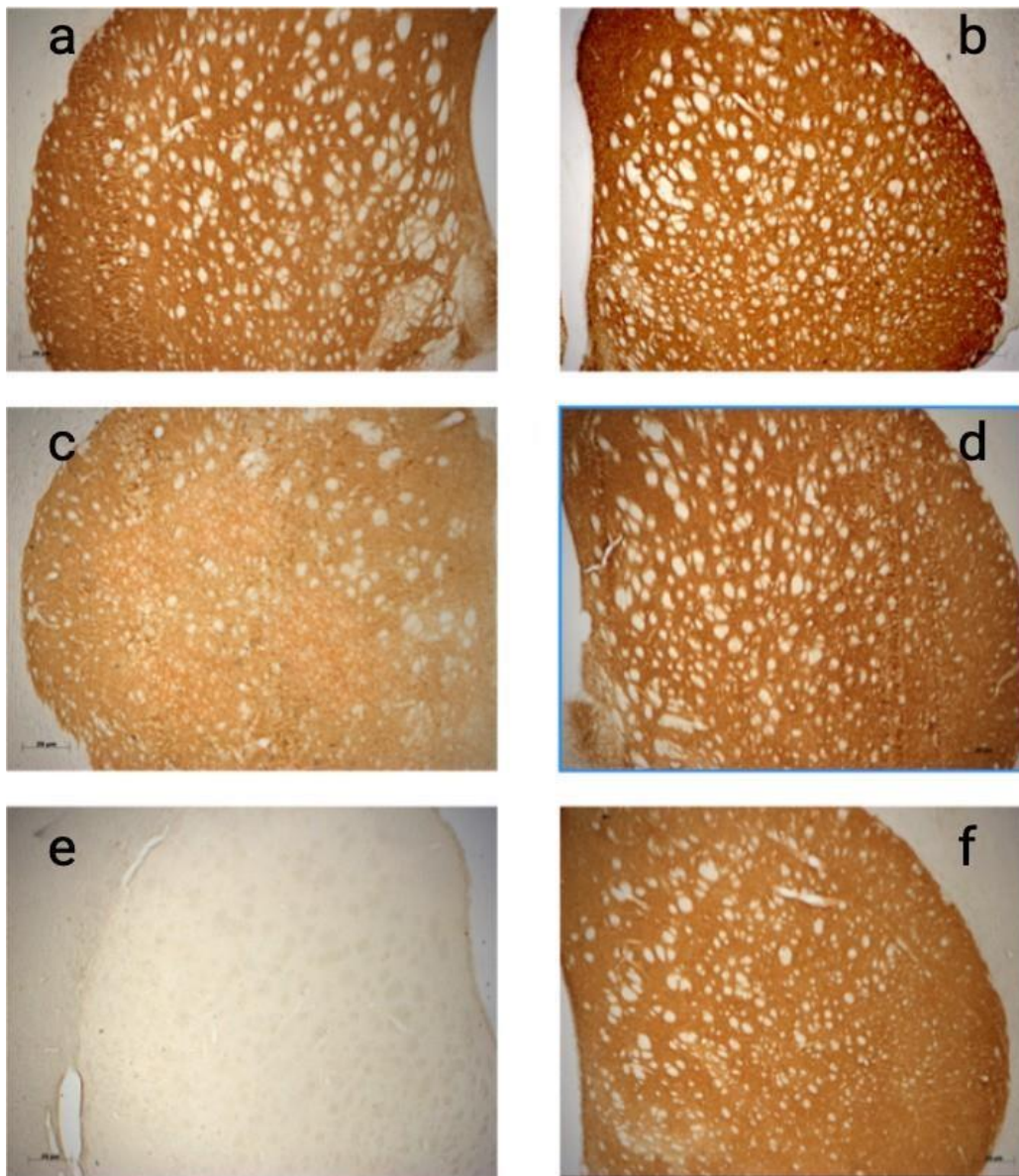


Figure 4. 9: TH immunostaining in the striatum of the saline treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) TH-positive fibers in the lesioned (a) and non-lesioned (b) striatum of animals that did not develop AIMs. (c–d) Lesioned (c) and non-lesioned (d) striatum from animals with partially developed AIMs. (e–f) Lesioned (e) and non-lesioned (f) striatum from animals with fully developed AIMs. Images captured at 2.5× magnification. Scale bar = 20 μ m.

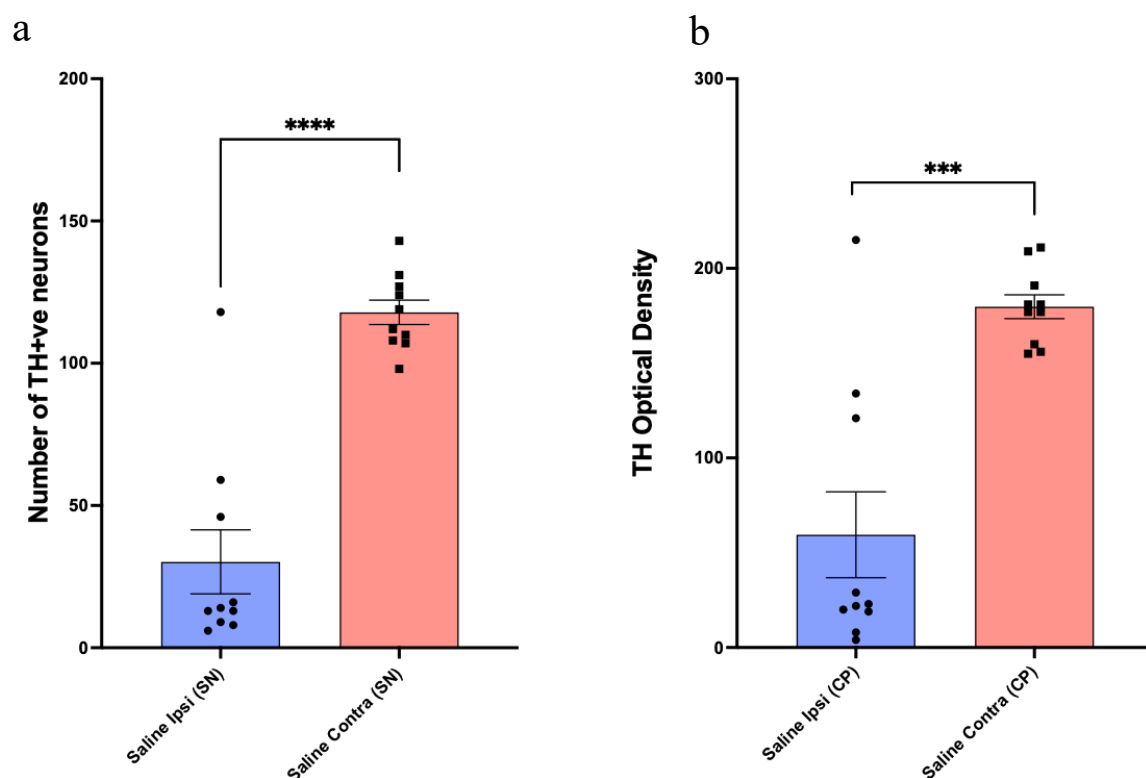


Figure 4.10: Quantification of TH positive neurons in the SNpc and TH optical density, expressed in arbitrary units, in the striatum of saline treated rats, comparing the control and lesioned hemispheres in rats. (a) Significant reduction in the number of TH positive neurons in the lesioned substantia nigra of rats. (b) Corresponding decrease in TH optical density in the lesioned striatum compared to the control hemisphere. ****P < 0.0001 (two-way ANOVA followed by Tukey's *post hoc* test).

4.3.7.2 Nicotine treated group

In the nicotine treated group (n = 9), which received levodopa and benserazide following 6-OHDA lesioning, animals exhibited a range of behavioral responses, reflected in varying severities of AIMs. Two rats failed to exhibit AIMs and displayed similar TH immunoreactivity in the lesioned substantia nigra pars compacta, indicating minimal dopaminergic cell loss (Figure 4.12a). A further two animals developed moderate AIMs, accompanied by a reduction of approximately 33% in TH-positive neurons in the SNpc (Figure 4.12c). In contrast, five animals presented with pronounced AIMs and exhibited severe

dopaminergic neurodegeneration, with over 90% loss of TH-positive cells in the lesioned SNpc (Figure 4.12e).

A similar trend was observed in the striatum, striatal TH fibre density also varied according to behavioural results. In animals without AIMs, dopaminergic terminal density in the lesioned striatum was similar to the contralateral side. Moderate AIM expression corresponded with visibly reduced TH labelling in the lesioned striatum, while severe AIM expression was associated with near complete loss of TH positive fibres, reflecting substantial dopaminergic denervation (Figure 4.13).

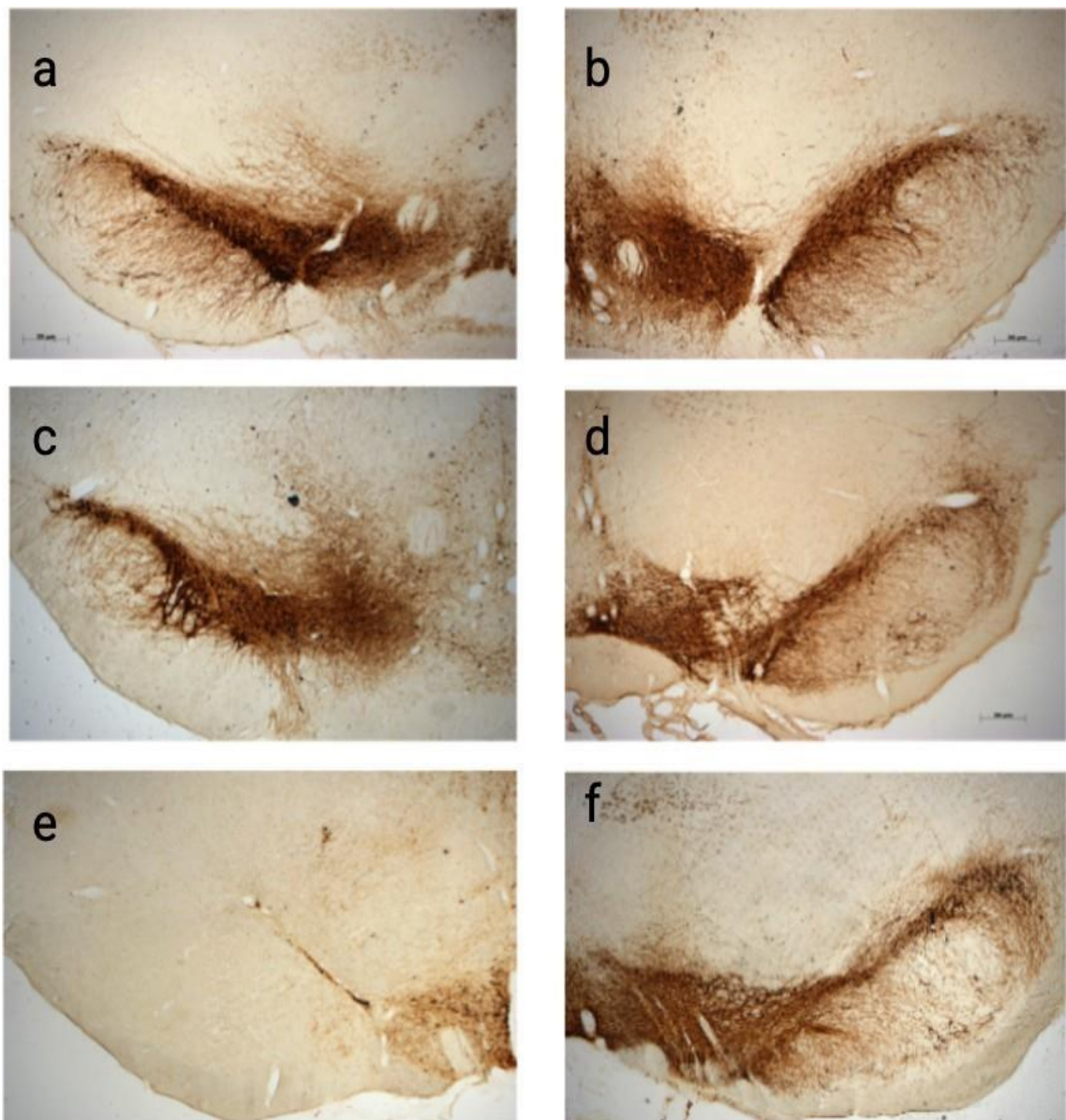


Figure 4. 11: TH immunostaining in the SNpc of the nicotine treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) TH-positive neurons in the lesioned (a) and non-lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) lesioned (c) and non-lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) lesioned (e) and non-lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 2.5× magnification. Scale bar = 20 μ m.

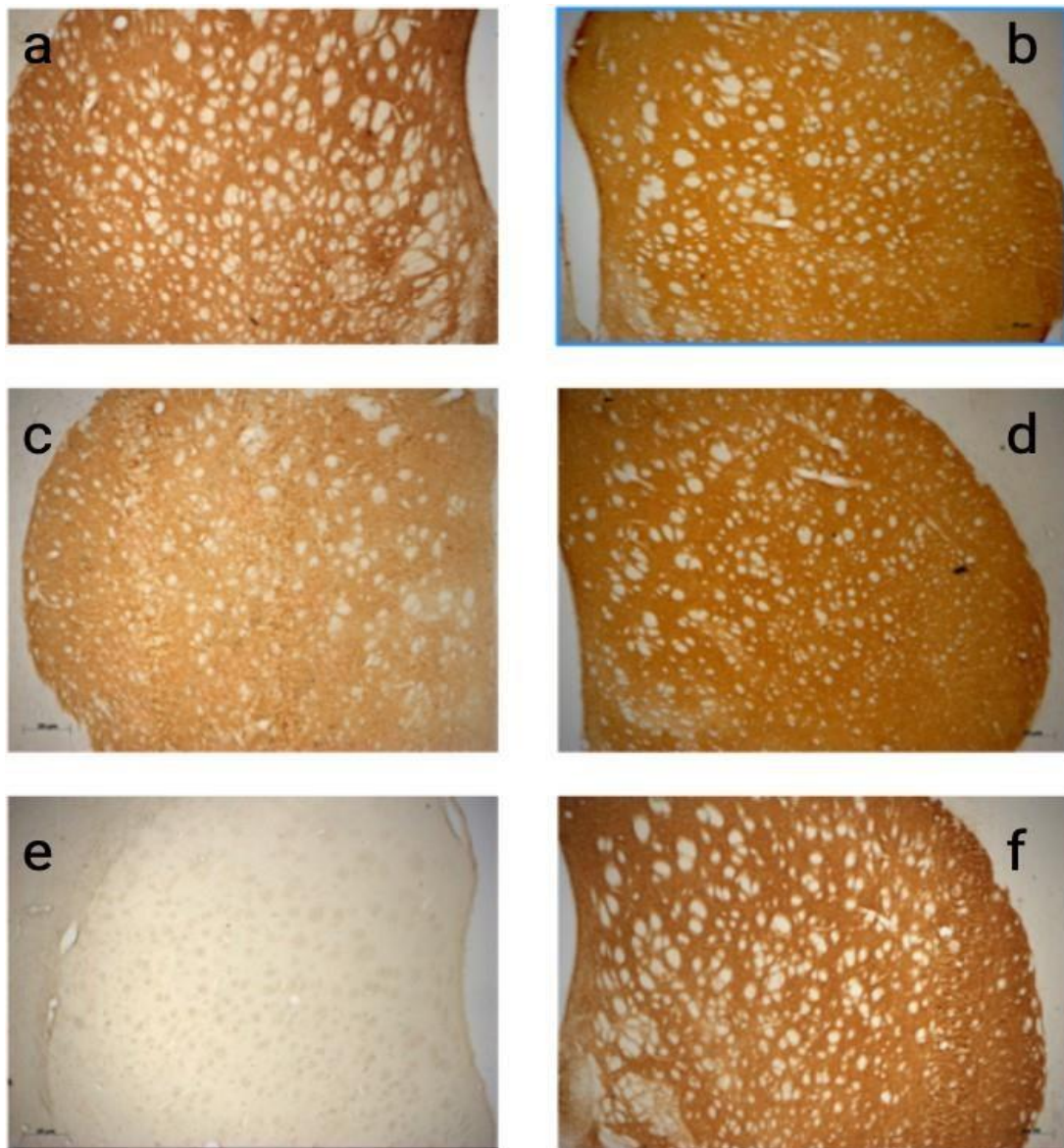


Figure 4. 12: TH immunostaining in the striatum of the nicotine treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) TH-positive fibers in the lesioned (a) and non-lesioned (b) striatum of animals that did not develop AIMs. (c–d) Lesioned (c) and non-lesioned (d) striatum from animals with partially developed AIMs. (e–f) Lesioned (e) and non-lesioned (f) striatum from animal with fully developed AIMs. Images captured at 2.5× magnification. Scale bar = 20 μ m.

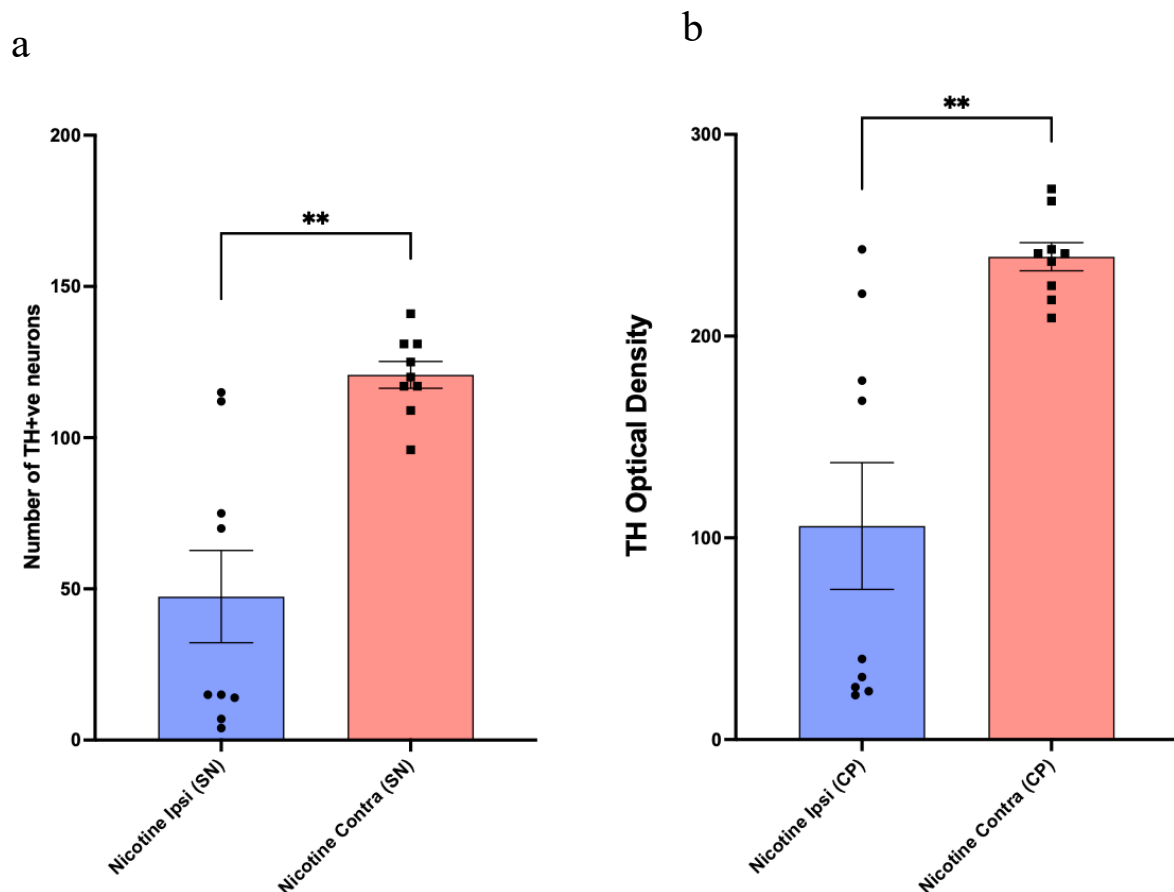


Figure 4.13: Quantification of TH positive neurons in the SNpc and TH optical density, expressed in arbitrary units, in the striatum of nicotine treated rats, comparing the control and 6-OHDA lesioned hemispheres in rats. (a) Significant reduction in the number of TH positive neurons in the lesioned substantia nigra of rats. (b) Corresponding decrease in TH optical density in the lesioned striatum compared to the control hemisphere. ** $P < 0.01$ (two-way ANOVA followed by Tukey's *post hoc* test).

4.3.7.3 Varenicline treated group

In the varenicline treated group ($n = 10$), which received varenicline in combination with levodopa and benserazide following 6-OHDA lesioning, similar to the saline and nicotine treated groups, animals displayed a range of AIM severities. One rat showed no evidence of AIMs and retained TH immunoreactivity in the lesioned SNpc similar to the contralateral side (Figure 4.15a). One animal exhibited moderate AIMs, accompanied by an estimated 67% reduction in TH-positive neurons (Figure 4.15c). The remaining eight animals developed high

AIM scores and showed marked dopaminergic cell loss in the lesioned SNpc, with over 90% reduction in TH immunoreactivity (Figure 4.15e).

Striatal TH labelling followed a similar pattern. The rat without AIMs exhibited similar dopaminergic terminal density between hemispheres. In the moderately affected animal, TH fiber density was visibly reduced in the lesioned striatum. Animals with pronounced AIMs displayed near complete loss of striatal TH-positive fibers (Figure 4.16).

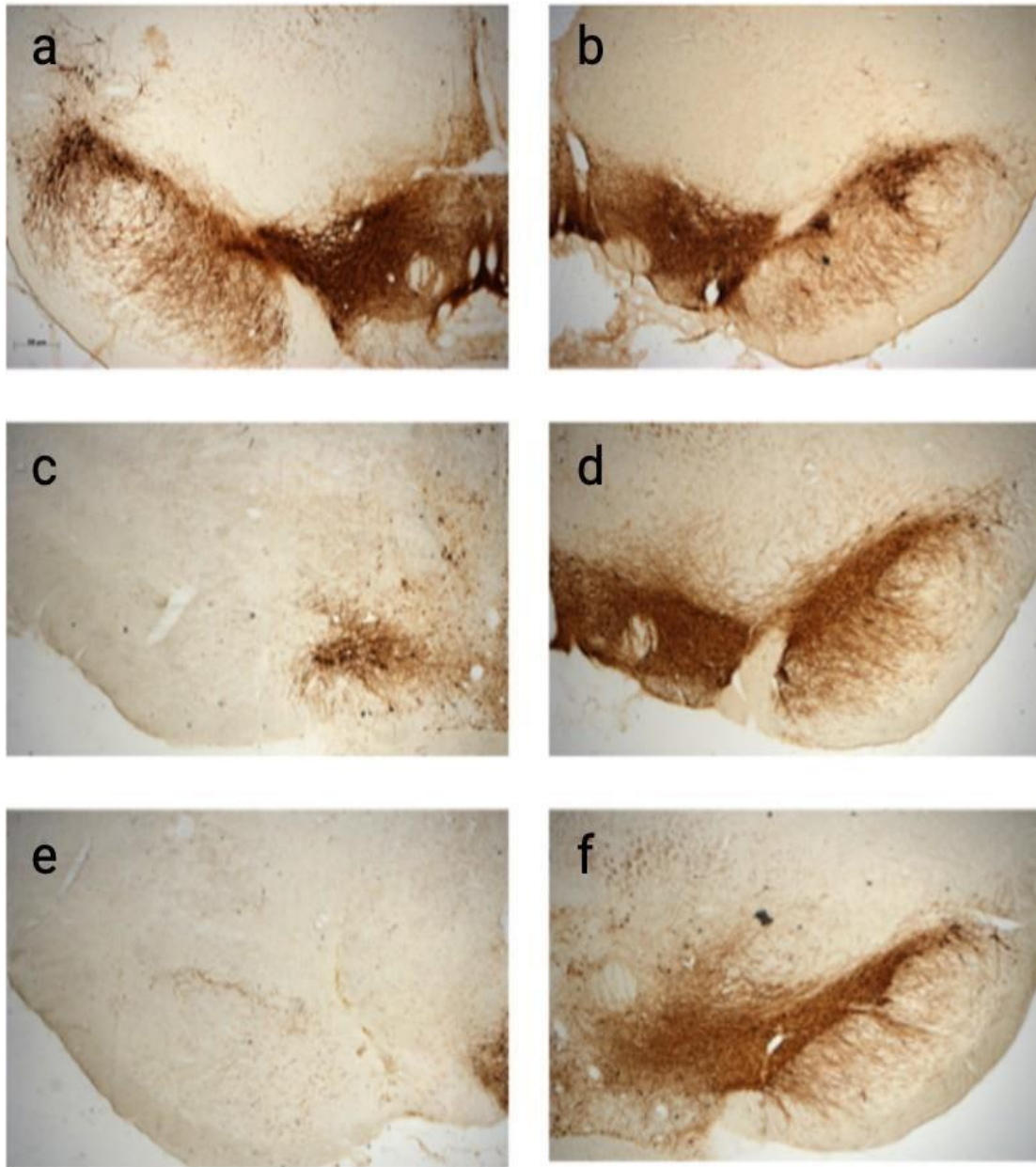


Figure 4. 14: TH immunostaining in the SNpc of the varenicline treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) TH-positive neurons in the lesioned (a) and non-lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) lesioned (c) and non-lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) lesioned (e) and non-lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 2.5× magnification. Scale bar = 20 μ m.

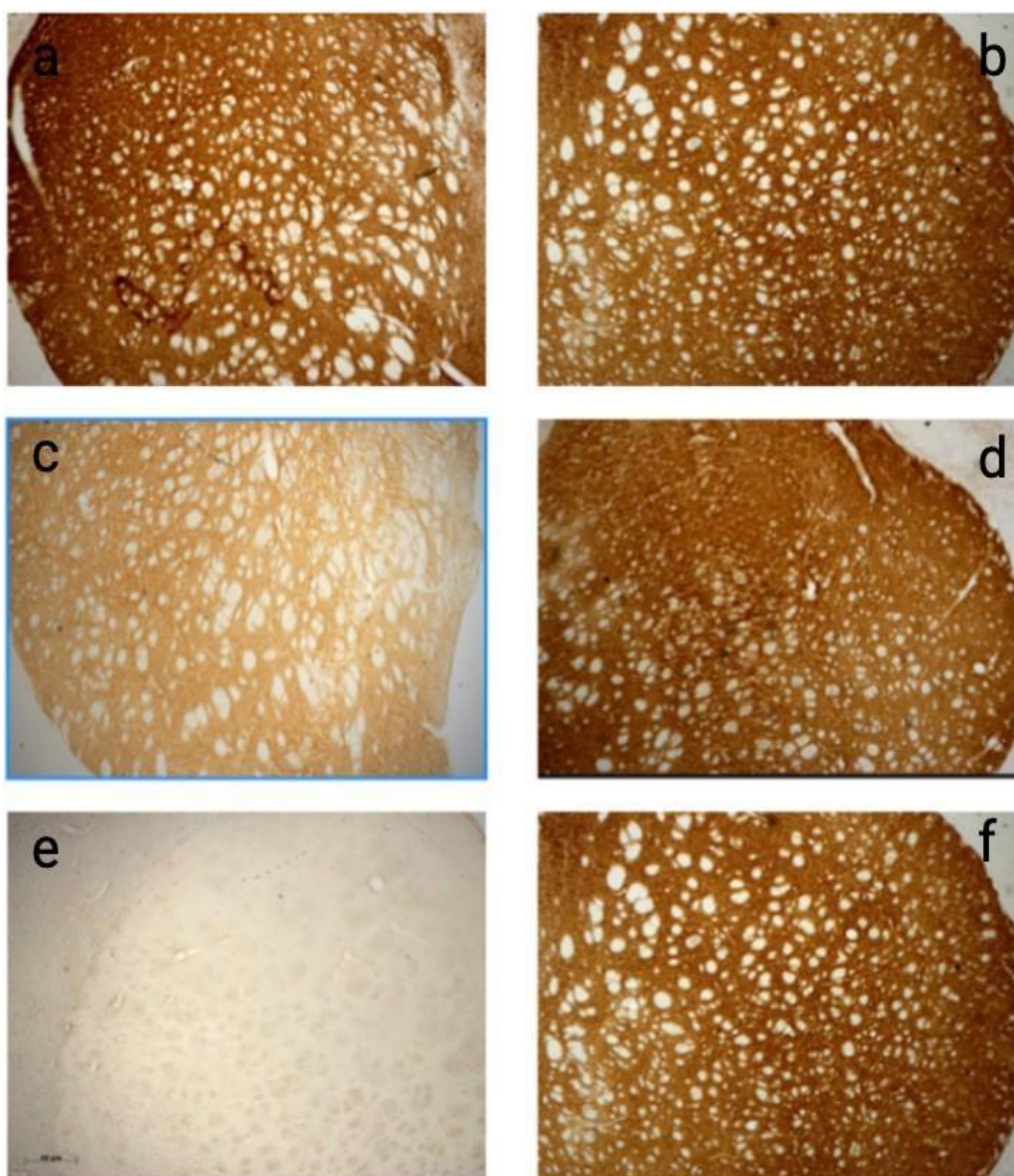


Figure 4.15: TH immunostaining in the striatum of the varenicline treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) TH-positive fibers in the lesioned (a) and non-lesioned (b) striatum of animals that did not develop AIMs. (c–d) Lesioned (c) and non-lesioned (d) striatum from animals with partially developed AIMs. (e–f) Lesioned (e) and non-lesioned (f) striatum from animals with fully developed AIMs. Images captured at 2.5× magnification. Scale bar = 20 μ m.

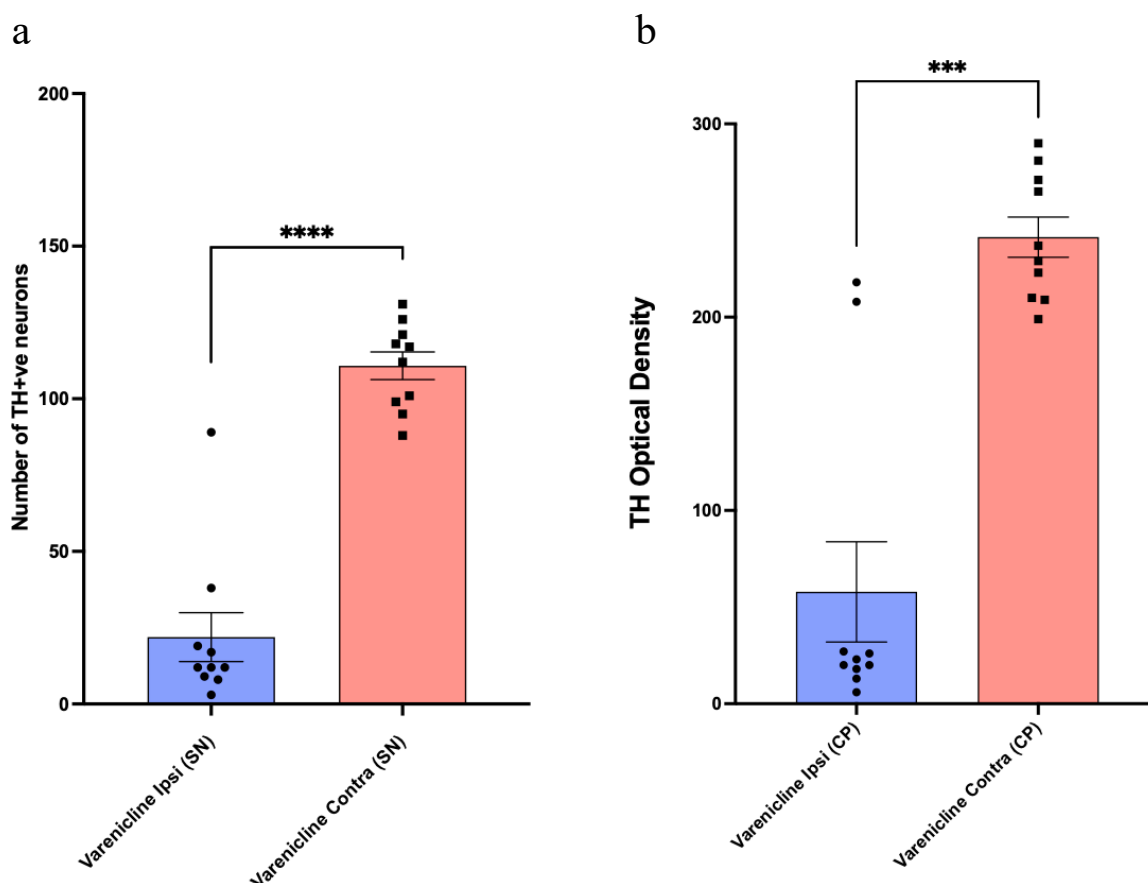


Figure 4. 16: Quantification of TH positive neurons in the SNpc and TH optical density, expressed in arbitrary units, in the striatum of Varenicline treated rats, comparing the control and lesioned hemispheres in rats. (a) Significant reduction in the number of TH positive neurons in the lesioned substantia nigra of rats. **(b)** Corresponding decrease in TH optical density in the lesioned striatum compared to the control hemisphere. *** $P < 0.01$ (two-way ANOVA followed by Tukey's *post hoc* test).

4.3.8 : Astrocytic activation in the striatum and substantia nigra following 6-OHDA lesioning

Immunohistochemical analysis of glial fibrillary acidic protein (GFAP) expression was conducted to evaluate astrocytic activation in the substantia nigra pars compacta and striatum following unilateral 6-OHDA lesioning. GFAP immunoreactivity was assessed in animals from the saline, nicotine, and varenicline treatment groups, each subdivided based on the

severity of abnormal involuntary movements into two behavioural categories: moderate AIMs and fully developed AIMs.

In the saline treated group, robust astrocytic activation was consistently observed in the lesioned hemisphere, as indicated by markedly increased GFAP staining in both the SNpc and striatum compared to the unlesioned side. This pronounced gliotic response was evident across both subgroups, regardless of AIM severity.

In contrast, animals treated with nicotine or varenicline exhibited a more similar pattern of GFAP expression between hemispheres. Across both AIM subgroups, GFAP immunoreactivity appeared relatively balanced, with minimal increase in astrocytic activation (Figures 4.18 to 4.26).

4.3.8.1 Saline treated group

In the saline treated group ($n = 10$), which received co-administration of levodopa and benserazide following 6-OHDA lesioning, animals exhibited varying degrees of AIMs. One animal did not develop AIMs, two animals showed partial expression, and the remaining seven animals developed robust AIMs. All animals were assessed for GFAP labelled astrocyte activation.

In animals with partial AIM expression, GFAP levels showed a moderate but statistically significant increase ($p = 0.0006$), same with the animals with fully developed AIMs demonstrated a highly significant upregulation of astrocyte activation in the substantia nigra ($p = 0.0002$; Figure 4.18).

Corresponding differences were observed in the striatum. Animals with partial AIM expression exhibited a significant increase in GFAP positive astrocyte density in the lesioned striatum ($p = 0.0004$). Animals with fully developed AIMs showed a markedly elevated GFAP signal in the striatum ($p < 0.0001$; Figure 4.19).

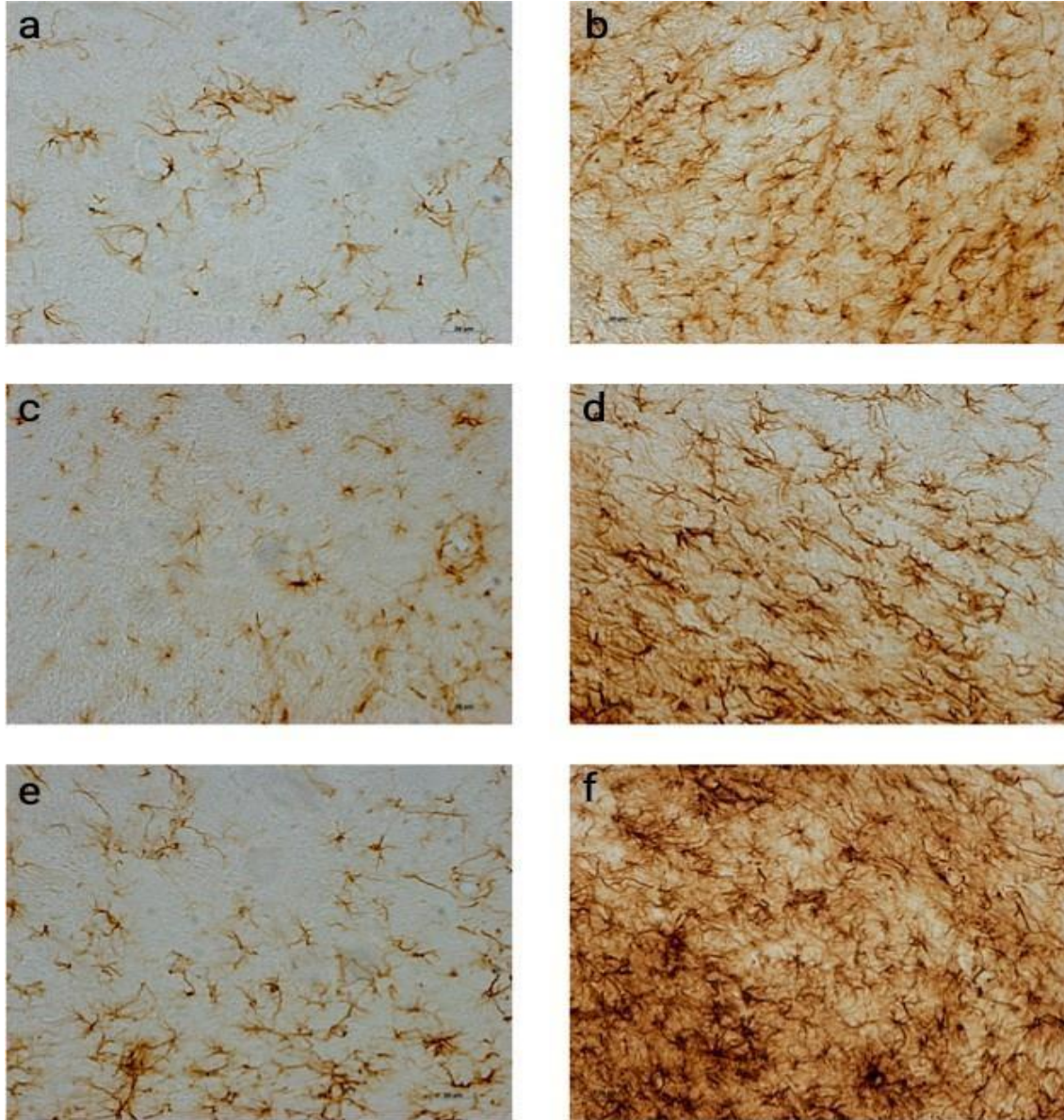


Figure 4. 17: GFAP-ir immunostaining in the SNpc of the saline treated group, comparing the non-lesioned and lesioned hemispheres in animals with varying severities of AIMs. (a–b) GFAP-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 20× magnification. Scale bar = 20 μ m.

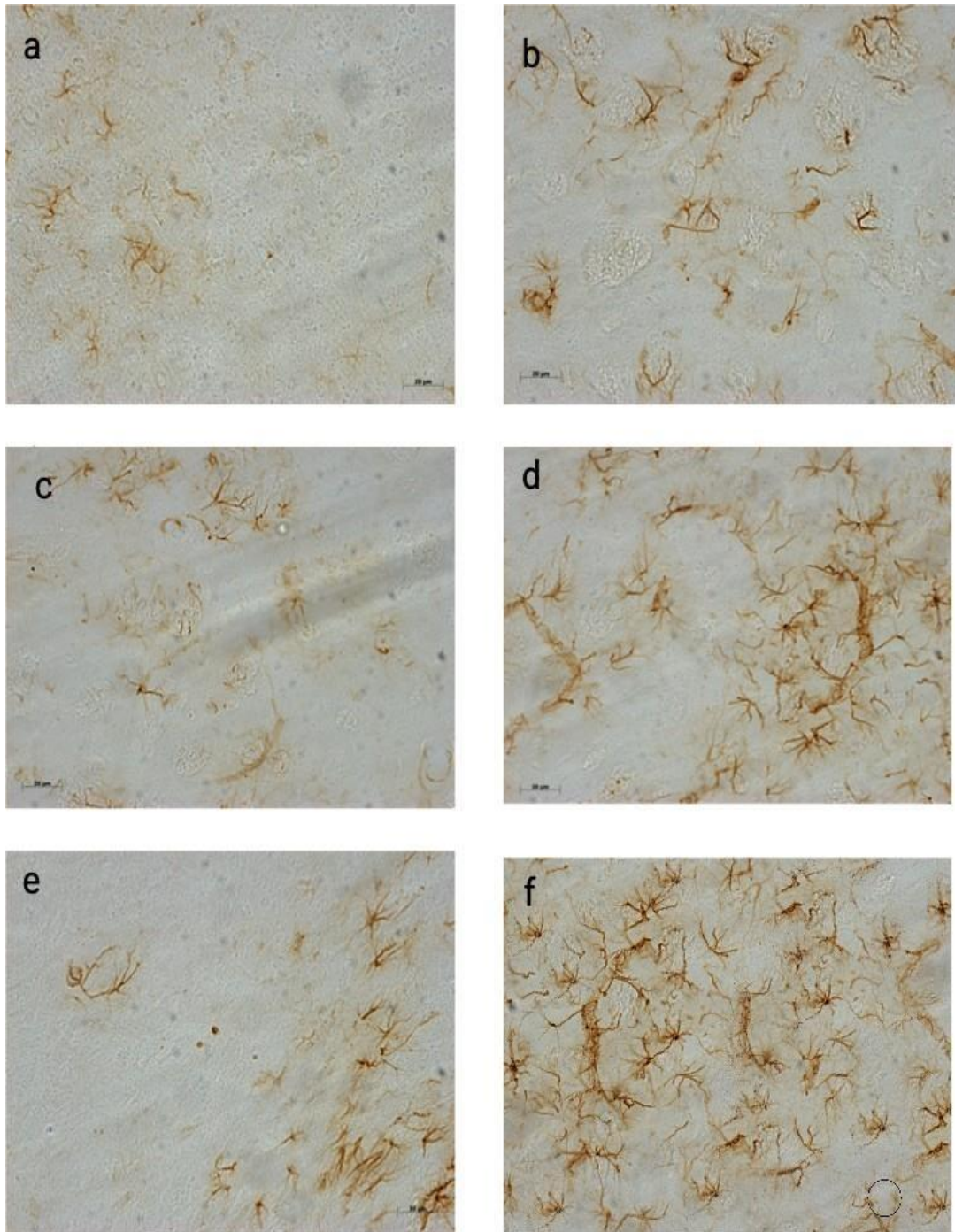


Figure 4.18: GFAP-ir immunostaining in the striatum of the saline treated group, comparing the non-lesioned and lesioned hemispheres in animals with varying severities of AIMs. (a–b) GFAP-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e– f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 20× magnification. Scale bar = 20 µm.

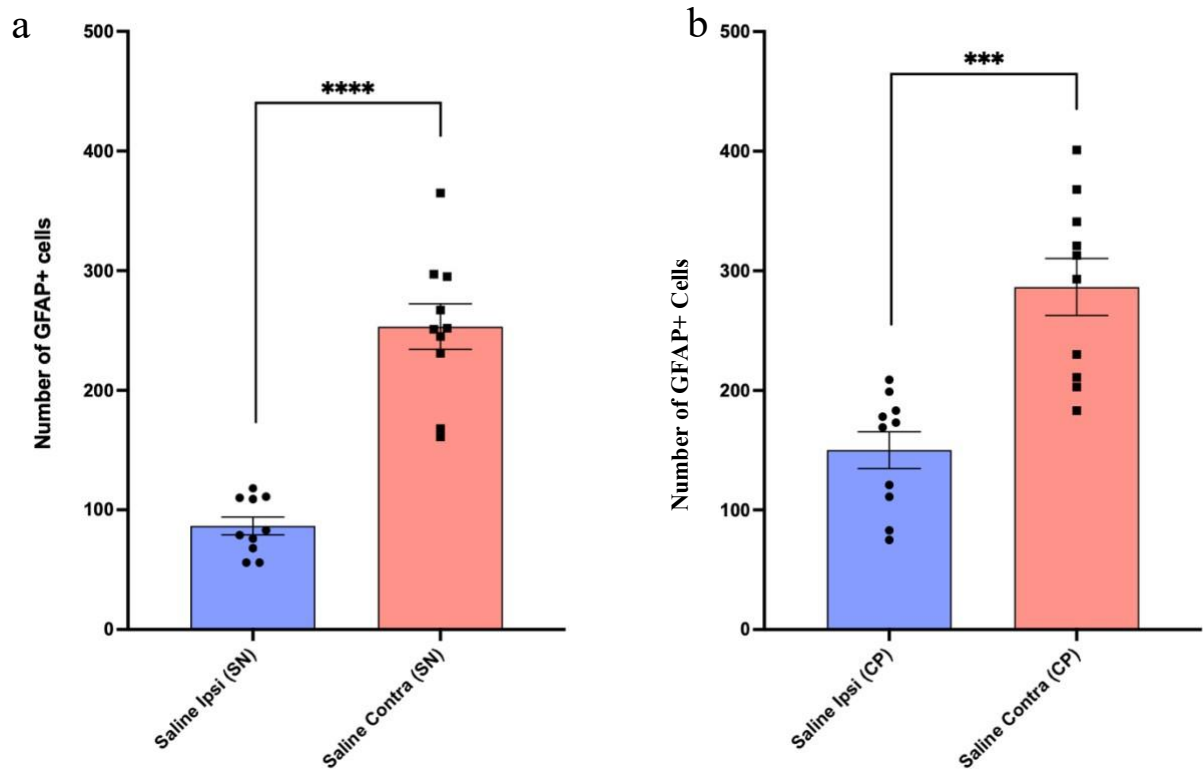


Figure 4. 19: Quantification of GFAP positive cells in the SNpc and GFAP optical density, expressed in arbitrary units, in the striatum of saline treated rats, comparing the control and lesioned hemispheres. (a) Significant increase in the number of GFAP positive cells in the lesioned substantia nigra of rats. (b) Corresponding elevation in GFAP optical density in the lesioned striatum compared to the unlesioned hemisphere. *P < 0.05, **P < 0.01, *P < 0.001 (two-way ANOVA followed by Tukey's *post hoc* test).**

4.3.8.2 Nicotine treated group

In the nicotine treated group (n = 9), two animals did not develop AIMs, two exhibited partial expression, and the remaining five developed robust AIMs. A statistically significant increase was observed in animals with partial AIM expression (p = 0.0014), those with fully developed AIMs showed an upregulation of astrocyte activation, although to a lesser extent than that observed in the saline treated group (p = 0.0128; Figure 4.21).

However, the results of striatum was slightly different. Neither animals with partial AIMs or fully developed did not display a significant GFAP increase in this region (Figure 4.22).

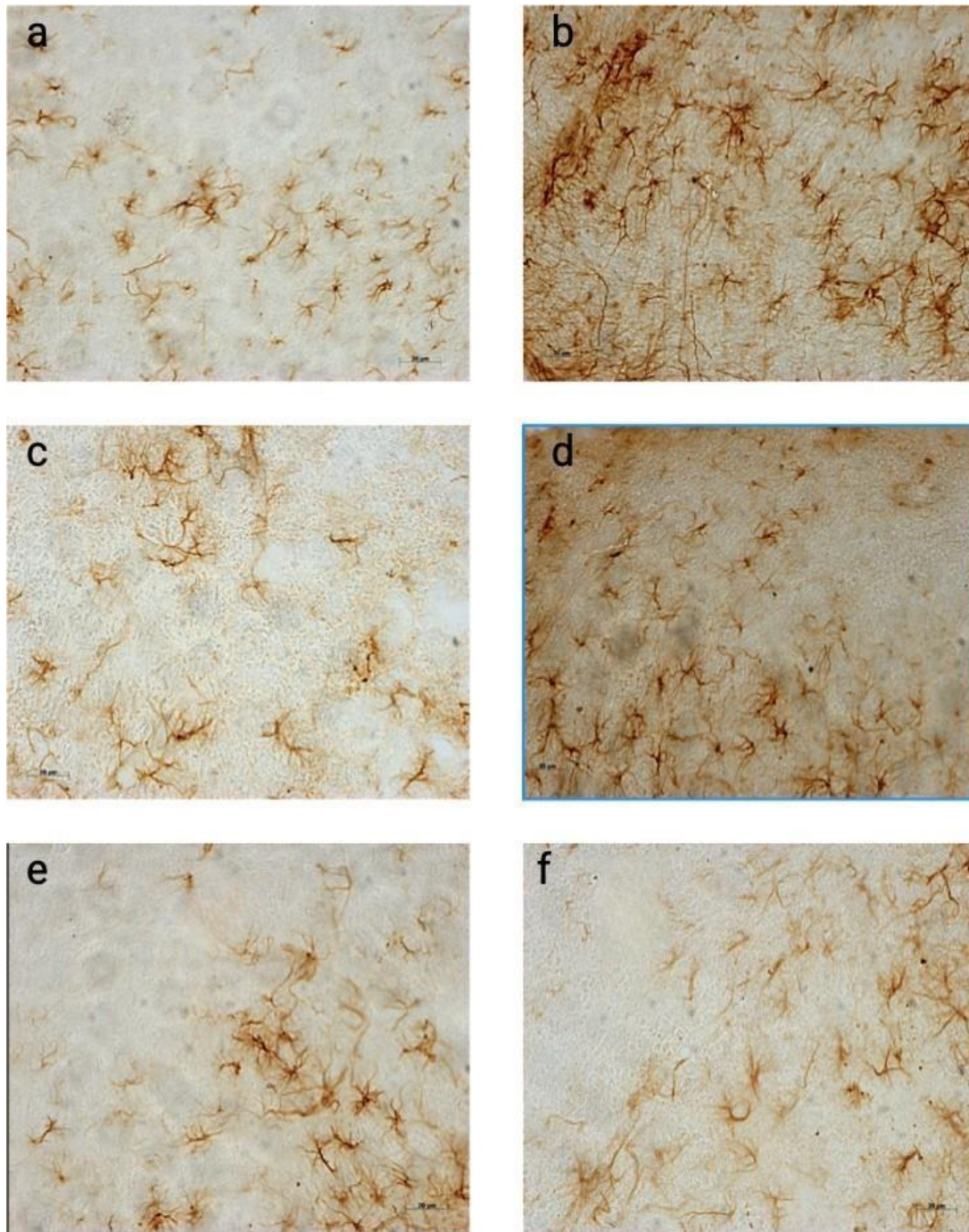


Figure 4. 20: GFAP-ir immunostaining in the SNpc of the nicotine treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) GFAP-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e– f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 40× magnification. Scale bar = 20 μ m.

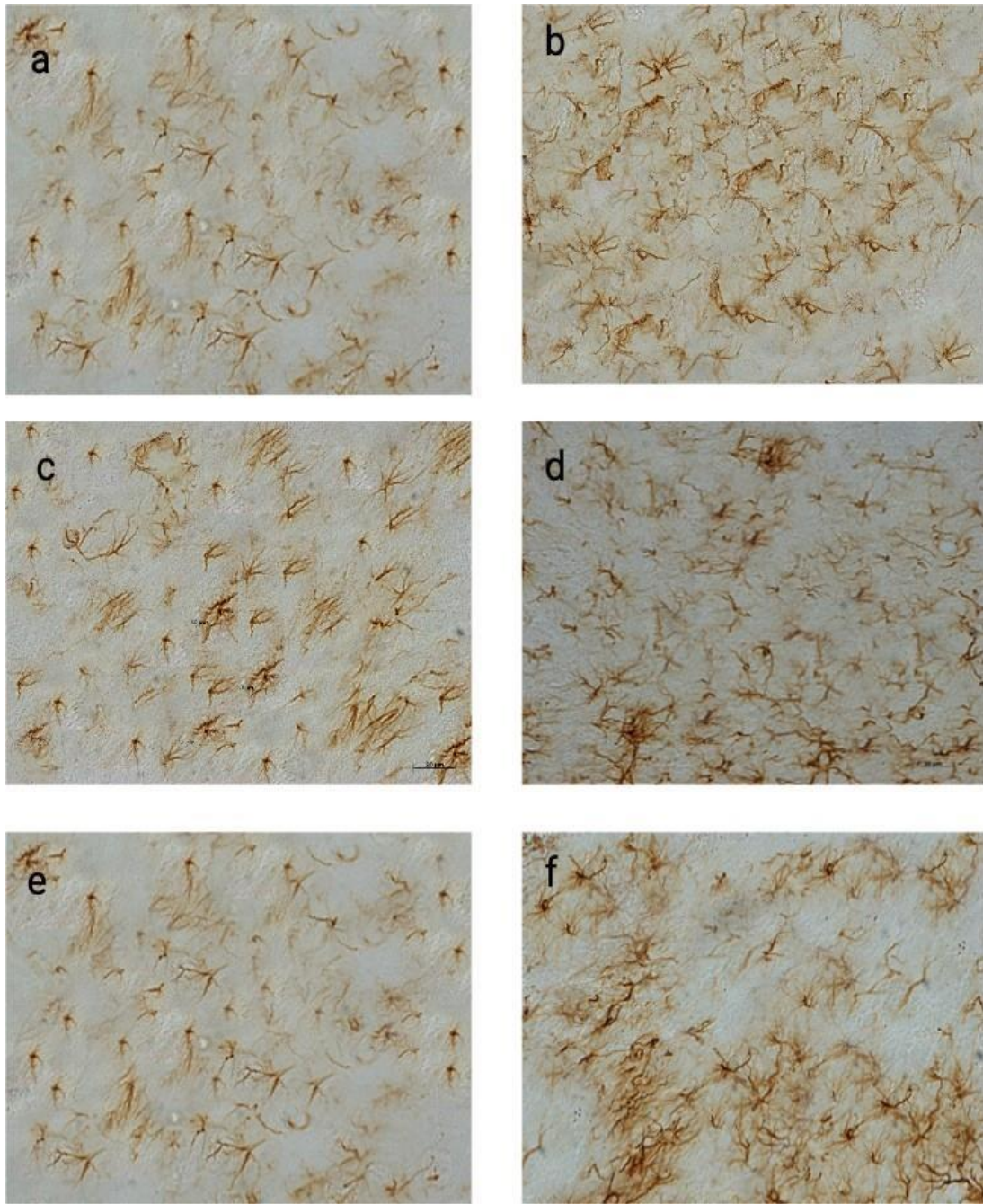


Figure 4. 21: GFAP-ir immunostaining in the striatum of the nicotine treated group, comparing the non-lesioned and lesioned hemispheres in animals with varying severities of AIMs. (a–b)GFAP-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e– f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 20× magnification. Scale bar = 20 μ m.

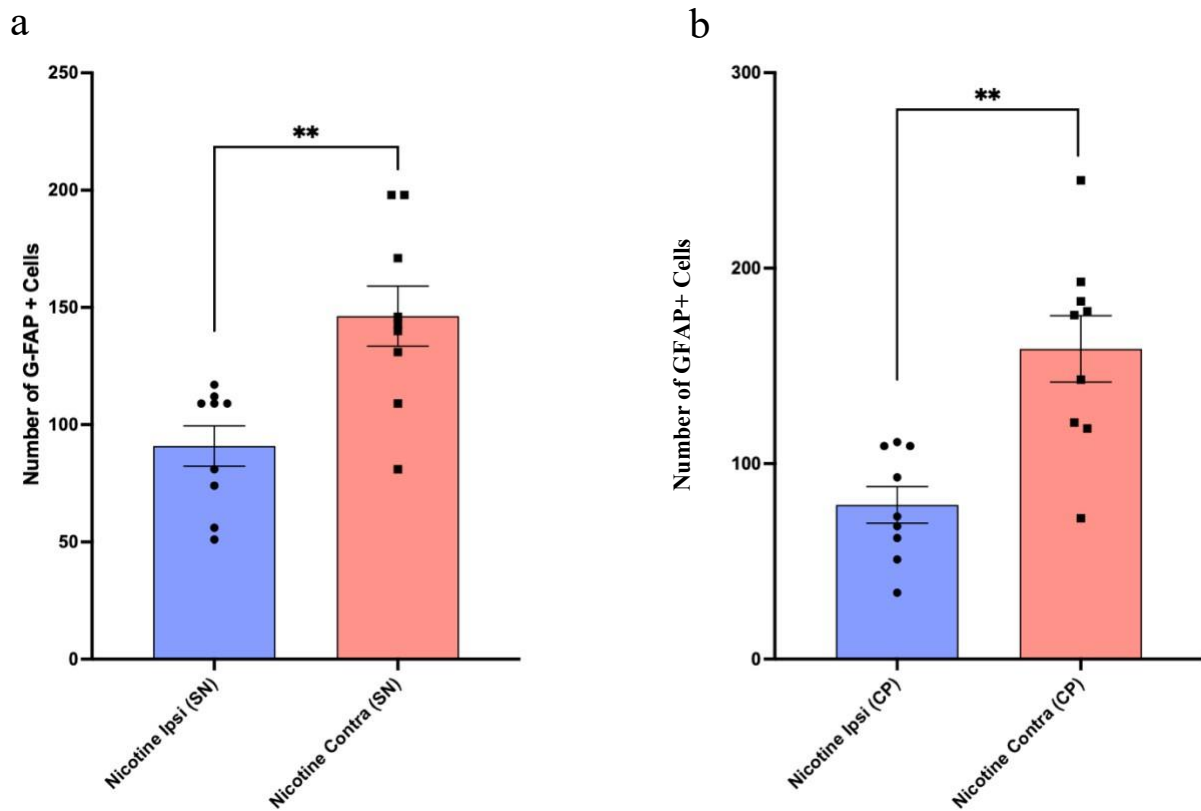


Figure 4.22: Quantification of GFAP positive cells in the SNpc and GFAP optical density, expressed in arbitrary units, in the striatum of nicotine treated rats, comparing the control and lesioned hemispheres. (a) Significant increase in the number of GFAP positive cells in the lesioned substantia nigra of rats. (b) Significant increase in GFAP optical density in the lesioned striatum compared to the unlesioned hemisphere. * $P < 0.05$, ** $P < 0.01$ (two-way ANOVA followed by Tukey's *post hoc* test).

4.3.8.3 Varenicline treated group

In the group treated with varenicline ($n = 10$), the severity of AIMs varied: one animal showed no signs of AIMs, one exhibited a partial response, and eight animals developed pronounced AIMs. A modest yet statistically significant elevation was detected in the animal with partial AIM expression ($p = 0.0027$). Animals with fully developed AIMs demonstrated increased astrocyte activation, although the response was lower compared to that in the saline treated group ($p = 0.0122$; Figure 4.24).

GFAP staining in the striatum showed corresponding changes. An increased GFAP positive astrocyte density was observed in the lesioned striatum of the animal with partial AIM

expression ($p = 0.0198$). However, In animals with robust AIMs, the significance of upregulation of GFAP signal was not detectable (Figure 4.25).

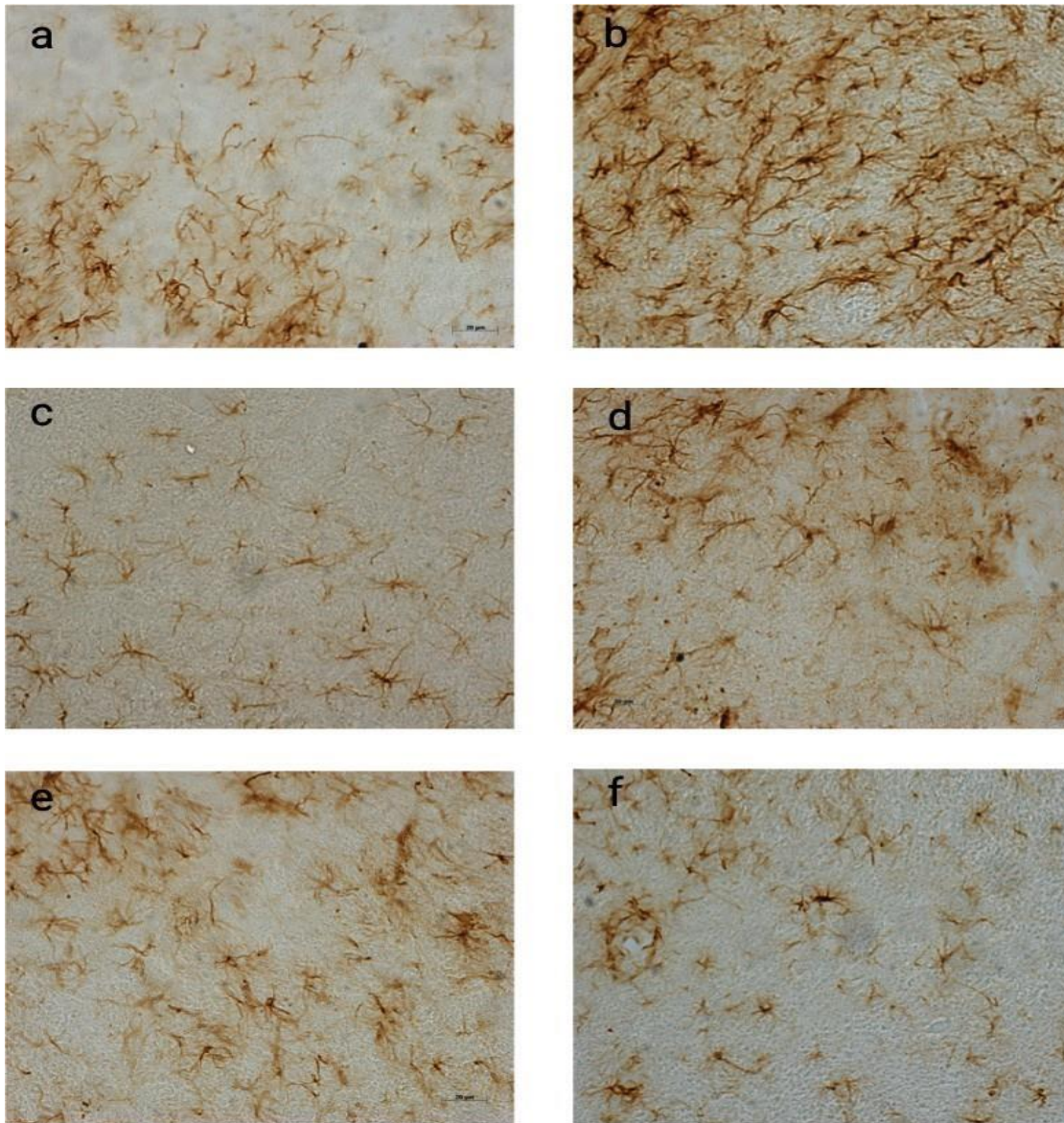


Figure 4. 23: GFAP-ir immunostaining in the SNpc of the varenicline treated group, comparing the non-lesioned and lesioned hemispheres in animals with varying severities of AIMs. (a–b) GFAP-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e– f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 40× magnification. Scale bar = 20 μ m.

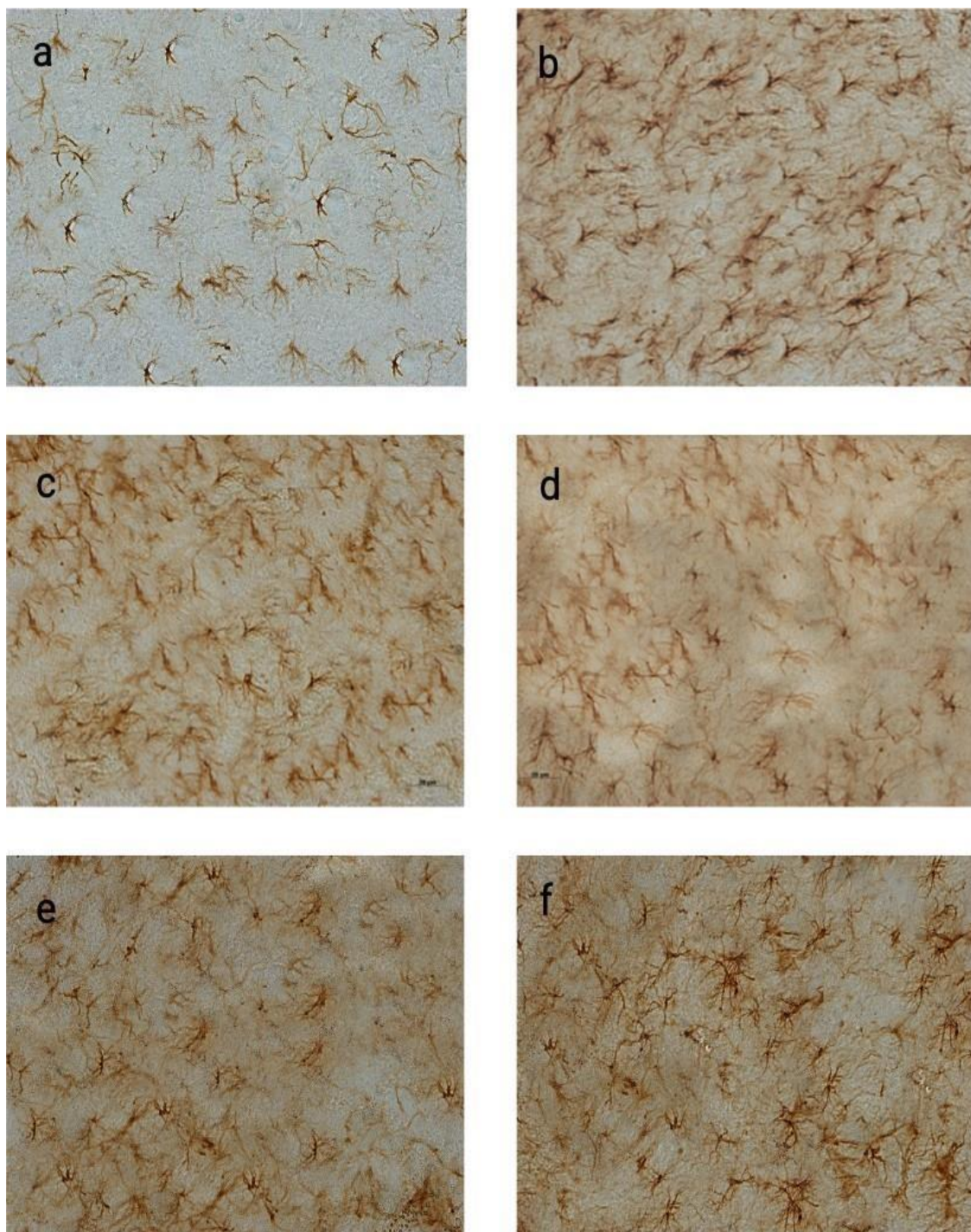


Figure 4. 24: GFAP-ir immunostaining in the striatum of the varenicline treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) GFAP-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 20× magnification. Scale bar = 20 μ m.

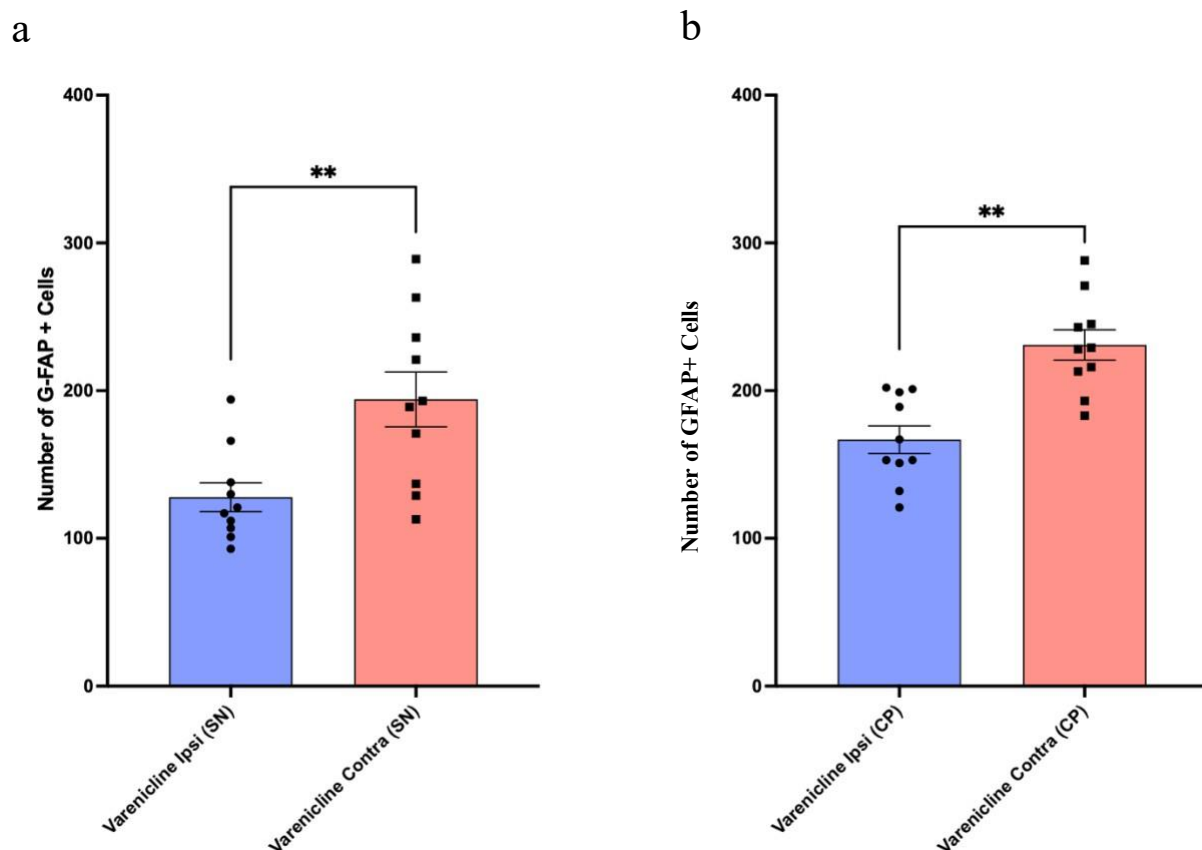


Figure 4. 25: Quantification of GFAP positive cells in the SNpc and GFAP optical density, expressed in arbitrary units, in the striatum of varenicline treated rats, comparing the control and lesioned hemispheres. (a) Significant increase in the number of GFAP positive cells in the lesioned substantia nigra of rats. (b) Corresponding elevation in GFAP optical density in the lesioned striatum compared to the unlesioned hemisphere. * $P < 0.05$, ** $P < 0.01$ (two-way ANOVA followed by Tukey's *post hoc* test).

4.3.9: 6-OHDA lesioning causes glial activation of levodopa induced dyskinetic rats in the striatum and substantia nigra (IBA-1)

Immunohistochemical analysis of ionised calcium binding adaptor molecule 1 (IBA-1) expression was performed to assess microglial activation in the substantia nigra pars compacta and striatum following unilateral 6-OHDA lesioning and subsequent levodopa priming. IBA-1 immunoreactivity was evaluated in animals from the saline, nicotine, and varenicline treatment groups, with each group further stratified according to abnormal involuntary movement severity into three behavioural categories: no AIMs, moderate AIMs, and fully

developed AIMs.

In the saline treated group, pronounced microglial activation was observed in the lesioned hemisphere, characterised by increased IBA-1 staining intensity and density in both the SNpc and striatum compared with the unlesioned side. This neuroinflammatory response was consistently present across all AIM subgroups, with the strongest activation typically evident in animals with fully developed AIMs.

By contrast, animals treated with nicotine or varenicline displayed a more reduced microglial response. IBA-1 expression between the lesioned and unlesioned hemispheres appeared more balanced, and microglial activation was less marked across the AIM subgroups. This reduction in neuroinflammation suggests that nicotine and varenicline treatment may lower the gliotic response associated with levodopa induced dyskinesia (Figures 4.27 to 4.35).

4.3.9.1 Saline treated group

In the saline treated group ($n = 10$), which received levodopa and benserazide following unilateral 6-OHDA lesioning, animals displayed varying degrees of AIMs. All animals were included in the analysis of microglial activation using IBA-1 immunohistochemistry.

Immunohistochemical quantification of IBA-1 revealed a lesion dependent and severity graded microglial response in the substantia nigra pars compacta. Compared with the contralateral, non-lesioned hemisphere, IBA-1 immunoreactivity in the lesioned SNpc was significantly elevated even in animals that exhibiting partial AIMs showed a larger and highly significant increase in IBA-1 expression ($p = 0.0003$). This effect was most pronounced in animals with fully developed AIMs, in which microglial density in the lesioned SNpc increased markedly ($p < 0.0001$; Figure 4.27).

IBA-1 immunoreactivity in the striatum demonstrated a differential pattern of microglial activation. In animals with moderate AIM expression displayed not significant rise in striatal microglial density. However, this effect was further accentuated in animals with robust dyskinetic behaviour, in which the lesioned striatum exhibited a pronounced increase in IBA-1 labelling compared with the contralateral hemisphere ($p < 0.0001$; Figure 4.28).

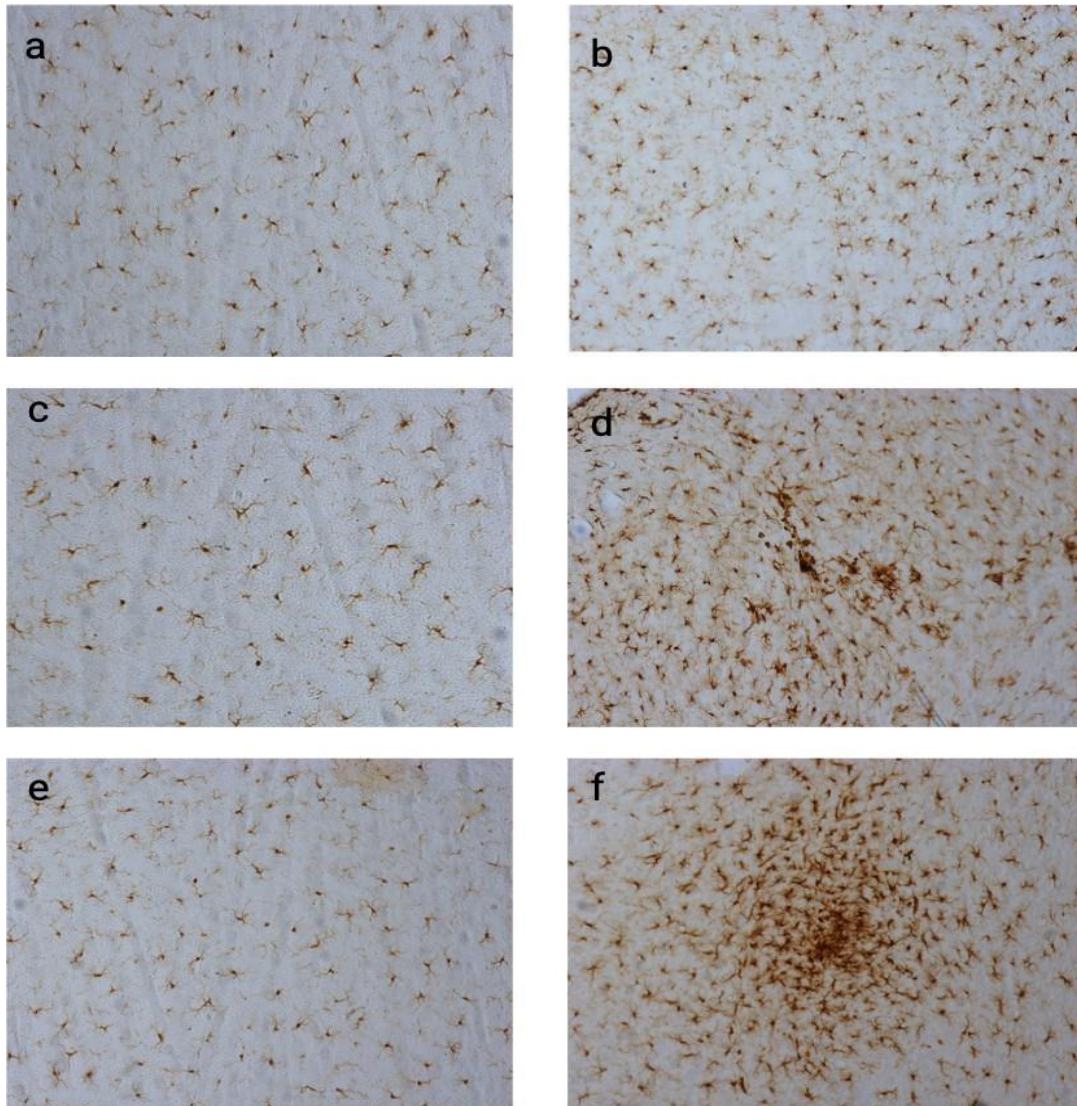


Figure 4. 26: IBA-1 immunostaining in the SNpc of the saline treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) IBA-1-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 40× magnification. Scale bar = 20 μ m.

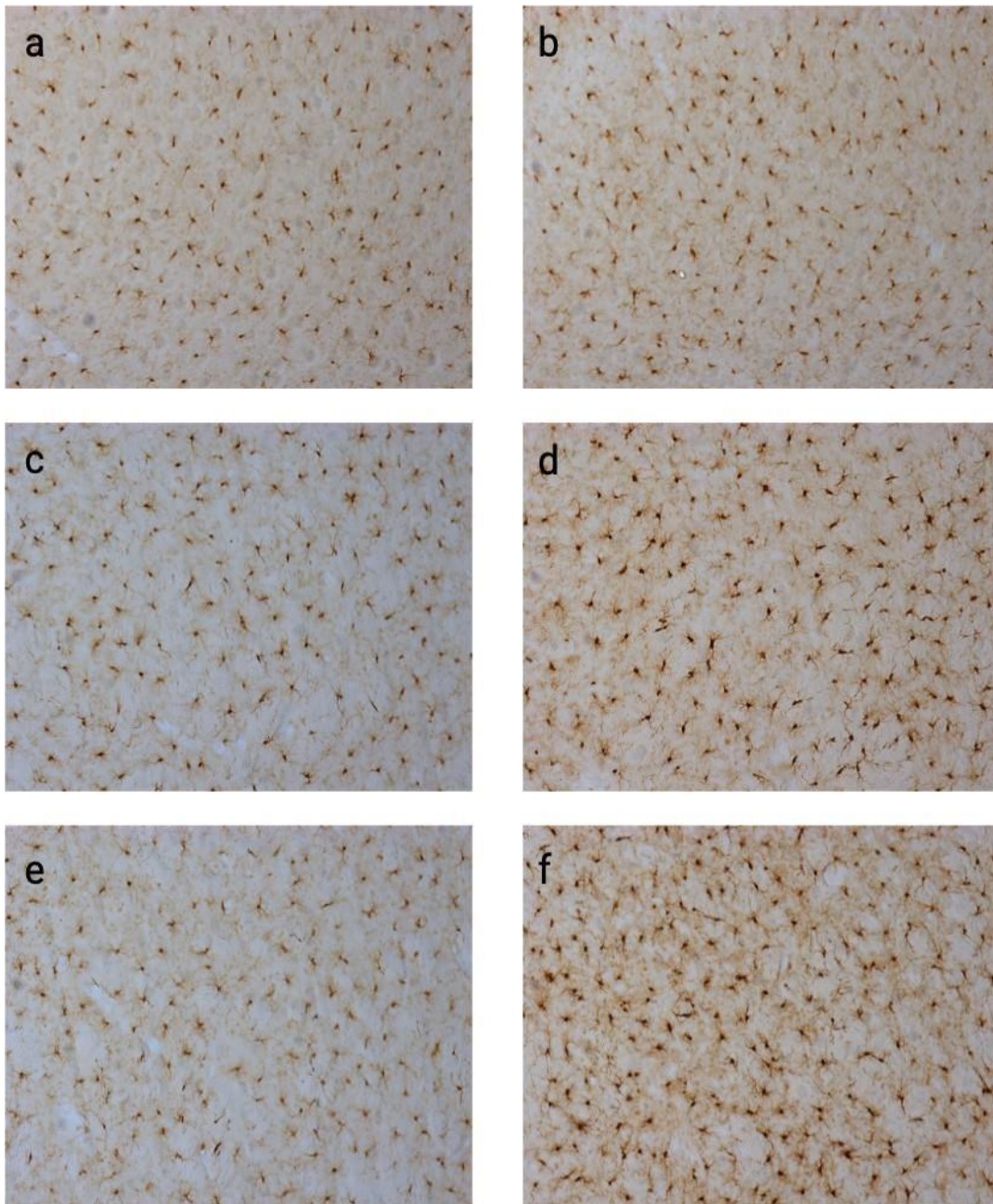


Figure 4. 27: IBA-1 immunostaining in the striatum of the saline treated group, comparing the non-lesioned and lesioned hemispheres in animals with varying severities of AIMs. (a–b) IBA-1-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 40× magnification. Scale bar = 20 μ m

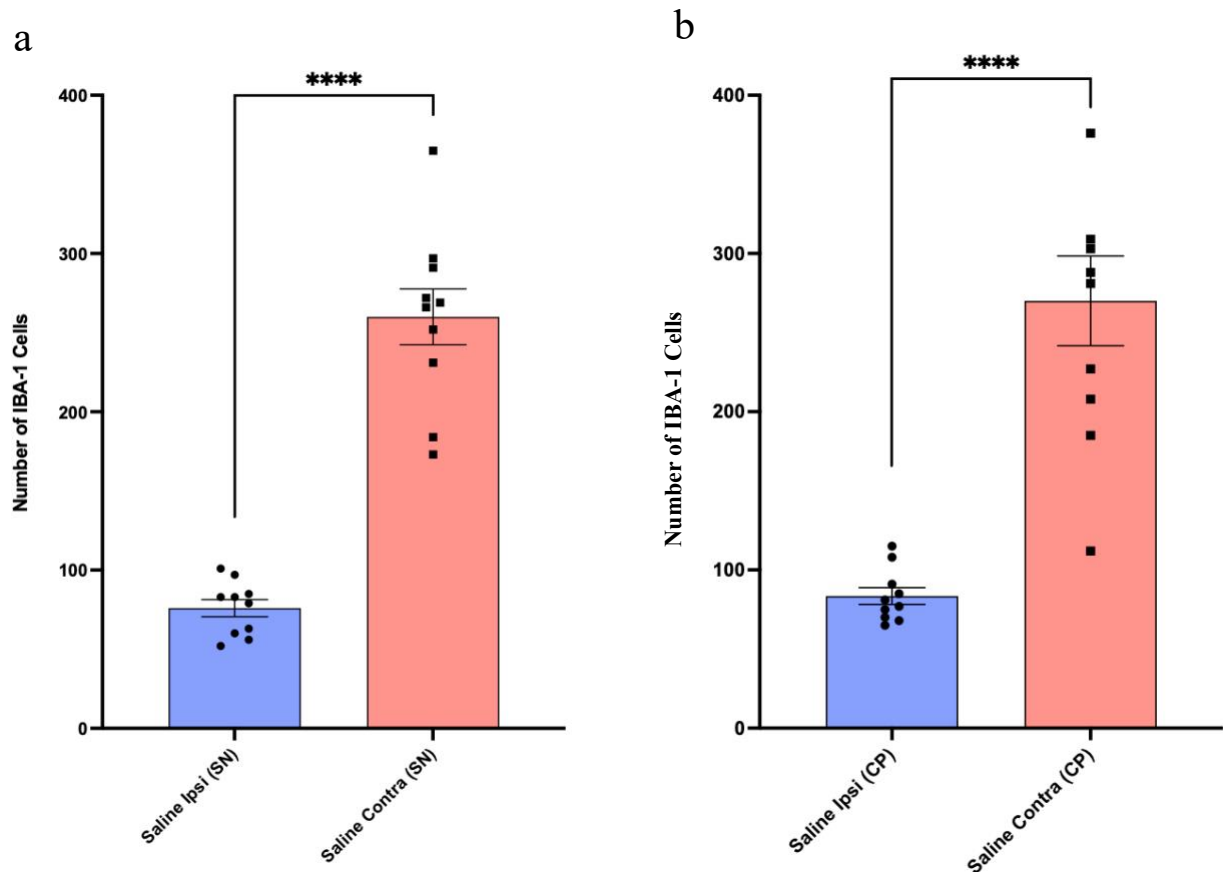


Figure 4.28: Quantification of IBA-1 cells in the SNpc and IBA-1 optical density, expressed in arbitrary units, in the striatum of saline treated rats, comparing the control and lesioned hemispheres in varying severities of AIMs. (a) Significant increase in the number of IBA-1-positive cells in the lesioned substantia nigra of rats with severe AIMs. (b) Corresponding elevation in IBA-1 optical density in the lesioned striatum compared to the unlesioned hemisphere. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (two-way ANOVA followed by Tukey's *post hoc* test).

4.3.9.2 Nicotine treated group

In the nicotine treated group ($n = 9$), IBA-1 immunoreactivity revealed a distinct pattern of microglial activation that differed from that observed in saline treated animals. In animals with moderate AIM expression, a statistically significant increase in microglial activation was observed ($p = 0.0024$). Interestingly, in animals with severe AIMs, no significant enhancement of IBA-1 signal was detected in the lesioned hemisphere. Although microglial presence was still evident, the response appeared reduced relative to that of saline treated animals at the same behavioural stage (Figure 4.30).

In the striatum of nicotine treated rats, the lesioned hemisphere showed a marked elevation in IBA-1 positive cell density in the animal with moderate AIMs compared to the contralateral side ($p = 0.0007$). By contrast, animals with fully developed AIMs demonstrated a significant, though less pronounced, increase in microglial density relative to the contralateral hemisphere ($p = 0.0025$; Figure 4.31).

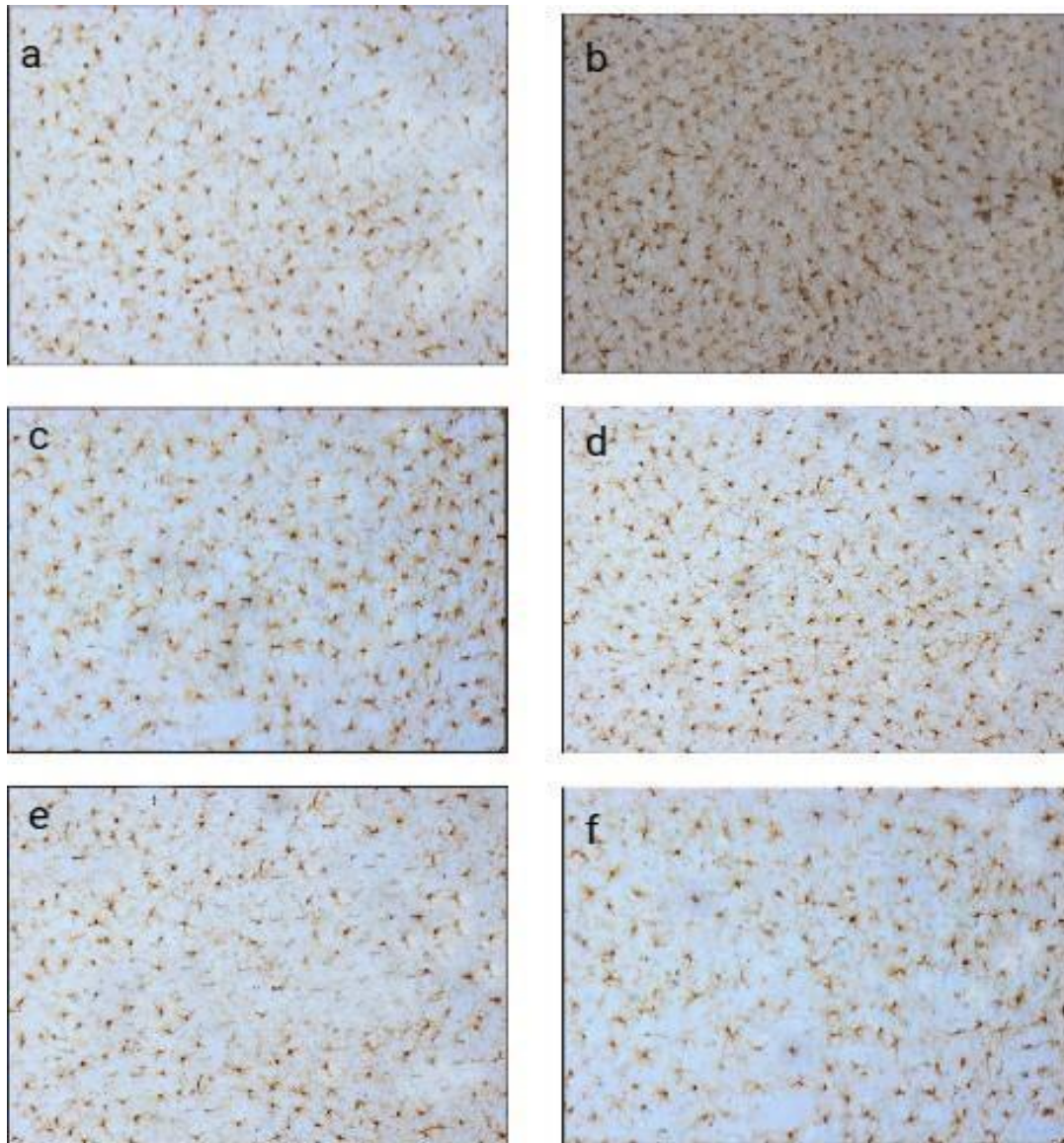


Figure 4. 29: IBA-1 immunostaining in the SNpc of the nicotine treated group, comparing the non-lesioned and lesioned hemispheres in animals with varying severities of AIMs. (a–b) IBA-1-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 40× magnification. Scale bar = 20 μ m.

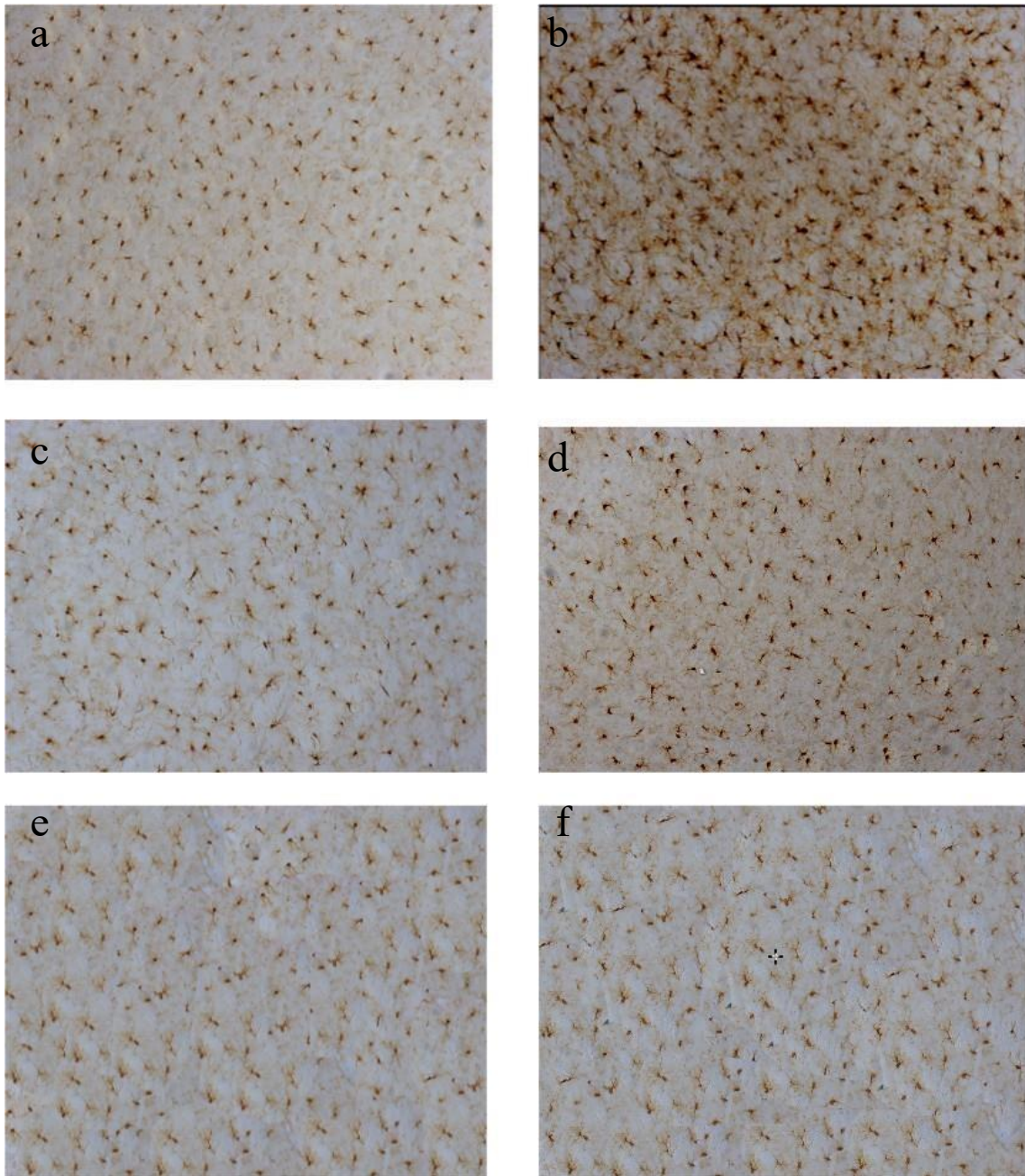


Figure 4.30: IBA-1 immunostaining in the striatum of the nicotine treated group, comparing the non-lesioned and lesioned hemispheres across animals with varying severities of AIMs. (a–b) IBA-1-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c–d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 40× magnification. Scale bar = 20 μ m.

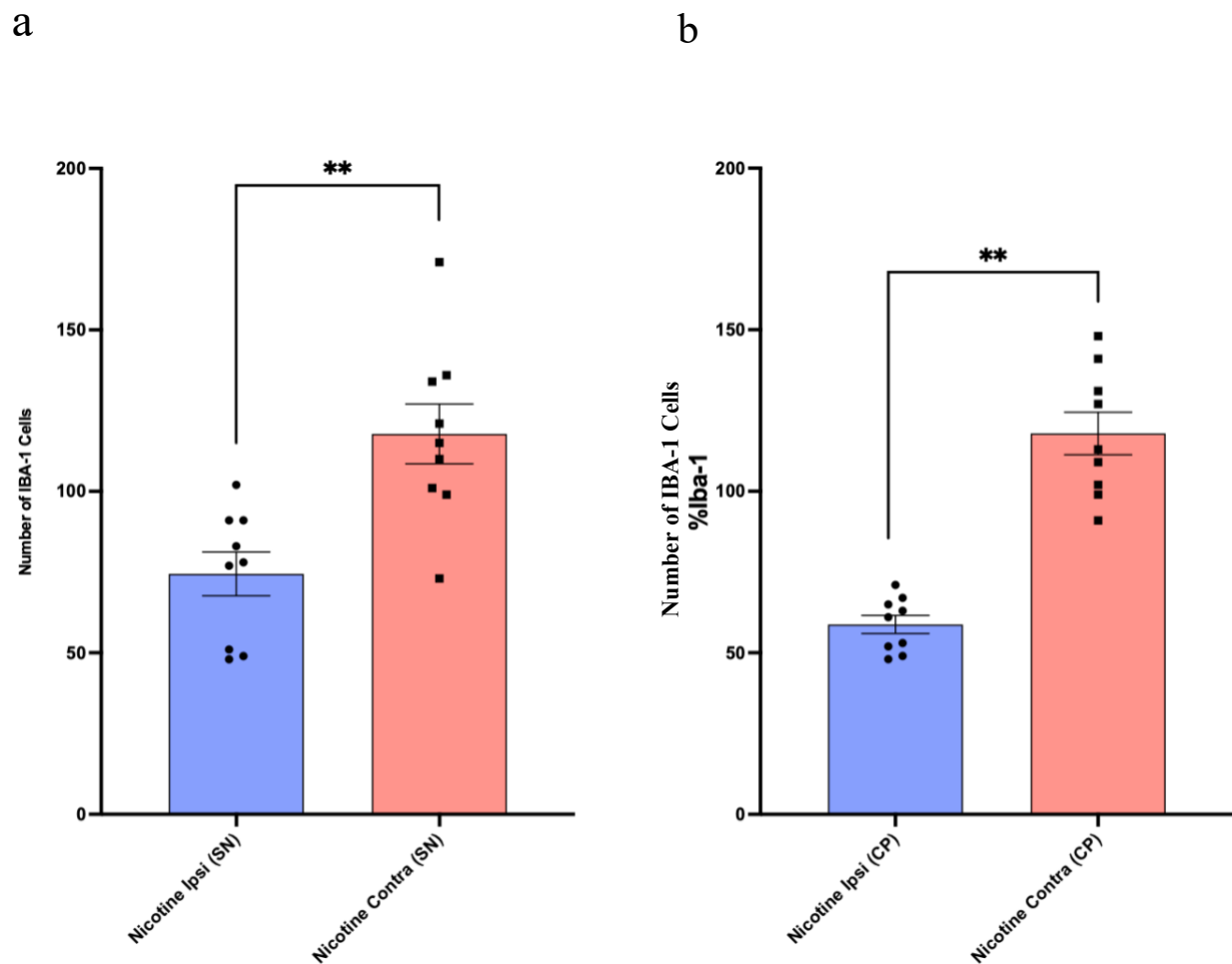


Figure 4.31: Quantification of IBA-1 positive cells in the SNpc and IBA-1 optical density, expressed in arbitrary units, in the striatum of nicotine treated rats, comparing the control and lesioned hemispheres across varying severities of AIMS. (a) Not Significant increase in the number of IBA-1 positive cells in the lesioned substantia nigra of rats with severe AIMS. (b) Elevation in IBA-1 optical density in the lesioned striatum compared to the unlesioned hemisphere. * $P < 0.05$, ** $P < 0.01$, * $P < 0.001$ (two-way ANOVA followed by Tukey's *post hoc* test).**

4.3.9.3 Varenicline treated group

Analysis of IBA-1 immunoreactivity in the varenicline treated cohort ($n = 10$) revealed a graded but comparatively reduced microglial response in the substantia nigra pars compacta (SNpc). Animals with partial AIM expression displayed a significant rise in IBA-1 signal ($p = 0.0289$). However, in animals with fully developed AIMS, microglial density didn't change significantly in the lesioned hemisphere (Figure 4.33).

A similar pattern emerged in the striatum, with varenicline treatment influencing the extent of lesion related microgliosis across behavioural groups. In the moderate dyskinetic animals, the lesioned striatum exhibited not significant elevation in IBA-1 expression compared to the contralateral side. This effect was also similar in those with severe dyskinesia, striatal IBA-1 labelling (Figure 4.34).

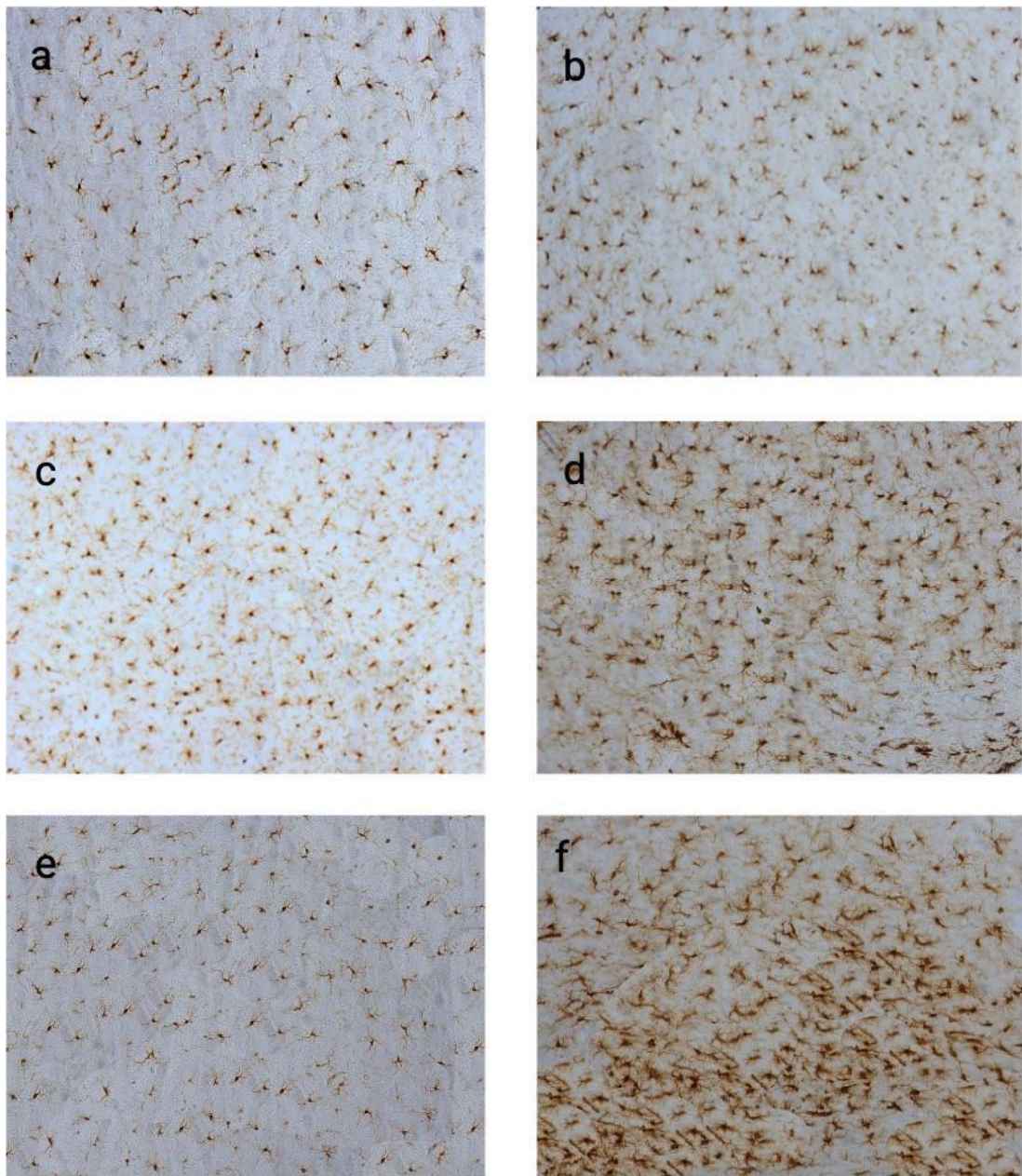


Figure 4. 32: IBA-1-ir immunostaining in the SNpc of the varenicline treated group, comparing the non-lesioned and lesioned hemispheres in animals with varying severities of AIMs. (a–b) IBA-1-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c– d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 40× magnification. Scale bar = 20 μ m.

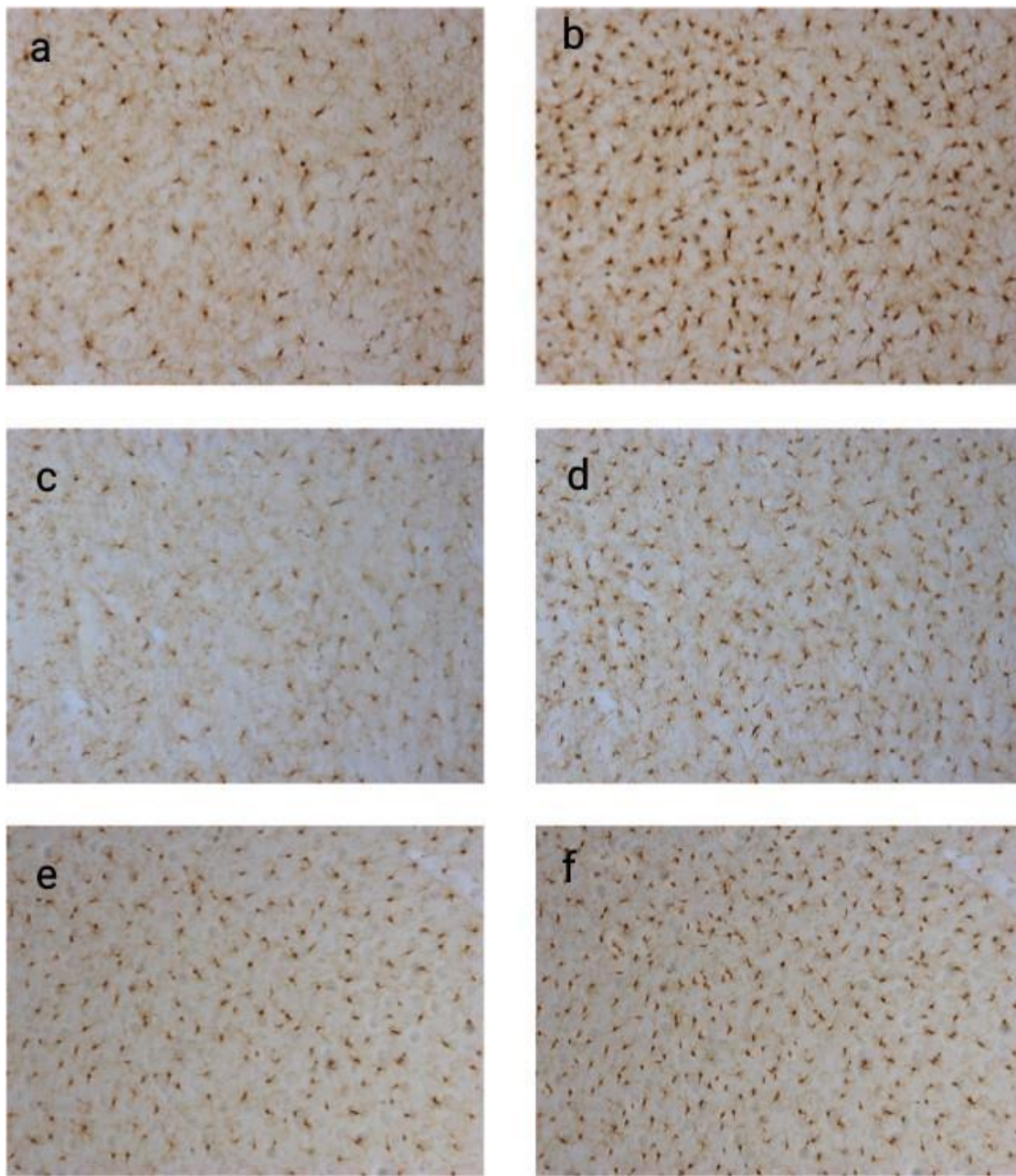


Figure 4.33: IBA-1 immunostaining in the striatum of the varenicline treated group, comparing the non-lesioned and lesioned hemispheres in animals with varying severities of AIMs. (a–b) IBA-1-ir in the non-lesioned (a) and lesioned (b) hemispheres of animals that did not develop AIMs. (c– d) non-lesioned (c) and lesioned (d) hemispheres from animals with partially developed AIMs. (e–f) non-lesioned (e) and lesioned (f) hemispheres from animals with fully developed AIMs. Images captured at 40× magnification. Scale bar = 20 μ m.

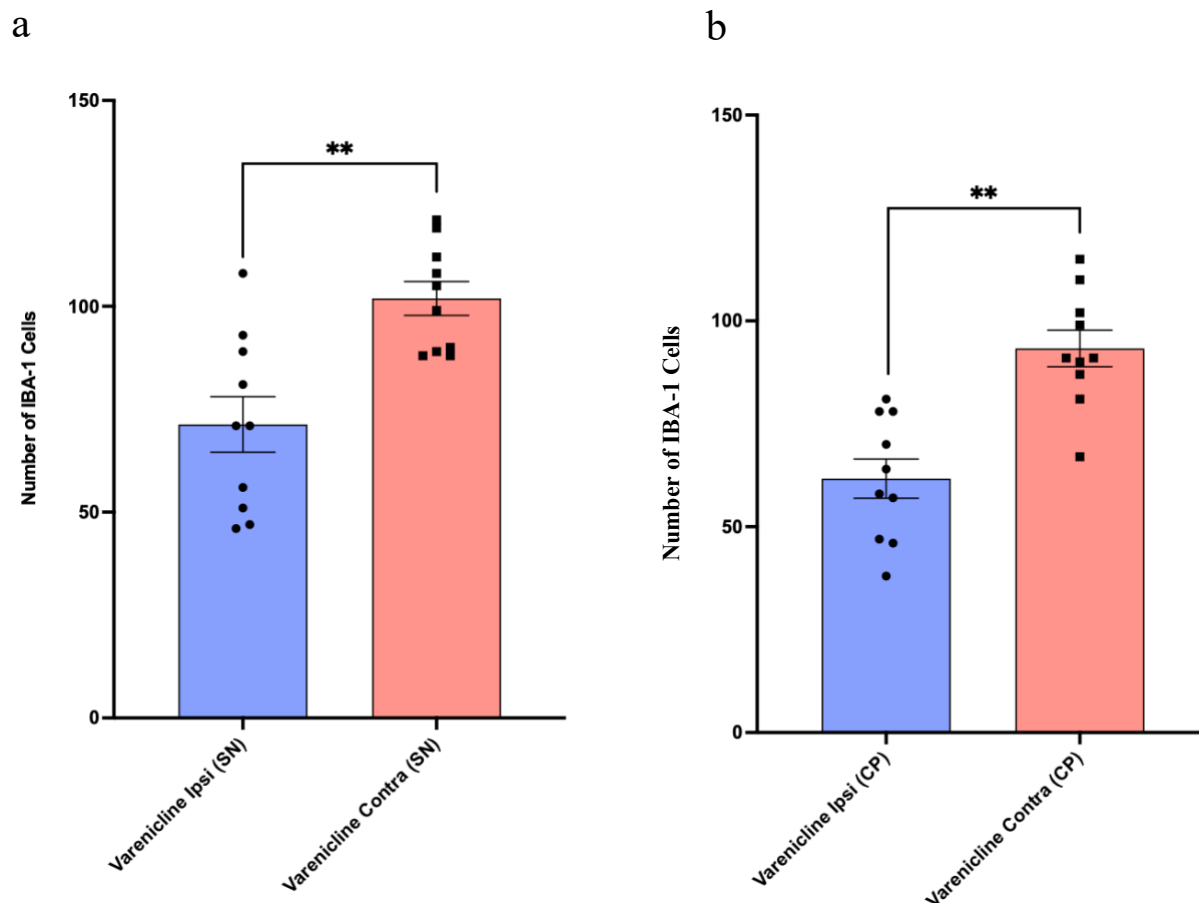


Figure 4. 34: Quantification of IBA-1 positive cells in the SNpc and IBA-1 optical density in the striatum of varenicline treated rats, comparing the control and lesioned hemispheres in varying severities of AIMS. (a) Significant increase in the number of IBA-1-positive cells in the lesioned substantia nigra of rats with severe AIMS. (b) not significant IBA-1 optical density in the lesioned striatum compared to the unlesioned hemisphere. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ (two-way ANOVA followed by Tukey's *post hoc* test).

4.4 Discussion

This study examined whether nicotinic acetylcholine receptor modulators, nicotine and varenicline administered during the early course of levodopa therapy, could reduce the development of levodopa induced AIMs in a 6-hydroxydopamine rat model of Parkinson's disease. While the antidyskinetic properties of these compounds have been previously demonstrated when given after dyskinesia is established (Quik et al., 2007; Bordia et al., 2008; Quik et al., 2013), the current findings provide evidence that targeting nicotinic receptors during the priming phase can prevent or reduce the initial pathological changes that give rise to LID.

In the saline treated group, repeated levodopa exposure led to a progressive increase in abnormal involuntary movements, reflecting the classical priming process associated with maladaptive plasticity in the striatum. These findings align with previous chapter demonstrating that pulsatile dopaminergic stimulation in the presence of nigrostriatal denervation sensitises motor circuits, leading to exaggerated responses upon subsequent levodopa administration (Cenci & Lundblad, 2006; Bastide et al., 2015).

Both nicotine and varenicline significantly suppressed the emergence and severity of AIMs across all behavioural subtypes axial, limb, and orolingual compared to the control group. Notably, reductions in AIMs were evident from the early sessions, suggesting that nAChR engagement during the sensitisation phase is capable of interrupting the molecular and synaptic changes that support LID development (Quik & Wonnacott, 2011). These results are consistent with earlier studies showing that $\beta 2$ -containing nAChRs particularly the $\alpha 4\beta 2$ and $\alpha 6\beta 2$ subtypes play a key role in modulating striatal dopamine release and can influence motor output when activated pharmacologically (Gotti et al., 2006; Quik et al., 2008).

Nicotine, a non-selective full agonist at multiple nAChR subtypes, produced a robust and consistent suppression of AIMs across sessions. Its mechanism of action likely involves activation followed by desensitisation of $\alpha 4\beta 2$ and $\alpha 6\beta 2$ receptors, stabilising dopaminergic signalling in the striatum and reducing the aberrant potentiation of corticostriatal pathways associated with dyskinesia (Quik et al., 2013). In addition, nicotine has been shown to modulate glutamatergic and GABAergic transmission within basal ganglia circuits (Quik & Wonnacott, 2011), which may further contribute to the observed behavioural stabilisation.

Varenicline, while also effective, displayed a distinct behavioural profile. As a partial agonist at $\alpha 4\beta 2$ and $\alpha 6\beta 2$ receptors, and a full agonist at $\alpha 7$ nAChRs, its mechanism of action involves more targeted receptor engagement (Mihalak et al., 2006; Bordia et al., 2012). The involvement of $\alpha 7$ receptors, which have been implicated in neuroinflammatory regulation and neuroprotection, may contribute to the broader neuromodulatory effects observed in this study (Zhang et al., 2013). The anti-inflammatory properties of $\alpha 7$ activation have previously been linked to improved neuronal resilience and reduced glial reactivity in neurodegenerative contexts (Shytle et al., 2004), supporting a plausible mechanism for varenicline's observed glial-suppressive effects.

Histological assessments further reinforced the behavioural findings. In the saline group, animals with pronounced AIMs exhibited extensive tyrosine hydroxylase loss in the substantia nigra pars compacta and reduced dopaminergic terminal density in the striatum. These results are in line with prior work showing that the severity of nigrostriatal degeneration is a strong predictor of dyskinesia vulnerability (Carta et al., 2008; Johnson et al., 2010). In contrast, several animals treated with nicotine or varenicline particularly those with little or no dyskinesia showed better preservation of TH-positive cells and terminals, suggesting a potential neuroprotective effect of early nAChR engagement.

Immunohistochemical analyses of glial markers provided additional mechanistic insight. GFAP and IBA1 staining revealed substantial astrocytic and microglial activation in

the lesioned hemispheres of saline treated rats, especially those with severe AIMs. This glial reactivity is consistent with previous reports implicating neuroinflammation in the pathogenesis of LID (Bastide et al., 2015; Morissette & Di Paolo, 2018). Both nicotine and varenicline reduced gliosis in the SNpc and striatum, with varenicline treated animals showing a particularly marked suppression of astrocytic activation. These effects may arise from $\alpha 7$ receptor activation, which modulates inflammatory responses by inhibiting pro-inflammatory cytokine release via the JAK2–STAT3 and NF- κ B pathways (Shytle et al., 2004; Wang et al., 2003). Thus, beyond dopaminergic modulation, nicotinic agents may exert therapeutic effects by altering the inflammatory milieu within vulnerable brain regions.

Although both drugs produced significant reductions in AIMs, their efficacy trajectories were not identical. Nicotine's effects appeared more stable across sessions, whereas varenicline-treated animals showed a transient elevation in orolingual AIMs early in the priming period. This may reflect differences in receptor desensitisation dynamics, downstream signalling, or pharmacokinetics. Nicotine's rapid desensitisation of $\beta 2$ -containing receptors may offer immediate stabilisation of synaptic transmission, while varenicline's partial agonist profile may result in more graded or delayed modulation. Nonetheless, both compounds demonstrated clear potential as pre-dyskinetic interventions.

Despite the promising results, several limitations should be noted. The study used a single dose of each compound and moderate group sizes, which may limit detection of subtle or dose-dependent effects. Moreover, AIMs scoring, while standardised, does not capture potential cognitive, emotional, or non-motor effects that may also be influenced by nAChR modulation. It remains uncertain whether the observed neuroprotective and anti-inflammatory effects are a direct consequence of drug action or reflect reduced dyskinetic severity. Finally, species differences in receptor distribution and pharmacodynamics must be considered before translating these findings to clinical settings.

4.5 Conclusion

In summary, this study provides compelling evidence that nicotine and varenicline, when administered during the early phase of levodopa treatment, can substantially reduce the development of LID. These results extend the therapeutic potential of nicotinic agents beyond symptomatic control and highlight their capacity to interfere with early plasticity and inflammatory cascades that underlie dyskinesia onset. By targeting $\beta 2$ -containing and $\alpha 7$ nAChRs, these compounds offer a dual mechanism, modulation of dopaminergic signalling and suppression of neuroinflammatory responses that may be critical in preventing motor complications in PD. These findings support the rationale for exploring nicotinic receptor agonists as early adjunctive therapies and provide a foundation for refining pharmacological strategies aimed at preventing LID in patients with Parkinson's disease.

Chapter 5

Effects of $\alpha 4\beta 2$ Nicotinic Receptor Modulation in AIMs in Unilaterally 6-OHDA Lesioned Rats

5.1 Introduction

Previous chapters demonstrated that nAChR agonists such as nicotine and varenicline can reduce LID in the 6-hydroxydopamine rat model of Parkinson's disease. Nicotine was effective when administered after LID had been established, while varenicline reduced the development of AIMs when given during the levodopa priming phase. Both agents achieved these effects without compromising levodopa's antiparkinsonian action. Together, these findings identified nAChRs, particularly $\beta 2$ containing subtypes such as $\alpha 4\beta 2$ and $\alpha 6\beta 2$, as key modulators of striatal dopaminergic function and dyskinetic behaviour. However, because both nicotine and varenicline act at multiple receptor subtypes, the specific mechanisms responsible for their antidyskinetic effects remain unclear.

Among $\beta 2$ containing receptors, the $\alpha 4\beta 2$ subtype is of particular interest due to its high expression in the striatum and its central role in regulating dopamine release from nigrostriatal terminals. Preclinical work suggests that selective modulation of $\alpha 4\beta 2$ nAChRs contributes significantly to nicotine's ability to reduce LID, yet the extent to which different modes of receptor engagement (activation, inhibition, or modulation) affect dyskinesia remains unknown. This chapter addresses that question directly by examining the role of $\alpha 4\beta 2$ nAChRs in LID using pharmacological tools with distinct mechanisms of action.

A85380 is a highly selective $\alpha 4\beta 2$ nAChR agonist with nanomolar affinity, making it a precise agent for evaluating the effects of $\alpha 4\beta 2$ activation. A85380 enhances dopaminergic neurotransmission and modulates dopamine release without significant activity at other nAChR subtypes (Baddick & Marks, 2011; Gotti et al., 2009). In preclinical studies, it exhibits high receptor subtype specificity, which minimises off-target effects and allows for controlled activation of the $\alpha 4\beta 2$ pathway. This selectivity provides an opportunity to assess whether

targeted receptor stimulation can produce antidyskinetic effects comparable to those seen with non-selective agonists such as nicotine, but through a cleaner mechanism.

DH β E (dihydro- β -erythroidine) is a potent, selective competitive antagonist of α 4 β 2 nAChRs and is widely used to define the functional role of this receptor subtype in the central nervous system (Harvey & Luetje, 1996; Gotti et al., 2009). By blocking α 4 β 2 activity, DH β E helps determine whether tonic receptor signaling contributes to the modulation of dyskinetic movements or dopamine release. Its inclusion provides an important control, clarifying whether the behavioral effects of agonists are indeed mediated through α 4 β 2 receptors.

NS9283 is a positive allosteric modulator (PAM) that selectively enhances α 4 β 2 receptor activity without directly activating the receptor. This mechanism enables NS9283 to potentiate the receptor's response to endogenous acetylcholine, producing more physiologically relevant and sustained modulation of synaptic transmission (Grupe et al., 2013; Olsen et al., 2013). Unlike full agonists, PAMs maintain receptor sensitivity and reduce the likelihood of desensitisation, offering a potentially safer therapeutic approach. Examining NS9283's effects allows the evaluation of whether indirect potentiation of α 4 β 2 receptors can reproduce the antidyskinetic outcomes seen with direct agonists.

Sazetidine-A is a partial agonist with high selectivity for α 4 β 2 nAChRs. It binds to and partially activates the receptor while promoting desensitisation, producing a functional state distinct from full agonism (Marks et al., 2007; Xiao et al., 2006). This pharmacological profile allows controlled modulation of receptor activity and may mimic the adaptive desensitisation associated with chronic nicotine exposure. Preclinical evidence shows that Sazetidine-A can alter cholinergic - dopaminergic interactions, making it a useful tool for assessing whether partial activation and receptor desensitisation are sufficient to reduce dyskinesia.

5.1.1 Aims and objectives

The aim of this chapter is to define how $\alpha 4\beta 2$ nicotinic acetylcholine receptors shape the expression of abnormal involuntary movements. To address this, the work is structured around three objectives: first, to establish and utilise the AIMS platform to examine dyskinesia in relation to nicotinic receptor function; second, to characterise the behavioural effects of selective pharmacological ligands targeting $\alpha 4\beta 2$ receptors during levodopa induced AIMS; and third, to conduct histological and pharmacological follow up studies to determine how these interventions influence receptor activity, local circuitry and downstream signalling associated with maladaptive plasticity.

Together, these objectives build on the findings of Chapters 3 and 4 by moving from broad nicotinic receptor involvement to a more defined, subtype specific framework. The novelty of this chapter lies in its focus on isolating $\alpha 4\beta 2$ receptor contributions and critically in distinguishing between different modes of receptor engagement rather than treating receptor activity as a uniform process. This approach addresses a key gap in the field by examining whether specific receptor states, rather than receptor presence alone, underlie nicotinic modulation of dyskinesia. In doing so, it provides a more precise mechanistic basis for evaluating $\alpha 4\beta 2$ nAChRs as targets for future nicotinic based therapies in Parkinson's disease.

5.2 Material and Methods

5.2.1 *Animals*

A cohort of 34 female Sprague Dawley rats (Sprague Dawley, UK), each weighing between 250–300 g, was allocated into two experimental groups. Five rats served as saline treated controls, while the remaining 29 rats received 6-OHDA. Animals were housed two per cage in standard plastic cages and maintained on a 12:12 hour light/dark cycle (lights on at 07:00, off at 19:00) in a temperature-controlled room (20 ± 1 °C). Food and water were available ad libitum.

5.2.2 Unilateral 6-OHDA Lesions

All surgical procedures, including acclimation, anesthesia, preparation of 6-OHDA, stereotaxic injections, post-operative care and analgesia, followed the protocol described in Chapter 2 (see Section 2.4 for full methodological details). All animals recovered uneventfully, and no adverse effects were observed. The complete procedural timeline is illustrated in Figure 5.1.

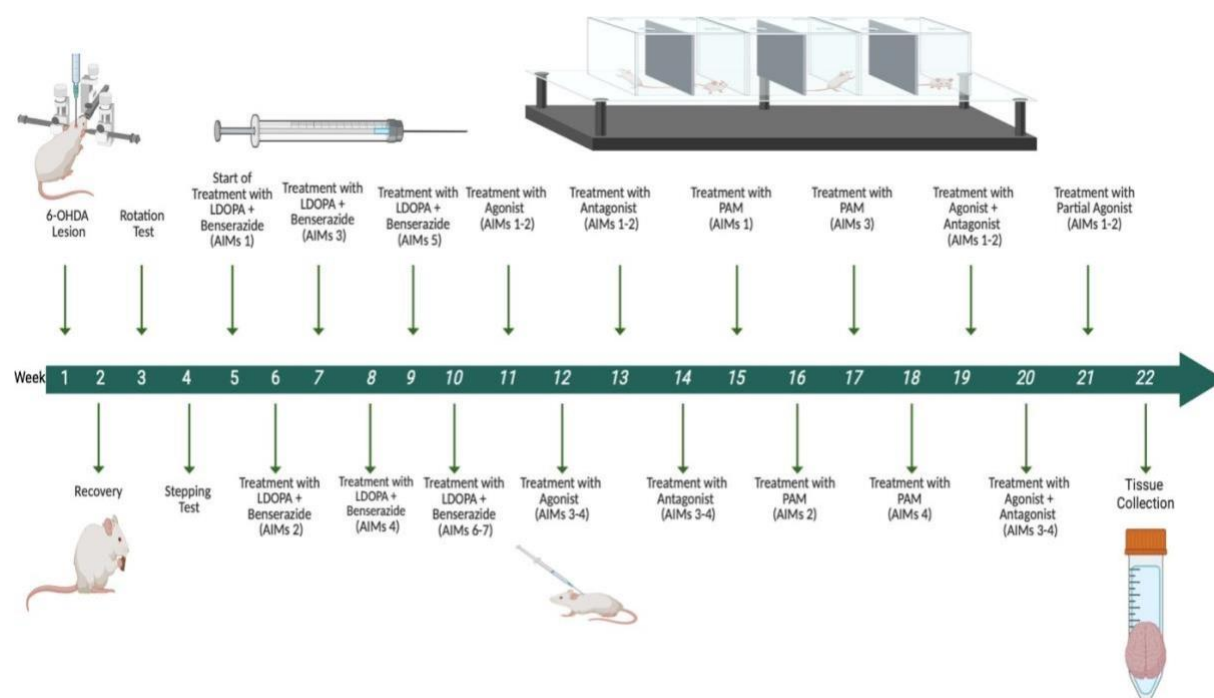


Figure 5. 1: Experimental timeline for the preparation of levodopa primed rats and the assessment of AIMs. Rats were unilaterally lesioned with (6-OHDA; 8 μ g) and allowed to recover for two weeks. In week 3, lesion efficacy was verified using amphetamine induced (5.0 mg/kg) and apomorphine induced (0.5 mg/kg) rotational behaviour tests, followed by stepping tests in week 4. From week 5, all animals received daily injections of levodopa (8 mg/kg) combined with benserazide (15 mg/kg) for five consecutive weeks. AIMs scoring began in the first week of levodopa administration and continued twice weekly until all animals were reliably primed. In week 11, twelve rats were tested with the α 4 β 2 agonist A85380 for two weeks (four AIM sessions). From week 13, the same cohort was tested with the α 4 β 2 antagonist Dh β E for two weeks (four AIM sessions, twice weekly). From week 15, animals were tested with the positive allosteric modulator NS9283 for four sessions, administered once weekly to allow sufficient washout between doses. In week 19, rats were tested with a combined agonist (A85380) and antagonist (Dh β E) treatment for four AIM sessions (twice weekly). Finally, in week 21, animals received the partial agonist Sazetidine-A for 1 week (two AIM sessions, twice weekly). At week 22, all animals were terminally perfused, and brain tissue was collected for subsequent immunohistochemical analysis.

5.2.3 Assessment of 6-OHDA induced Motor Asymmetry (rotational behaviour)

Rotational behaviour was assessed using the method described in Chapter 2.6. Unilateral 6-OHDA lesioned rats were tested with amphetamine or apomorphine and those displaying at least 6 ipsiversive or contraversive turns per minute were classified as fully lesioned. Of the 34 rats that received unilateral 6-OHDA lesions, 12 met this criterion and were included in the experimental cohort.

5.2.4 Assessment of 6-OHDA induced Motor Asymmetry (stepping test)

Following priming, all lesioned animals were assessed for forelimb akinesia using the stepping test, as described in Chapter 2, Section 2.5.

5.2.5 Levodopa Priming for Inducing Abnormal Involuntary Movements

Abnormal involuntary movements were assessed once weekly for five weeks using a modified ALO (axial, limb, orolingual) rating scale, as described in Chapter 2 (Section 2.7). At each observation time point following levodopa administration, AIM subtypes were scored on a severity scale at regular intervals. For each animal, subtype scores were summed across all time points to generate cumulative scores for axial, limb, and orolingual AIMs, with the total ALO score representing the combined sum of all three subtypes across the full observation period. Only animals that met the predefined lesion criteria and subsequently developed stable dyskinesia were included in behavioural analyses (n = 12).

5.2.6 Experimental Protocol

Experimental procedures for assessing drug effects on levodopa induced dyskinesia followed the methods described in Section 3.2.5.

5.2.7 Drugs

Levodopa methyl ester [L-DOPA methyl ester; 8 mg/kg; Tocris; Biotechnie, UK] and benserazide [Benserazide - HCL; Tocris; Biotechnie, UK] were dissolved in 5% w/v sucrose prepared in deionised water and administered orally in combination (8.0 mg/kg levodopa plus 15.0 mg/kg benserazide). The dosing point was designated as time 0 min.

A85380 [3-[2(S)-azetidinylmethoxy]pyridine dihydrochloride; Sigma, UK] served as the agonist. The compound was dissolved in saline and delivered subcutaneously using a Latin square schedule across four groups of rats ($n = 3$ per group). Doses tested were 0.001, 0.003, 0.01, and 0.03 mg/kg.

To investigate antagonism, dihydro- β -erythroidine hydrobromide (DH β E) was prepared in saline and injected intraperitoneally. Twelve rats were assigned to four cohorts ($n = 3$ each) that received 1, 3, or 10 mg/kg, or saline as control. A Latin square design ensured each animal was systematically exposed to all treatment conditions.

A combined treatment paradigm was then implemented, in which the same cohort of twelve rats underwent two sessions testing A85380 (0 or 0.01 mg/kg) in combination with DH β E (0 or 1 mg/kg). The study was double blinded, and treatment order was counterbalanced with a Latin square arrangement so that every rat experienced each drug combination.

For experiments with the positive allosteric modulator NS9283 [(S)-3-(3,5-dichlorophenyl)-3-(pyridin-3-yl)propionamide], escalating doses of 0.3, 1, and 3 mg/kg were administered intraperitoneally. The compound was formulated in a vehicle consisting of 1% DMSO, 30% PEG-300, and 1% Tween 80. All dosing followed a double blind, Latin square schedule in twelve rats.

Finally, desensitisation effects were assessed with Sazetidine-A [(E)-N-methyl-4-(5-(pyridin-3-yl)pyridin-3-yl)but-3-en-1-amine; Bio-Techne, UK]. Twelve animals received

subcutaneous injections of either 0 or 1 mg/kg across two sessions. A double blind, Latin square design was applied to balance dosing order, ensuring each rat experienced both treatment conditions.

A sufficient washout period was implemented between successive drug administrations to minimise potential carryover effects. This interval exceeded the expected pharmacokinetic clearance of all compounds tested, based on their reported half-lives, ensuring that each treatment condition was pharmacologically independent. As such, while a Latin square design was employed to structure dosing order, additional counterbalancing to control for residual drug effects was not required.

Table 5. 1: Range of orthostatic and allosteric nAChR ligands to be evaluated.

Drug	Action at receptor	Doses (mg/kg)
A85380	Agonist	0.001, 0.003, 0.01, 0.03
Dh β E	Antagonist	1.0, 3.0 , 10.0
NS9283	Positive Allosteric Modulator	0.3, 1.0 , 3.0
Sazetidine-A	Agonist Desensitizer	0, 1.0

5.2.8 Behavioural Assessments

Abnormal Involuntary Movements were evaluated twice a week with regards to the washing period of the drugs, using a rat dyskinesia scale fully describe in chapter 2.

5.2.9 Histological Analysis

All immunohistochemical procedures followed the methods described in Chapter 2. TH staining was performed using the same dilutions noted in Section 3.2.8. GFAP and IBA1 staining were carried out with primary antibodies at 1:5000 and 1:5000, respectively, followed by standard secondary labelling and DAB development. Quantification of TH, GFAP and IBA1 (optical density, area fraction and cell counts) was conducted in ImageJ, and statistical analysis followed the procedures outlined in Chapter 2.

5.2.10 Statistical Analysis

Statistical analyses followed the procedures outlined in Section 3.2.9.

5.3 Results

5.3.1 *Amphetamine induced ipsiversive rotation test*

The assessment of amphetamine induced rotational behaviour was carried out 10 days after 6-hydroxydopamine lesioning to evaluate the degree of dopaminergic neuronal loss. Rats were administered (+)-amphetamine (5 mg/kg, intraperitoneally), which produced marked ipsilateral rotations towards the lesioned hemisphere. Behaviour was monitored over a 90 minute period to capture the temporal profile of responses. The 6-OHDA group displayed a pronounced and sustained increase in ipsilateral turning, with an early peak that gradually stabilised over the recording period, whereas the saline/SHAM group maintained consistently low rotation counts throughout the session. Statistical analysis confirmed a significant lesion effect on rotational behaviour [$F(18, 540) = 2.478$, $p = 0.0007$], with levels of significance ranging from $p < 0.05$ to $p < 0.0001$ (Graph 5.2).

Figure 5.3 presents the mean rate of amphetamine induced rotations per minute, highlighting a clear distinction between 6-OHDA lesioned animals and saline/SHAM controls. Lesioned rats displayed markedly elevated rotational activity, exceeding an average of five rotations per minute, while control animals remained close to baseline with values approaching zero. This represents a substantial increase in ipsilateral turning behaviour following lesioning. Statistical analysis confirmed this difference to be significant ($**p < 0.01$), indicating a robust and consistent effect of dopaminergic denervation on motor asymmetry.

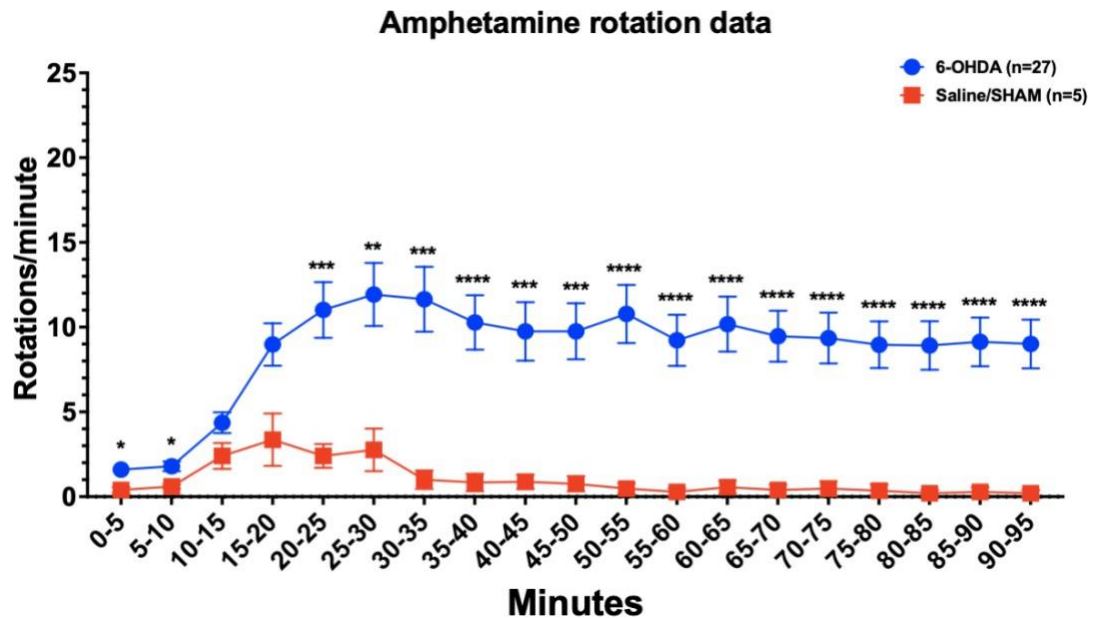


Figure 5. 2: Amphetamine Rotation Data Over Time. Ipsilateral rotational behaviour in unilaterally lesioned rats treated with 8 μ g 6-OHDA compared to saline/sham treated controls. Rats were assessed for lesion integrity prior to levodopa priming by measuring the number of ipsiversive rotations in response to 5 mg/kg (+)-amphetamine. 6-OHDA treated rats (n=27) showed a significant increase in rotational behaviour compared to saline controls (n=5) over a 90-minute period, starting from beginning of post amphetamine administration. Rats with ≥ 6 turns/min were included for priming. Statistical analysis was performed using a two-way ANOVA. Error bars represent mean $\pm$ SEM. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

rotational behaviour that gradually declined over time, but remained higher than control levels at most time points. The effect was most prominent during the early phase of the session, where rotational frequency was greatest and statistical significance strongest, before showing a progressive reduction in magnitude as the session continued. Despite this decline, lesioned animals continued to exhibit a sustained increase in contralateral turning relative to controls, which remained close to baseline throughout.

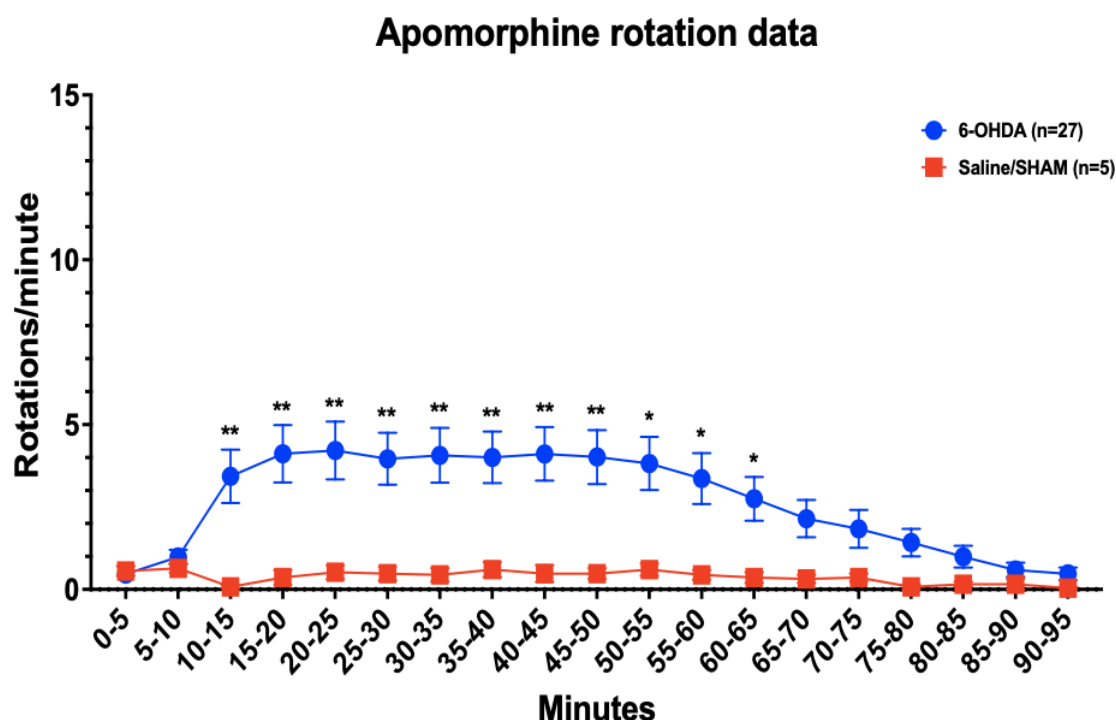


Figure 5. 4: Apomorphine Rotation Data Over Time. Contralateral rotational behaviour induced by apomorphine (0.5 mg/kg) in saline treated controls and 6-OHDA lesioned rats (n=27). A significant increase in rotational behaviour was observed in the 6-OHDA group compared to the saline/sham group (n=5), with rotations peaking at 10 minutes post injection and persisting for the 60 minute observation period. Lesion integrity was assessed prior to levodopa priming, and rats with ≥ 6 turns/min were included for priming. Statistical analysis was conducted using a two-way ANOVA. Error bars represent mean $\pm$ SEM. P is significant when * $p \leq 0.05$ and ** $p < 0.01$.

contralateral forelimb stepping, indicating a pronounced impairment in motor function on the affected side, while ipsilateral stepping remained relatively preserved. In contrast, non-turners and control animals exhibited more balanced stepping behaviour between forelimbs, with minimal asymmetry.

This deficit in the turner group was substantial and consistent across animals, aligning with their established rotational phenotype. Statistical analysis confirmed a highly significant effect ($n = 12$; **** $p \leq 0.0001$), as determined by Tukey's *post hoc* test.

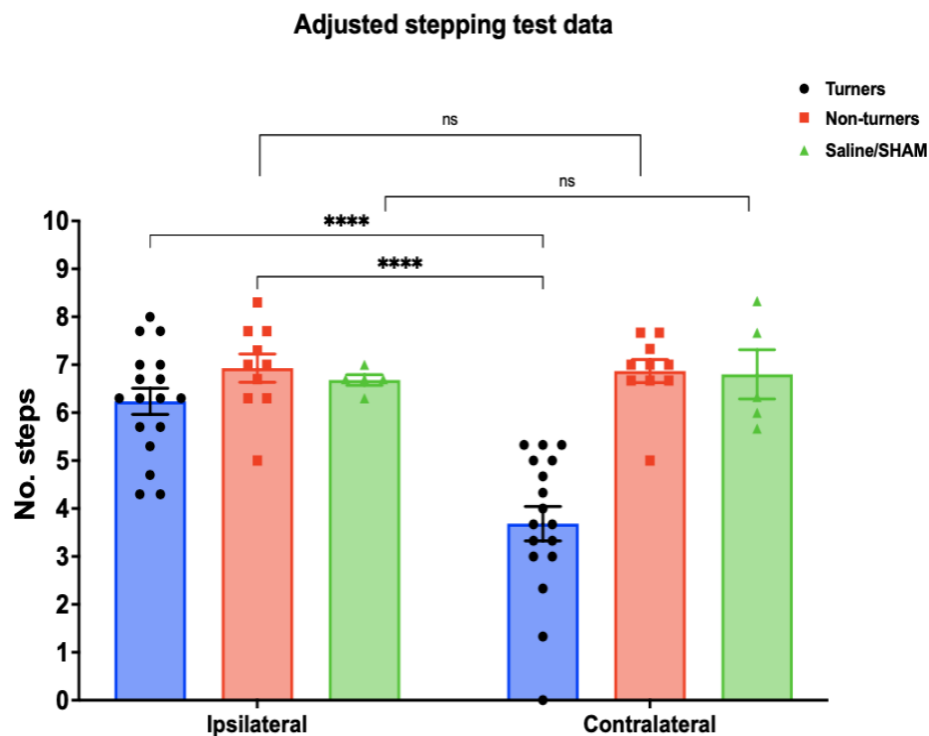


Figure 5. 6: Comparative Analysis of Stepping Test Performance in Unilaterally 6-OHDA Lesioned Rats. Following amphetamine and apomorphine rotational behaviour, all animals tested for lesion integrity using stepping test. 6-OHDA treated group ($n=29$) produced greater level of limb asymmetry respectively comparing to Saline/sham ($n=5$) treated group of but just $n=12$ was used based on the expression of AIMs forelimb akinesia in the stepping test. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$, mean $\pm$ SEM.

5.3.4 Levodopa + Benserazide AIMs Sessions (ALO development)

All experimental rats (turners) were assessed over the course of 35 days with chronic treatment. The treatment consisted of acute daily administration of L-DOPA (8 mg/kg) in conjunction with benserazide (16mg/kg). Dyskinetic movements induced by this treatment regimen were then assessed in 7 sessions (twice a week) to monitor the progression of LID using a standardized rating scale, with observations made by three experimentally blinded observers to eliminate bias.

The graph 5.7 shows the progression of ALO (axial, limb and orolingual) abnormal involuntary movements that was assessed during the AIM sessions with chronic L-DOPA and benserazide treatment that consistently induced AIMs across all sessions in turners group. The stability of the AIMs scores across sessions may imply that the treatment regimen has led to a stable induction of LID in turner group.

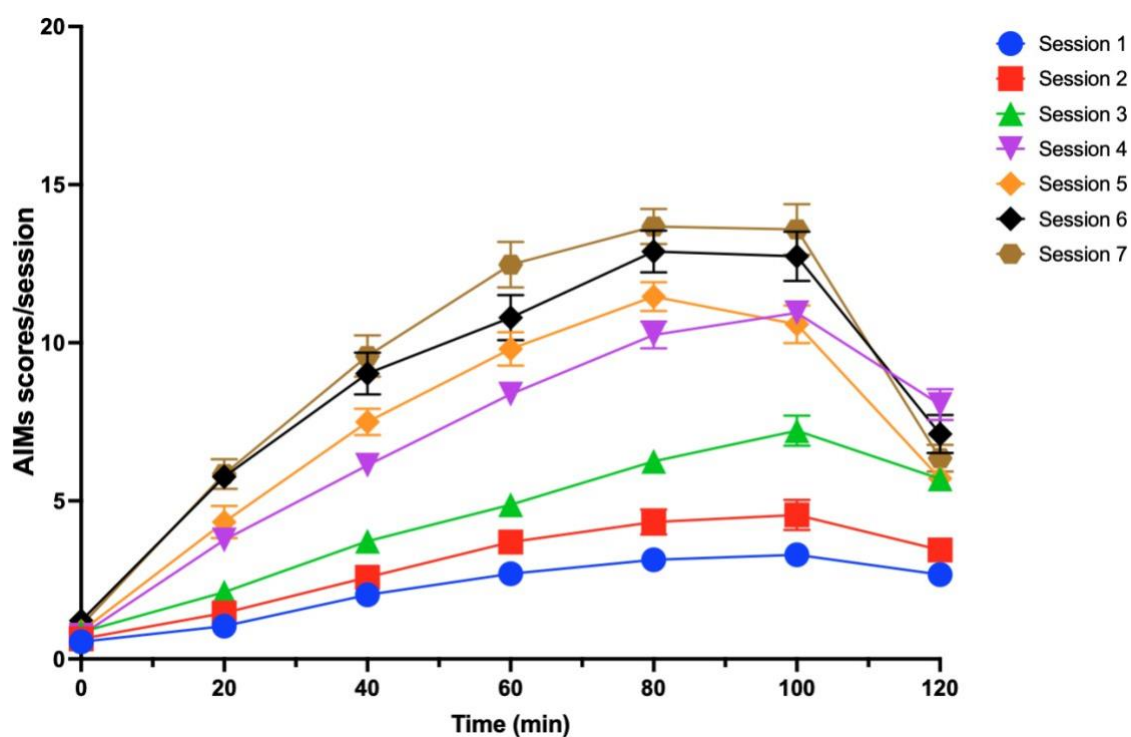


Figure 5. 7: Progression of AIMs expression following levodopa, benserazide treatment in 6-OHDA lesioned rats. Across all seven assessment sessions, co-administration of levodopa (8 mg/kg) and benserazide (15 mg/kg) induced AIMs in 6-OHDA lesioned rats ($n = 12$), with peak dyskinesia consistently occurring between 40 and 100 minutes post-injection. Data is analysed using two-way ANOVA followed by Tukey's *post-hoc* test. Error bars represent the standard error of the mean (SEM).

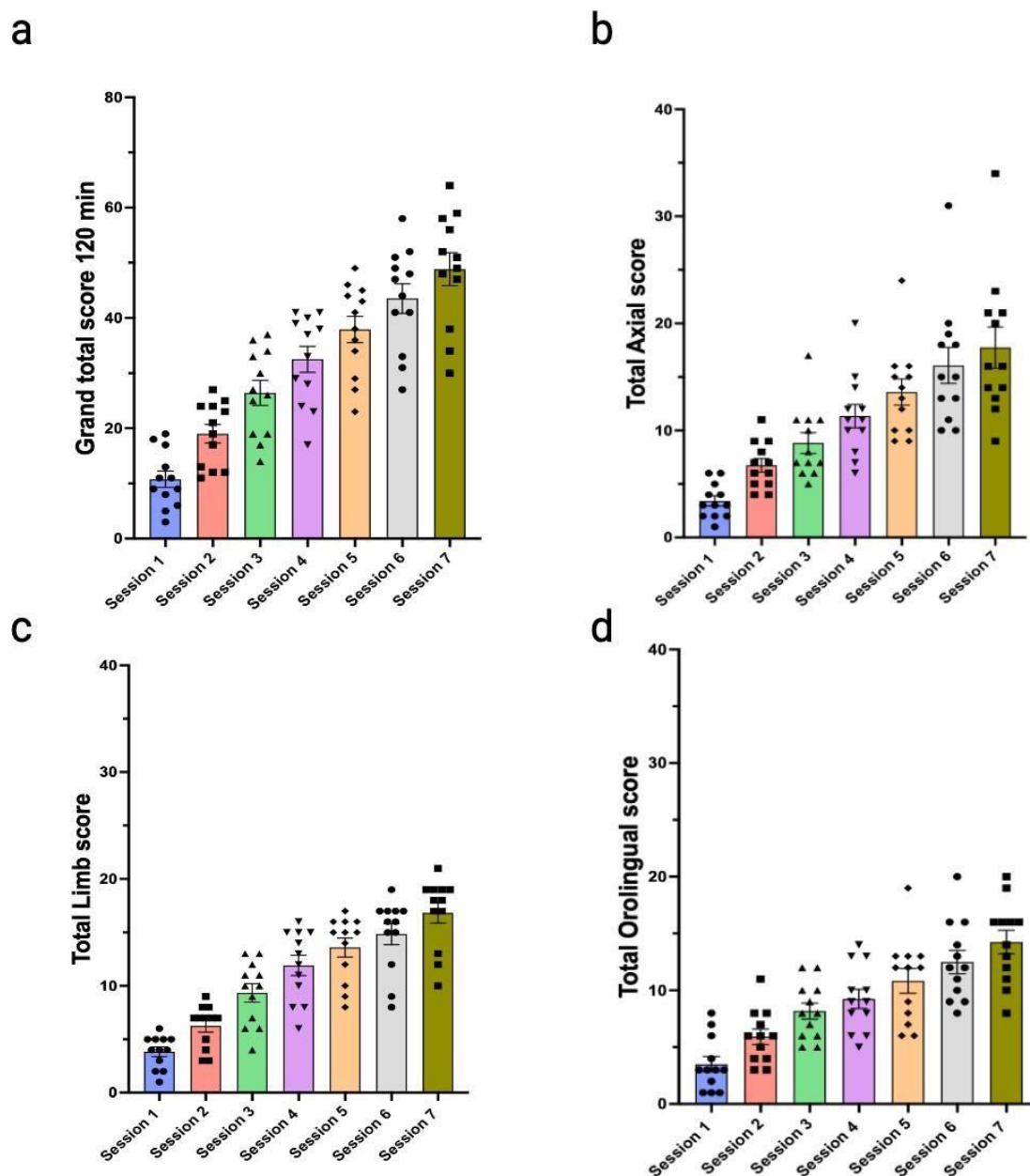


Figure 5. 8: Development of AIMs over chronic treatment with levodopa and benserazide across seven sessions. The bar graph represents the progression of L-DOPA induced dyskinesia in rats receiving a daily dose of 8 mg/kg L-DOPA in combination with 15 mg/kg benserazide across seven sessions conducted twice weekly over three weeks. Panel (a) shows the grand total AIMs score across all sessions. Panels (b), (c) and (d) present the axial, limb, and orolingual components of AIMs, respectively. Error bars represent mean $\pm$ SEM.

5.3.5 The agonist A85380 has no effect on L-DOPA induced locomotive AIMs

Locomotive AIM scores over time are shown for rats administered the $\alpha 4\beta 2$ agonist A85380 at doses of 0, 0.001, 0.003, and 0.01 mg/kg. Analysis of the agonist's effect on locomotive AIMs across the 120 minute period revealed a significant main effect of time [$F(3.175, 34.93) = 6.559, p = 0.0010$], indicating that locomotive activity varied over the course of the session.

However, there was no significant main effect of dose [$F(1.660, 18.26) = 0.7174, p = 0.4765$] and no significant dose $\times$ time interaction [$F(3.758, 41.34) = 1.499, p = 0.2224$] (Figure 5.9a). Across all treatment groups, locomotive AIMs followed a similar temporal profile, with no clear separation between A85380 treated animals and vehicle controls at any time point.

Figure 5.9b presents the cumulative locomotive AIM scores over the full 120 minute session. Comparison of summed scores between vehicle and A85380 treatment groups (0.001, 0.003, and 0.01 mg/kg) revealed no statistically significant differences, as all p-values exceeded 0.05. The magnitude of locomotive AIMs remained comparable across all doses, with no observable trend towards either an increase or reduction in activity. These data were analysed using repeated measures one-way ANOVA (Dunnett's *post hoc* test) and are expressed as mean $\pm$ SEM.

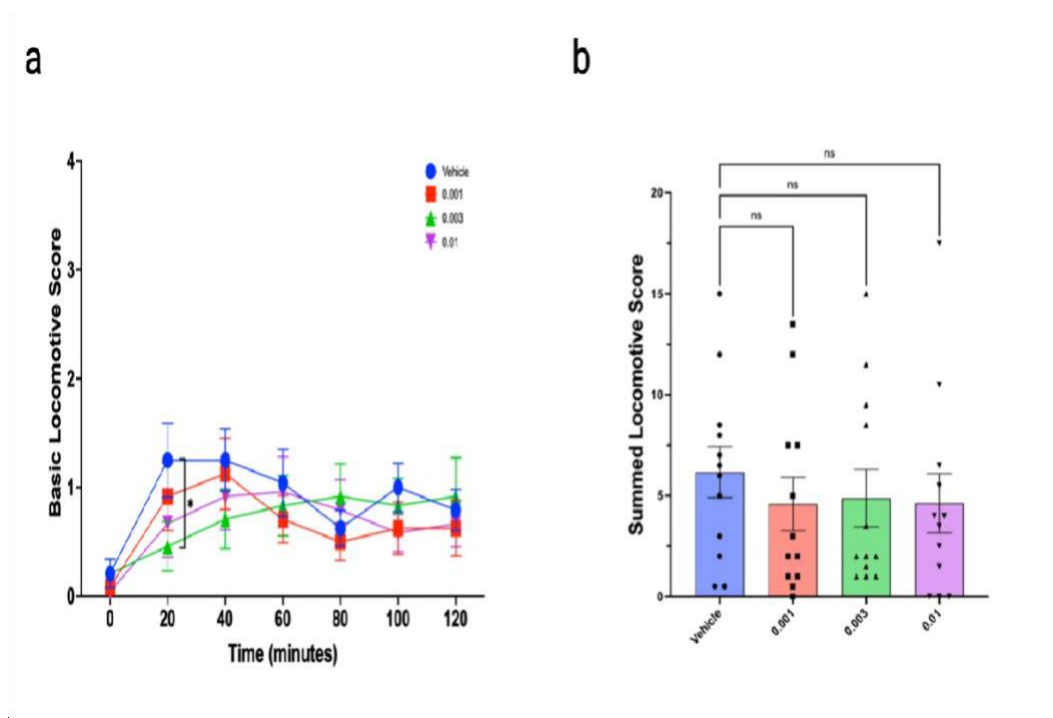


Figure 5.9: Effects of administration of A85380 on locomotive AIMs in 6-OHDA lesioned primed rats. (a) Time course of locomotive AIMs over 120 minutes following de novo administration of A85380. A transient reduction was observed 20 minutes post administration at the 0.003 mg/kg dose (* $p < 0.05$), although no sustained or dose dependent effect was detected. (b) Comparison of summed locomotive AIMs across doses, showing no statistically significant differences as confirmed by one-way ANOVA ($p = 0.4765$). Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ($n=12$).

5.3.6 A85380 Reduces Basic AIMs

An analysis of the effects of A85380 on individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is shown in Figure 5.10 (panels a–d). The total ALO scores (panel a) represent the combined measure of axial, limb, and orolingual movements. Statistical analysis identified a significant reduction in total levodopa induced AIMs at the dose of 0.01 mg/kg compared with vehicle ($p = 0.0319$), indicating an overall attenuation of dyskinetic behaviour at this dose.

Panel (b) illustrates the effect of A85380 on axial AIMs. Although a reduction was observed across increasing doses, this effect was modest and did not reach statistical significance relative to vehicle, suggesting a limited impact of A85380 on axial dyskinesia.

In contrast, the effect on limb AIMs (panel c) was more pronounced. Significant reductions in limb movement scores were detected at doses of 0.001 mg/kg ($p = 0.0313$) and 0.01 mg/kg ($p = 0.0440$) compared with vehicle. This reduction was consistent across the observation period, indicating a clear and sustained suppression of limb dyskinesia, although the absence of significance at the intermediate dose suggests a non-linear dose response.

Finally, orolingual AIMs (panel d), reflecting repetitive jaw and tongue movements, showed a clear dose related effect. A85380 significantly reduced orolingual scores at 0.01 mg/kg ($p = 0.0045$), representing the most robust subtype specific response observed. This effect was marked in magnitude and consistent across animals, indicating strong sensitivity of orolingual dyskinesia to $\alpha 4\beta 2$ receptor activation. All data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

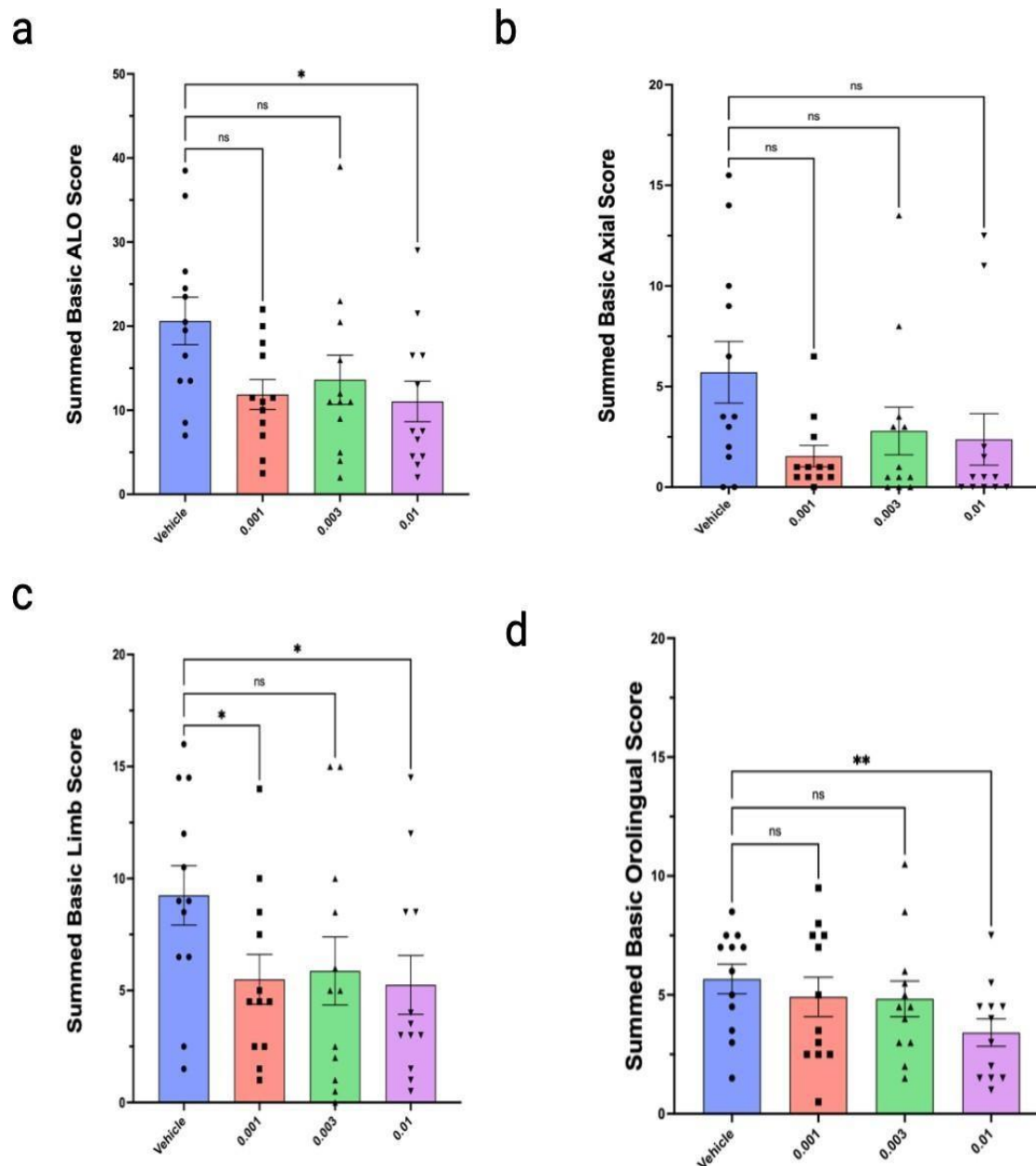


Figure 5. 10: Effects of administration of A85380 on basic AIM subtypes in lesioned primed rats. Summed scores represent the cumulative total of AIM subtype ratings across all observation time points following levodopa administration. Panel (a) shows total ALO AIM scores, where A85380 produced a significant overall reduction compared with vehicle ($p = 0.0319$). Panel (b) illustrates axial AIMs, which exhibited a non-significant decrease across doses. Panel (c) presents limb AIMs, demonstrating significant reductions at doses of 0.001 mg/kg and 0.01 mg/kg relative to vehicle. Panel (d) shows orolingual AIMs, where a marked reduction was observed at the 0.01 mg/kg dose ($p = 0.0045$) compared with vehicle. Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ** $p \leq 0.01$; ($n=12$).

5.3.7 Effects of A85380 on Basic AIMs Across a 120 Minute Period

An analysis of the effects of A85380 on individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.11 (panels a–d). Examination of total ALO scores over time revealed a significant main effect of time [$F(2.948, 32.43) = 7.981, p = 0.0004$] and a significant main effect of dose [$F(1.915, 21.06) = 3.612, p = 0.0465$], indicating that overall dyskinesia varied across the session and was modulated by A85380 treatment. However, the interaction between dose and time did not reach statistical significance [$F(4.755, 52.31) = 2.075, p = 0.0864$], suggesting that the temporal profile of AIMs was broadly similar across doses.

Panel (b) illustrates the effect of A85380 on axial AIMs. Analysis revealed a significant main effect of time [$F(3.252, 35.77) = 6.389, p = 0.0011$], indicating fluctuations in axial dyskinesia over the session. However, there was no significant main effect of dose [$F(2.180, 23.98) = 2.197, p = 0.1295$], nor a significant dose $\times$ time interaction [$F(5.111, 56.23) = 1.419, p = 0.2308$], suggesting that A85380 produced no consistent or measurable reduction in axial AIMs across the doses tested.

In contrast, the effect on limb AIMs (panel c) was more pronounced. A significant main effect of time [$F(3.469, 38.16) = 10.52, p < 0.0001$] and a significant main effect of dose [$F(2.224, 24.46) = 4.079, p = 0.026$] were observed, indicating both temporal variation and a clear treatment effect. Although the dose $\times$ time interaction did not reach significance [$F(5.460, 60.06) = 2.030, p = 0.0815$], *post hoc* comparisons showed that A85380 significantly reduced limb AIM scores at 0.003 mg/kg and 0.01 mg/kg after 20 minutes, and at 0.001 mg/kg after 60 minutes ($p < 0.05$). These reductions indicate a consistent suppression of limb dyskinesia, with effects emerging at distinct time points across doses rather than following a uniform temporal pattern.

Finally, panel (d) illustrates the effect of A85380 on orolingual AIMs. Analysis showed no significant main effect of time [$F(2.880, 31.68) = 2.062, p = 0.1272$] and no significant dose dependent effect [$F(2.570, 28.27) = 2.755, p = 0.0685$]. Similarly, the dose $\times$ time interaction was

not significant [$F(6.081, 66.89) = 1.332, p = 0.2549$]. Orolingual AIMs remained relatively stable across the session, with no clear or consistent separation between treatment groups, indicating a limited effect of A85380 on this subtype under these conditions. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

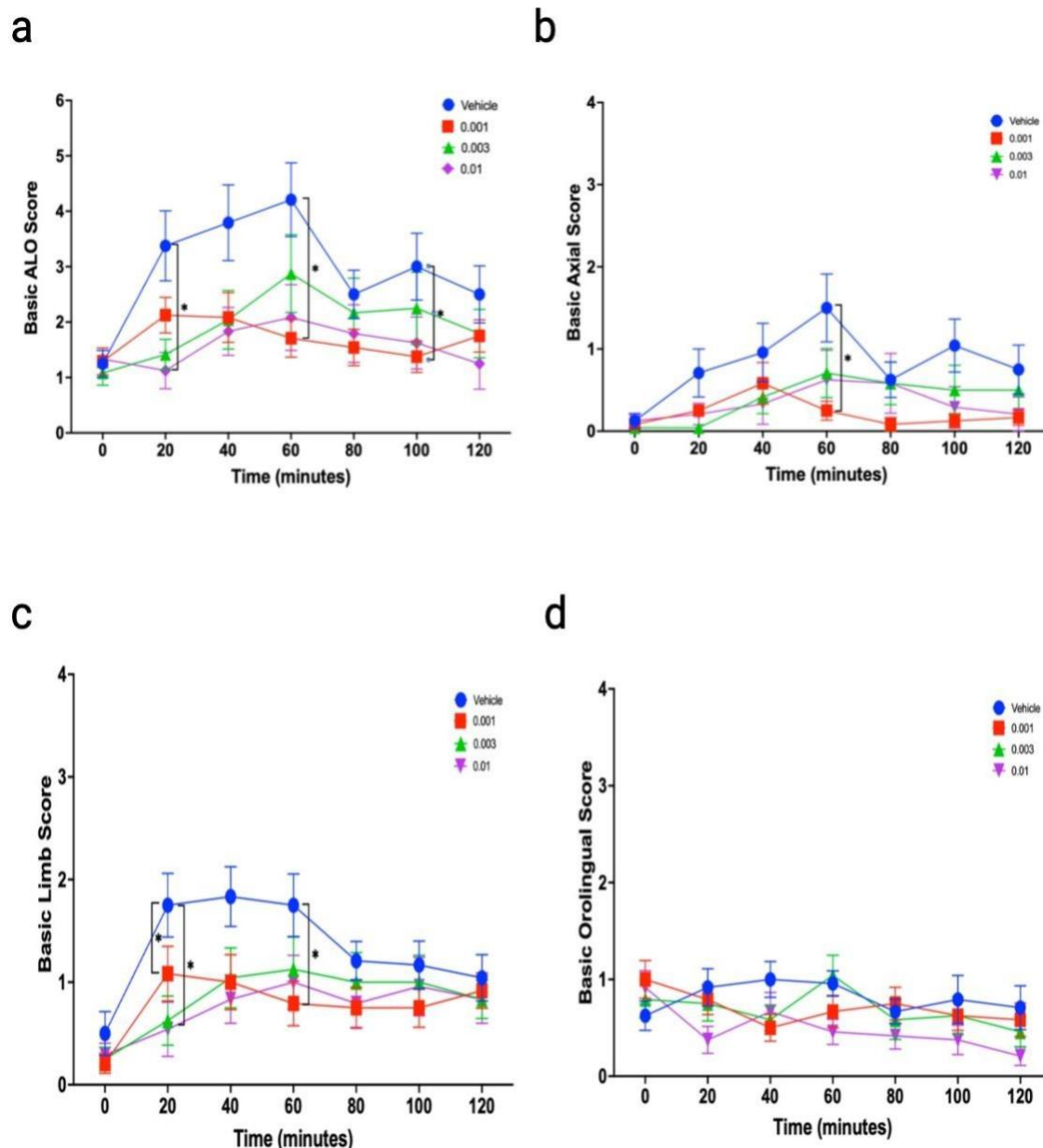


Figure 5. 11: Effects of administration of A85380 on basic AIM subtypes lesioned primed rats. Panel (a) shows total ALO AIM scores, where A85380 produced a significant main effect of both time and dose, indicating an overall reduction in AIMs compared with vehicle. Panel (b) illustrates axial AIMs, which showed a time dependent variation but no significant dose related effects. Panel (c) presents limb AIMs, demonstrating clear reductions at doses of 0.003 mg/kg and 0.01 mg/kg after 20 minutes, and at 0.001 mg/kg after 60 minutes. Panel (d) shows orolingual AIMs, which were not significantly affected by A85380 across the 120 minute period. Data are expressed as mean $\pm$ SEM; * p < 0.05, (n=12).

5.3.8 A85380 Reduces the Amplitude AIMS

When the effect of A85380 on ALO scores was examined, there was a significant main effect of time [$F(2.942, 32.36) = 7.589, p = 0.0006$] and a significant main effect of dose [$F(1.940, 21.34) = 3.833, p = 0.0389$], indicating that the amplitude of levodopa induced AIMS varied over the session and was reduced by A85380 treatment. However, the interaction between dose and time was not statistically significant [$F(5.306, 58.37) = 1.717, p = 0.1411$], suggesting that the reduction in total AIM amplitude followed a broadly similar temporal profile across doses.

Panel (b) illustrates the effect of A85380 on axial AIMS. Analysis revealed a significant main effect of time [$F(3.204, 35.24) = 5.294, p = 0.0034$], indicating fluctuations in axial movement severity over the session. However, the main effect of dose was not significant [$F(1.962, 21.58) = 2.375, p = 0.1177$], and no significant dose $\times$ time interaction was observed [$F(4.555, 50.11) = 1.027, p = 0.4084$], suggesting that A85380 produced no consistent reduction in axial AIM amplitude.

The effect on limb AIMS (panel c) was more pronounced. Significant main effects of both time [$F(3.518, 38.70) = 9.806, p < 0.0001$] and dose [$F(2.050, 22.55) = 3.835, p = 0.0360$] were identified, indicating both temporal variation and a clear treatment effect. Although the interaction between dose and time was not statistically significant [$F(5.964, 65.60) = 1.334, p = 0.2550$], *post hoc* comparisons demonstrated significant reductions in limb AIM amplitude at 0.01 mg/kg between 20 and 40 minutes after administration ($p < 0.05$). This indicates a temporally defined reduction in limb movement severity, with the effect most evident during the early phase of the session.

Finally, panel (d) shows the effects of A85380 on orolingual AIMS. The analysis showed that the effect of time was not significant [$F(3.544, 38.99) = 1.124, p > 0.05$] and although a trend toward a dose dependent effect was observed, this did not reach statistical significance [$F(2.208, 24.29) = 2.962, p = 0.0661$]. The dose $\times$ time interaction also failed to reach significance [$F(5.518, 60.70) = 1.176, p = 0.3315$]. Orolingual AIM amplitude remained relatively stable across the session, with no clear or consistent separation between treatment groups. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

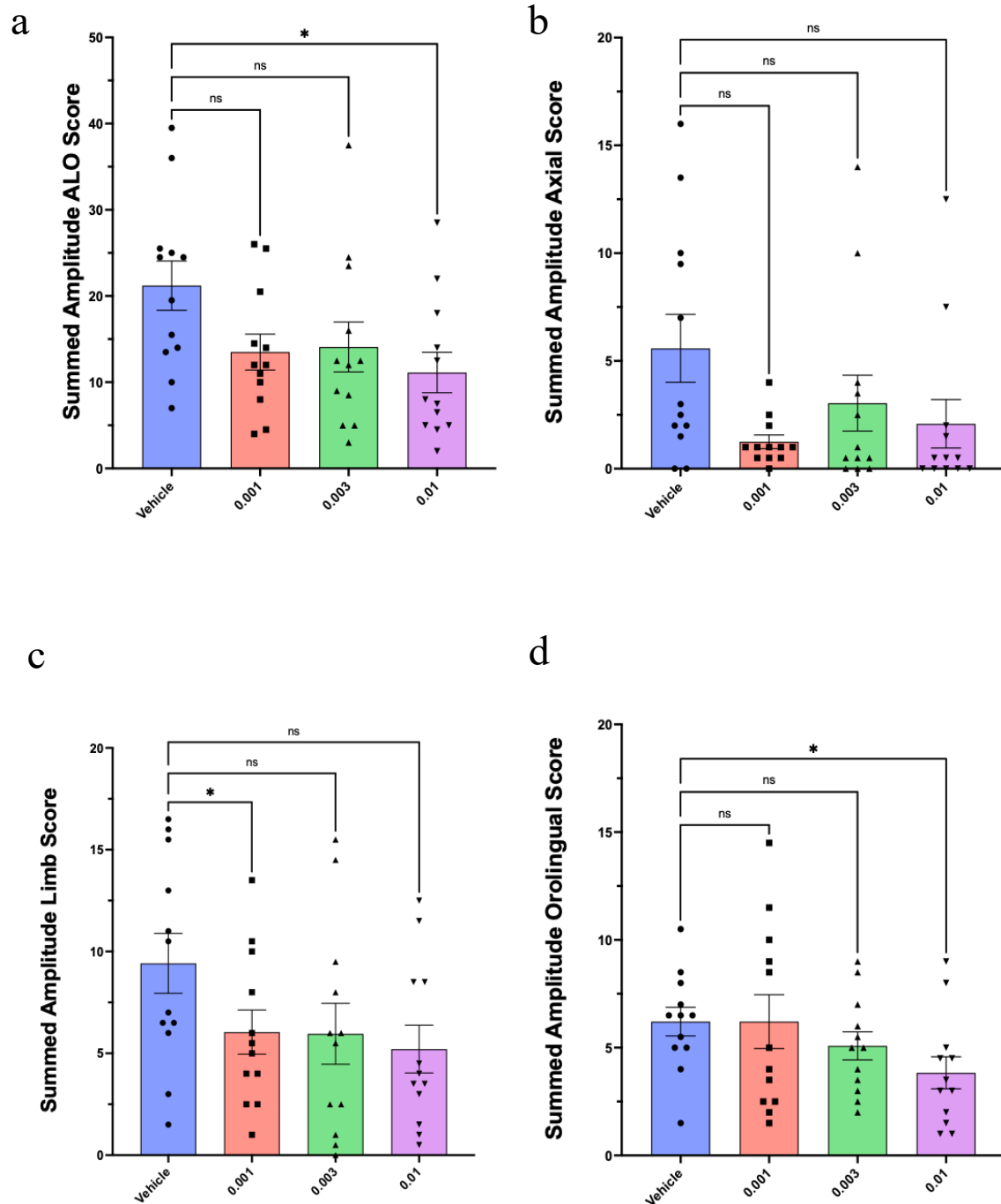


Figure 5.12: Effects of administration of A85380 on the amplitude of AIM lesioned, primed rats. Panel (a) shows total ALO AIM scores, where A85380 produced a significant reduction in overall AIM severity compared with vehicle ($p = 0.0223$). Panel (b) illustrates axial AIMs, which showed a non-significant decrease across doses. Panel (c) presents limb AIMs, demonstrating a significant reduction in amplitude at the 0.001 mg/kg dose ($p = 0.0497$) relative to vehicle. Panel (d) shows orolingual AIMs, where a significant reduction was observed at the 0.01 mg/kg dose ($p = 0.0233$) compared with vehicle. Data are expressed as mean $\pm$ SEM. * $p < 0.05$, ($n=12$).

5.3.9 Effects of A85380 on Amplitude AIMs Across a 120 Minute Period

An analysis of the effects of A85380 on the amplitude of individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.13 (panels a–d).

Examination of total ALO scores revealed a significant main effect of time [$F(2.942, 32.36) = 7.589, p = 0.0006$] and a significant main effect of dose [$F(1.940, 21.34) = 3.833, p = 0.0389$], indicating that AIM amplitude varied across the session and was reduced by A85380 treatment. However, the absence of a significant dose $\times$ time interaction [$F(5.306, 58.37) = 1.717, p = 0.1411$] suggests that this reduction occurred without a marked shift in the temporal profile of dyskinesia.

Panel (b) illustrates the effect of A85380 on axial AIMs. A significant main effect of time was observed [$F(3.204, 35.24) = 5.294, p = 0.0034$], indicating variability in axial movement severity over the session. However, neither a main effect of dose [$F(1.962, 21.58) = 2.375, p = 0.1177$] nor a dose $\times$ time interaction [$F(4.555, 50.11) = 1.027, p = 0.4084$] was detected, suggesting that A85380 did not produce a consistent or measurable reduction in axial AIM amplitude.

The effect on limb AIMs (panel c) was more evident. Significant main effects of time [$F(3.518, 38.70) = 9.806, p < 0.0001$] and dose [$F(2.050, 22.55) = 3.835, p = 0.0360$] were observed, indicating both temporal variation and a clear treatment related reduction in movement severity. Although the dose $\times$ time interaction was not significant [$F(5.964, 65.60) = 1.334, p = 0.2550$], post hoc analysis demonstrated that A85380 significantly reduced limb AIM amplitude at 0.01 mg/kg between 20 and 40 minutes after administration ($p < 0.05$). This indicates a defined reduction in limb dyskinesia that is most apparent during the early phase of the session.

Finally, panel (d) shows the effects of A85380 on orolingual AIMs. No significant main effect of time was observed [$F(3.544, 38.99) = 1.124, p > 0.05$] and although a trend toward a dose dependent reduction was apparent, this did not reach statistical significance [$F(2.208, 24.29) = 2.962,$

$p = 0.0661$]. The dose $\times$ time interaction was also not significant [$F(5.518, 60.70) = 1.176$, $p = 0.3315$]. Overall, orolingual AIM amplitude remained relatively stable across conditions, with only a modest, non-significant tendency towards reduction at higher doses. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

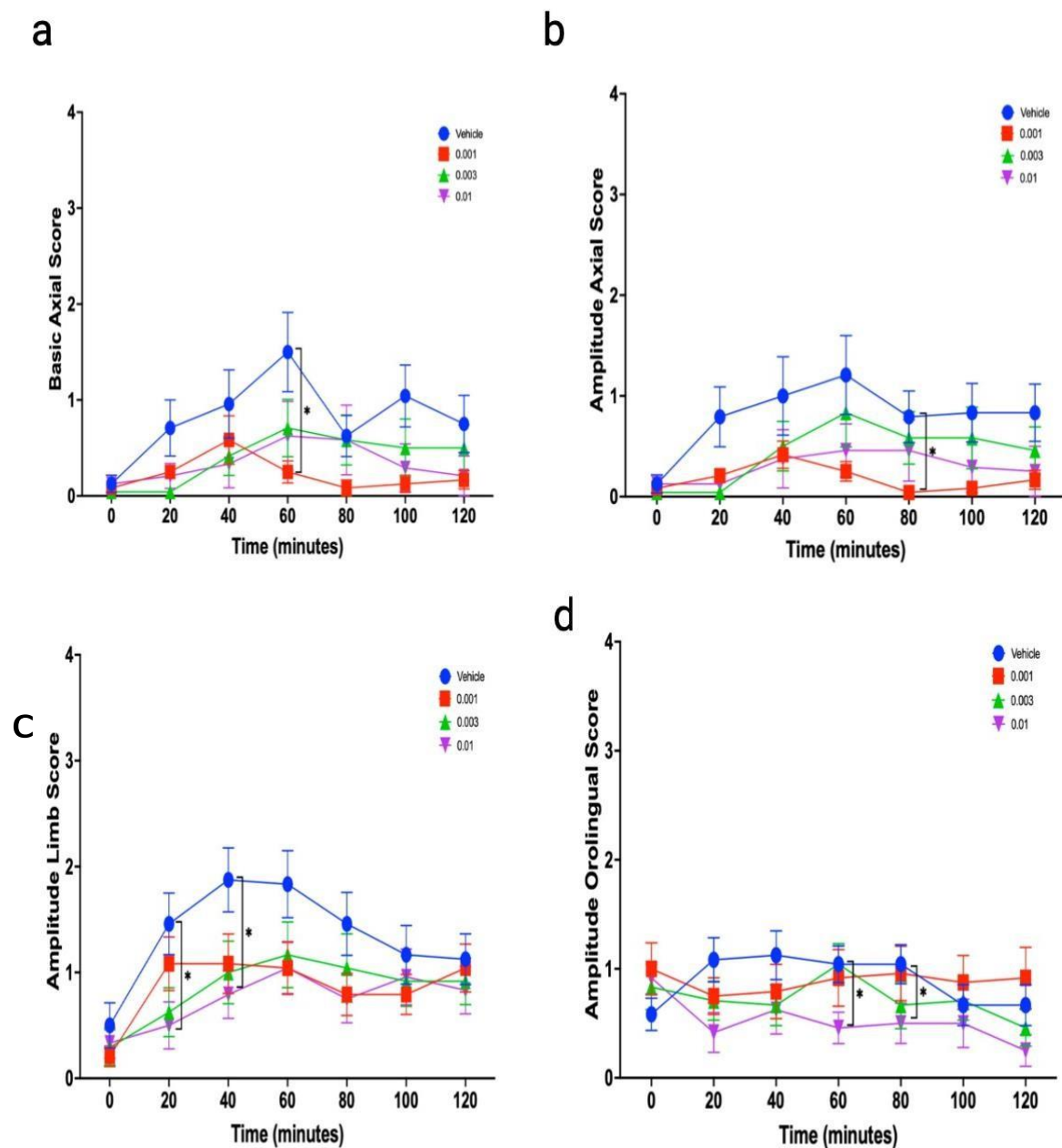


Figure 5. 13: Effects of administration of A85380 on the amplitude of AIMs on lesioned, primed rats. Panel (a) shows total ALO AIM scores, where A85380 produced significant main effects of both time and dose, indicating an overall reduction in the amplitude of levodopa induced AIMs compared with vehicle. Panel (b) illustrates axial AIMs, which displayed significant time dependent variation but no significant dose related changes. Panel (c) presents limb AIMs, showing a clear dose dependent reduction in amplitude, with significant decreases observed at 0.01 mg/kg between 20–40 minutes post administration. Panel (d) shows orolingual AIMs, which showed a non-significant trend toward dose dependent reduction but did not reach statistical significance across the 120-minute session. Data are expressed as mean $\pm$ SEM; * $p < 0.05$, (n=12).

5.3.10 A85380 Reduces the Global AIMS

The global AIM score, representing the combined total of both basic (frequency) and amplitude (severity) ratings across the axial, limb, and orolingual subtypes, provides an integrated measure of overall dyskinetic activity. An analysis of the effects of A85380 on global and individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.14 (panels a–d).

Panel (a) shows the total global ALO AIM scores, incorporating both the basic and amplitude components across all movement subtypes. Although the overall comparison across doses did not reach statistical significance ($p = 0.0650$), a clear reduction was observed at the highest dose, with A85380 (0.01 mg/kg) producing a significant decrease compared with vehicle ($p = 0.0308$). This indicates that, at sufficient dose, A85380 is capable of reducing overall dyskinetic burden when both frequency and severity are considered together.

Panel (b) presents the effect of A85380 on axial AIMS. While reductions in both amplitude and frequency were observed, these effects were modest and did not achieve statistical significance relative to vehicle, suggesting a limited contribution of axial movements to the overall treatment effect. Panel (c) illustrates the effects on limb AIMS, where a more evident dose dependent reduction was observed. A85380 significantly decreased global limb AIM scores at doses of 0.001 mg/kg ($p = 0.0303$) and 0.01 mg/kg ($p = 0.0478$), indicating a consistent suppression of both the frequency and severity of limb dyskinesia across the session. This shows the prominent sensitivity of limb AIMS to $\alpha 4\beta 2$ receptor modulation.

Finally, orolingual AIMS (panel d) also showed a significant reduction at the 0.01 mg/kg dose ($p = 0.0266$) compared with vehicle. This effect, while less pronounced than that observed for limb AIMS, indicates that higher doses of A85380 are capable of reducing both the frequency and severity of orolingual movements. All data are expressed as mean $\pm$ standard error and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

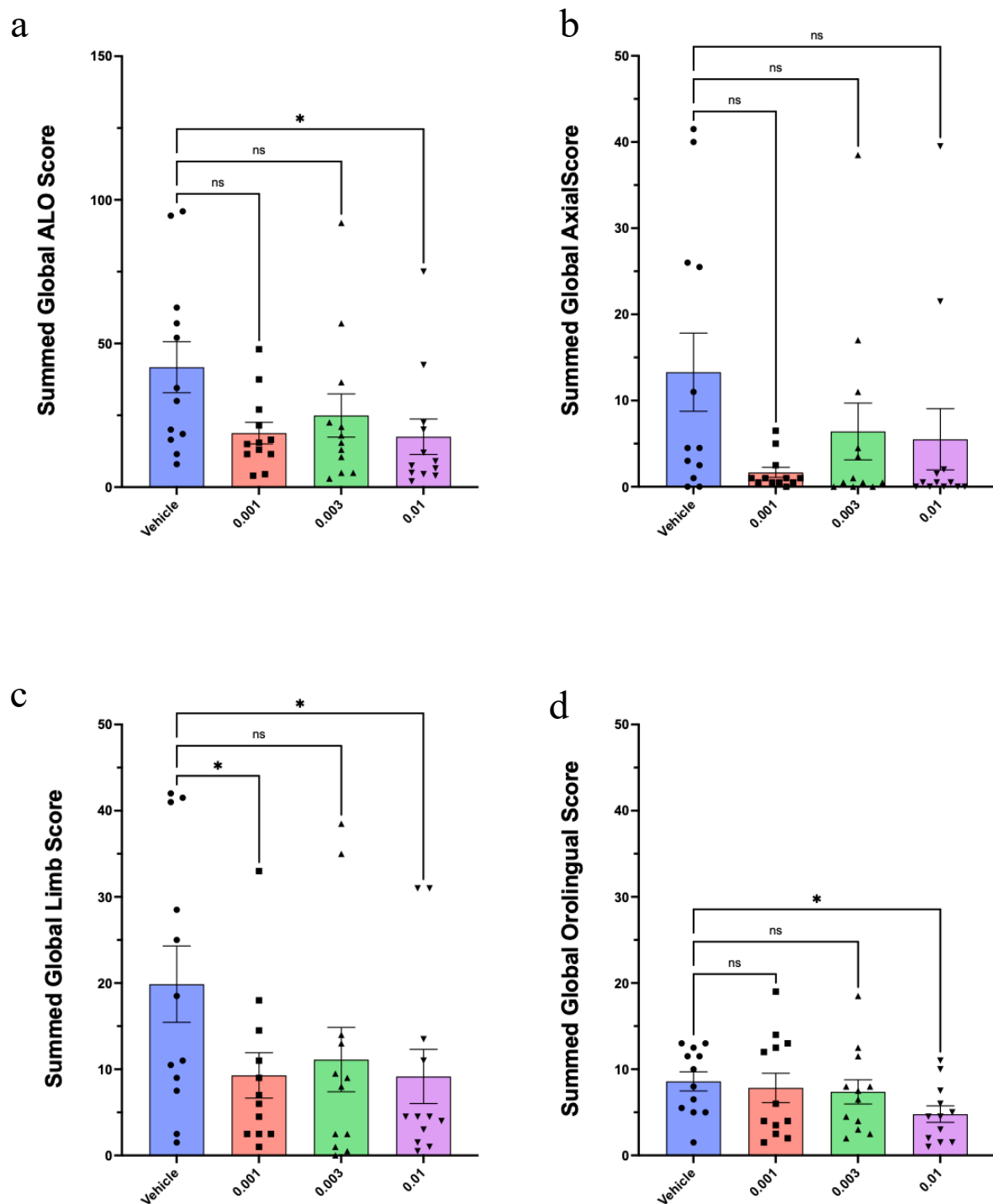


Figure 5. 14: Effects of administration A85380 on global AIMs in lesioned, primed rats. Panel (a) shows total global ALO scores, where A85380 produced a significant reduction compared with vehicle, with the highest dose (0.01 mg/kg) approaching significance ($p = 0.0308$). Panel (b) illustrates axial AIMs, which displayed a downward trend without reaching statistical significance. Panel (c) presents limb AIMs, showing significant reductions at 0.001 mg/kg ($p = 0.0303$) and 0.01 mg/kg ($p = 0.0478$). Panel (d) shows orolingual AIMs, where a significant decrease was observed at the 0.01 mg/kg dose ($p = 0.0266$) relative to vehicle. Data are expressed as mean $\pm$ standard deviation; * $p < 0.05$, ($n=12$).

5.3.11 Effects of A85380 on Global AIMs Across a 120 Minute Period

An analysis of the effects of A85380 on the global AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.15 (panels a–d).

When the effect of A85380 on total global ALO scores was examined (panel a), there was a significant main effect of time [$F(3.209, 35.30) = 6.602, p = 0.0009$] and a significant main effect of dose [$F(1.522, 16.74) = 4.950, p = 0.0276$], indicating that overall dyskinetic activity varied across the session and was reduced by A85380 treatment. However, the absence of a significant dose $\times$ time interaction [$F(4.333, 47.66) = 1.751, p = 0.1500$] suggests that the reduction occurred without a marked alteration in the temporal pattern of AIM expression. Panel (b) illustrates the effect of A85380 on global axial AIMs. A significant main effect of time was observed [$F(3.118, 34.29) = 5.451, p = 0.0032$], indicating fluctuations in axial dyskinesia over the session. However, the main effect of dose did not reach significance [$F(2.004, 22.04) = 2.043, p = 0.1534$], nor did the dose $\times$ time interaction [$F(4.471, 49.18) = 1.018, p = 0.4126$], suggesting that A85380 did not produce a consistent reduction in axial AIMs when both frequency and severity were considered.

The effect on global limb AIMs (panel c) was more prominent. Significant main effects of both time [$F(3.333, 36.67) = 8.609, p = 0.0001$] and dose [$F(1.743, 19.18) = 3.987, p = 0.0405$] were observed, indicating both temporal variation and a clear treatment related reduction. Although the dose $\times$ time interaction was not significant [$F(4.385, 48.23) = 1.748, p = 0.1497$], the overall pattern demonstrates a dose related decrease in limb AIMs across the session, reinforcing the sensitivity of this subtype to A85380. Finally, global orolingual AIMs (panel d) showed no significant time dependent changes [$F(2.889, 31.78) = 0.8562, p = 0.4702$] and no significant dose dependent effects [$F(2.544, 27.98) = 2.110, p = 0.1296$]. The dose $\times$ time interaction was also not significant [$F(5.812, 63.93) = 1.198, p = 0.3193$]. Orolingual AIMs remained relatively stable across the observation period, with no clear or consistent modulation by A85380 at the doses tested. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

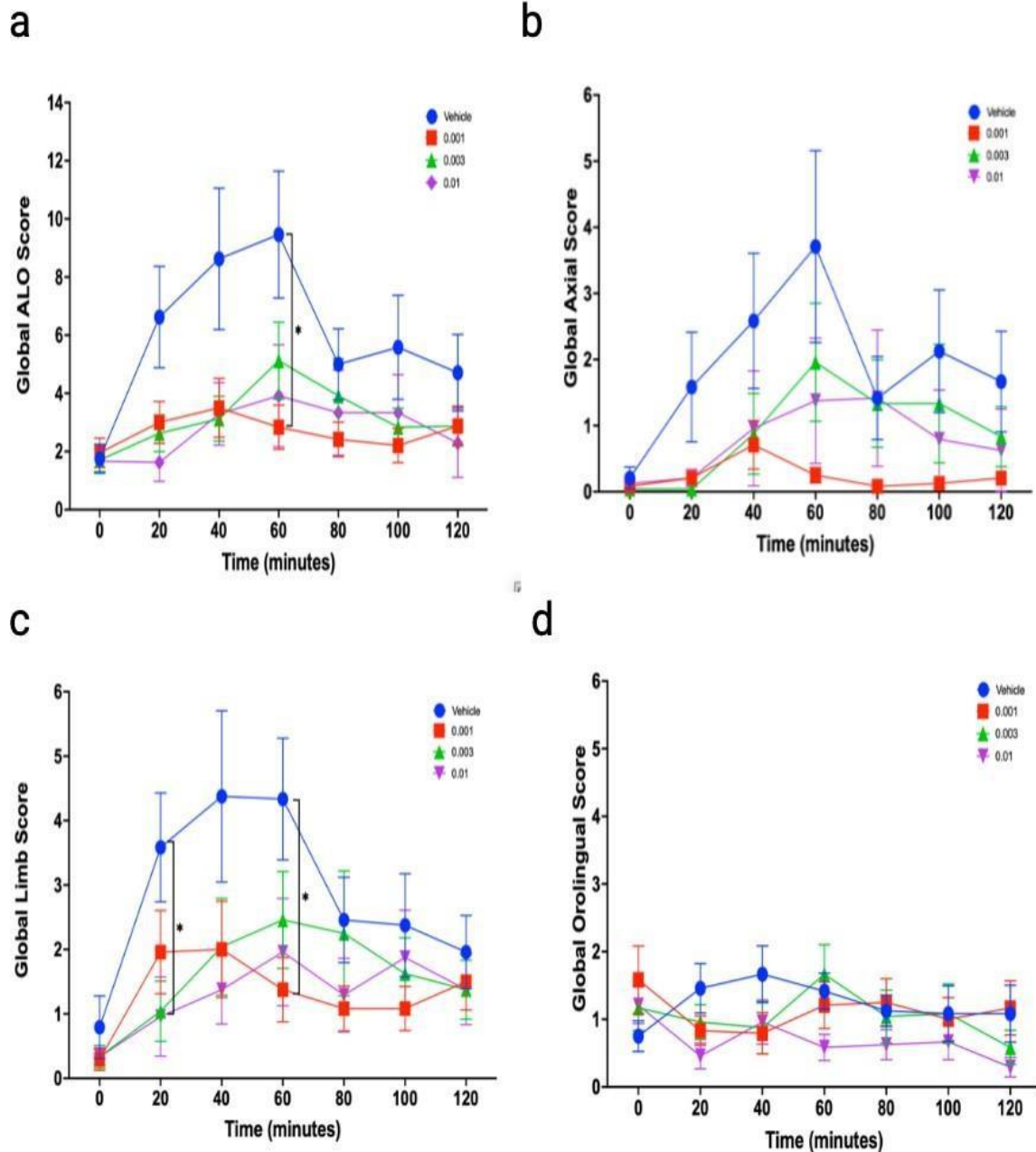


Figure 5.15: Effects of administration of A85380 on global AIMs on lesioned, primed rats. Panel (a) shows total global ALO AIM scores, where A85380 produced a significant main effect of both time and dose, indicating an overall reduction in AIMs compared with vehicle. Panel (b) illustrates global axial AIMs, which showed a significant time effect but no significant dose related change. Panel (c) presents global limb AIMs, demonstrating significant dose dependent reductions over time, particularly at 0.001 mg/kg and 0.01 mg/kg doses. Panel (d) shows global orolingual AIMs, which did not exhibit significant changes across the 120 minute observation period. Data are expressed as mean $\pm$ SEM; * $p < 0.05$, ($n = 12$).

5.3.12 Effect of the Dh β E on locomotive AIMs

Locomotive AIM scores over time are shown for rats administered the selective $\alpha 4\beta 2$ nicotinic receptor antagonist dihydro- β -erythroidine (Dh β E) at doses of 1.0, 3.0, and 10.0 mg/kg to evaluate its effects on levodopa induced AIMs. Analysis of the antagonist's effect on locomotive AIMs across the 120 minute observation period revealed a significant main effect of time [$F(3.320, 36.52) = 7.145, p = 0.0005$], indicating that locomotive activity varied over the course of the session.

However, there was no significant main effect of dose [$F(1.274, 14.01) = 0.07962, p = 0.8395$] and no significant dose $\times$ time interaction [$F(3.908, 42.99) = 0.3418, p = 0.8441$] (Figure 5.16a). Across all treatment groups, locomotive AIMs followed a similar temporal pattern, with no clear separation between Dh β E treated animals and vehicle controls at any time point.

Figure 5.16b presents the summed locomotive AIM scores over the 120 minute session. Comparison of cumulative scores between the vehicle and Dh β E treated groups (1.0, 3.0, and 10.0 mg/kg) revealed no statistically significant differences, with all p-values exceeding 0.05. The magnitude of locomotive AIMs remained comparable across all doses, with no observable trend towards either an increase or reduction in activity. Data were analysed using repeated measures one-way ANOVA (Dunnett's *post hoc* test) and are expressed as mean $\pm$ standard error.

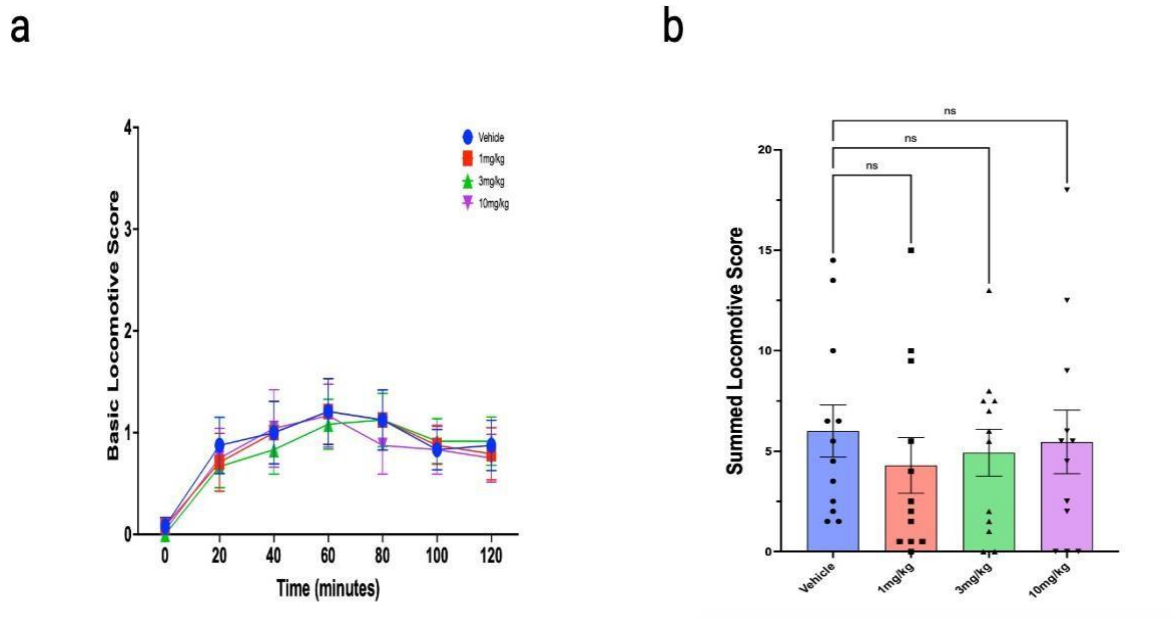


Figure 5.16: Effects of administration of DhβE on locomotive AIMs on lesioned, levodopa primed rats. (a) Time course of locomotive AIMs over 120 minutes following DhβE administration at doses of 1.0, 3.0, and 10.0 mg/kg. Although a significant main effect of time was detected ($p = 0.0005$), DhβE produced no dose dependent or interaction effects on AIM expression. (b) Summed locomotive AIM scores across the 120 minute session showed no statistically significant differences between vehicle and DhβE treated groups, as confirmed by one-way ANOVA. Data are expressed as mean $\pm$ standard error ($n = 12$).

5.3.13 DhβE Does Not Significantly Alter Basic AIMs

An analysis of the effects of DhβE on the basic area under the curve of individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.17 (panels a–d).

The total ALO scores (panel a), representing the combined basic measure of axial, limb, and orolingual movements, showed a modest reduction compared with vehicle; however, this effect did not reach statistical significance, as determined by one-way ANOVA. Panel (b) illustrates the effect of DhβE on axial AIMs, where reductions in basic scores were observed across all tested doses, but these changes were small in magnitude and not statistically significant relative to vehicle. Panel (c) shows the effect on limb AIMs, which similarly did not differ significantly between groups. Although a slight downward trend was apparent at higher doses, this reduction was inconsistent and

did not reach statistical significance, indicating no clear effect of Dh β E on limb dyskinesia during the 120 minute session.

Finally, orolingual AIMs (panel d) demonstrated no significant changes in basic scores across all doses compared with vehicle. Scores remained relatively stable across treatment conditions, with no evident dose related pattern or consistent deviation from control levels.

All data are expressed as mean $\pm$ standard error and were analysed using one-way ANOVA.

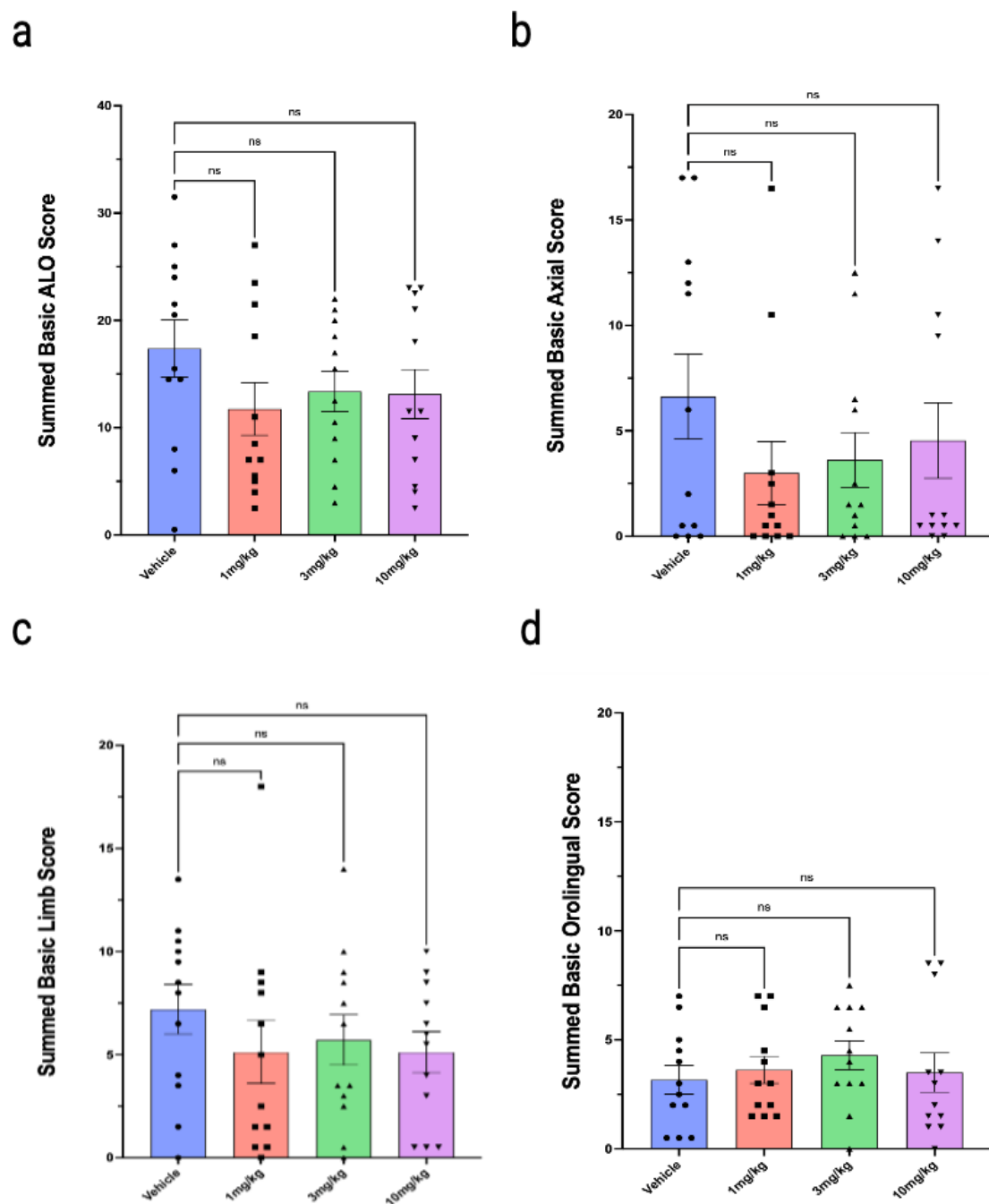


Figure 5. 17: Effects of administration of Dh β E on basic AIMs on lesioned, primed rats. Panel (a) shows total ALO AIM scores, where Dh β E produced a non-significant reduction compared with vehicle. Panel (b) illustrates axial AIMs, which showed no statistically significant changes across the tested doses (1, 3, and 10 mg/kg). Panel (c) presents limb AIMs, where reductions in scores were observed but did not reach significance compared with vehicle. Panel (d) shows orolingual AIMs, which were also unaffected by Dh β E treatment (n = 12). Data are expressed as mean $\pm$ standard error and were analysed using one-way ANOVA.

5.3.14 Effects of Dh β E on Basic AIMs Across a 120 Minute Period

An analysis of the effects of Dh β E on the basis of individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.18 (panels a–d).

When the effect of Dh β E on total ALO scores was examined (panel a), a significant main effect of time was observed [$F(2.549, 28.04) = 10.12, p = 0.0002$], reflecting the expected fluctuation in dyskinesia severity across the observation period. Although no significant main effect of dose [$F(2.191, 24.10) = 1.188, p = 0.3254$] or dose $\times$ time interaction [$F(6.096, 67.05) = 0.6427, p = 0.6980$] was detected, total ALO scores remained broadly comparable across treatment groups, with no clear dose-dependent pattern emerging relative to vehicle. Panel (b) illustrates the effect of Dh β E on axial AIMs, where a significant main effect of time was again observed [$F(2.441, 26.85) = 7.084, p = 0.0021$], indicating that axial movements followed a similar temporal profile, with variation in severity over the 120 minute period. In contrast, neither a significant dose effect [$F(1.794, 19.73) = 1.447, p = 0.2581$] nor a dose $\times$ time interaction [$F(4.268, 46.95) = 1.266, p = 0.2963$] was observed, and axial AIM scores appeared to follow a similar trajectory across all groups.

The effect of Dh β E on limb AIMs (panel c) also showed a significant main effect of time [$F(3.374, 37.11) = 10.67, p < 0.0001$], consistent with time dependent changes in limb AIM severity, typically characterised by an initial increase followed by a gradual decline. However, no significant main effect of dose [$F(2.392, 26.31) = 1.187, p = 0.3274$] or dose $\times$ time interaction [$F(6.403, 70.43) = 0.8531, p = 0.5396$] was observed, with limb AIM scores showing no consistent separation between Dh β E treated and vehicle groups across the session. Lastly, orolingual AIMs (panel d) showed no significant effects of time [$F(2.264, 24.91) = 1.474, p = 0.2483$], dose [$F(2.119, 23.31) = 0.6350, p = 0.5476$], or dose $\times$ time interaction [$F(6.169, 67.86) = 1.049, p = 0.4029$], with scores remaining relatively stable and low in magnitude throughout the observation period, suggesting minimal modulation of this subtype under these conditions. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

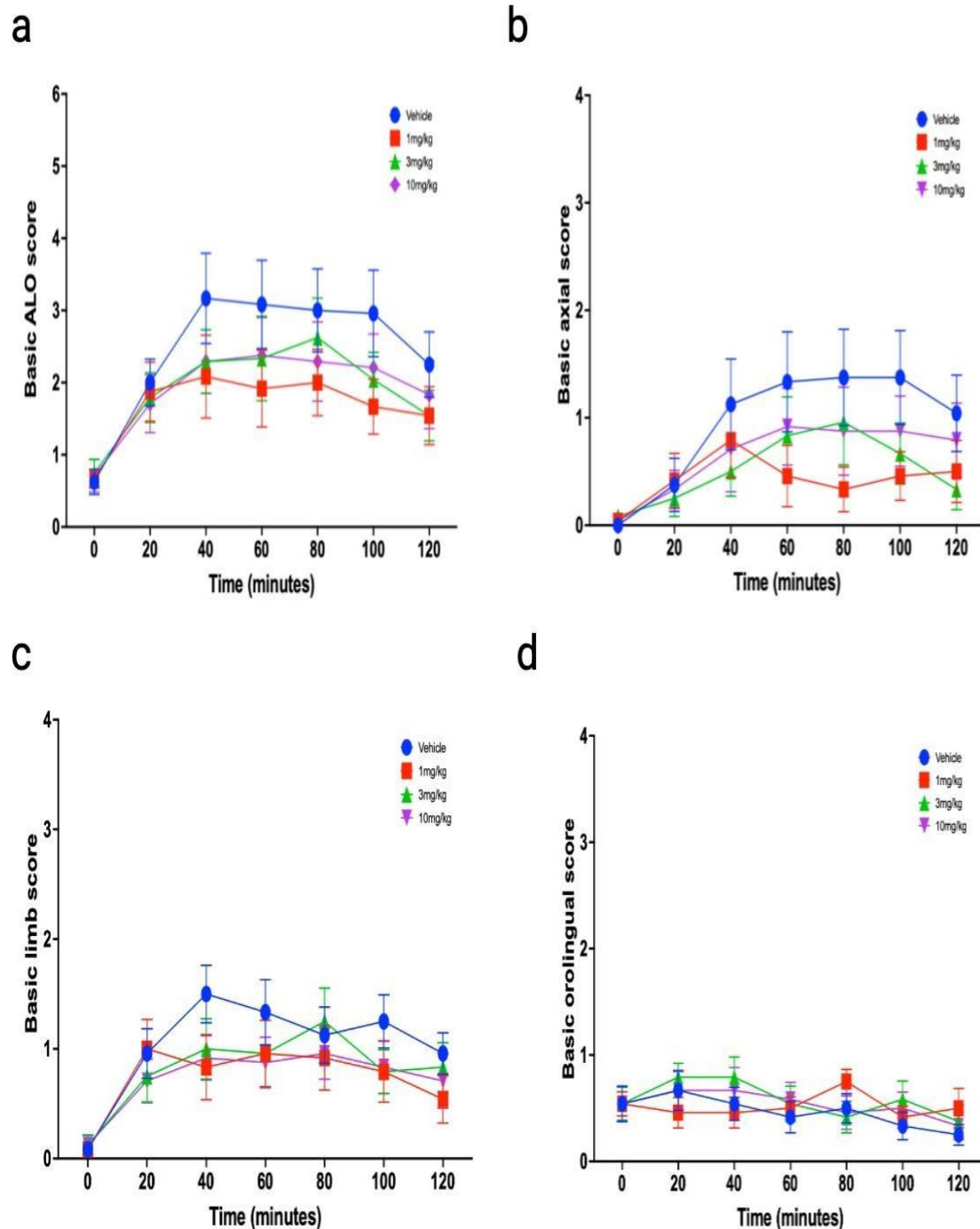


Figure 5.18: Effects of administration of Dh β E on basic AIMs on lesioned, levodopa primed rats. Panel (a) shows total ALO AIM scores, where Dh β E produced a significant main effect of time but no significant dose related changes or dose $\times$ time interaction. Panel (b) illustrates axial AIMs, which showed a significant time dependent effect and a transient reduction at the 1 mg/kg dose at 80 minutes ($p < 0.05$), though overall dose effects were not significant. Panel (c) presents limb AIMs, showing a significant effect of time but no influence of dose or interaction, suggesting Dh β E did not modify limb AIMs. Panel (d) depicts orolingual AIMs, where no significant effects of time, dose, or interaction were detected, indicating the antagonist had no measurable impact on this subtype. Data are expressed as mean $\pm$ SEM; ($n = 12$).

5.3.15 Dh β E Does Not Significantly Reduce the Amplitude AIMs

An analysis of the effects of Dh β E on the amplitude of individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.19 (panels a–d).

Total ALO scores (panel a) showed no statistically significant reductions across all tested doses when compared with vehicle, as confirmed by one-way ANOVA ($p > 0.05$), although small, non-significant decreases were observed at certain doses. Panel (b) illustrates the effect of Dh β E on axial AIMs, where no significant differences were detected between treatment groups and vehicle over the 120 minute session, with axial AIM amplitude showing only minor fluctuations, including slight increases and decreases across doses. Similarly, panel (c) presents limb AIMs, where no significant reductions in AIM severity were observed at any dose relative to vehicle, although limb AIM amplitude showed small, inconsistent decreases at some doses without a clear pattern. Finally, orolingual AIMs (panel d) showed no significant changes across all tested doses, with amplitude remaining largely stable, showing only minimal increases or decreases throughout. All data are expressed as mean $\pm$ standard error and were analysed using one-way ANOVA followed by Dunnett's post *hoc* test.

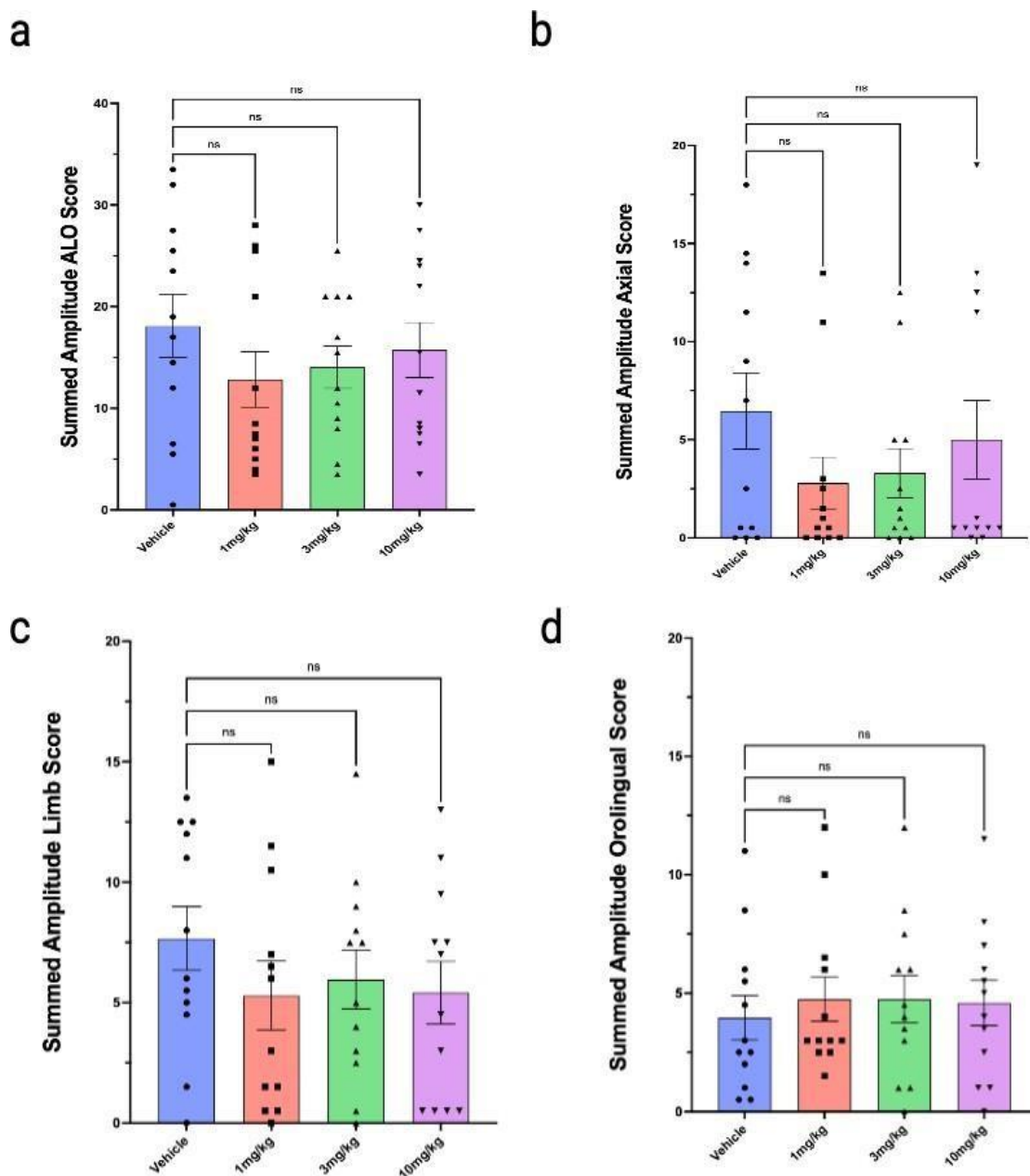


Figure 5.19: Effects of DhβE on the amplitude of AIMs on lesioned, primed rats. Panel (a) shows total ALO AIM scores, where DhβE produced no statistically significant reductions across all tested doses compared with vehicle. Panel (b) illustrates axial AIMs, which exhibited a non-significant decrease across doses, indicating limited modulation by the antagonist. Panel (c) presents limb AIMs, where no significant changes in amplitude were detected across the 120 minute period. Panel (d) shows orolingual AIMs, which similarly displayed no significant reductions in amplitude at any dose relative to vehicle. Data are expressed as mean $\pm$ SEM; (n = 12).

5.3.16 Effects of Dh β E on Amplitude AIMs Across a 120 Minute Period

An analysis of the effects of Dh β E on the amplitude of individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.20 (panels a–d).

When the effect of Dh β E on total ALO AIM scores was examined, the analysis revealed a significant main effect of time [$F(2.291, 25.20) = 10.95, p = 0.0002$], reflecting time dependent increases followed by decreases in AIM severity over the session, whereas the main effect of dose was not significant [$F(2.185, 24.03) = 1.052, p = 0.3703$]. Similarly, the dose $\times$ time interaction did not reach statistical significance [$F(5.650, 62.15) = 0.7805, p = 0.5815$], indicating that while AIM scores increased and decreased over time, the antagonist did not significantly alter the overall severity of total AIMs. Panel (b) illustrates the effect of Dh β E on axial AIMs. The analysis demonstrated a significant main effect of time [$F(2.148, 23.63) = 7.663, p = 0.0023$], with axial AIMs showing increases at earlier time points followed by a decline, but no significant effect of dose [$F(1.900, 20.90) = 1.576, p = 0.2306$] or dose $\times$ time interaction [$F(4.173, 45.91) = 1.500, p = 0.2161$], with only minor increases or decreases observed across doses.

Panel (c) presents the effect of Dh β E on limb AIMs, where the analysis showed a significant main effect of time [$F(2.886, 31.74) = 10.14, p < 0.0001$], again reflecting increases in severity followed by decreases over time, but no significant main effect of dose [$F(1.966, 21.62) = 1.425, p = 0.2621$] or dose $\times$ time interaction [$F(6.551, 72.06) = 0.8451, p = 0.5476$], with limb AIM amplitude showing small, inconsistent increases and decreases across treatment groups. Finally, panel (d) shows orolingual AIMs, where the analysis revealed no significant main effect of time [$F(3.105, 34.15) = 1.360, p = 0.2713$], dose [$F(2.087, 22.95) = 0.5608, p = 0.5855$], or dose $\times$ time interaction [$F(5.885, 64.74) = 1.151, p = 0.3433$], with scores remaining relatively stable, showing only slight increases or decreases throughout the observation period. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

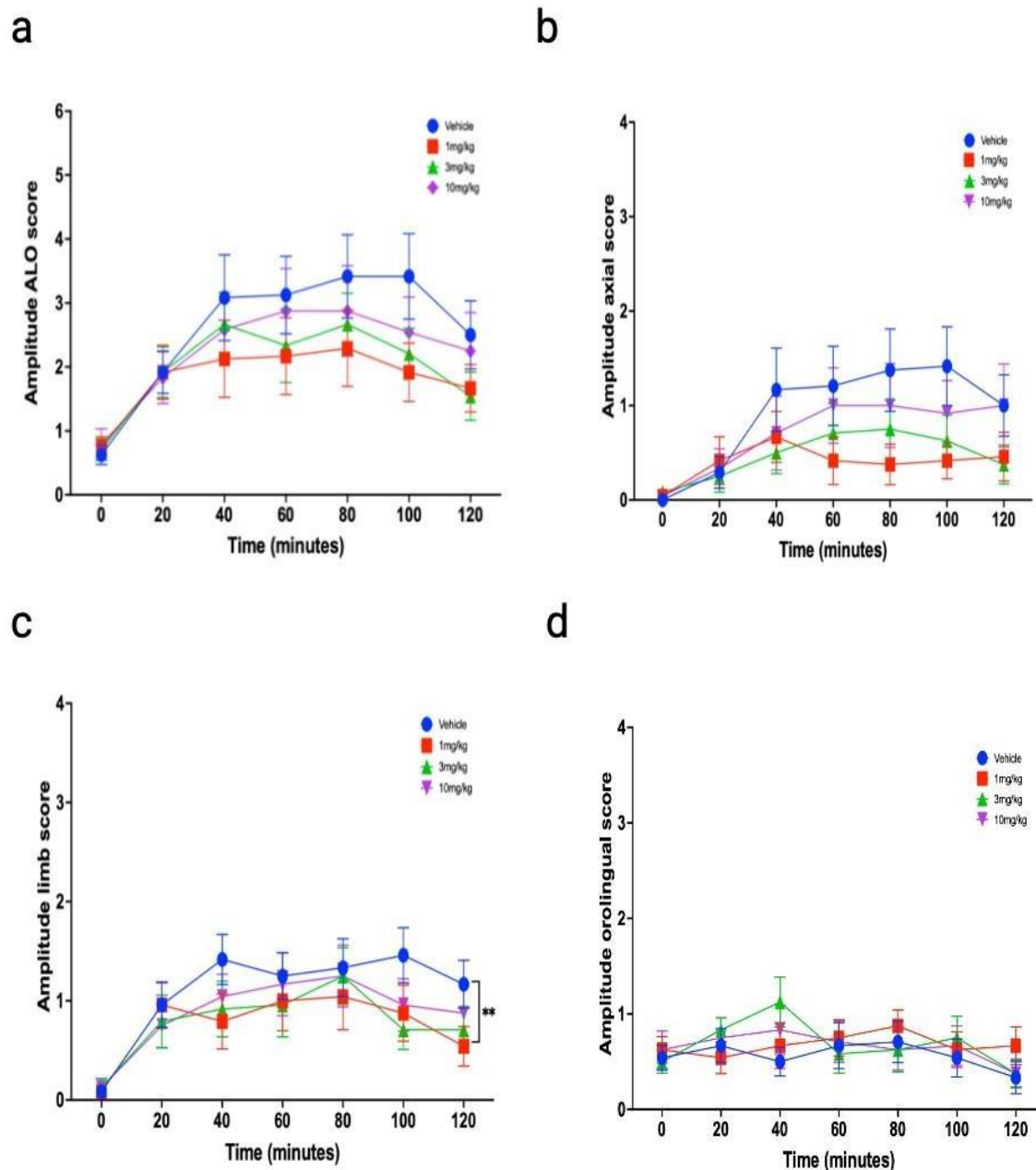


Figure 5. 20: Effects of administration of DhβE on the amplitude of AIMs subtypes on lesioned, primed rats. Panel (a) shows total ALO AIM scores, where DhβE produced a significant main effect of time but no significant dose related changes. Panel (b) illustrates axial AIMs, which showed a significant time dependent variation but no significant dose or dose $\times$ time effect. Panel (c) presents limb AIMs, which also showed a significant effect of time but no overall dose related change. Panel (d) shows orolingual AIMs, where no main effects of time, dose, or their interaction were detected. Data are expressed as mean $\pm$ SEM; * $p \leq 0.05$, ** $p \leq 0.01$, ($n = 12$).

5.3.17 Dh β E Does Not Significantly Alter the Global AIMs

An analysis of the effects of Dh β E on global and individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.21 (panels a–d).

Panel (a) shows the total global ALO AIM scores, where statistical analysis revealed that reductions across all tested doses were not statistically significant compared to vehicle ($p > 0.05$), although small decreases were observed at certain doses, indicating that Dh β E had no clear overall effect on total dyskinetic activity over the 120 minute period. Panel (b) presents the effect of Dh β E on axial AIMs, where reductions across doses did not reach statistical significance, with axial AIMs showing only minor increases and decreases across treatment groups, suggesting minimal effect of the antagonist on this subtype. Panel (c) illustrates the effects of Dh β E on limb AIMs, where a one-way ANOVA confirmed no significant reductions across any dose relative to vehicle, although limb AIMs showed small, inconsistent decreases at some doses without a clear pattern.

Finally, panel (d) shows orolingual AIMs, where Dh β E similarly failed to produce any significant reduction in overall AIM scores across the 120 minute observation period, with values remaining relatively stable and showing only slight increases or decreases. All data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

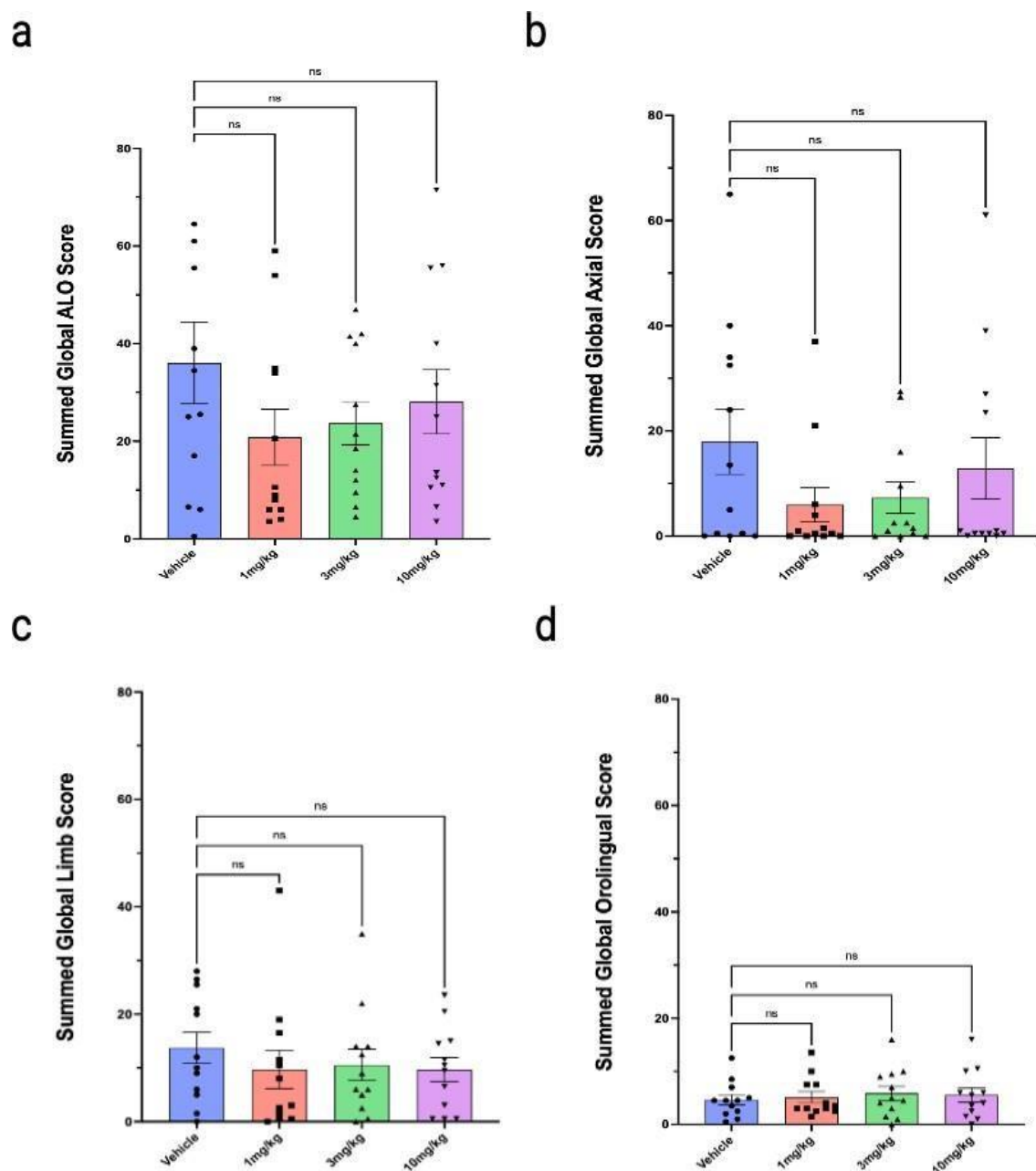


Figure 5. 21: Effects of administration of DhβE on global AIMs in lesioned, primed rats. Panel (a) shows total global ALO AIM scores, where DhβE produced no statistically significant reductions compared with vehicle across the 120 minute session. Panel (b) illustrates axial AIMs, which displayed a similar non-significant trend across doses. Panel (c) presents limb AIMs, where DhβE failed to produce any significant changes in global scores relative to vehicle. Panel (d) shows orolingual AIMs, which also exhibited no significant dose dependent reductions following DhβE administration. Data are expressed as mean ± standard error; (n = 12).

5.3.18 Dh β E Produces Time Dependent Reductions in Global AIMs

An analysis of Dh β E on the global AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.22 (panels a–d). When the effect of Dh β E on total global ALO AIM scores was examined (panel a), there was a significant main effect of time [$F(2.664, 29.31) = 11.29, p < 0.0001$], reflecting increases in AIM severity followed by decreases over time, while the main effect of dose did not reach statistical significance [$F(2.059, 22.65) = 1.220, p = 0.3150$], nor did the dose $\times$ time interaction [$F(4.487, 49.35) = 0.9227, p = 0.4666$], with only minor increases and decreases observed across doses.

Panel (b) illustrates the effect of Dh β E on global axial AIMs, which showed a significant main effect of time [$F(2.421, 26.64) = 7.427, p = 0.0017$], again reflecting an increase followed by a decline over the session, but not of dose [$F(1.792, 19.72) = 1.688, p = 0.2117$], and the dose $\times$ time interaction was non-significant [$F(3.391, 37.30) = 1.461, p = 0.2382$], with axial AIMs showing small, inconsistent increases and decreases across treatment groups. Panel (c) shows the effect of Dh β E on global limb AIMs, where a significant main effect of time was detected [$F(3.451, 37.96) = 9.402, p < 0.0001$], reflecting increases in severity followed by decreases, but neither dose [$F(2.162, 23.78) = 0.7613, p = 0.4876$] nor the dose $\times$ time interaction [$F(5.428, 59.71) = 0.7459, p = 0.6025$] reached significance, with limb AIMs showing minor fluctuations across doses. However, at 120 minutes, the 1 mg/kg dose significantly reduced limb AIM amplitude compared to the vehicle (* $p < 0.05$).

Lastly, panel (d) presents global orolingual AIMs, which showed no significant main effects of time [$F(2.789, 30.68) = 1.286, p = 0.2962$], dose [$F(1.977, 21.75) = 0.9322, p = 0.4079$], or dose $\times$ time interaction [$F(5.046, 55.51) = 1.314, p = 0.2714$], with scores remaining relatively stable and showing only slight increases or decreases throughout. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

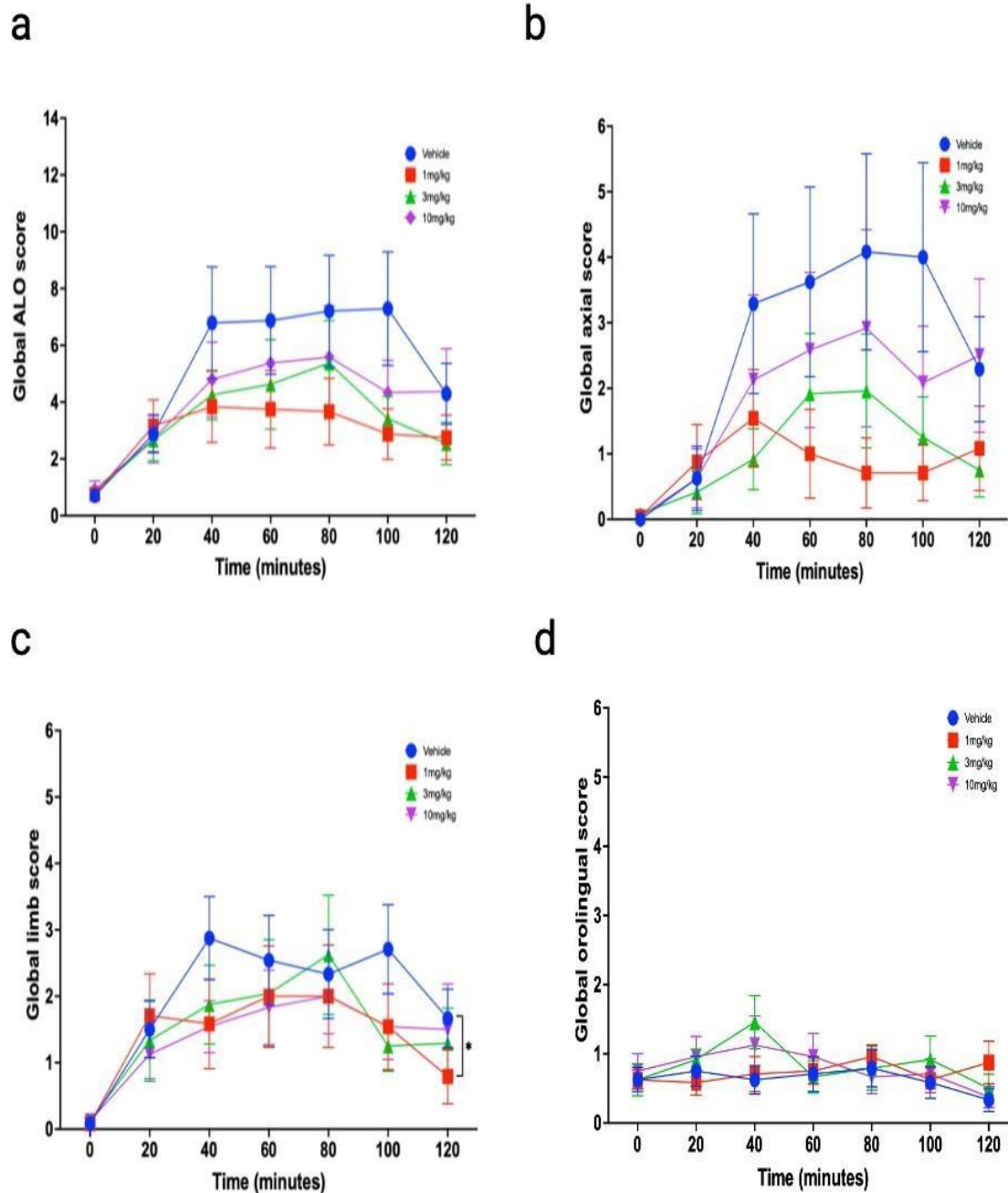


Figure 5.22: Effects of administration of Dh β E on global AIMs in lesioned, primed rats. Panel (a) shows total global ALO AIM scores, where Dh β E produced a significant main effect of time but no significant effect of dose or dose $\times$ time interaction. Panel (b) illustrates global axial AIMs, which also showed a significant time effect but no significant dose dependent changes. Panel (c) presents global limb AIMs, where Dh β E produced a significant time effect but no main dose effect, with a notable decrease at 1 mg/kg after 120 minutes ($p < 0.05$). Panel (d) shows global orolingual AIMs, which exhibited no significant effects of time, dose, or interaction. Data are expressed as mean $\pm$ SEM; * $p < 0.05$; ($n = 12$).

5.3.19 Co-administration of A85380 and DhβE has no significant effect on Locomotive AIMs

Locomotive AIM scores over time are shown for rats co-administered with A85380 (0.01 mg/kg) and the antagonist DhβE (1 mg/kg), as well as for groups receiving each drug individually, to assess the interactive effects of receptor activation and inhibition on levodopa induced AIMs. Analysis across the 120 minute observation period revealed a significant main effect of time [$F(6, 66) = 12.97, p < 0.0001$], indicating time dependent increases in locomotive AIM expression followed by decreases over the session. However, neither the main effect of dose [$F(3, 33) = 0.7298, p = 0.5416$] nor the dose $\times$ time interaction [$F(18, 198) = 0.5128, p = 0.9502$] reached statistical significance, with locomotive AIM scores showing only minor increases and decreases across treatment groups (Figure 5.23a).

Figure 5.23b presents the summed locomotive AIM scores over the 120 minute session. Comparison of cumulative scores among the vehicle, A85380 (0.01 mg/kg), DhβE (1 mg/kg) and combination treatment groups revealed no statistically significant differences, as confirmed by one-way ANOVA ($p > 0.05$), with total scores remaining broadly similar across groups without clear increases or decreases. Data are expressed as mean $\pm$ standard error.

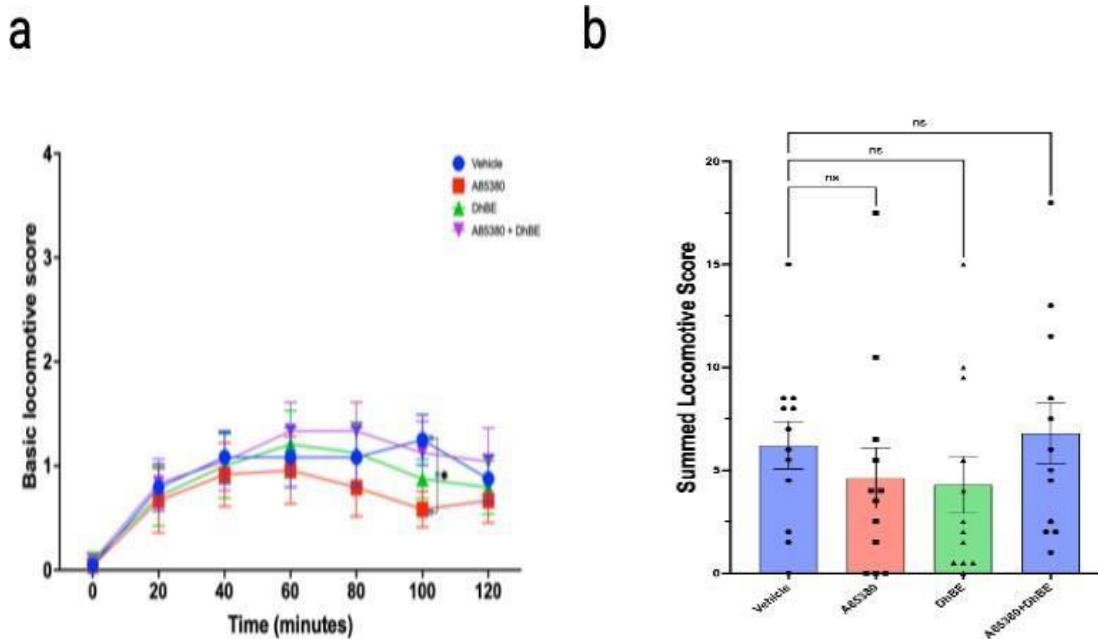


Figure 5. 23: Effects of co-administration A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on locomotive AIMs in lesioned, primed rats. (a) Time course of locomotive AIMs over a 120 minute period following administration of A85380, DhβE, their combination, or vehicle. A significant main effect of time was observed ($p < 0.0001$), indicating time dependent variation in AIM expression, but no significant dose dependent or interaction effects were detected. (b) Summed locomotive AIM scores across the 120 minute session revealed no significant differences between treatment groups, as confirmed by one-way ANOVA ($*p > 0.05$). Data are expressed as $\pm$ SEM; (n = 12).

5.3.20 Co-administration of A85380 and DhβE Does Not Significantly Change

Basic AIMs

An analysis of the effects of co-administration of A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on the basic area under the curve of individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats (n = 12) is presented in Figure 5.24 (panels a–d).

Panel (a) shows total ALO AIM scores, representing the combined basic frequency of axial, limb, and orolingual movements. One-way ANOVA revealed no significant overall differences between the vehicle, A85380, DhβE, or co-administration groups, with total scores showing only

minor increases and decreases across treatments, indicating that simultaneous receptor activation and inhibition did not alter total dyskinetic frequency. Panel (b) illustrates axial AIMs, which displayed no significant reductions across treatment groups compared with vehicle, with axial scores showing small increases and decreases without a consistent pattern. The co-administration of A85380 and Dh β E did not produce any clear additive or opposing effects on axial AIM expression. Panel (c) presents limb AIMs, where all treatments, including co-administration, failed to produce significant changes relative to vehicle. Although a slight decrease was observed in the A85380 alone and co-treatment groups, this did not reach statistical significance and was not consistently maintained across groups.

Finally, orolingual AIMs (panel d) showed no significant group differences, with both individual and combined treatments producing comparable scores to the vehicle group, and only minimal increases or *decreases* observed across conditions. All data are expressed as mean $\pm$ standard error and were analysed using one-way ANOVA with Dunnett's *post hoc* test.

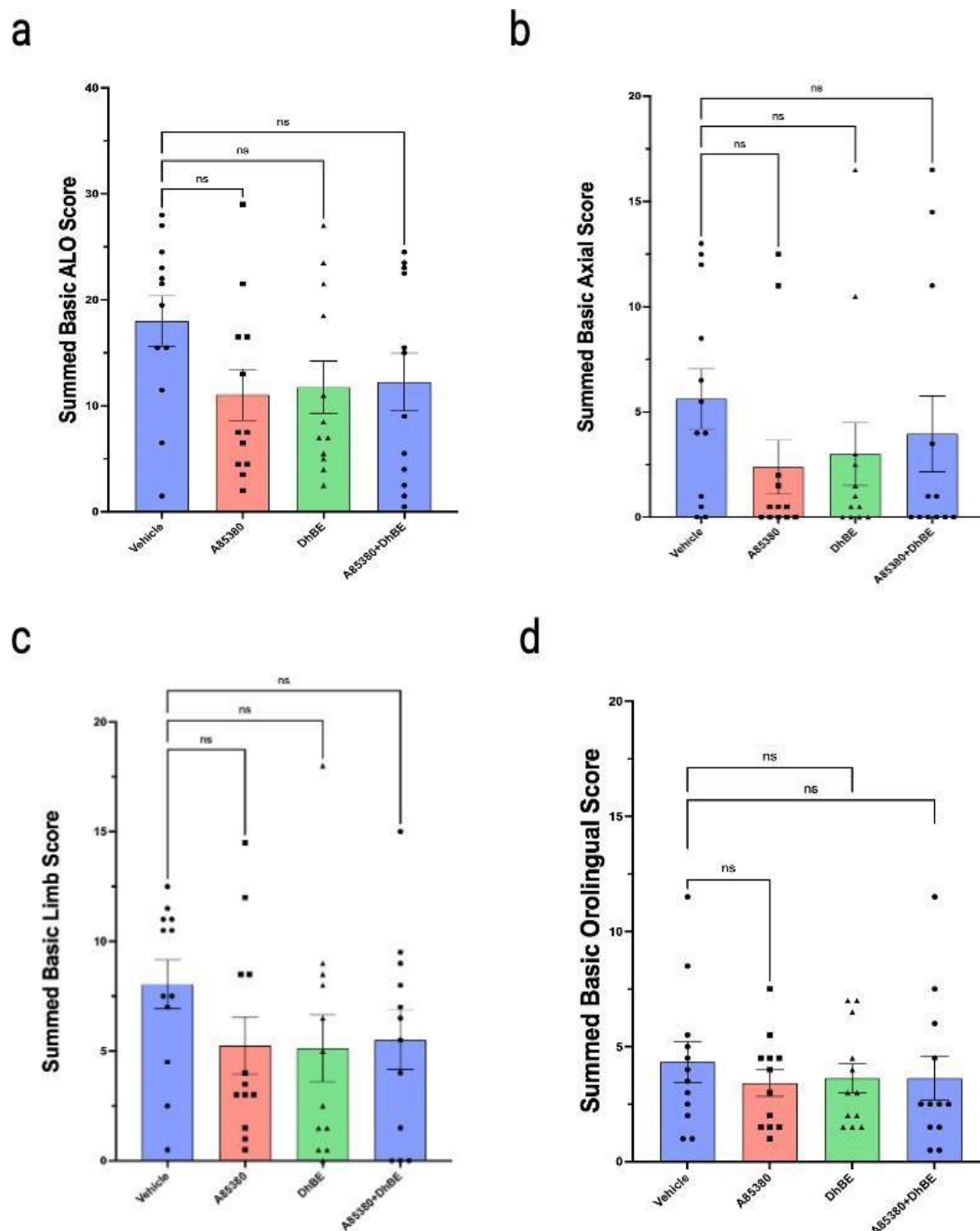


Figure 5. 24: Effects of co-administration of A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on basic AIM in lesioned, primed rats. Panel (a) shows total ALO AIM scores, where co-administration produced no significant changes compared with vehicle, indicating no combined effect on overall dyskinetic frequency. Panel (b) illustrates axial AIMs, which showed no significant alterations across treatment groups. Panel (c) presents limb AIMs, where minor reductions were observed in the A85380 and co-treatment groups but did not reach statistical significance. Panel (d) shows orolingual AIMs, which were unaffected by either individual or combined drug administration. Data are expressed as mean $\pm$ standard error were analysed using one-way ANOVA; (n = 12).

5.3.21 Effects of Co-administration of A85380 and DhβE on Basic AIMs Across a 120 Minute

An analysis of the effects of co-administration of A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on the frequency of individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.25 (panels a–d).

When the effect of co-administration on total ALO AIM scores was examined (panel a), the analysis revealed a significant main effect of time [$F(2.974, 32.72) = 9.749, p < 0.0001$], indicating time dependent increases in dyskinetic expression followed by decreases over the session. However, no significant main effect of dose [$F(2.627, 28.90) = 2.144, p = 0.1230$] or dose $\times$ time interaction [$F(4.293, 47.22) = 2.121, p = 0.0886$] was observed, with scores showing minor increases and decreases across groups. Post hoc comparisons showed significant reductions in basic ALO scores at specific time points: A85380 reduced scores at $t = 0, 100$, and 120 minutes ($*p \leq 0.05$); DhβE reduced scores at $t = 100$ and 120 minutes ($*p \leq 0.05$); and co-administration reduced scores only at $t = 40$ minutes ($*p \leq 0.05$). Panel (b) illustrates the effect on axial AIMs, where a significant main effect of time was observed [$F(6, 66) = 7.333, p < 0.0001$], reflecting increases followed by decreases over time, but no significant effects of dose [$F(3, 33) = 0.9493, p = 0.4281$] or dose $\times$ time interaction [$F(18, 198) = 1.466, p = 0.1057$] were detected, with axial AIMs showing small increases and decreases across treatments. Notably, A85380 significantly reduced scores at $t = 40$ and 100 minutes ($*p \leq 0.05$), while DhβE reduced scores at $t = 80$ and 100 minutes ($**p \leq 0.001$).

Panel (c) presents limb AIMs, where the analysis revealed a significant main effect of time [$F(6, 66) = 9.053, p < 0.0001$], again reflecting increases in severity followed by decreases, but neither the main effect of dose [$F(3, 33) = 2.536, p = 0.0736$] nor the dose $\times$ time interaction [$F(18, 198) = 1.412, p = 0.1289$] reached statistical significance, with limb AIMs showing minor

fluctuations across groups. Post hoc tests indicated that A85380 significantly reduced limb AIM scores at $t = 40$ ($***p \leq 0.0001$) and $t = 80$ ($*p \leq 0.05$), Dh β E at $t = 40$ ($***p \leq 0.0001$), $t = 100$, and $t = 120$ ($*p \leq 0.05$), and the co-administration at $t = 40$ and $t = 80$ ($*p \leq 0.05$). Lastly, orolingual AIMS (panel d) showed no significant main effects of time [$F(2.925, 32.17) = 0.9038$, $p = 0.4479$], dose [$F(2.075, 22.83) = 2.032$, $p = 0.1528$], or dose $\times$ time interaction [$F(6.389, 70.28) = 1.341$, $p = 0.2483$], with scores remaining relatively stable and showing only slight increases or decreases throughout the observation period. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

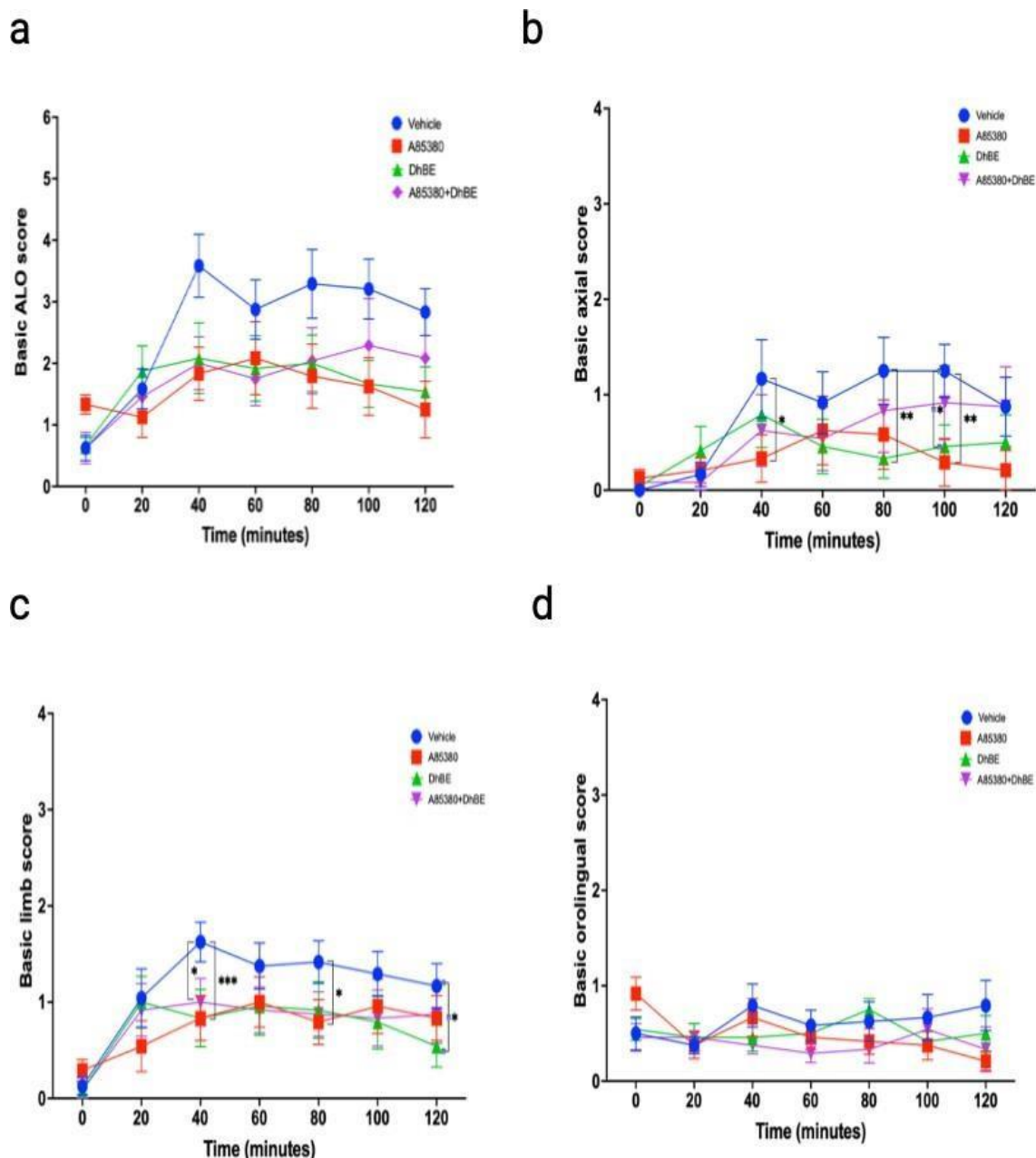


Figure 5. 25: Effects of co-administration of A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on basic AIMs in lesioned, primed rats. Panel (a) shows total ALO AIM scores, where a significant main effect of time was observed ($p < 0.0001$), but no significant dose related effects or dose $\times$ time interactions were detected. Significant reductions were observed at time points: A85380 at 0, 100, and 120 minutes; DhβE at 100 and 120 minutes; and the co-administration at 40 minutes ($*p \leq 0.05$). Panel (b) illustrates axial AIMs, showing a significant effect of time ($p < 0.0001$) but no dose dependent or interaction effects. A85380 significantly reduced scores at 40 and 100 minutes ($*p \leq 0.05$), while DhβE reduced them at 80 and 100 minutes ($**p \leq 0.001$). Panel (c) presents limb AIMs, which exhibited a significant main effect of time ($p < 0.0001$) but no dose or interaction effects. A85380 reduced limb scores at 40 ($***p \leq 0.0001$) and 80 minutes ($*p \leq 0.05$), DhβE at 40 ($***p \leq 0.0001$), 100, and 120 minutes ($*p \leq 0.05$), and the co-administration at 40 and 80 minutes ($*p \leq 0.05$). Panel (d) shows orolingual AIMs, which did not show significant effects of time, dose, or interaction, indicating no measurable influence of either treatment or their combination. Data are expressed as mean $\pm$ SEM; $*p < 0.05$, $**p < 0.001$, $***p < 0.0001$; ($n = 12$).

5.3.22 A85380 and Dh β E Co-administration Significantly Reduces the Amplitude AIMs

An analysis of the effects of co-administration of A85380 (0.01 mg/kg) and Dh β E (1 mg/kg) on the amplitude of individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.26 (panels a–d). The total ALO scores (panel a) showed a significant reduction in amplitude compared with the vehicle, as confirmed by one-way ANOVA ($p = 0.0187$), indicating a decrease in the overall severity of levodopa induced AIMs, driven primarily by A85380 treatment.

Panel (b) illustrates the effect on axial AIMs, where one-way ANOVA revealed no significant overall reductions in amplitude compared to the vehicle, with axial AIMs showing only minor increases and decreases across treatment groups, suggesting that neither A85380, Dh β E, nor their combination significantly altered the severity of axial movements. Panel (c) presents limb AIMs, where one-way ANOVA confirmed significant overall reductions in amplitude across all tested treatment groups relative to vehicle, demonstrating a clear decrease in limb AIM severity following both individual and combined treatments.

Finally, orolingual AIMs (panel d) showed significant reductions in amplitude only under co-administration of A85380 and Dh β E ($p = 0.0343$) compared with vehicle, indicating a decrease in the severity of repetitive jaw and tongue movements under combined treatment, while individual treatments showed little change. All data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

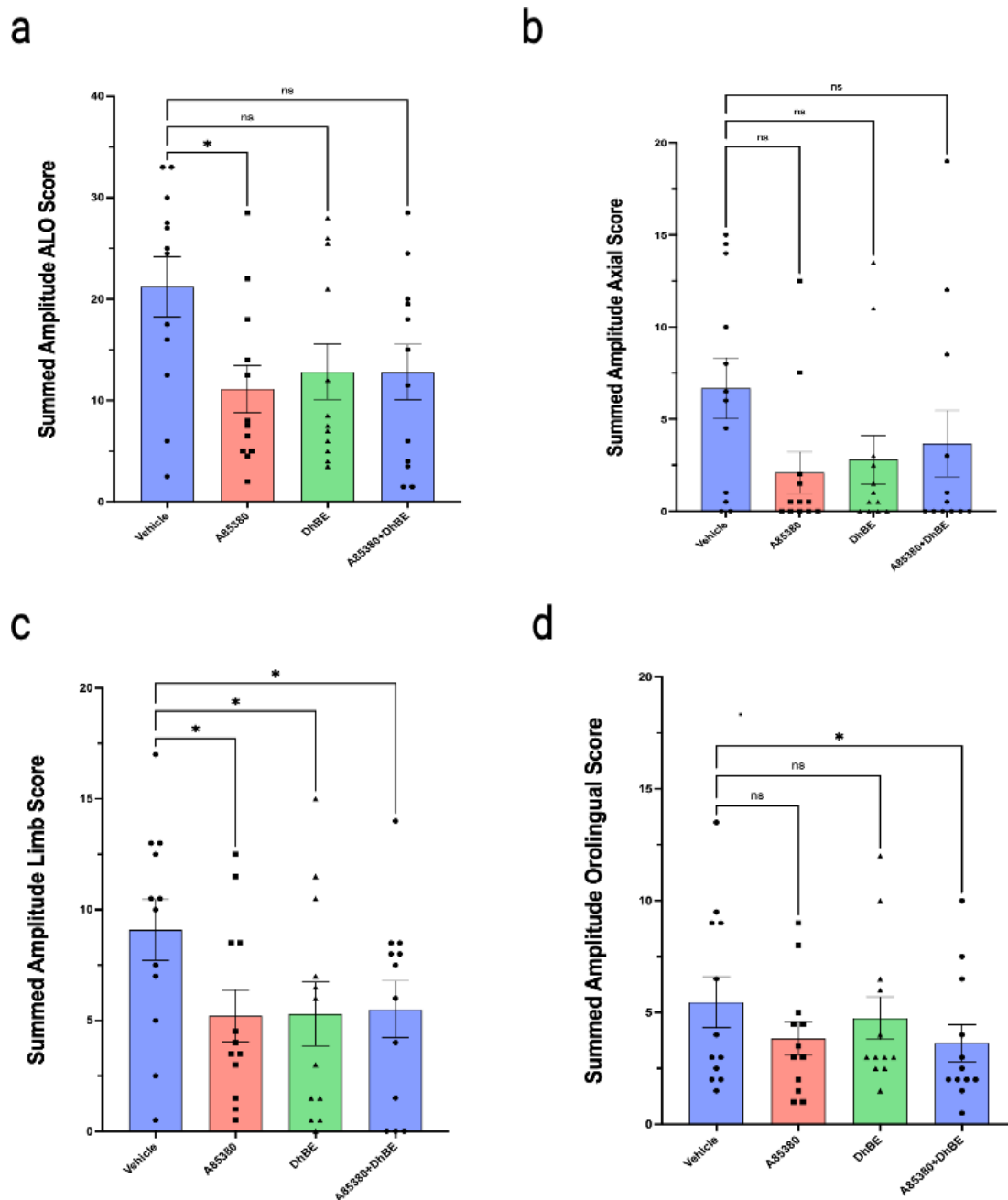


Figure 5. 26: Effects of co-administration of A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on the amplitude AIMs in lesioned, primed rats. Panel (a) shows total ALO AIM scores, where A85380 produced a significant reduction in amplitude compared with vehicle ($p = 0.0187$). Panel (b) illustrates axial AIMs, which showed no significant changes across treatment groups. Panel (c) presents limb AIMs, where all treatment groups showed significant overall reductions compared with vehicle. Panel (d) shows orolingual AIMs, which displayed a significant reduction only under co-administration of A85380 and DhβE ($p = 0.0343$). Data are expressed as $\pm$ SEM**; * $p < 0.05$; ($n = 12$).

5.3.23 Co-administration of A85380 and DhβE Significantly Reduces the Amplitude AIMS Over a 120 Minute Period

An analysis of the effects of co-administration of A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on the amplitude of individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.27 (panels a–d).

When the effect of co-administration on total ALO AIM scores was examined (panel a), the analysis revealed a significant main effect of time [$F(3.216, 35.37) = 10.16, p < 0.0001$], reflecting increases in AIM severity followed by decreases over time, and a significant main effect of dose [$F(2.694, 29.63) = 3.961, p = 0.0203$], indicating an overall decrease in AIM amplitude across treatment groups. However, no significant dose $\times$ time interaction [$F(4.808, 52.89) = 2.113, p = 0.0806$] was observed, with scores showing similar increases and decreases across groups. Significant reductions in ALO AIM severity were observed at specific time points: A85380 reduced scores at $t = 80, t = 100$, and $t = 120$ ($*p \leq 0.05$), DhβE reduced scores at $t = 40, t = 100$ ($*p \leq 0.05$), and $t = 120$ ($**p \leq 0.001$), while co-administration reduced scores only at $t = 40$ ($*p \leq 0.05$). Panel (b) illustrates the effect on axial AIMS, where the analysis showed a significant effect of time [$F(6, 66) = 7.575, p < 0.0001$], reflecting increases followed by decreases over the session, but no significant effect of dose [$F(3, 33) = 1.981, p = 0.1360$] or dose $\times$ time interaction [$F(18, 198) = 2.005, p = 0.0110$], with axial AIMS showing small increases and decreases across treatments. Both A85380 and DhβE produced transient decreases at distinct time points, notably at $t = 40, 80$, and 100 minutes ($*p \leq 0.05; **p \leq 0.001$).

Panel (c) presents limb AIMS, which showed a significant main effect of time [$F(6, 66) = 9.186, p < 0.0001$], reflecting increases followed by decreases, and a significant main effect of dose [$F(3, 33) = 4.205, p = 0.0126$], indicating an overall reduction in limb AIM amplitude across

treatments, though the dose $\times$ time interaction did not reach significance [$F(18, 198) = 2.068$, $p = 0.0083$], with similar temporal increases and decreases observed across groups. Significant reductions were seen at multiple time points: A85380 at $t = 40$ ($****p \leq 0.00001$), $t = 80$ ($***p \leq 0.0001$), and $t = 100$ ($*p \leq 0.05$); Dh β E at $t = 40$ ($****p \leq 0.00001$), $t = 80, 100$ ($*p \leq 0.05$), and $t = 120$ ($***p \leq 0.0001$); and the co-administration at $t = 40$ ($***p \leq 0.0001$), $t = 60$ ($*p \leq 0.05$), $t = 100$ ($**p \leq 0.001$), and $t = 120$ ($*p \leq 0.05$). Finally, panel (d) shows orolingual AIMs, where no significant main effect of time [$F(3.764, 41.41) = 0.7176$, $p = 0.5768$] was detected, though a significant main effect of dose was observed [$F(2.067, 22.74) = 3.658$, $p = 0.0407$]. The dose $\times$ time interaction was not significant [$F(6.745, 74.20) = 1.143$, $p = 0.3464$]. Importantly, a significant reduction in orolingual AIM amplitude was observed only during co-administration at $t = 120$ ($*p \leq 0.05$). All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

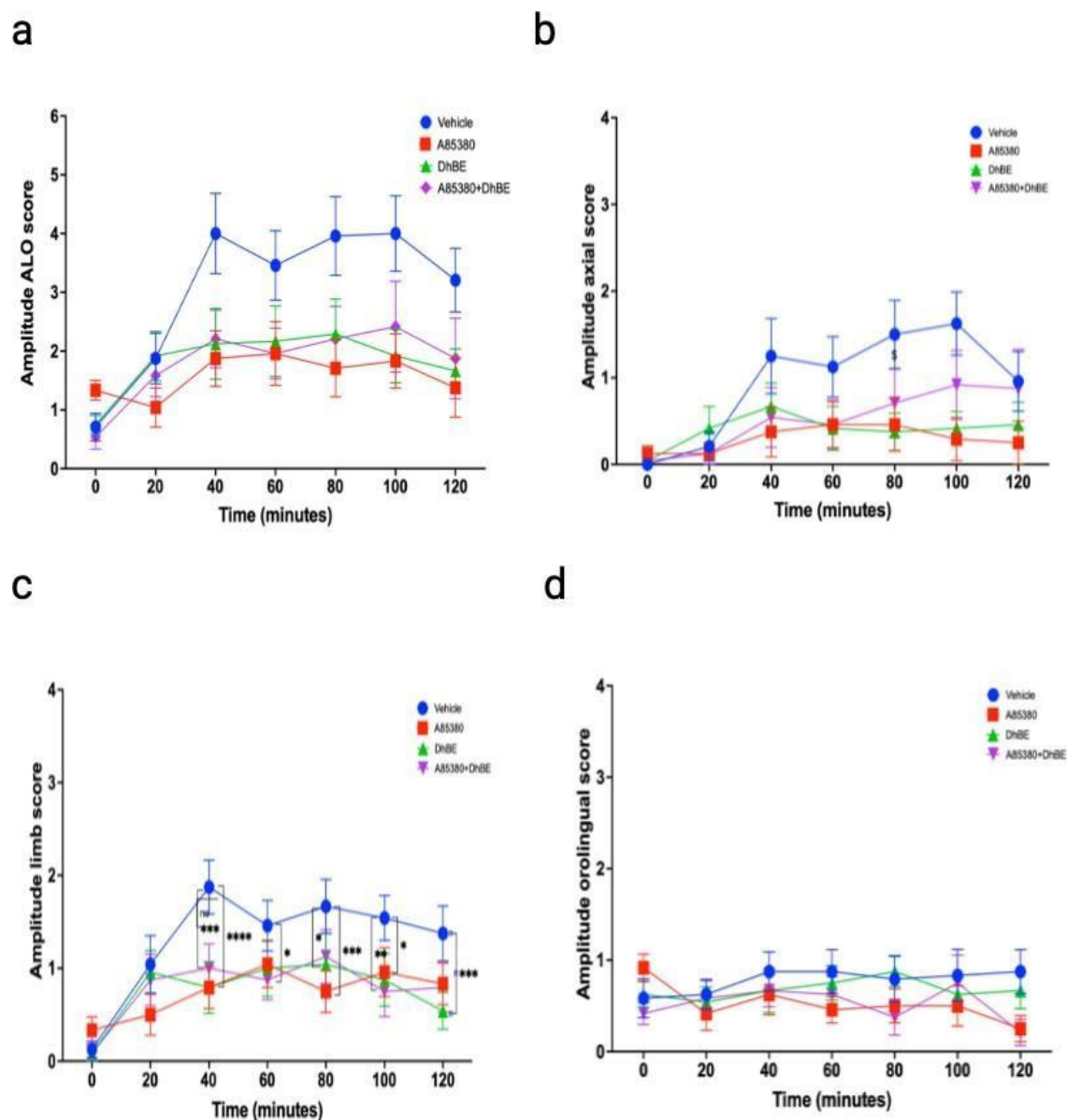


Figure 5. 27: Effects of co-administration of A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on the amplitude of various AIMs in lesioned, primed rats. Panel (a) shows total ALO AIM scores, where both A85380 and DhβE produced significant main effects of time and dose, indicating overall reduction of AIM severity, while the combined treatment reduced scores only at $t = 40$ minutes ($*p \leq 0.05$). Panel (b) illustrates axial AIMs, which showed a significant effect of time but no significant dose or interaction effects, though transient reductions were observed at several time points, particularly with DhβE at $t = 80$ and 100 minutes ($**p \leq 0.001$). Panel (c) presents limb AIMs, where significant effects of both time and dose were found, with marked reductions at multiple time points for all treatments, including co-administration ($*p \leq 0.05$, $**p \leq 0.001$, $***p \leq 0.0001$). Panel (d) shows orolingual AIMs, where only the combined A85380 and DhβE treatment significantly reduced amplitude at $t = 120$ minutes ($*p \leq 0.05$). Data are expressed as mean $\pm$ SEM; $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, $****p \leq 0.0001$; ($n = 12$).

5.3.24 Co-administration of A85380 and Dh β E Does Not Significantly Alter the Global AIMs

An analysis of the effects of co-administration of A85380 (0.01 mg/kg) and Dh β E (1 mg/kg) on global and individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.28 (panels a–d).

Panel (a) shows the total global ALO AIM scores, where one-way ANOVA revealed that reductions across all tested groups (A85380, Dh β E, and co-administration) were not statistically significant compared with vehicle ($p > 0.05$), with total scores showing only minor decreases and occasional increases, suggesting no overall effect on total dyskinetic activity. Panel (b) illustrates global axial AIMs, where none of the treatments produced significant reductions relative to vehicle, with axial AIMs showing small increases and decreases across groups, indicating that both A85380 and Dh β E, individually or combined, did not meaningfully affect this subtype. Panel (c) presents global limb AIMs, which also showed no significant reductions across treatment groups, with limb AIMs remaining relatively stable and showing only slight increases and decreases following $\alpha 4\beta 2$ receptor modulation.

Finally, panel (d) shows global orolingual AIMs, where no significant changes were observed across any treatment condition compared with vehicle, with scores remaining low and showing minimal increases or decreases, indicating that co-administration did not modify overall orolingual dyskinetic expression. All data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

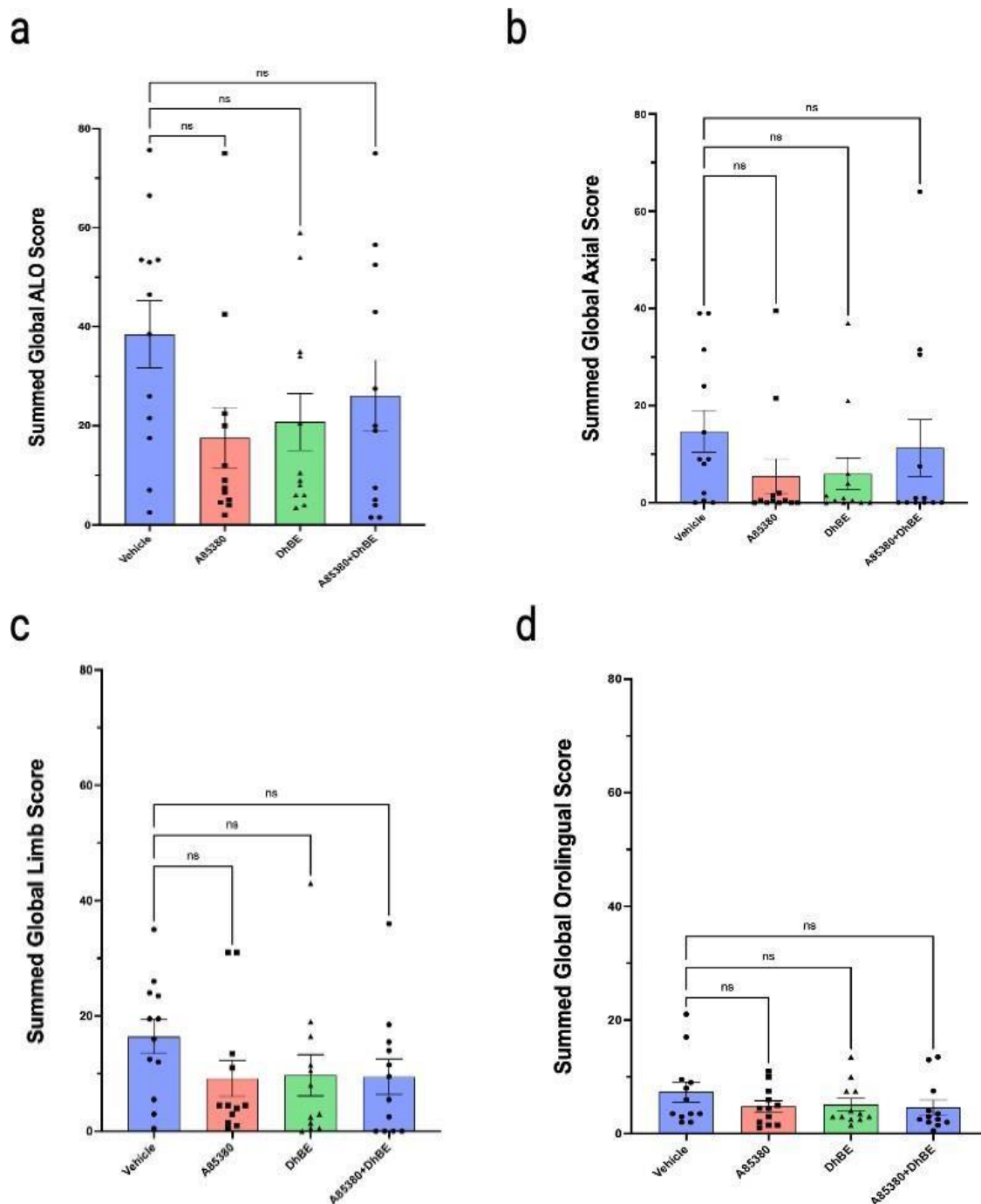


Figure 5. 28: Effects of co-administration of A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on global AIMs in lesioned, primed rats. Panel (a) shows total global ALO AIM scores, where none of the treatments (A85380, DhβE, or their co-administration) produced statistically significant reductions compared with vehicle across the 120-minute session. Panel (b) illustrates global axial AIMs, which displayed a similar non-significant trend across all treatment conditions. Panel (c) presents global limb AIMs, where no significant changes were detected in any treatment group relative to vehicle. Panel (d) shows global orolingual AIMs, which also exhibited no significant dose related reductions following either individual or combined administration. Data are expressed as mean $\pm$ SEM; (n = 12).

5.3.25 Co-administration of A85380 and Dh β E Produces Time Dependent Reductions on Global AIMs

An analysis of the effects of co-administration of A85380 (0.01 mg/kg) and Dh β E (1 mg/kg) on global AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.29 (panels a–d).

When the effect on total global ALO AIM scores was examined (panel a), a significant main effect of time was observed [$F(3.628, 39.91) = 9.162, p < 0.0001$], reflecting increases in AIM severity followed by decreases over time, while the main effect of dose [$F(2.633, 28.97) = 2.064, p = 0.1333$] and the dose $\times$ time interaction [$F(3.851, 42.36) = 1.503, p = 0.2200$] were not significant, with scores showing only minor increases and decreases across treatment groups. Panel (b) illustrates the effect on global axial AIMs, which demonstrated a significant main effect of time [$F(6, 66) = 6.968, p < 0.0001$], again reflecting increases followed by decreases over the session, but no significant dose effect [$F(3, 33) = 1.050, p = 0.3833$] or dose $\times$ time interaction [$F(18, 198) = 1.208, p = 0.2571$], with axial AIMs showing small increases and decreases across groups. A85380 significantly reduced axial AIM scores at $t = 40$ and $t = 100$ minutes ($*p \leq 0.05$), while Dh β E reduced scores at $t = 80$ and $t = 100$ minutes ($**p \leq 0.001$). Panel (c) shows global limb AIMs, where the analysis revealed significant main effects of both time [$F(6, 66) = 6.381, p < 0.0001$], reflecting increases followed by decreases, and dose [$F(3, 33) = 3.017, p = 0.0437$], indicating an overall decrease in limb AIM amplitude across treatments, but no significant dose $\times$ time interaction [$F(18, 198) = 1.496, p = 0.0943$], with similar temporal increases and decreases across groups. At specific time points, A85380 reduced limb AIMs at $t = 40$ ($****p \leq 0.00001$) and $t = 80$ ($**p \leq 0.001$), Dh β E reduced scores at $t = 40$ ($***p \leq 0.0001$) and $t = 120$ ($*p \leq 0.05$), while co-administration produced reductions at $t = 40$ ($***p \leq 0.0001$).

Lastly, global orolingual AIMs (panel d) showed no significant main effects of time [$F(3.229, 35.52) = 0.8076$, $p = 0.5059$], dose [$F(1.832, 20.15) = 2.718$, $p = 0.0939$], or interaction [$F(5.228, 57.51) = 1.209$, $p = 0.3164$], with scores remaining relatively stable and showing only slight increases or decreases, indicating that neither A85380, Dh β E, nor their combination altered orolingual AIMs across the session. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

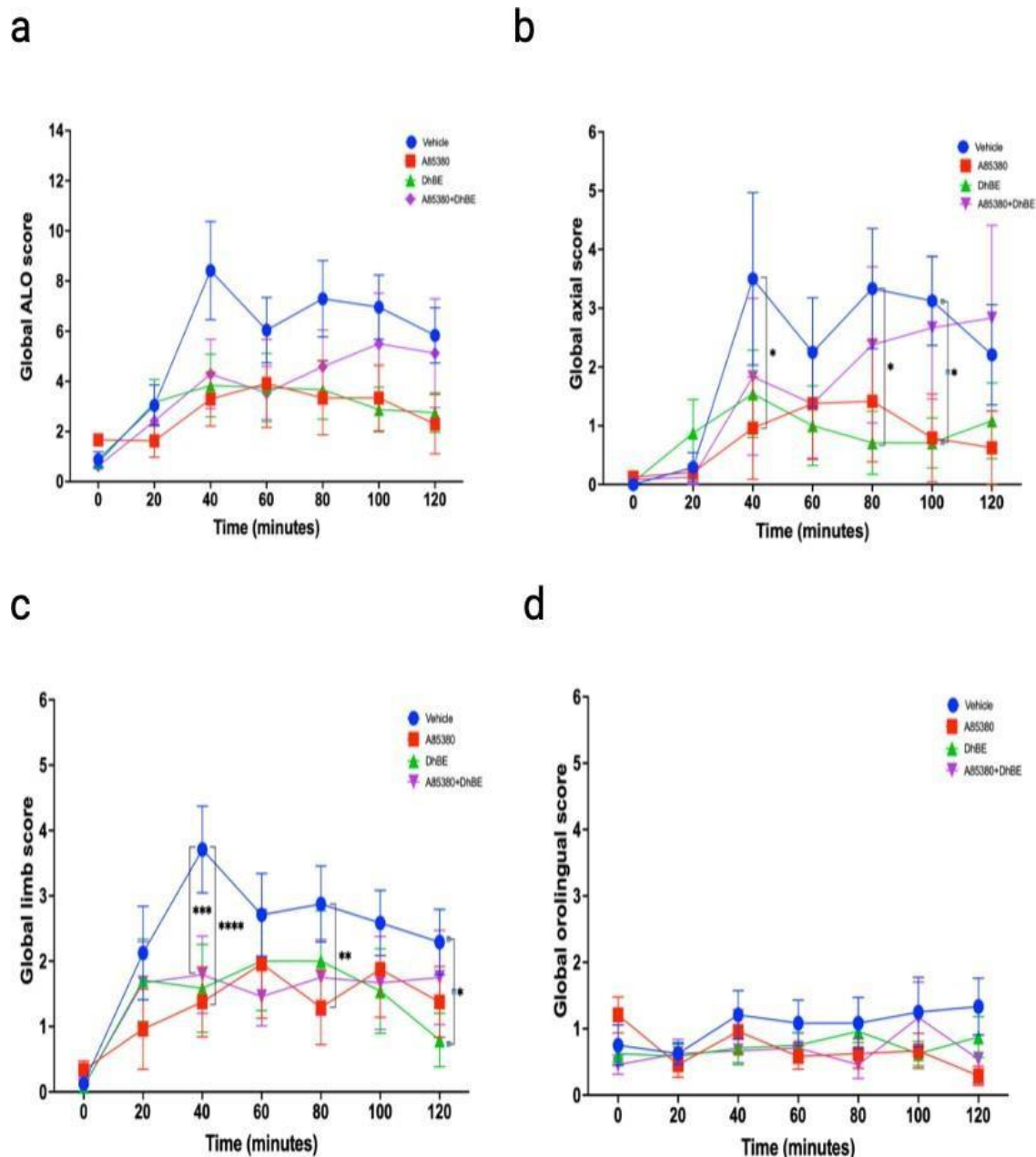


Figure 5. 29: Effects of co-administration of A85380 (0.01 mg/kg) and DhβE (1 mg/kg) on global AIMs in lesioned, primed rats. Panel (a) shows total global ALO AIM scores, where a significant main effect of time was detected ($p < 0.0001$), but no significant effect of dose or dose $\times$ time interaction was observed. Panel (b) illustrates global axial AIMs, which also showed a significant main effect of time ($p < 0.0001$) but no dose dependent changes, with transient reductions observed at $t = 40$ – 100 minutes for A85380 and $t = 80$ – 100 minutes for DhβE. Panel (c) presents global limb AIMs, where both time ($p < 0.0001$) and dose ($p = 0.0437$) had significant effects, though the dose $\times$ time interaction did not reach significance. Marked reductions occurred at $t = 40$ minutes across all treatments, with DhβE and A85380 each producing additional decreases at later time points. Panel (d) shows global orolingual AIMs, which displayed no significant effects of time, dose, or interaction, indicating that neither treatment nor their combination altered orolingual AIMs across the session. Data are expressed as $\pm$ SEM; * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$; ($n = 12$).

5.3.26 Administration of NS9283 Does Not Significantly Affect Locomotive AIMs

Locomotive AIM scores over time are shown for rats treated with varying doses of the positive allosteric modulator (PAM) NS9283 to assess its effects on levodopa induced AIMs. Analysis across the observation period revealed a significant main effect of time [$F(3.210, 35.31) = 7.029$, $p = 0.0006$], indicating time dependent increases in locomotive AIM expression followed by decreases over the session. However, neither the main effect of dose [$F(1.639, 18.03) = 0.1122$, $p = 0.8568$] nor the dose $\times$ time interaction [$F(3.406, 37.47) = 1.160$, $p = 0.3408$] reached statistical significance, with scores showing only minor increases and decreases across doses (Figure 5.30 panel a).

Figure 5.30b presents the summed locomotive AIM scores across treatment conditions. Post hoc analysis of area under the curve values using one-way ANOVA followed by Dunnett's test revealed no significant reductions in cumulative locomotive AIM scores at any tested dose of NS9283 compared to the vehicle group ($p > 0.05$), with total scores remaining broadly similar and showing only slight increases or decreases across groups. Data are expressed as mean $\pm$ standard error.

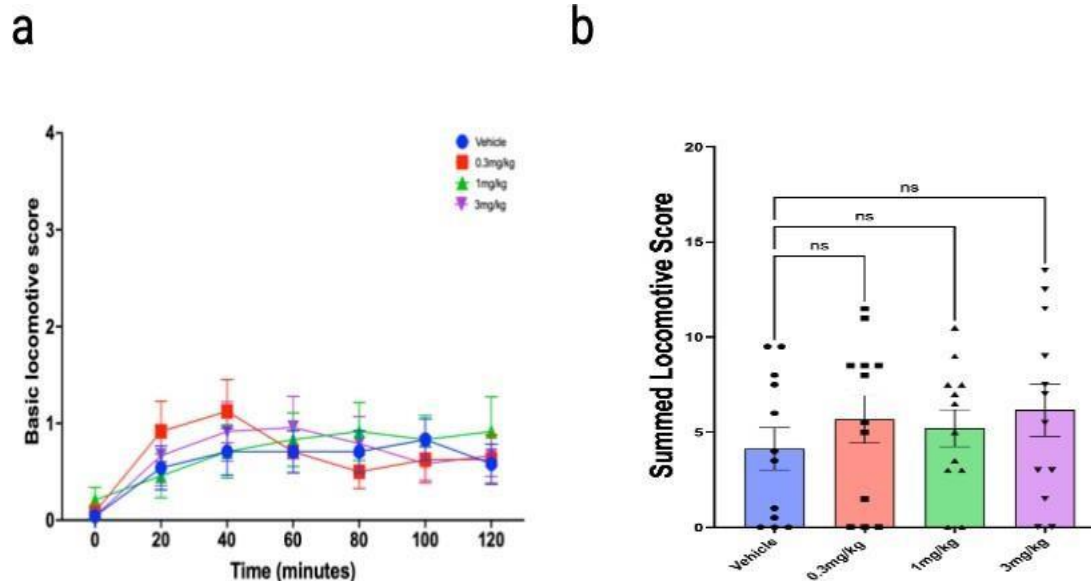


Figure 5. 30: Effects of NS9283 on locomotive AIMs in lesioned, primed rats. (a) Time course of locomotive AIMs following administration of NS9283 at varying doses or vehicle control. A significant main effect of time was observed whereas no significant effects of dose or dose $\times$ time interaction were detected ($p > 0.05$). (b) Summed locomotive AIM scores expressed as area under the curve showed no significant differences among treatment groups, as confirmed by one-way ANOVA followed by Dunnett's *post hoc* test ($p > 0.05$). Data are presented as mean $\pm$ SEM; ($n = 12$).

5.3.27 Administration of the NS9283 Does Not Significantly Alter Basic AIMs

An analysis of the effects of NS9283 on the basic area under the curve of individual AIM subtypes over the observation period in unilaterally 6-OHDA lesioned, levodopa primed rats (n = 12) is presented in Figure 5.31 (panels a–d).

Panel (a) shows total ALO AIM scores, representing the combined basic frequency of axial, limb, and orolingual movements. One-way ANOVA revealed no significant overall reductions in total ALO AIMs at any tested dose of NS9283 compared with the vehicle group, with total scores showing only minor increases and decreases across doses, indicating that PAM administration did not alter the overall dyskinetic frequency. Panel (b) illustrates axial AIMs, where one-way ANOVA confirmed that NS9283 did not produce any significant reductions across tested doses compared to vehicle, with axial AIMs showing small increases and decreases without a consistent pattern. Panel (c) presents limb AIMs, which showed no significant reductions at any tested dose relative to vehicle, with limb AIMs displaying slight increases and decreases across doses, indicating no dose dependent modulation. Panel (d) displays orolingual AIMs, which also showed no significant reductions across doses compared to vehicle, with scores remaining relatively stable and showing only minimal increases or decreases. All data are expressed as mean $\pm$ standard error and were analysed using one-way ANOVA with Dunnett's *post hoc* test.

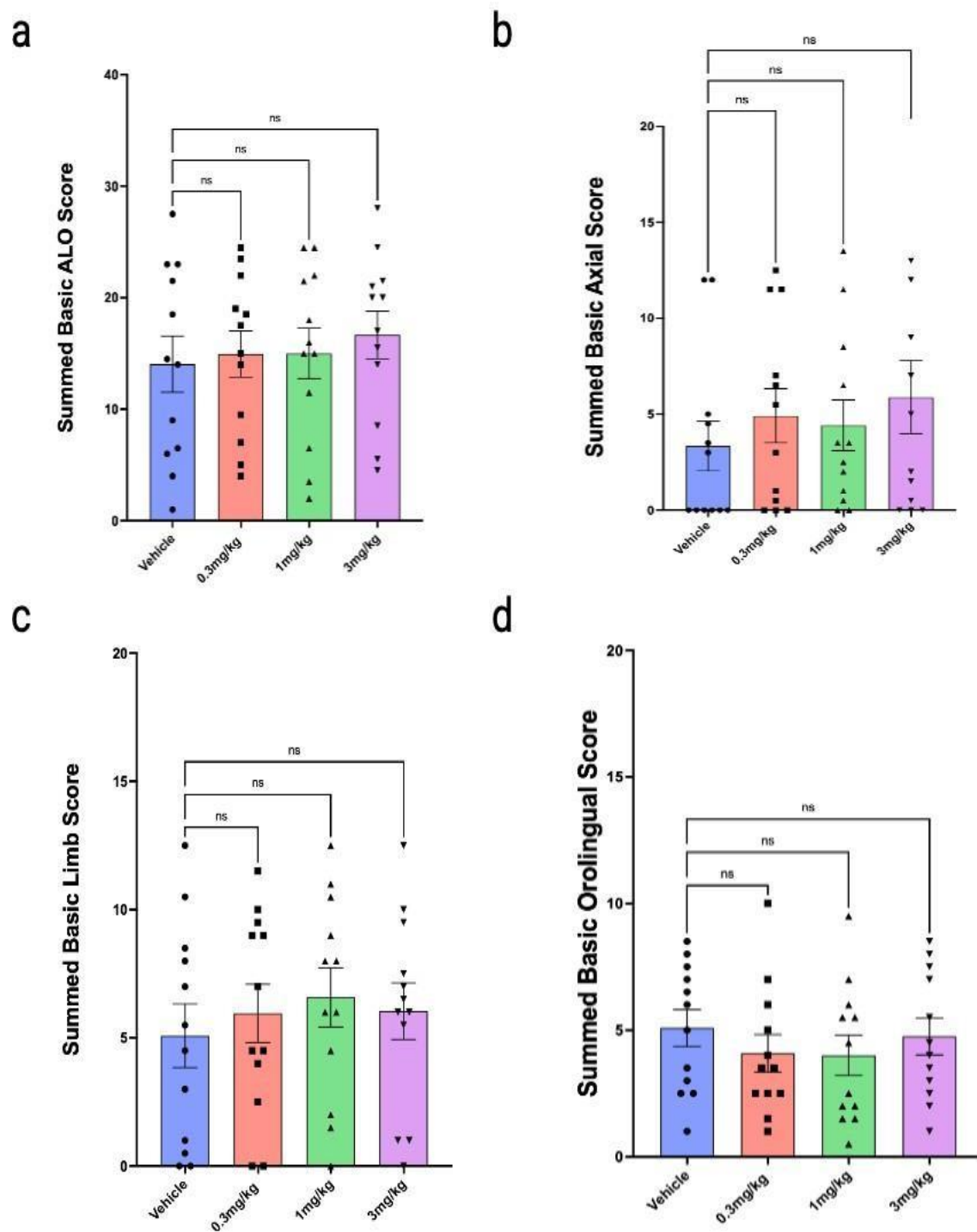


Figure 5. 31: Effects of NS9283 on basic AIMs in lesioned, primed rats. Panel (a) shows total ALO AIM scores which no significant reductions were observed at any tested dose of NS9283 compared with the vehicle group. Panel (b) illustrates axial AIMs, which showed no significant differences across doses. Panel (c) presents limb AIMs, where all tested doses of NS9283 produced no significant reductions relative to vehicle. Panel (d) displays orolingual AIMs, which were also unaffected by NS9283 administration. Data are expressed as mean $\pm$ standard error and were analysed using one-way ANOVA with Dunnett's *post hoc* test; (n = 12).

5.3.28 Effects of the NS9283 on Basic AIMs Across the Observation Period

An analysis of the effects of NS9283 on the frequency of individual AIM subtypes over the observation period (120 minutes) in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.32 (panels a–d).

When the effect of NS9283 on total ALO AIM scores was examined (panel a), a significant main effect of time was detected [$F(3.196, 35.15) = 14.85, p < 0.0001$], indicating time dependent increases in AIM expression followed by decreases over the session. However, neither the main effect of dose [$F(2.434, 26.77) = 0.3859, p = 0.7232$] nor the dose $\times$ time interaction [$F(5.895, 64.85) = 0.5130, p = 0.7934$] reached statistical significance, with scores showing only minor increases and decreases across doses. Panel (b) illustrates the effect of NS9283 on axial AIMs, where a significant main effect of time was observed [$F(3.082, 33.90) = 7.357, p = 0.0006$], reflecting increases followed by decreases over time. In contrast, there were no significant effects of dose [$F(1.721, 18.93) = 0.5498, p = 0.5603$] or dose $\times$ time interaction [$F(5.215, 57.36) = 0.4869, p = 0.7919$], with axial AIMs showing small increases and decreases across treatment groups.

Panel (c) presents limb AIMs, showing a significant main effect of time [$F(3.310, 36.41) = 9.500, p < 0.0001$], again reflecting increases followed by decreases, but no significant effects of dose [$F(2.228, 24.51) = 0.7517, p = 0.4955$] or dose $\times$ time interaction [$F(6.166, 67.83) = 1.080, p = 0.3836$], with limb AIMs showing minor fluctuations across doses. *Post hoc* analysis revealed that NS9283 at 1 and 3 mg/kg significantly reduced limb AIM scores at $t = 120$ minutes ($*p \leq 0.05$).

Finally, panel (d) displays orolingual AIMs, which showed no significant main effects of time [$F(3.471, 38.18) = 2.031, p = 0.1177$], dose [$F(2.706, 29.77) = 2.035, p = 0.1353$], or dose $\times$ time interaction [$F(6.745, 74.20) = 0.4937, p = 0.8303$], with scores remaining relatively stable and showing only slight increases or decreases, indicating that NS9283 did not significantly affect orolingual movement frequency during the observation period. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

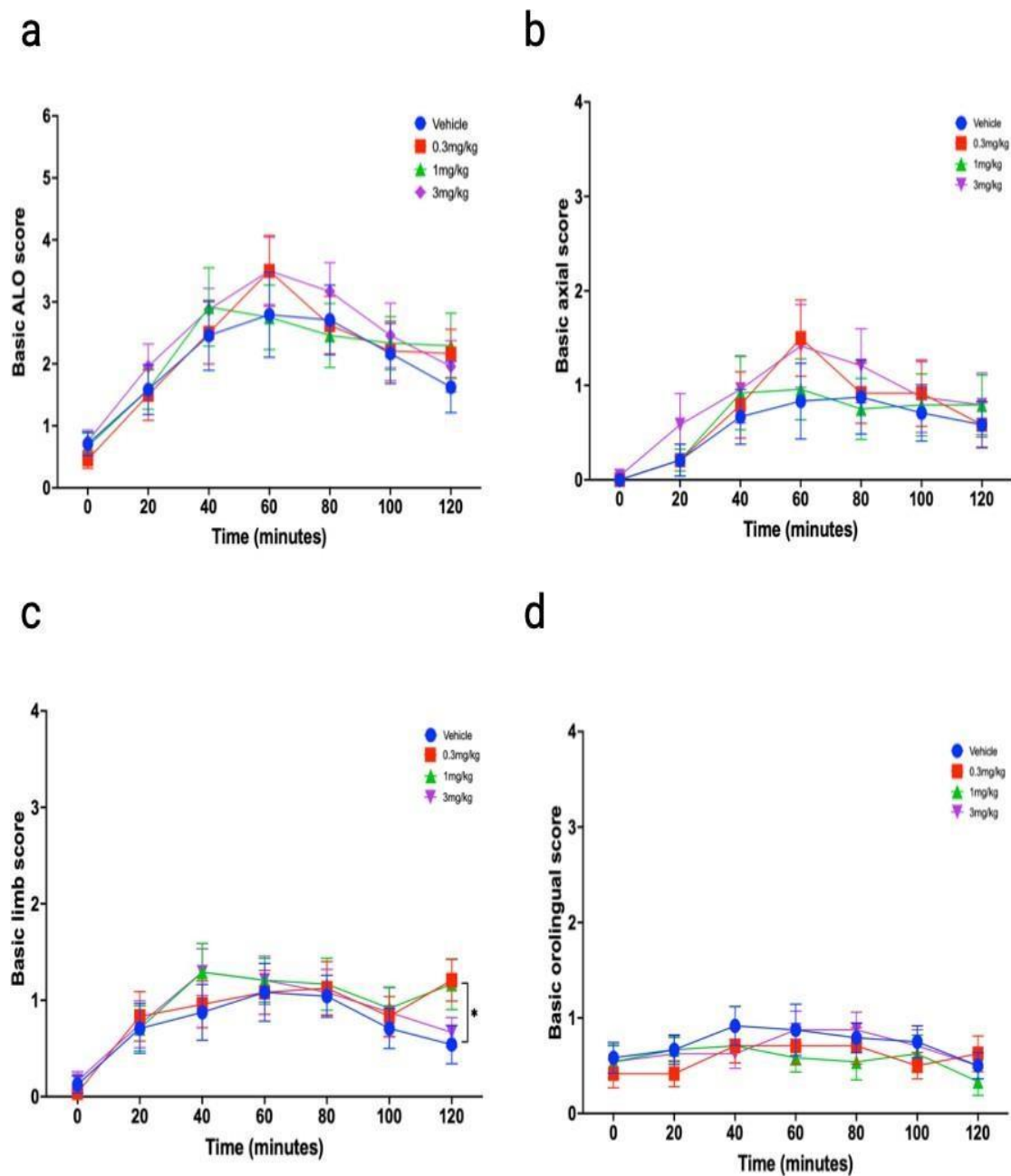


Figure 5.32: Effects of NS9283 on basic AIMs in 6-OHDA lesioned, primed rats. Panel (a) shows total ALO AIM scores, where a significant main effect of time was observed ($p < 0.0001$), but no significant dose related effects or dose $\times$ time interactions were detected. Panel (b) illustrates axial AIMs, which also showed a significant effect of time ($p = 0.0006$) but no significant effects of dose or interaction. Panel (c) presents limb AIMs, displaying a significant main effect of time ($p < 0.0001$) but no dose dependent or interaction effects. *Post hoc* analysis revealed that NS9283 at 1 and 3 mg/kg significantly reduced limb AIM scores at $t = 120$ minutes ($*p \leq 0.05$). Panel (d) shows orolingual AIMs, which exhibited no significant main effects of time, dose, or dose $\times$ time interaction, indicating no measurable effect of NS9283 on orolingual movement frequency. Data are expressed as mean $\pm$ SEM; ($n = 12$).

5.3.29 Administration of the NS9283 Does Not Significantly Alter the Amplitude AIMs

An analysis of the effects of NS9283 on the amplitude of individual AIM subtypes in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.33 (panels a–d).

The total ALO scores (panel a) showed no significant reductions in AIM amplitude across any tested dose of NS9283 compared with the vehicle group, as confirmed by one-way ANOVA, with total scores showing only minor increases and decreases across doses, indicating that PAM administration did not alter the overall severity of AIMs. Panel (b) illustrates the effect on axial AIMs, where one-way ANOVA revealed no significant reductions in amplitude at any tested dose of NS9283, with axial AIMs showing small increases and decreases across treatment groups, suggesting that the treatment did not modify the severity of axial movements. Panel (c) presents limb AIMs, which also showed no significant overall reductions in amplitude across doses compared with the vehicle, with limb AIMs displaying slight increases and decreases across doses, confirming the absence of a dose dependent effect on severity. Finally, orolingual AIMs (panel d) showed no significant differences in amplitude across treatment groups, as determined by one-way ANOVA, with scores remaining relatively stable and showing only minimal increases or decreases, indicating that NS9283 did not influence the severity of orolingual movements. All data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

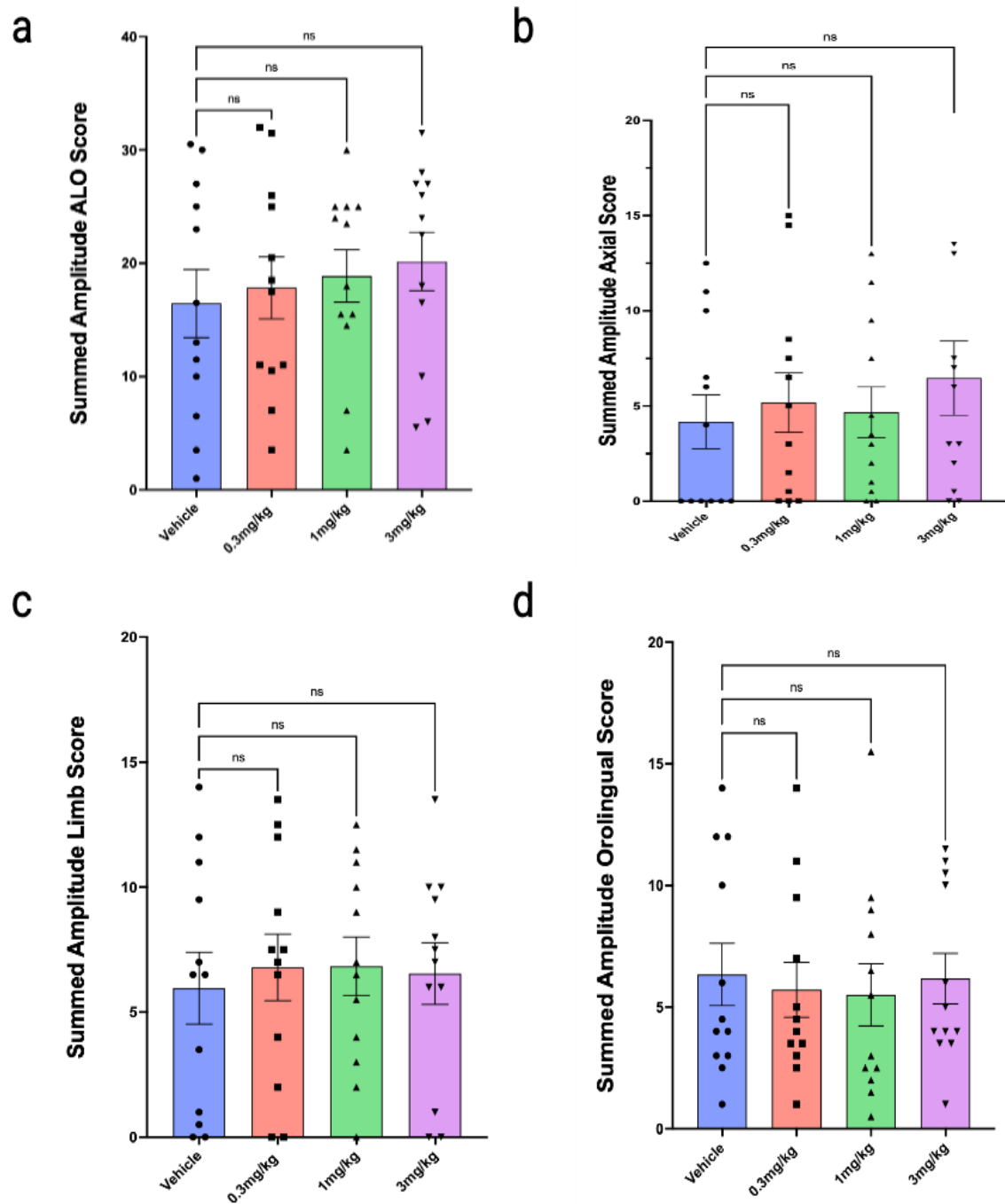


Figure 5.33: Effects of NS9283 on the amplitude of various AIMs in lesioned, primed rats. Panel (a) shows total ALO AIM scores, where one-way ANOVA revealed no significant reductions in amplitude at any tested dose of NS9283 compared with the vehicle group. Panel (b) illustrates axial AIMs, which showed no significant changes in amplitude across treatment groups. Panel (c) presents limb AIMs, where no significant overall reductions in amplitude were observed across doses relative to vehicle. Panel (d) shows orolingual AIMs, which were also unaffected by NS9283 treatment, indicating no measurable impact on movement severity. Data are expressed as mean $\pm$ SEM; (n = 12).

5.3.30 Administration of the NS9283 Does Not Significantly Affect the Amplitude AIMs Over the 120 Minutes Period

An analysis of the effects of NS9283 on the amplitude of individual AIM subtypes over the observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.34 (panels a–d).

When the effect of NS9283 on total ALO AIM scores was examined (panel a), the analysis revealed a significant main effect of time [$F(3.306, 36.37) = 13.75, p < 0.0001$], indicating time dependent increases in AIM amplitude followed by decreases over the session. However, neither the main effect of dose [$F(2.398, 26.38) = 0.4274, p = 0.6922$] nor the dose $\times$ time interaction [$F(5.633, 61.97) = 0.5516, p = 0.7564$] reached statistical significance, with scores showing only minor increases and decreases across doses. Panel (b) illustrates the effect of NS9283 on axial AIMs, where a significant main effect of time was observed [$F(3.323, 36.56) = 7.680, p = 0.0003$], reflecting increases followed by decreases over time. In contrast, there were no significant effects of dose [$F(1.855, 20.40) = 0.8017, p = 0.4536$] or dose $\times$ time interaction [$F(4.576, 50.34) = 0.5805, p = 0.7003$], with axial AIMs showing small increases and decreases across treatment groups. Panel (c) presents limb AIMs, which showed a significant main effect of time [$F(3.463, 38.09) = 10.99, p < 0.0001$], again reflecting increases followed by decreases, but no significant effects of dose [$F(2.539, 27.93) = 0.2890, p = 0.8007$] or dose $\times$ time interaction [$F(5.256, 57.82) = 0.4462, p = 0.8228$], with limb AIMs showing minor fluctuations across doses.

Finally, panel (d) shows orolingual AIMs, where a significant main effect of time was detected [$F(2.890, 31.79) = 3.037, p = 0.0450$], reflecting slight increases followed by decreases over time, but no significant effects of dose [$F(2.426, 26.68) = 0.5931, p = 0.5909$] or dose $\times$ time interaction [$F(6.503, 71.54) = 0.4602, p = 0.8485$], with scores showing only small increases and decreases, indicating that NS9283 did not significantly alter orolingual AIM amplitude across time.

All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

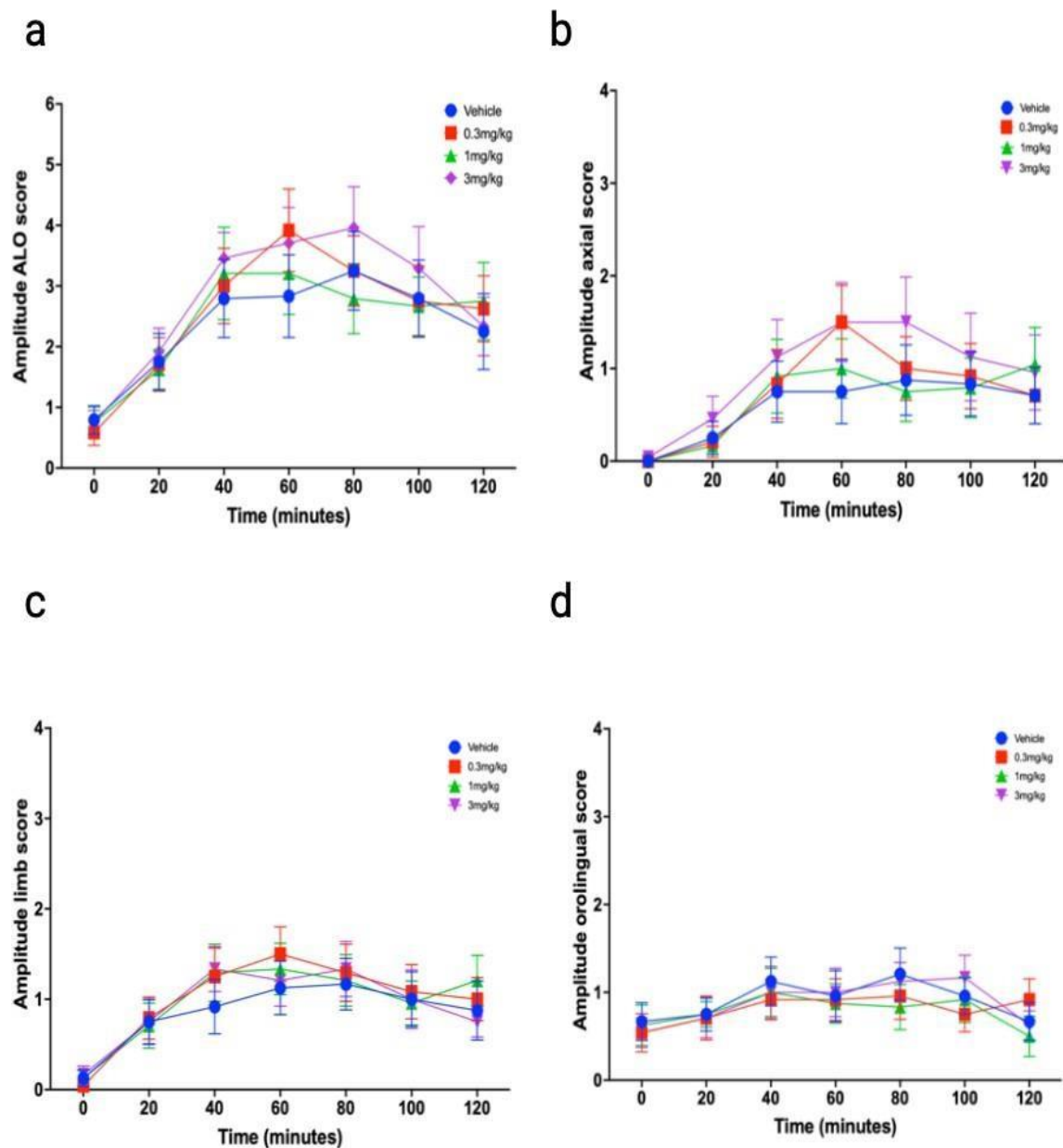


Figure 5.34: Effects of NS9283 on the amplitude of AIMs in lesioned, primed rats. Panel (a) shows total ALO AIM scores, where a significant main effect of time was observed ($p < 0.0001$), but no significant effects of dose or dose $\times$ time interaction were detected. Panel (b) illustrates axial AIMs, which also showed a significant main effect of time ($p = 0.0003$), while neither dose nor dose $\times$ time interaction reached statistical significance. Panel (c) presents limb AIMs, where a significant effect of time was detected ($p < 0.0001$), but no significant effects of dose or interaction were found. Panel (d) shows orolingual AIMs, where a significant effect of time was observed ($p = 0.0450$), but no significant dose dependent or interaction effects were identified, indicating that NS9283 did not significantly alter the amplitude of orolingual movements. Data are expressed as mean $\pm$ SEM; ($n = 12$).

5.3.31 Administration of the NS9283 Does Not Significantly Alter the Global AIMs

An analysis of the effects of NS9283 on global and individual AIM subtypes in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.35 (panels a–d).

Panel (a) shows the total global ALO AIM scores, where one-way ANOVA confirmed that NS9283 did not produce any significant reductions at any tested dose compared with the vehicle group ($p > 0.05$), with total scores showing only minor increases and decreases across doses, indicating no overall modulation of total dyskinetic activity. Panel (b) illustrates global axial AIMs, where one-way ANOVA revealed no significant reductions at any tested dose relative to vehicle, with axial AIMs showing small increases and decreases across groups, suggesting that NS9283 had no detectable effect on axial AIMs. Panel (c) presents global limb AIMs, which also showed no significant reductions across doses compared with the vehicle group, with limb AIMs remaining relatively stable and showing only slight increases and decreases following PAM administration.

Finally, panel (d) shows global orolingual AIMs, where one-way ANOVA confirmed no significant reductions at any tested dose compared with vehicle, with scores showing minimal increases and decreases across groups, demonstrating that NS9283 did not influence overall orolingual AIMs. All data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

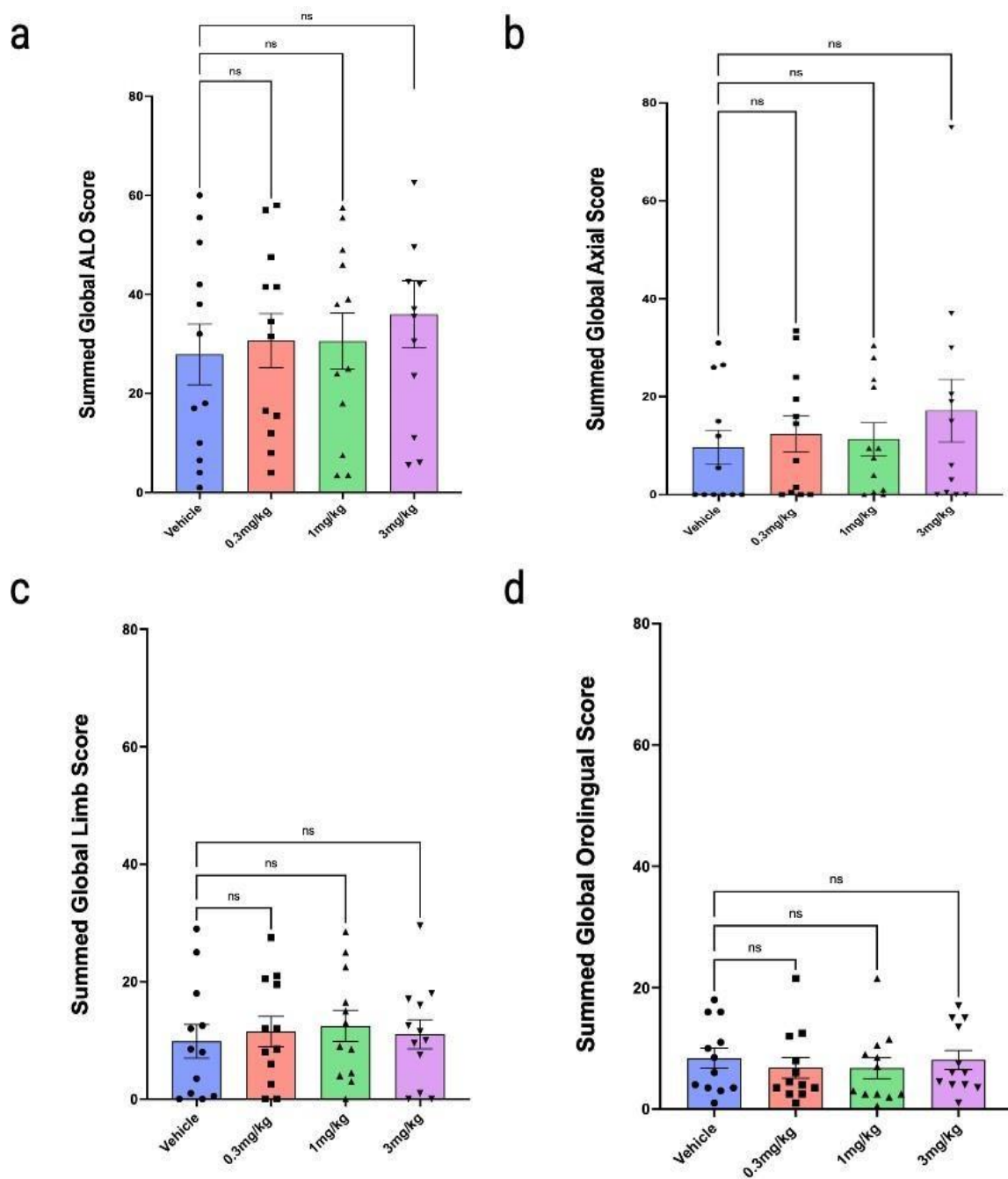


Figure 5.35: Effects of NS9283 on global AIMs in lesioned, primed rats. Panel (a) shows total global ALO AIM scores, where one-way ANOVA revealed no significant reductions at any tested dose compared with the vehicle group. Panel (b) illustrates global axial AIMs, which showed no significant differences across doses, indicating that NS9283 did not alter axial dyskinetic activity. Panel (c) presents global limb AIMs, where no significant changes were observed across treatment groups relative to vehicle. Panel (d) shows global orolingual AIMs, which also displayed no significant reductions across doses, confirming that NS9283 administration did not affect orolingual dyskinetic severity. Data are expressed as mean ± SEM; (n = 12).

5.3.32 NS9283 Produces Time Dependent Effects on Global AIMS

When the effect of NS9283 on total global ALO AIM scores was examined (panel a), a significant main effect of time was observed [$F(3.362, 36.98) = 11.59, p < 0.0001$], indicating time dependent increases in overall AIMS severity followed by decreases over the session. However, neither the main effect of dose [$F(1.930, 21.23) = 0.4263, p = 0.6514$] nor the dose $\times$ time interaction [$F(5.010, 55.11) = 0.6694, p = 0.6486$] reached statistical significance, with scores showing only minor increases and decreases across doses.

Panel (b) illustrates the effect on global axial AIMS, which showed a significant main effect of time [$F(3.118, 34.30) = 6.822, p = 0.0009$], reflecting increases followed by decreases over time, while no significant effects were detected for dose [$F(1.474, 16.22) = 0.7027, p = 0.4680$] or dose $\times$ time interaction [$F(4.858, 53.44) = 0.6604, p = 0.6508$], with axial AIMS showing small increases and decreases across treatment groups. Panel (c) presents global limb AIMS, where analysis revealed a significant main effect of time [$F(3.797, 41.77) = 8.439, p < 0.0001$], again reflecting increases followed by decreases, but no significant effects of dose [$F(2.234, 24.57) = 0.3778, p = 0.7118$] or dose $\times$ time interaction [$F(5.643, 62.07) = 0.5775, p = 0.7368$], with limb AIMS showing minor fluctuations across doses.

Lastly, panel (d) shows global orolingual AIMS, which also demonstrated a significant main effect of time [$F(2.419, 26.61) = 3.673, p = 0.0319$], reflecting slight increases followed by decreases over time, but no significant effects of dose [$F(2.637, 29.00) = 1.283, p = 0.2975$] or dose $\times$ time interaction [$F(5.527, 60.80) = 0.5697, p = 0.7394$], with scores showing only small increases and decreases across groups. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

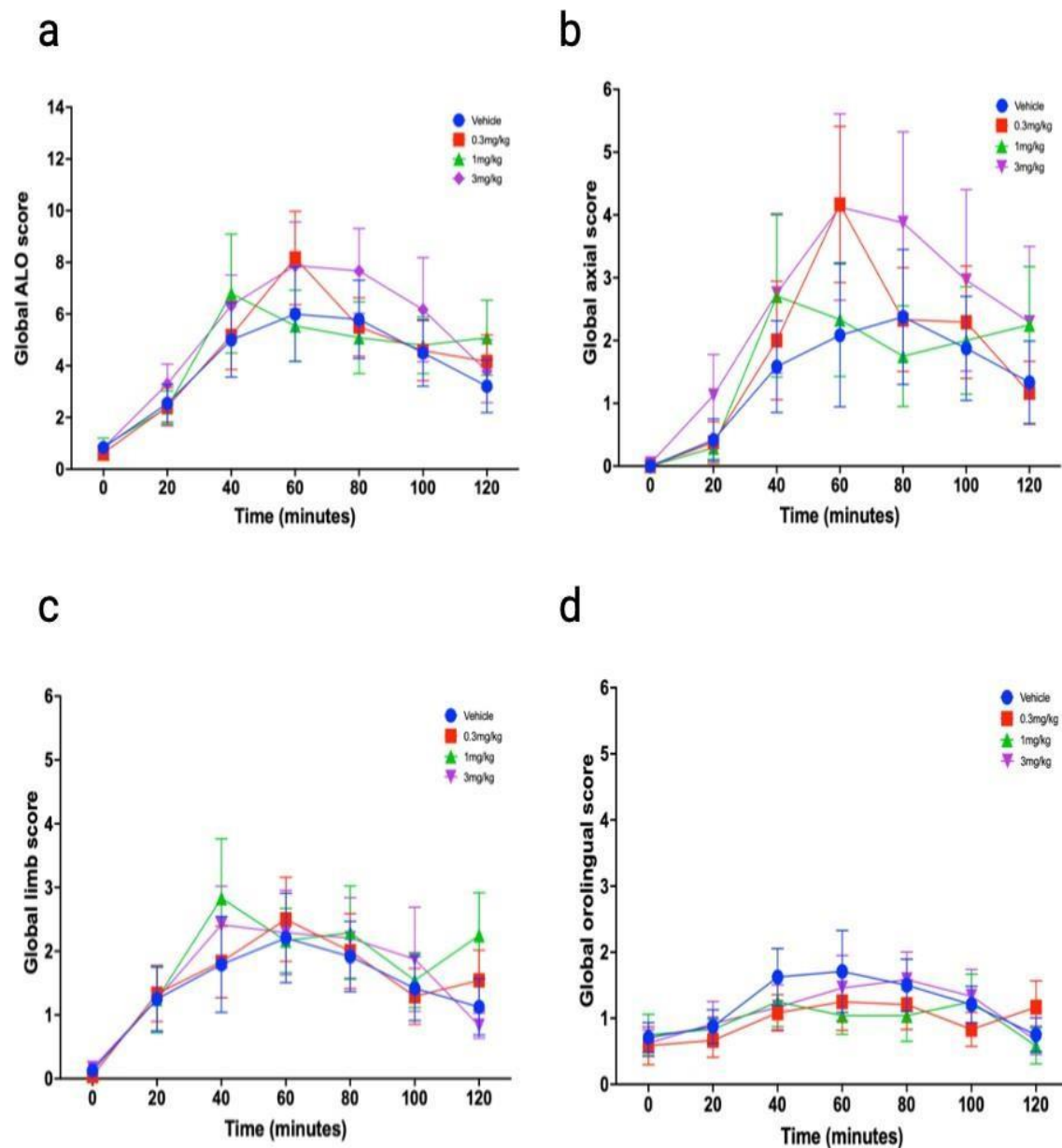


Figure 5.36: Effects of NS9283 on global AIMs in lesioned, primed rats. Panel (a) shows total global ALO AIM scores, where a significant main effect of time was observed ($p < 0.0001$), but no significant effects of dose or dose $\times$ time interaction were detected. Panel (b) illustrates global axial AIMs, which also showed a significant main effect of time ($p = 0.0009$) but no significant effects of dose or interaction. Panel (c) presents global limb AIMs, where a significant main effect of time was found ($p < 0.0001$), while neither dose nor the dose $\times$ time interaction reached significance. Panel (d) shows global orolingual AIMs, where a significant main effect of time was detected ($p = 0.0319$), but no significant effects of dose or interaction were observed, indicating that NS9283 did not significantly alter orolingual AIMs severity across the session. Data are expressed as mean $\pm$ SEM; ($n = 12$).

5.3.33 Administration of Sazetidine-A Does Not Significantly Affect Locomotive

AIMs

Sazetidine-A, a selective partial agonist of $\alpha 4\beta 2$ nicotinic acetylcholine receptors, plays a critical role in the investigation of levodopa induced AIMs. The importance of this compound lies in its ability to modulate $\alpha 4\beta 2$ nAChR activity, which has been implicated in the dysregulated cholinergic signalling associated with LID. By providing partial activation of these receptors, Sazetidine-A offers a mechanism to restore balance in cholinergic neurotransmission without overstimulating the system, thereby potentially reducing dyskinetic symptoms. This study tested Sazetidine-A at doses of 0 and 1 mg/kg to evaluate its therapeutic potential in mitigating LID. These doses were chosen to assess the efficacy of a partial agonist that can fine tune receptor activity without causing excessive activation, which might lead to adverse effects.

Locomotive AIM scores over time are shown for rats treated with the partial $\alpha 4\beta 2$ nicotinic receptor agonist Sazetidine-A (1 mg/kg) to evaluate its effects on levodopa induced AIMs. Analysis across the 120 minute observation period using a two-way ANOVA revealed no significant main effects of time [$F(2.970, 32.67) = 1.670, p = 0.1930$], dose [$F(1.000, 11.00) = 0.6018, p = 0.4542$], or dose $\times$ time interaction [$F(2.716, 29.87) = 0.7074, p = 0.5420$], with locomotive AIM scores showing only minor increases and decreases over time and between groups (Figure 5.37a). Figure 5.37b presents the summed locomotive AIM scores expressed as AUC. Statistical comparison using the Wilcoxon matched pairs signed rank test confirmed that Sazetidine-A (1 mg/kg) did not significantly alter cumulative locomotive AIM scores compared with the vehicle group ($p > 0.05$), with total scores remaining broadly similar and showing only slight increases or decreases. Data are expressed as mean $\pm$ standard error, with $n = 12$ animals per treatment group.

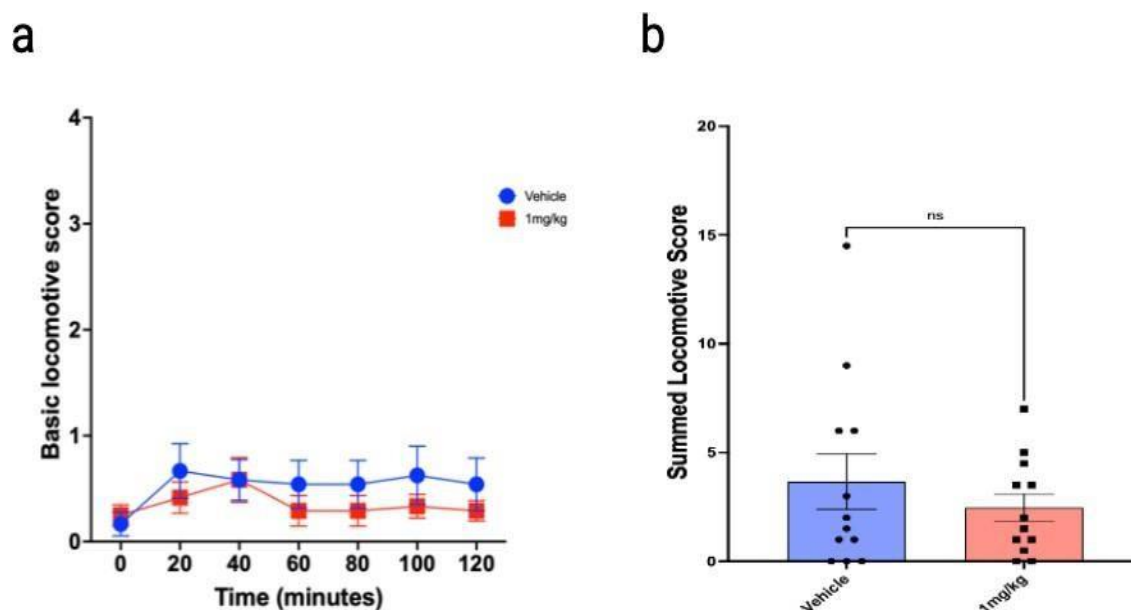


Figure 5.37: Effects of the Sazetidine-A on locomotive AIMs in lesioned, primed rats. (a) Time course of locomotive AIMs following administration of Sazetidine-A (1 mg/kg) or vehicle. No significant main effects of time, dose, or dose $\times$ time interaction were detected ($p > 0.05$). (b) Summed locomotive AIM scores expressed as area under the curve showed no significant differences between Sazetidine-A and vehicle groups, as determined by the Wilcoxon matched pairs signed rank test ($p > 0.05$). Data are presented as mean $\pm$ standard error; ($n = 12$).

5.3.34 Administration of the Sazetidine-A Significantly Reduces Basic ALO AIMs

An analysis of the effects of Sazetidine-A on the basic area under the curve of individual AIM subtypes over the observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.38 (panels a–d).

Panel (a) shows total ALO AIM scores, representing the combined basic frequency of axial, limb, and orolingual movements. Analysis using the Wilcoxon matched pairs signed rank test revealed a significant reduction in total ALO AIM scores at the 1 mg/kg dose compared with the vehicle group ($p = 0.0085$), indicating a decrease in overall dyskinetic frequency. Panel (b) illustrates axial AIMs, where the Wilcoxon test confirmed no significant differences between the 1 mg/kg Sazetidine-A group and vehicle, with axial AIMs showing only minor increases and decreases, suggesting no detectable effect on axial movement frequency.

Panel (c) presents limb AIMS, which showed a significant overall reduction in basic AIM scores at the 1 mg/kg dose ($p = 0.0137$) compared with vehicle, indicating a decrease in the frequency of limb AIMS. Panel (d) displays orolingual AIMS, where Sazetidine-A produced no significant changes in basic AIM frequency relative to vehicle, with scores remaining relatively stable and showing only slight increases or decreases. All data are expressed as mean $\pm$ standard error and were analysed using the Wilcoxon matched pairs signed rank test.

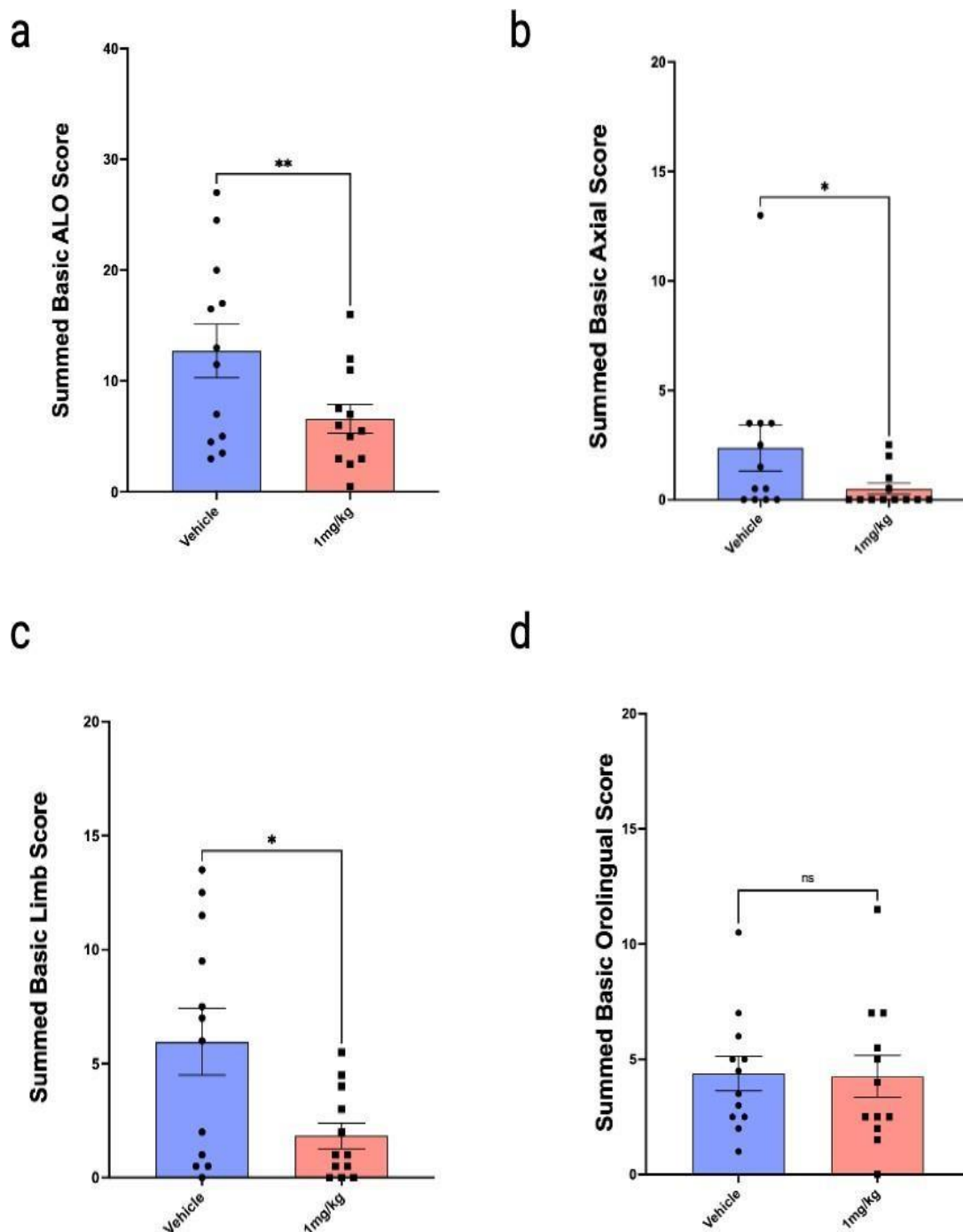


Figure 5.38: Effects of the Sazetidine-A on basic AIMs in levodopa primed rats.

Panel (a) shows total ALO AIM scores, where administration of Sazetidine-A (1 mg/kg) significantly reduced basic ALO AIMs compared with the vehicle group ($p = 0.0085$). Panel (b) illustrates axial AIMs, which showed no significant difference between Sazetidine-A and vehicle treatment. Panel (c) presents limb AIMs, where Sazetidine-A produced a significant reduction in basic AIM scores compared with vehicle ($p = 0.0137$). Panel (d) displays orolingual AIMs, which were unaffected by Sazetidine-A administration. Data are expressed as mean $\pm$ standard error and were analysed using the Wilcoxon matched pairs signed rank test. Data are expressed as $\pm$ SEM; * $p < 0.05$ and ** $P < 0.001$; ($n = 12$).

5.3.35 Effects of Sazetidine-A on Basic AIMs Across the 120 Minute Period

An analysis of the effects of Sazetidine-A on the frequency of individual AIM subtypes over the 120 minutes observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.39 (panels a–d).

When the effect of Sazetidine-A on total ALO AIM scores was examined (panel a), significant main effects of time [$F(3.323, 36.55) = 3.501, p = 0.0216$], dose [$F(1.000, 11.00) = 10.24, p = 0.0085$], and a significant dose $\times$ time interaction [$F(3.495, 38.45) = 3.940, p = 0.0116$] were observed, reflecting time dependent increases in AIM frequency followed by decreases, alongside an overall decrease in scores with treatment. Post hoc analysis revealed that Sazetidine-A significantly reduced basic ALO AIM scores at specific time points from $t = 60$ to $t = 120$ minutes ($*p \leq 0.05$). Panel (b) illustrates the effect on axial AIMs, where two-way ANOVA showed no significant main effects of time [$F(3.139, 34.53) = 1.987, p = 0.1318$], dose [$F(1.000, 11.00) = 3.741, p = 0.0792$], or dose $\times$ time interaction [$F(3.334, 36.68) = 1.429, p = 0.2480$], with axial AIMs showing small increases and decreases across time and treatment groups, indicating no measurable influence of Sazetidine-A on axial movement frequency. Panel (c) presents limb AIMs, where analysis revealed significant main effects of time [$F(3.137, 34.51) = 3.051, p = 0.0395$], dose [$F(1.000, 11.00) = 8.571, p = 0.0137$], and a significant dose $\times$ time interaction [$F(2.857, 31.43) = 4.954, p = 0.0069$], reflecting increases followed by decreases over time and an overall reduction in limb AIM frequency with treatment. *Post hoc* comparisons showed that Sazetidine-A significantly reduced limb AIM scores at $t = 0, t = 60, t = 80, t = 100$, and $t = 120$ minutes ($*p \leq 0.05$).

Finally, panel (d) shows orolingual AIMs, which exhibited no significant main effects of time [$F(3.722, 40.95) = 1.954, p = 0.1239$], dose [$F(1.000, 11.00) = 0.01235, p = 0.9135$], or dose $\times$ time interaction [$F(3.101, 34.11) = 1.014, p = 0.4005$], with scores remaining relatively stable and showing only slight increases or decreases, indicating that Sazetidine-A did not significantly affect the frequency of orolingual movements. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

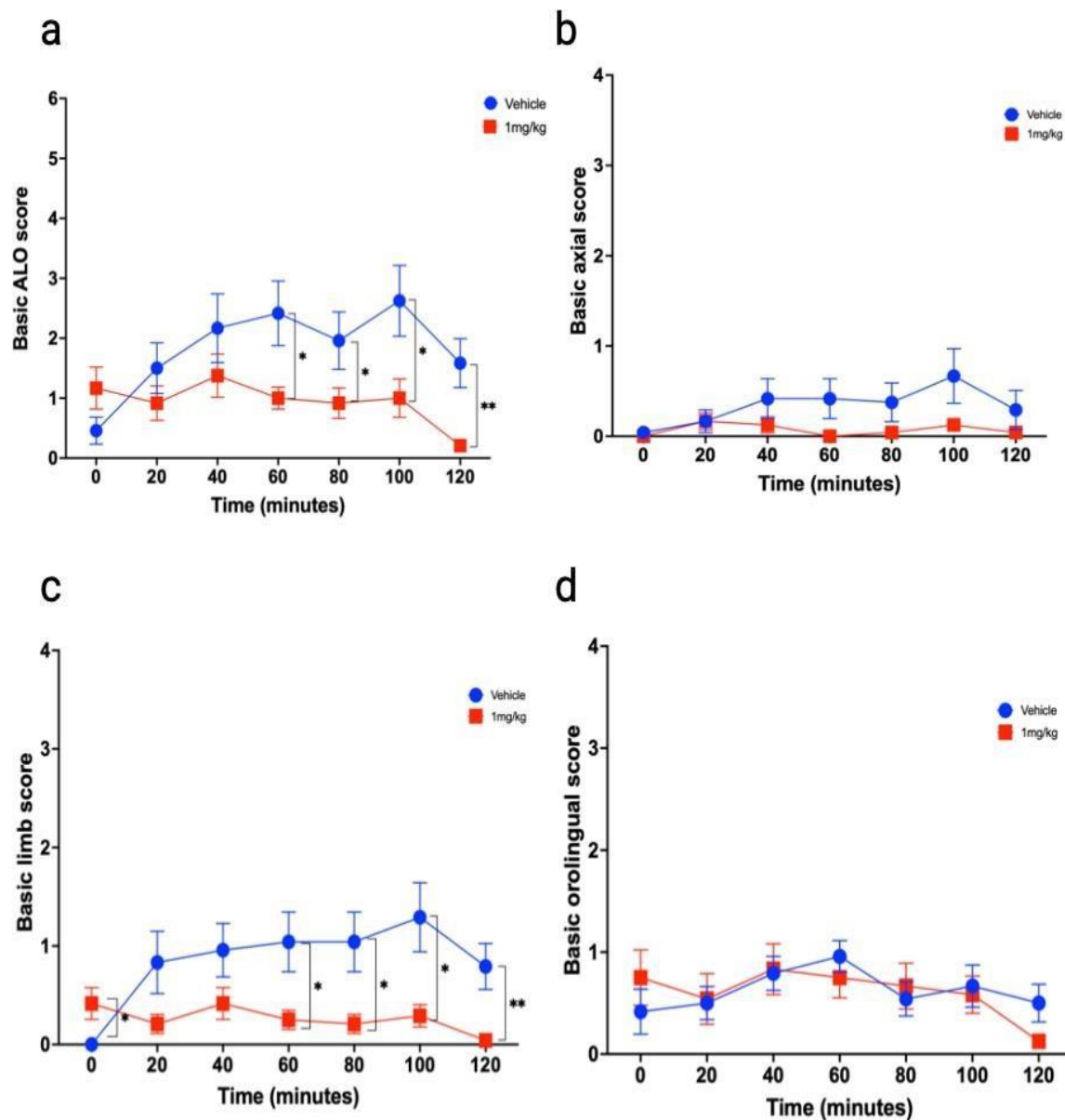


Figure 5.39: Effects of the Sazetidine-A on basic AIMs in lesioned, levodopa primed rats. Panel (a) shows total ALO AIM scores, where significant main effects of time ($p = 0.0216$), dose ($p = 0.0085$), and dose $\times$ time interaction ($p = 0.0116$) were observed. *Post hoc* analysis revealed that Sazetidine-A significantly reduced ALO AIM scores at time points between $t = 60$ and $t = 120$ minutes ($*p \leq 0.05$). Panel (b) illustrates axial AIMs, which showed no significant main effects of time, dose, or interaction. Panel (c) presents limb AIMs, which exhibited significant effects of time ($p = 0.0395$), dose ($p = 0.0137$), and dose $\times$ time interaction ($p = 0.0069$), with *post hoc* analysis showing reductions at $t = 0$, 60, 80, 100, and 120 minutes ($*p \leq 0.05$). Panel (d) displays orolingual AIMs, where no significant effects of time, dose, or interaction were detected. Data are expressed as mean $\pm$ SEM; $*p < 0.05$, $**P < 0.001$; ($n = 12$).

5.3.36 Sazetidine-A Significantly Reduces the Amplitude of ALO and Limb AIMs

An analysis of the effects of Sazetidine-A on the amplitude of individual AIMs in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 40 (panels a–d).

Panel (a) shows total ALO AIM scores, where the 1 mg/kg dose of Sazetidine-A significantly reduced AIM amplitude compared with the vehicle group ($p = 0.0177$), indicating a decrease in overall AIMs severity. Panel (b) illustrates axial AIMs, which showed no significant difference in amplitude between treatment groups, with axial AIMs showing only minor increases and decreases, suggesting that Sazetidine-A did not alter the severity of axial movements. Panel (c) presents limb AIMs, where a significant reduction in amplitude was observed at the 1 mg/kg dose ($p = 0.0382$) compared with vehicle, indicating a decrease in the severity of limb AIMs. Finally, panel (d) shows orolingual AIMs, where no significant differences were detected across treatment groups, with scores remaining relatively stable and showing only slight increases or decreases, demonstrating that Sazetidine-A did not affect the severity of orolingual movements. All data are expressed as mean $\pm$ SEM and were analysed using the Wilcoxon matched pairs signed rank test.

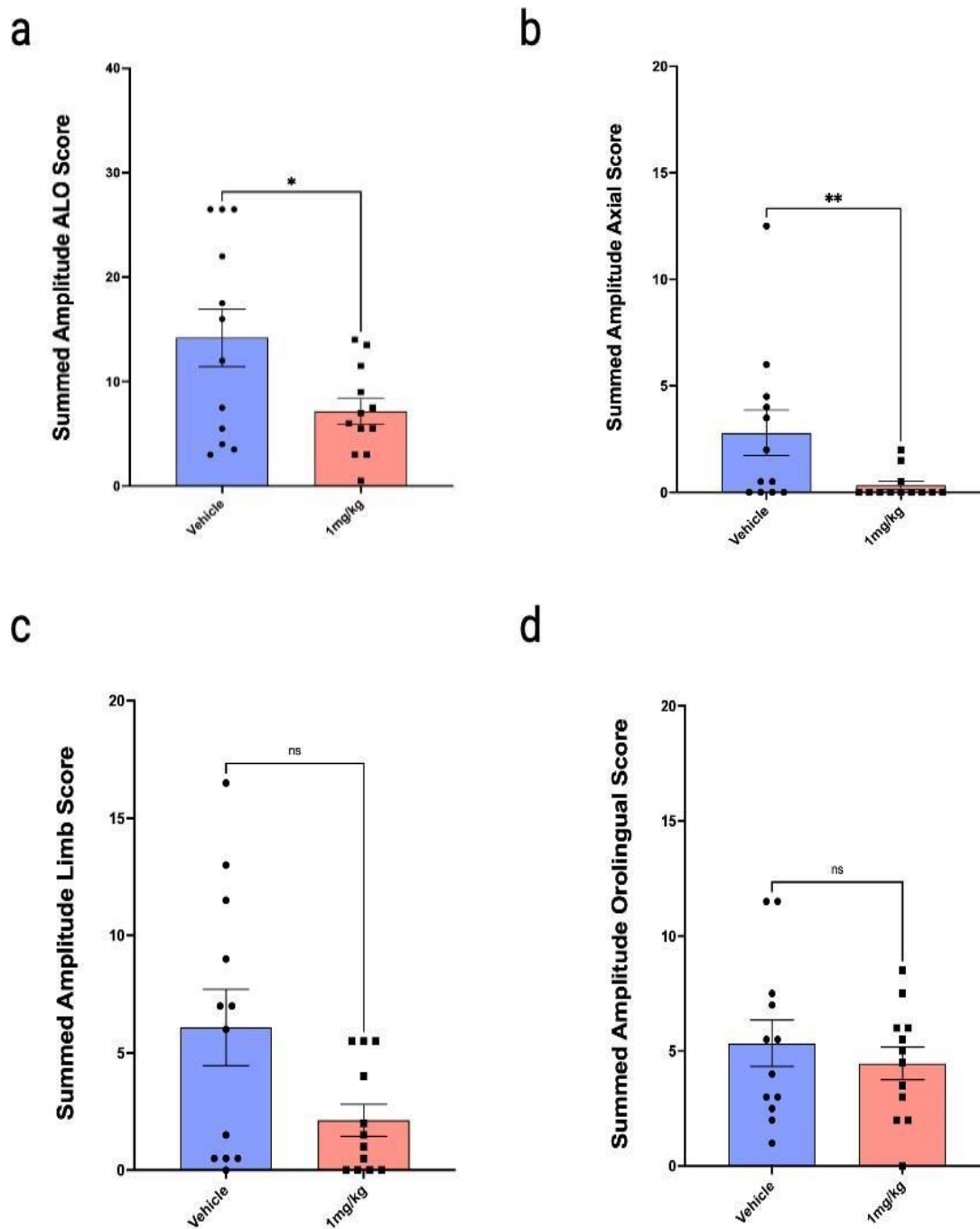


Figure 5. 40: Effects of Sazetidine-A on the amplitude AIMs in lesioned, levodopa primed rats. Panel (a) shows total ALO AIM scores, where the 1 mg/kg dose of Sazetidine-A significantly reduced AIM amplitude compared with the vehicle group ($p = 0.0177$). Panel (b) illustrates axial AIMs, which showed no significant changes in amplitude across treatment groups. Panel (c) presents limb AIMs, where Sazetidine-A significantly reduced AIM amplitude at the 1 mg/kg dose ($p = 0.0382$) relative to vehicle. Panel (d) shows orolingual AIMs, which were unaffected by treatment. Data are expressed as mean $\pm$ SEM and were analysed using the Wilcoxon matched-pairs signed-rank test; * $p < 0.05$, ** $P < 0.001$; ($n = 12$).

5.3.37 Sazetidine-A Significantly Reduces the Amplitude of ALO and Limb AIMs Over the 120 Minute Period

An analysis of the effects of Sazetidine-A on the amplitude of individual AIM subtypes over the 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.41 (panels a–d).

When the effect of Sazetidine-A on total ALO AIM scores was examined (panel a), the analysis revealed significant main effects of time [$F(3.642, 40.06) = 4.168, p = 0.0079$], dose [$F(1.000, 11.00) = 7.771, p = 0.0177$], and dose $\times$ time interaction [$F(3.560, 39.16) = 3.176, p = 0.0278$], reflecting time dependent increases in AIM amplitude followed by decreases, alongside an overall decrease with treatment. Post hoc analysis showed significant reductions in ALO AIM amplitude at $t = 60, t = 100$, and $t = 120$ minutes ($*p \leq 0.05$). Panel (b) illustrates axial AIMs, where no significant main effect of time was observed [$F(3.053, 33.59) = 2.216, p = 0.1034$], with axial AIMs showing small increases and decreases over time; however, a significant main effect of dose was detected [$F(1.000, 11.00) = 5.629, p = 0.0370$], indicating a general decrease in axial AIM amplitude across the session. The dose $\times$ time interaction was not significant [$F(3.242, 35.66) = 1.516, p = 0.2250$]. Panel (c) presents limb AIMs, which showed significant main effects of time [$F(3.145, 34.60) = 2.980, p = 0.0426$], dose [$F(1.000, 11.00) = 5.541, p = 0.0382$] and a significant dose $\times$ time interaction [$F(2.831, 31.14) = 4.024, p = 0.0172$], reflecting increases followed by decreases over time and an overall reduction in limb AIM amplitude with treatment. *Post hoc* comparisons revealed significant reductions in AIM amplitude at $t = 0, t = 60, t = 80, t = 100$, and $t = 120$ minutes ($*p \leq 0.05$).

Finally, panel (d) shows orolingual AIMs, where a significant main effect of time was found [$F(3.954, 43.49) = 2.718, p = 0.0423$], reflecting slight increases followed by decreases over time, but neither the main effect of dose [$F(1.000, 11.00) = 0.4965, p = 0.4957$] nor the dose $\times$ time interaction [$F(4.289, 47.18) = 1.453, p = 0.2292$] reached significance. Notably, Sazetidine-A significantly reduced orolingual AIM amplitude at $t = 40$ minutes ($*p \leq 0.05$). All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

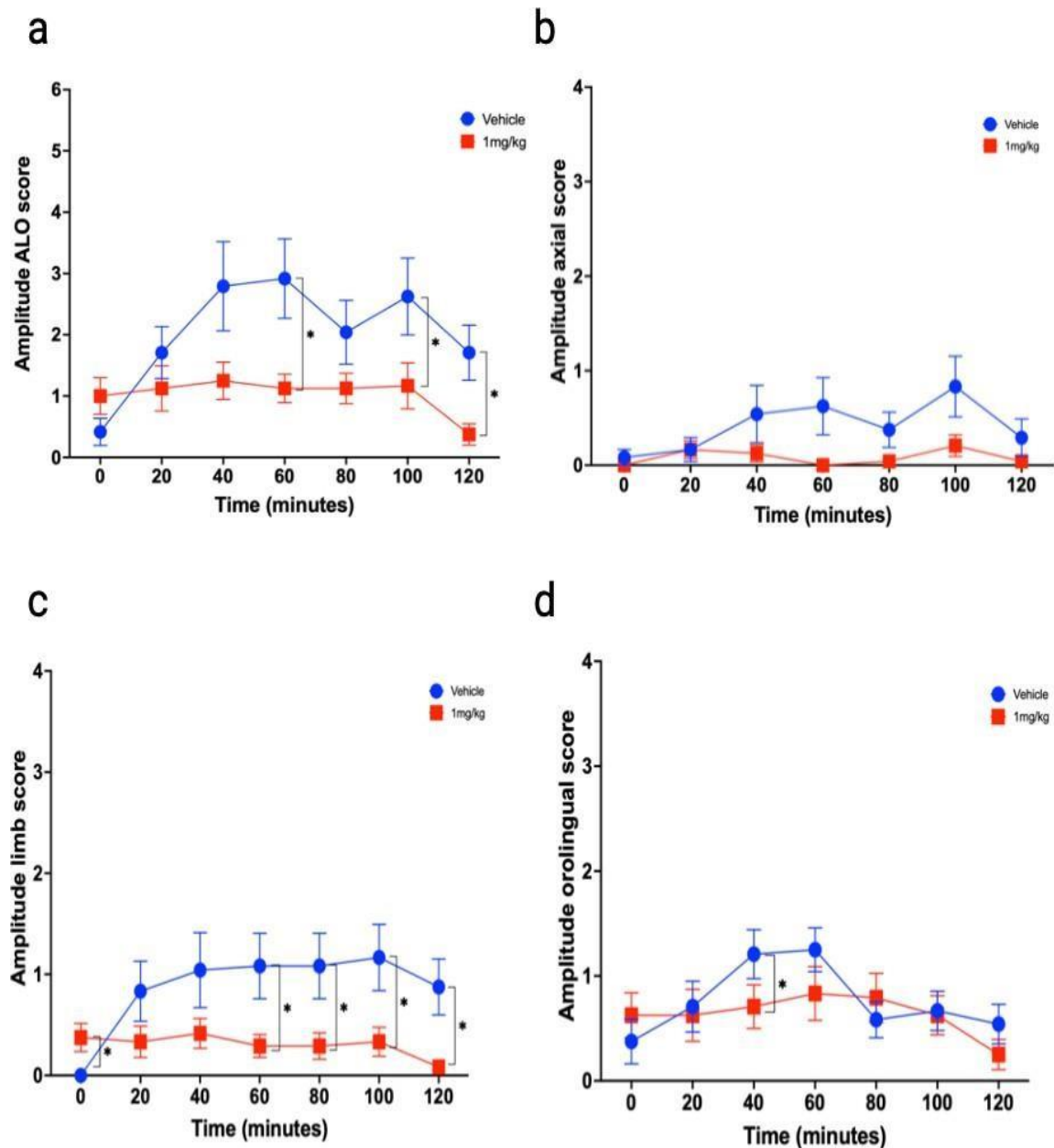


Figure 5. 41: Effects of Sazetidine-A on the amplitude of AIMs in lesioned, primed rats. Panel (a) shows total ALO AIM scores, where significant main effects of time ($p = 0.0079$), dose ($p = 0.0177$), and dose $\times$ time interaction ($p = 0.0278$) were observed. *Post hoc* analysis revealed that Sazetidine-A significantly reduced ALO AIM amplitude at $t = 60, 100$, and 120 minutes ($*p \leq 0.05$). Panel (b) illustrates axial AIMs, which showed a significant main effect of dose ($p = 0.0370$) but no significant effects of time or dose $\times$ time interaction. Panel (c) presents limb AIMs, which exhibited significant main effects of time ($p = 0.0426$), dose ($p = 0.0382$), and dose $\times$ time interaction ($p = 0.0172$). *Post hoc* analysis indicated significant reductions at $t = 0, 60, 80, 100$, and 120 minutes ($*p \leq 0.05$). Panel (d) shows orolingual AIMs, where a significant main effect of time ($p = 0.0423$) was detected, but no significant effects of dose or interaction were observed. A time-specific reduction was noted at $t = 40$ minutes ($*p \leq 0.05$). Data are expressed as mean $\pm$ SEM; $*p < 0.05$; ($n = 12$).

5.3.38 Sazetidine-A Significantly Reduces Global ALO, Axial and Limb AIMs

An analysis of Sazetidine-A on global and individual AIM subtypes in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.42 (panels a–d).

Panel (a) shows total global ALO AIM scores, where the 1 mg/kg dose of Sazetidine-A significantly reduced total global ALO AIMs compared with the vehicle group ($p = 0.0125$), indicating a decrease in overall dyskinetic activity. Panel (b) illustrates global axial AIMs, where Sazetidine-A treatment produced a significant change relative to the vehicle ($p = 0.0391$), indicating a decrease in axial AIM amplitude, despite small increases and decreases observed across individual animals. Panel (c) presents global limb AIMs, where a significant reduction in scores was observed following administration of Sazetidine-A at 1 mg/kg ($p = 0.0200$), indicating a decrease in overall limb related dyskinetic activity.

Finally, panel (d) shows global orolingual AIMs, where no significant differences were detected between Sazetidine-A and vehicle groups, with scores remaining relatively stable and showing only slight increases or decreases, suggesting that the partial agonist did not alter orolingual AIMs. All data are expressed as mean $\pm$ SEM and were analysed using the Wilcoxon matched pairs signed rank test.

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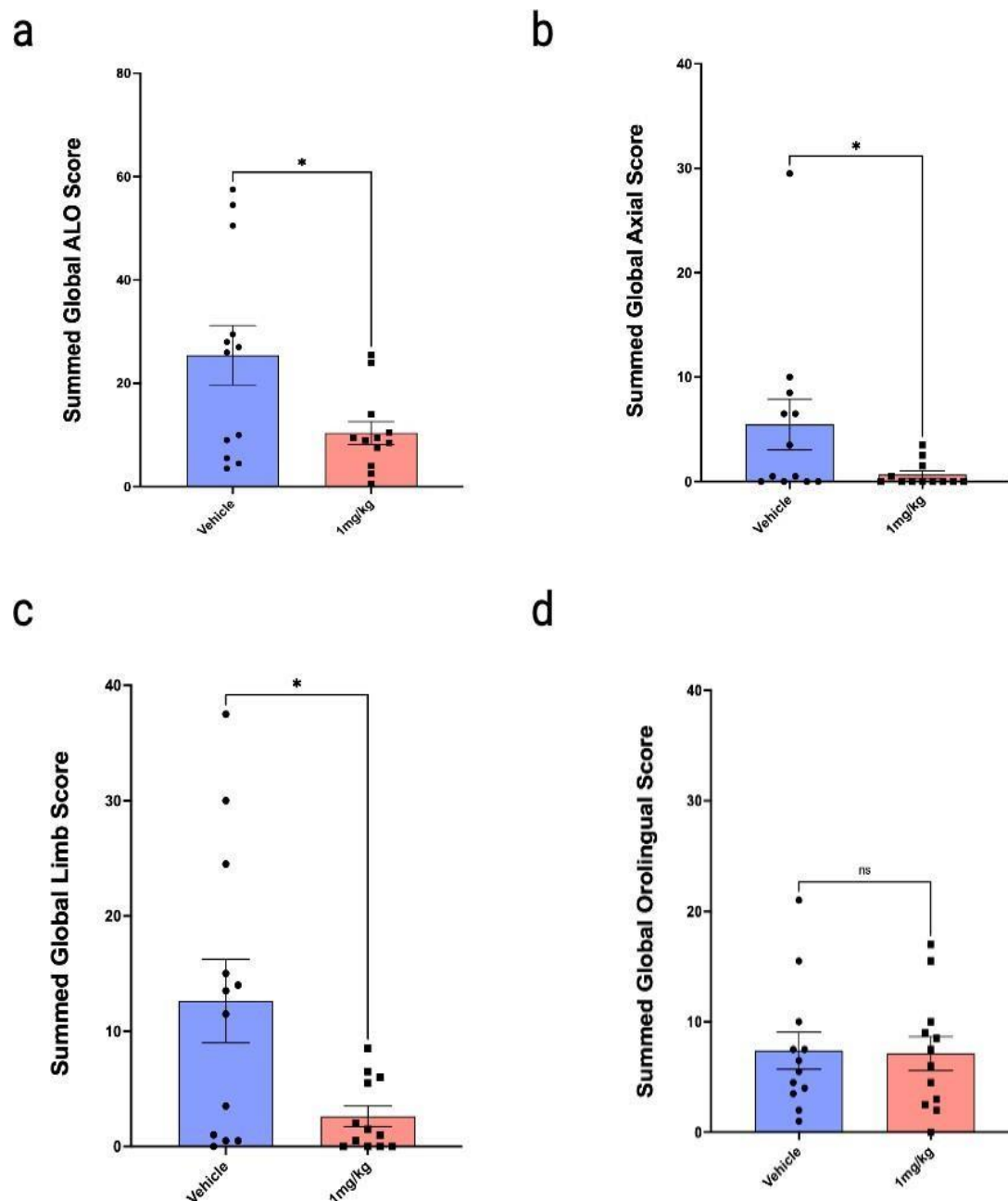


Figure 5. 42: Effects of Sazetidine-A on global AIMs in lesioned, levodopa primed rats. Panel (a) shows total global ALO AIM scores, a significant reduction produced following administration of Sazetidine-A at 1 mg/kg compared with the vehicle group ($p = 0.0125$). Panel (b) illustrates global axial AIMs, which showed significant differences between Sazetidine-A and vehicle ($p = 0.0391$). Panel (c) presents global limb AIMs, where a significant reduction was observed at the 1 mg/kg dose ($p = 0.0200$). Panel (d) shows global orolingual AIMs, which were not significantly altered by Sazetidine-A treatment compared with vehicle. Data are expressed as mean $\pm$ SEM; * $p < 0.05$; ($n = 12$).

5.3.39 Sazetidine-A Produces Time Dependent Reductions in Global AIMs

An analysis of Sazetidine-A on global AIM subtypes over the 120 minutes observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$) is presented in Figure 5.43 (panels a–d).

When the effect of Sazetidine-A on total global ALO AIM scores was examined (panel a), a significant main effect of time was observed [$F(3.293, 36.22) = 3.119, p = 0.0340$], reflecting increases in AIM severity followed by decreases over time, along with a significant main effect of dose [$F(1.000, 11.00) = 8.891, p = 0.0125$], indicating an overall decrease in AIM amplitude with treatment. The dose $\times$ time interaction trended toward significance [$F(3.025, 33.27) = 2.514, p = 0.0748$], with similar patterns of increase and decrease across groups. Post hoc analysis revealed significant reductions in global ALO scores from $t = 60$ to $t = 120$ ($*p \leq 0.05$), indicating that Sazetidine-A reduced overall AIMs severity across these time points. Panel (b) illustrates the effect on global axial AIMs, where no significant main effects of time [$F(2.823, 31.06) = 1.715, p = 0.1866$], dose [$F(1.000, 11.00) = 4.226, p = 0.0643$], or dose $\times$ time interaction [$F(2.776, 30.53) = 1.262, p = 0.3037$] were observed, with axial AIMs showing small increases and decreases across time and treatment groups, indicating no clear effect of Sazetidine-A.

Panel (c) presents global limb AIMs, where no significant main effect of time was detected [$F(2.953, 32.48) = 2.487, p = 0.0788$], but both the dose $\times$ time interaction [$F(2.661, 29.28) = 3.398, p = 0.0353$] and the main effect of dose [$F(1.000, 11.00) = 7.387, p = 0.0200$] were significant, indicating an overall decrease in limb AIM amplitude with treatment alongside time dependent increases and decreases. Post hoc analysis revealed reductions at $t = 0, t = 60, t = 80, t = 100$, and $t = 120$ ($*p \leq 0.05$), suggesting that Sazetidine-A reduced limb AIMs amplitude across multiple time points. Finally, panel (d) shows global orolingual AIMs, where a significant main effect of time was found [$F(4.053, 44.58) = 2.632, p = 0.0459$], reflecting slight increases followed by decreases over time, but no significant main effect of dose [$F(1.000, 11.00) = 0.01138, p = 0.9170$] or dose $\times$ time interaction [$F(3.390, 37.29) = 0.8455, p = 0.4896$] was observed, with scores showing only minor

increases and decreases. These results indicate that Sazetidine-A did not significantly alter the expression of orolingual AIMS over time. All data are expressed as mean $\pm$ SEM and were analysed using two-way ANOVA followed by Dunnett's *post hoc* test.

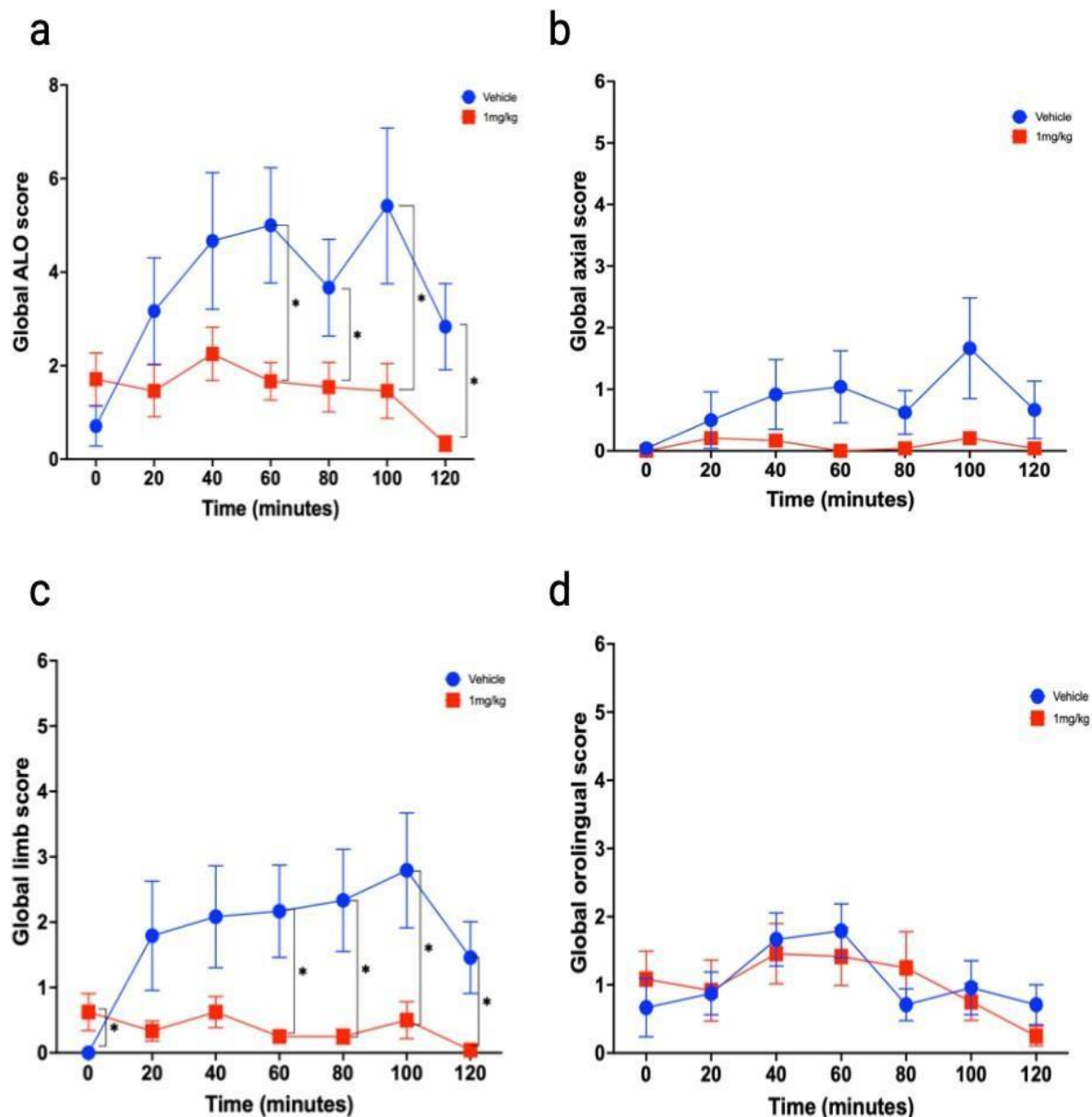


Figure 5. 43: Effects of Sazetidine-A on global AIM subtypes in lesioned, primed rats. Panel (a) shows total global ALO AIM scores, where significant main effects of time ($p = 0.0340$) and dose ($p = 0.0125$) were observed, with a trending dose $\times$ time interaction ($p = 0.0748$). *Post hoc* analysis revealed significant reductions in global ALO scores from $t = 60$ to $t = 120$ minutes ($*p \leq 0.05$). Panel (b) illustrates global axial AIMs, which showed no significant effects of time, dose, or dose $\times$ time interaction ($p > 0.05$). Panel (c) presents global limb AIMs, which demonstrated a significant main effect of dose ($p = 0.0200$) and a significant dose $\times$ time interaction ($p = 0.0353$), with Sazetidine-A reducing limb AIM scores at multiple time points ($t = 0, 60, 80, 100$, and 120 minutes; $*p \leq 0.05$). Panel (d) shows global orolingual AIMs, where a significant main effect of time ($p = 0.0459$) was detected, but neither dose nor interaction effects reached significance ($p > 0.05$). Data are expressed as mean $\pm$ SEM; $*p < 0.05$; ($n = 12$).

5.4.40 : 6-OHDA lesioning induces over 90% dopaminergic cell loss in the striatum and substantia nigra

Immunohistochemical analysis of tyrosine hydroxylase expression was performed to assess dopaminergic neurodegeneration in the substantia nigra pars compacta and the loss of dopaminergic terminal density in the striatum following unilateral 6-hydroxydopamine lesioning. Unilateral 6-OHDA injection resulted in variable degrees of TH-positive neuronal loss in the SNpc as well as different levels of TH fibre density reduction in the striatum.

In animals that did not exhibit abnormal involuntary movements, TH immunoreactivity appeared largely preserved in both the SNpc and striatum, with minimal difference between hemispheres. In contrast, animals that developed pronounced AIMs exhibited extensive loss of TH-positive neurons in the SNpc (greater than 90%) and a marked decrease in TH fibre density in the striatum. These observations are illustrated in the representative images below (figure 5.44 to 5.50).

5.4.40.1 Saline treated group

In the saline treated control group ($n = 5$), which received saline during the lesioning phase, animals did not develop AIMs and showed no signs of parkinsonism. The lesioned hemisphere remained intact and comparable to the intact side, with no apparent dopaminergic cell loss as evidenced by preserved TH immunoreactivity in the substantia nigra pars compacta (Figure 5.44, a-b).

Correspondingly, in the striatum of these animals, TH fibre density was maintained across both hemispheres, indicating preserved dopaminergic terminal innervation and no measurable depletion of TH-positive fibres in the lesioned striatum (Figure 5.44, c-d).

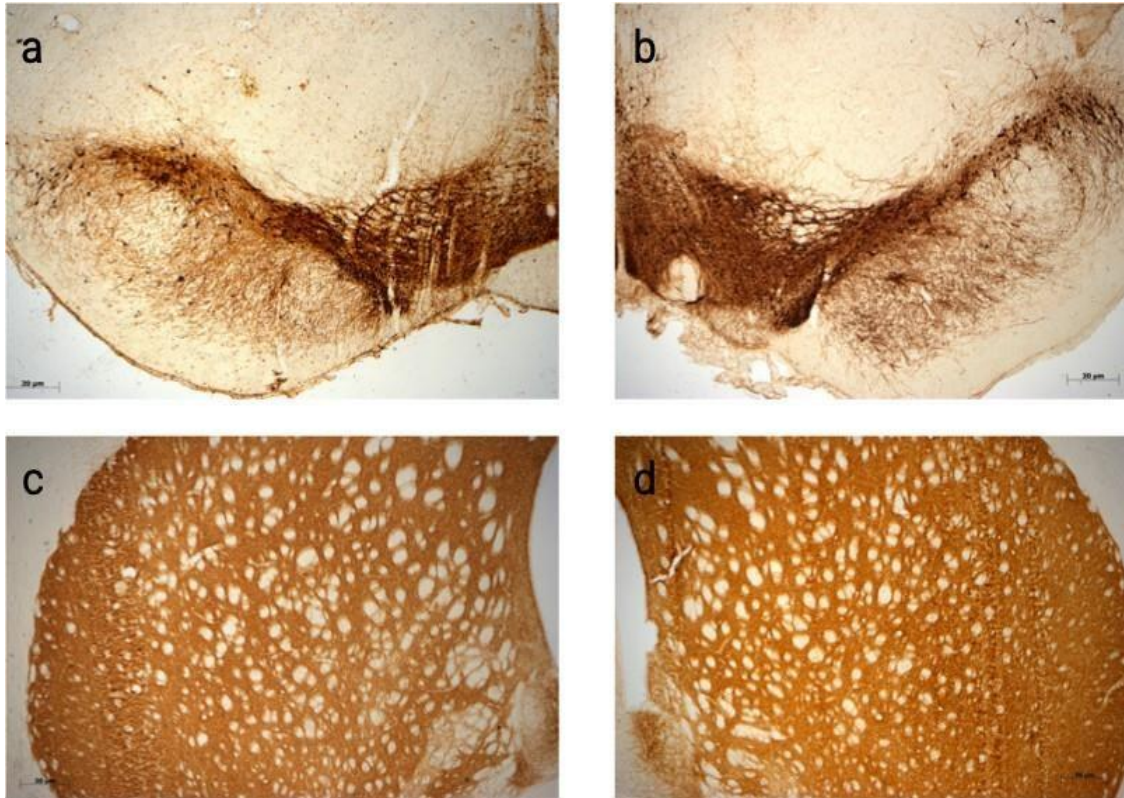


Figure 5. 44: TH immunostaining in the SNpc and striatum of the saline treated group (n=5), showing comparison between the left and right hemispheres. (a–b) TH-positive neurons in the SNpc of the left (a) and right (b) hemispheres. (c–d) TH-positive fibre density in the striatum of the left (c) and right (d) hemispheres. Images captured at 2.5× magnification. Scale bar = 20 μm.

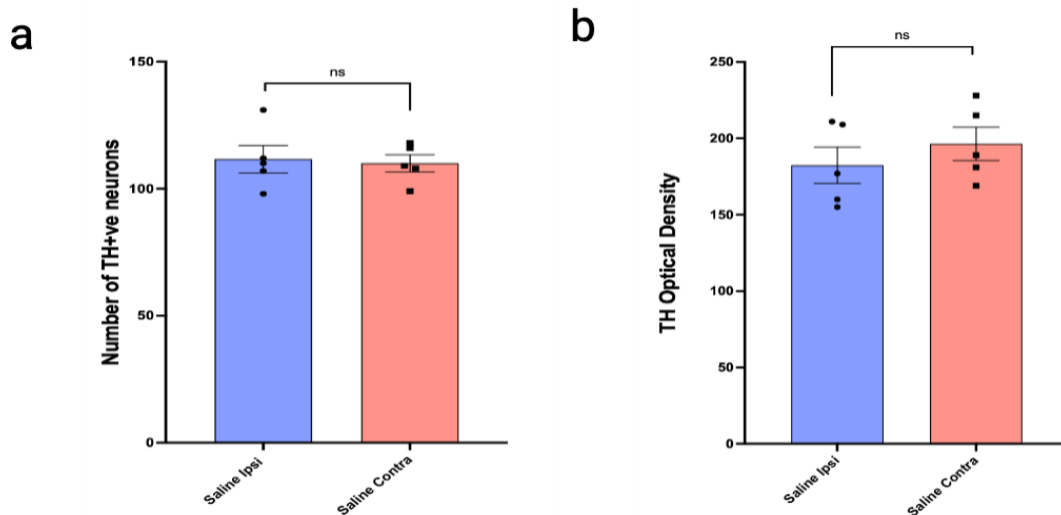


Figure 5. 45: Quantification of TH-positive neurons in the SNpc and TH optical density , expressed in arbitrary units, in the striatum of saline treated rats. (a) No significant reduction in the number of TH-positive neurons between the left and right substantia nigra in saline treated rats. (b) Correspondingly, no significant difference in TH optical density in the striatum between hemispheres. Statistical analysis was performed using two-way ANOVA followed by Tukey’s *post hoc* test.

5.4.40.2 6-OHDA treated group

In the 6-OHDA treated group (n = 29), which received levodopa and benserazide following 6-OHDA lesioning, animals displayed a range of behavioural results, reflected in varying severities of AIMs.

Seventeen animals did not develop severe AIM scores and were classified as the non-dyskinetic group. These animals exhibited preserved TH immunoreactivity in the lesioned substantia nigra pars compacta, indicating minimal dopaminergic neuronal loss (Figure 5.46). A corresponding pattern was observed in the striatum. In non-dyskinetic animals, TH-positive fibre density in the lesioned striatum was comparable to the contralateral hemisphere, suggesting preserved dopaminergic terminal innervation (Figure 5.47). In contrast, twelve animals developed robust AIMs and showed extensive dopaminergic neurodegeneration, with greater than 90% reduction in TH-positive neurons in the lesioned SNpc (Figure 5.48).

Similarly, animals with severe AIM expression exhibited a marked depletion of striatal TH-positive fibres, indicating substantial dopaminergic denervation (Figure 5.49).

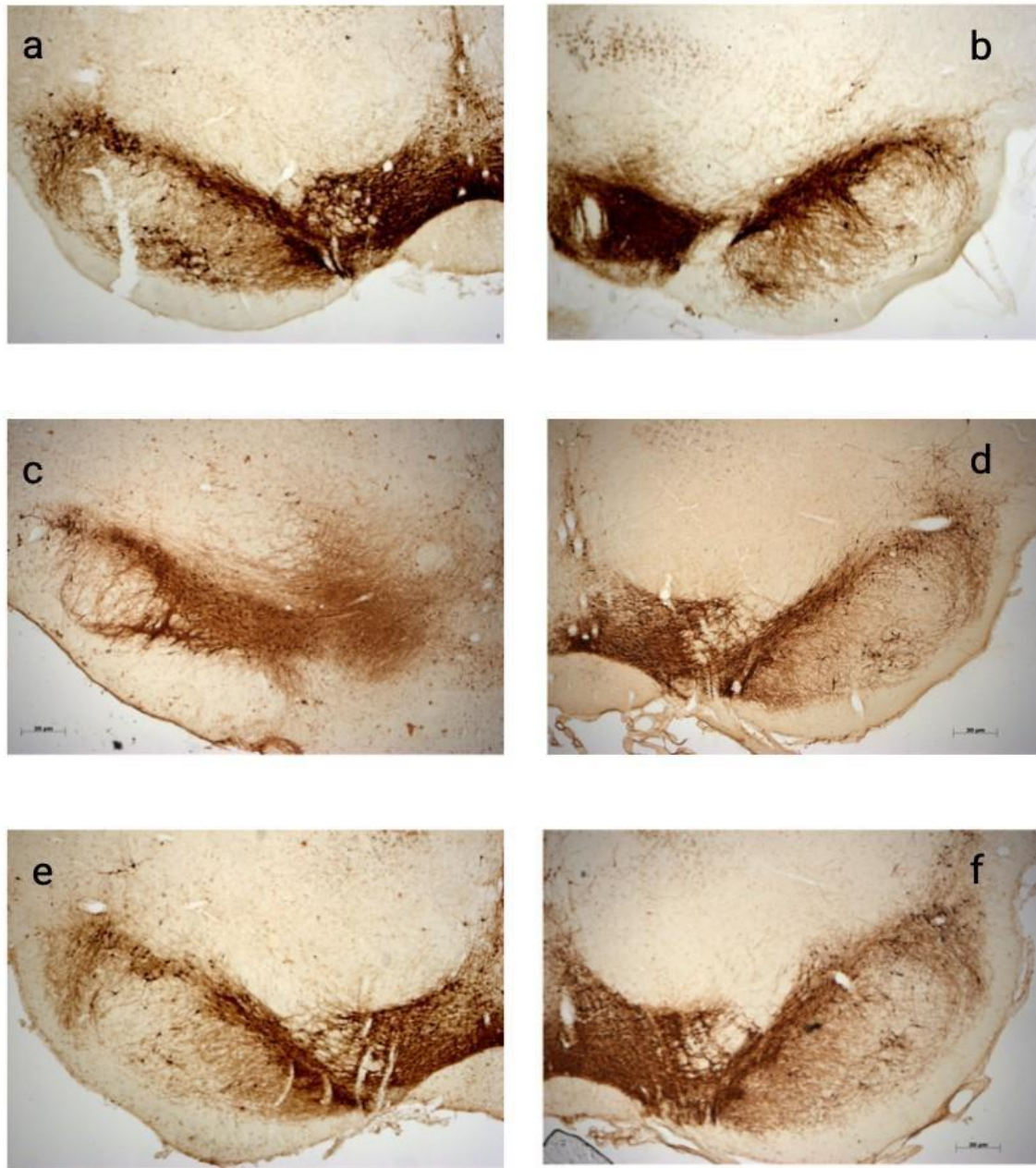


Figure 5. 46: TH immunostaining in the SNpc of 6-OHDA treated animals which didn't develop AIMS. In all animals, TH-positive neurons are preserved bilaterally, indicating no dopaminergic cell loss or asymmetry following 6-OHDA administration. Images captured at 2.5× magnification. Scale bar = 20 μ m.

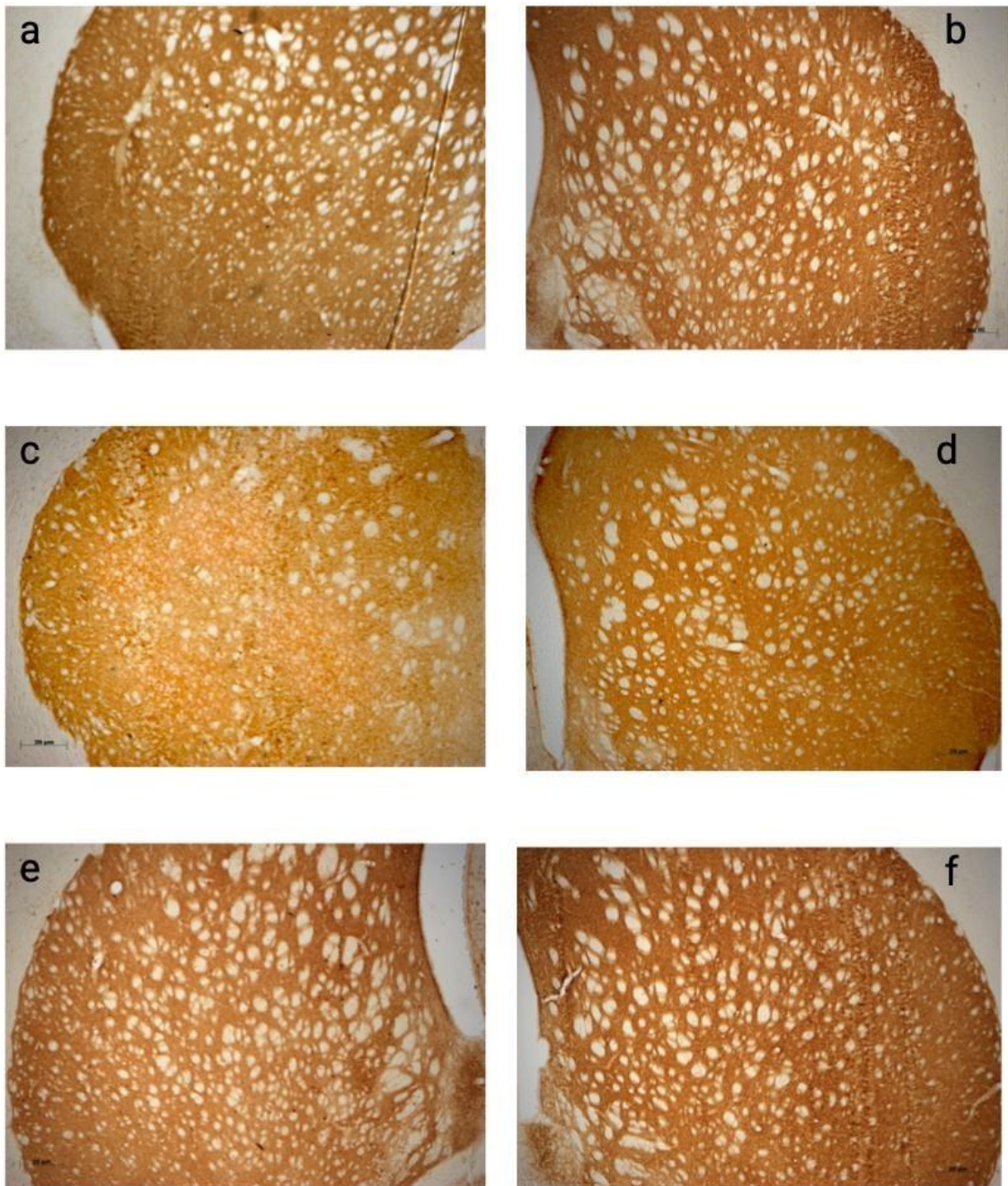


Figure 5. 47: TH immunostaining in the striatum of 6-OHDA treated animals which didn't develop AIMS. No reduction in dopaminergic terminal innervation is observed, indicating preserved striatal dopaminergic input following 6-OHDA administration. Images captured at 2.5× magnification. Scale bar = 20 μm.

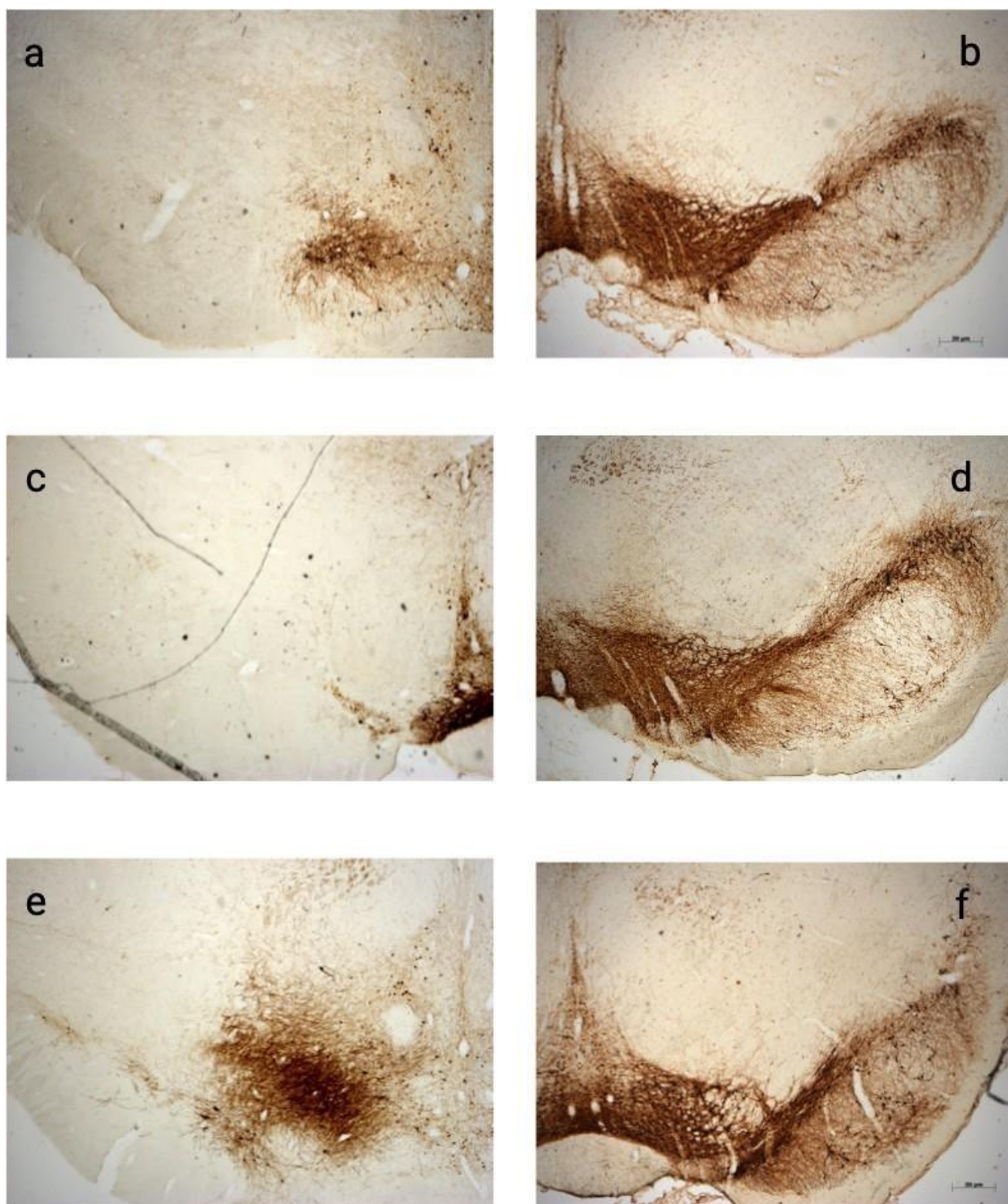


Figure 5. 48: Tyrosine hydroxylase immunostaining in the SNpc of 6-OHDA treated animals showing severe dopaminergic neurodegeneration. In 12 animals, the lesioned hemisphere exhibits a marked reduction (>90%) in TH-positive neurons compared to the intact hemisphere, indicating extensive loss of dopaminergic cell bodies following 6-OHDA administration. Images captured at 2.5× magnification. Scale bar = 20 μm.

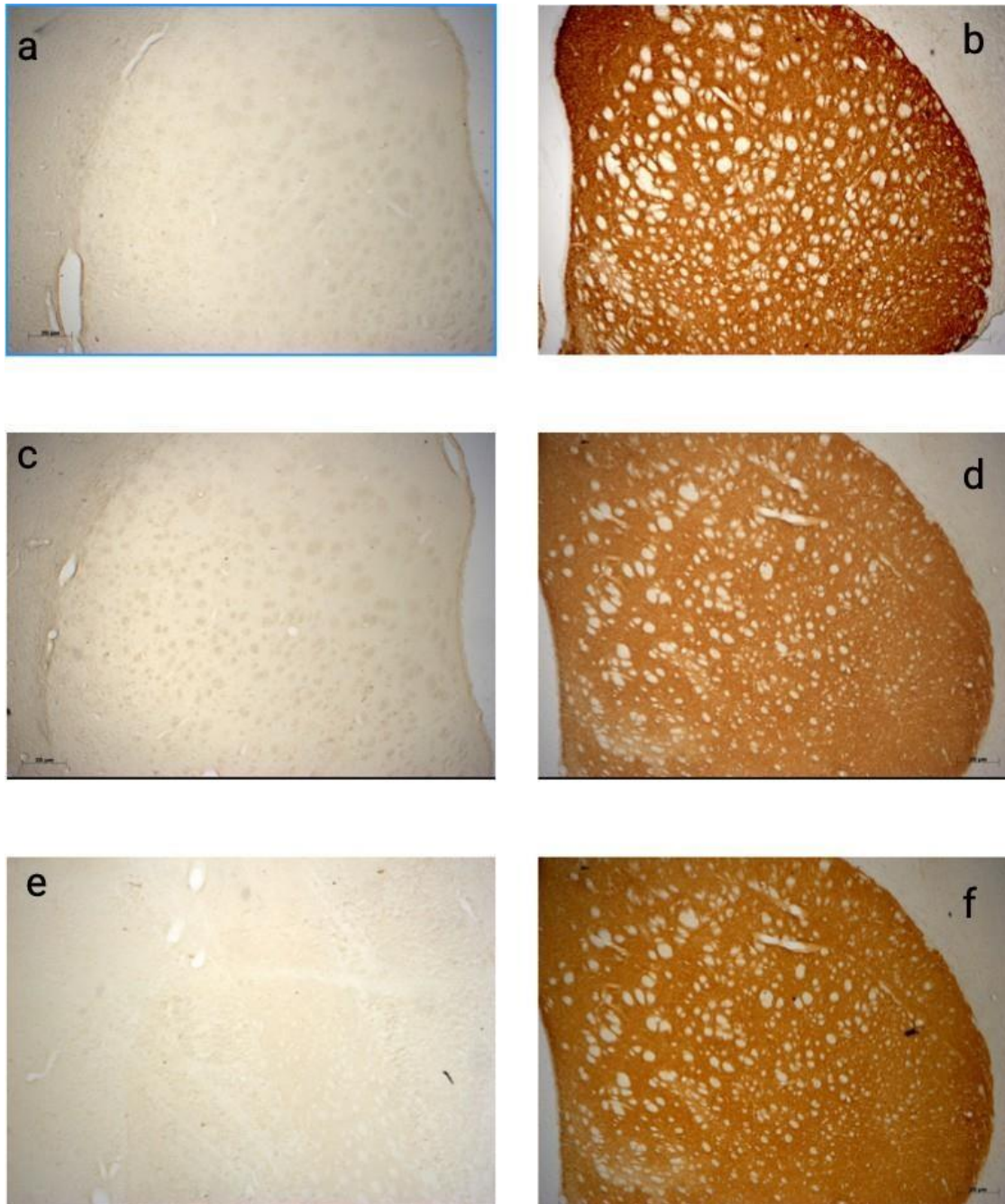


Figure 5. 49: TH immunostaining in the striatum of 6-OHDA treated animals showing extensive dopaminergic denervation. In 12 animals, the lesioned hemisphere displays a profound reduction in TH-positive fibre density, consistent with near complete loss of dopaminergic input (>90%), whereas the contralateral hemisphere remains intact. Images captured at 2.5× magnification. Scale bar = 20 µm.

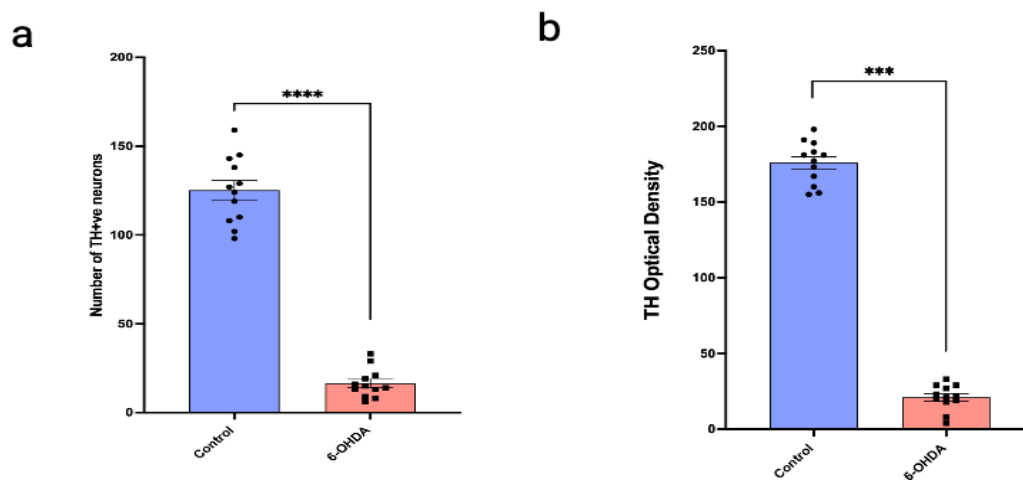


Figure 5. 50: Quantification of TH-positive neurons in the SNpc and TH optical density , expressed in arbitrary units, in the striatum of the intact hemisphere and 6-OHDA lesioned hemisphere. (a) Significant reduction in the number of TH-positive neurons in the lesioned substantia nigra of rats with severe AIMs. (b) Corresponding decrease in TH optical density in the lesioned striatum compared to the intact hemisphere. * $P < 0.001$, **** $P < 0.0001$ (two-way ANOVA followed by Tukey's *post hoc* test).**

5.4.41 : Astrocytic activation in the striatum and substantia nigra following 6-OHDA lesioning

Immunohistochemical analysis of glial fibrillary acidic protein (GFAP) expression was conducted to evaluate astrocytic activation in the substantia nigra pars compacta and striatum following unilateral 6-OHDA lesioning. GFAP immunoreactivity was assessed across three groups: saline treated controls, non-dyskinetic 6-OHDA lesioned animals (non-turners), and dyskinetic 6-OHDA lesioned animals (turners).

In the saline control group, GFAP expression was low and symmetrical between hemispheres in both the SNpc and striatum, indicating the absence of astrocytic activation or neuroinflammatory changes (figure 5.51).

In non-dyskinetic 6-OHDA lesioned animals, a mild increase in GFAP immunoreactivity was observed in the lesioned hemisphere. This was characterised by subtle astrocytic hypertrophy and limited gliosis in both the SNpc and striatum, reflecting a modest neuroinflammatory response in the absence of dyskinetic behaviours (figures 5.53 and 5.54).

In dyskinetic (turner) animals, GFAP expression was markedly increased in the lesioned hemisphere. Pronounced astrocytic activation was evident in both the SNpc and striatum, with dense GFAP positive staining and hypertrophic astrocytes. This strong gliotic response corresponded with the presence of AIMs and more severe nigrostriatal degeneration (figures 5.55 and 5.56).

5.4.41.1 Saline treated group

In the saline treated control group ($n = 5$), which received saline during the lesioning phase, animals did not develop AIMs and showed no signs of parkinsonism. The substantia nigra pars compacta and striatum appeared bilaterally intact, with no evidence of neuroinflammation or astrocytic reactivity.

GFAP immunostaining showed low and symmetrical expression between hemispheres. No astrocytic hypertrophy, gliosis, or increased GFAP positive staining was observed in the SNpc (Figure 5.51, a–b), confirming the absence of astrocytic activation. Similarly, in the striatum, GFAP expression remained minimal and comparable across both hemispheres, indicating preserved tissue integrity and the absence of lesion induced gliosis (Figure 5.51, c–d).

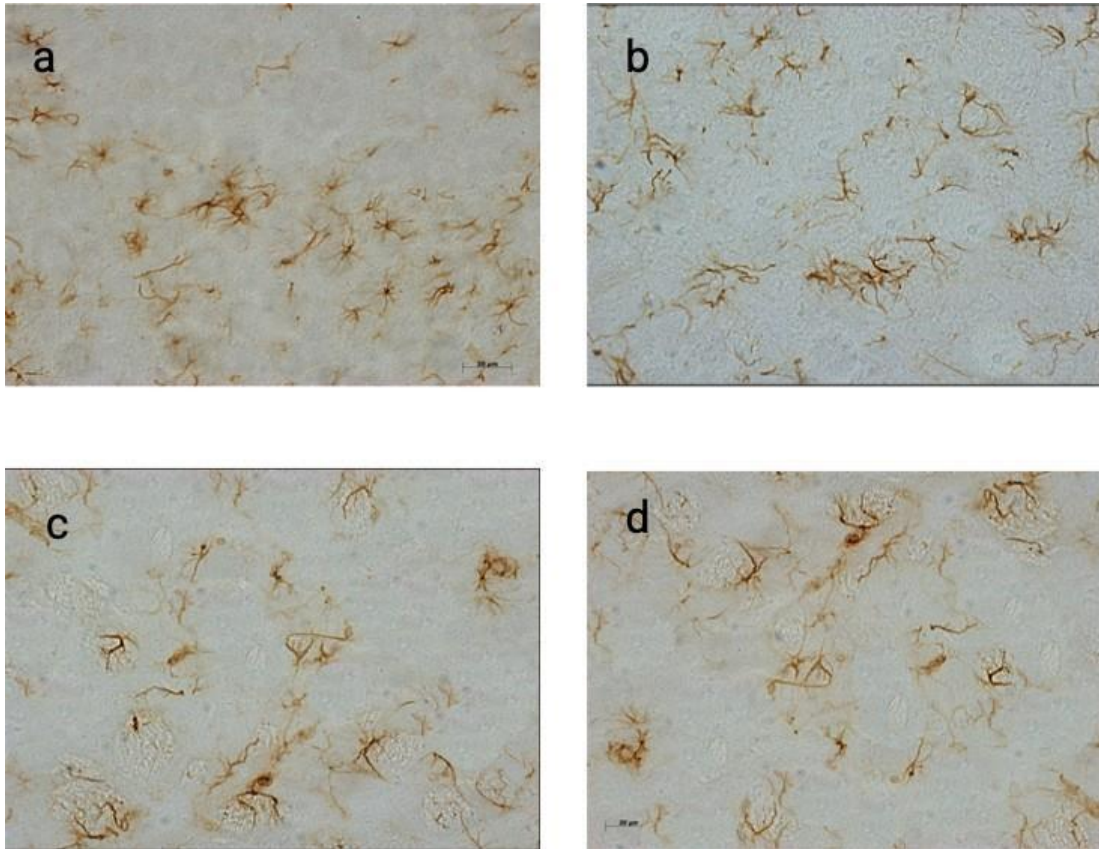


Figure 5. 51: GFAP-ir immunostaining in the SNpc and striatum of the saline treated group, comparing the left and right hemispheres. (a–b) GFAP-ir in the SNpc of the left (a) and right (b) hemispheres, showing minimal astrocytic activation and symmetrical staining in the absence of lesioning with 6-OHDA or AIMs. (c–d) GFAP-ir in the striatum of the left (c) and right (d) hemispheres, demonstrating similarly low astrocytic activity and preserved tissue integrity. Images captured at 20× magnification. Scale bar = 20 μm.

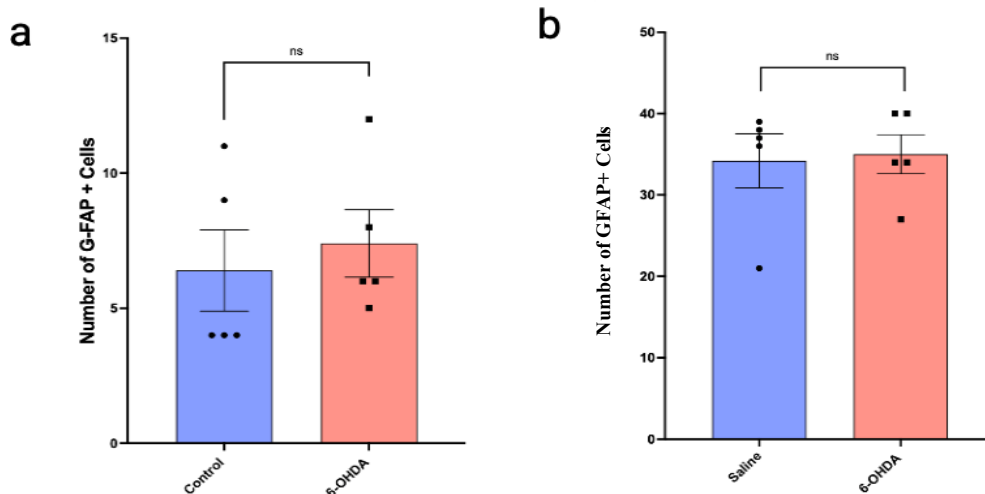


Figure 5.52: Quantification of GFAP expression in the SNpc and striatum of saline treated rats.

(a) No significant difference in GFAP-positive astrocytic profiles between the left and right substantia nigra, indicating absence of astrocytic activation in saline controls. (b) Similarly, GFAP optical density, expressed in arbitrary units, in the striatum showed no significant difference between hemispheres, confirming the lack of gliosis or neuroinflammatory response. Statistical analysis was performed using two-way ANOVA followed by Tukey's *post hoc* test.

5.4.41.2 6-OHDA treated group

In the 6-OHDA treated group ($n = 29$), which received levodopa and benserazide following unilateral 6-OHDA lesioning, seventeen animals did not develop AIMs and were classified as the non-dyskinetic group. In these animals, GFAP immunoreactivity in the lesioned substantia nigra pars compacta remained relatively low and comparable to the contralateral side, indicating limited astrocytic activation and a milder neuroinflammatory response (Figure 5.53). A similar pattern was observed in the striatum, where GFAP-positive fibre density did not differ markedly between hemispheres, suggesting an absence of substantial gliosis (Figure 5.54).

In contrast, twelve animals developed pronounced dyskinesia and were classified as the dyskinetic group. These animals displayed a significant increase ($p = 0.0004$) in GFAP

expression within the lesioned SNpc, characterised by hypertrophic astrocytes and enhanced glial reactivity (Figure 5.55). Correspondingly, in the striatum, dyskinetic animals exhibited marked astrocytic activation ($p = 0.0009$) with increased GFAP-positive fibre density in the lesioned hemisphere, consistent with a heightened neuroinflammatory response following dopaminergic denervation (Figure 5.56).

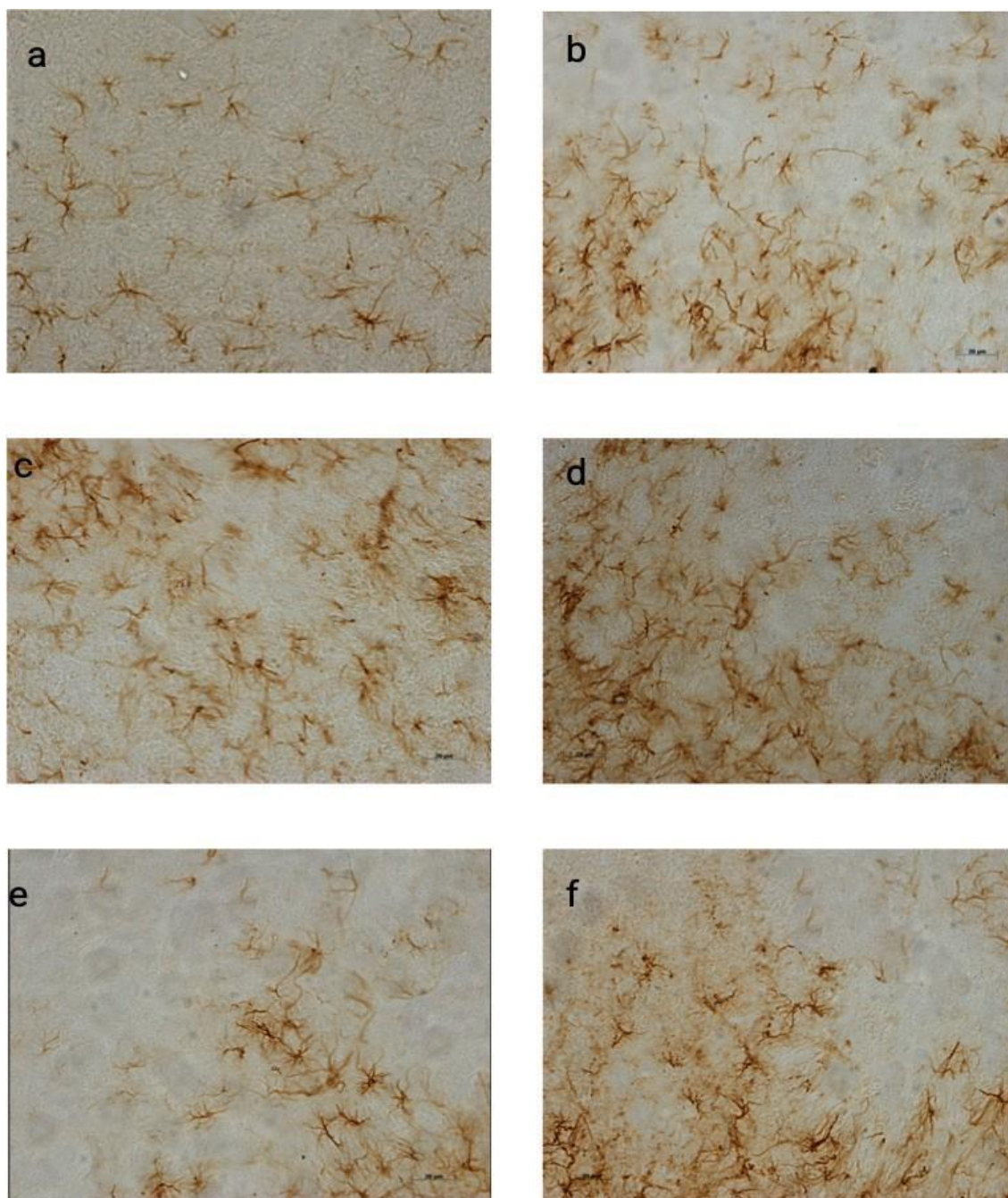


Figure 5. 53: GFAP immunostaining in the SNpc of non-dyskinetic 6-OHDA treated animals, showing comparable astrocytic profiles between the lesioned and intact hemispheres. GFAP expression remains low and symmetrical in both hemispheres, indicating minimal astrocytic activation and absence of significant gliosis despite 6-OHDA administration in animals that did not develop AIMs. Images captured at 40× magnification. Scale bar = 20 μ m.

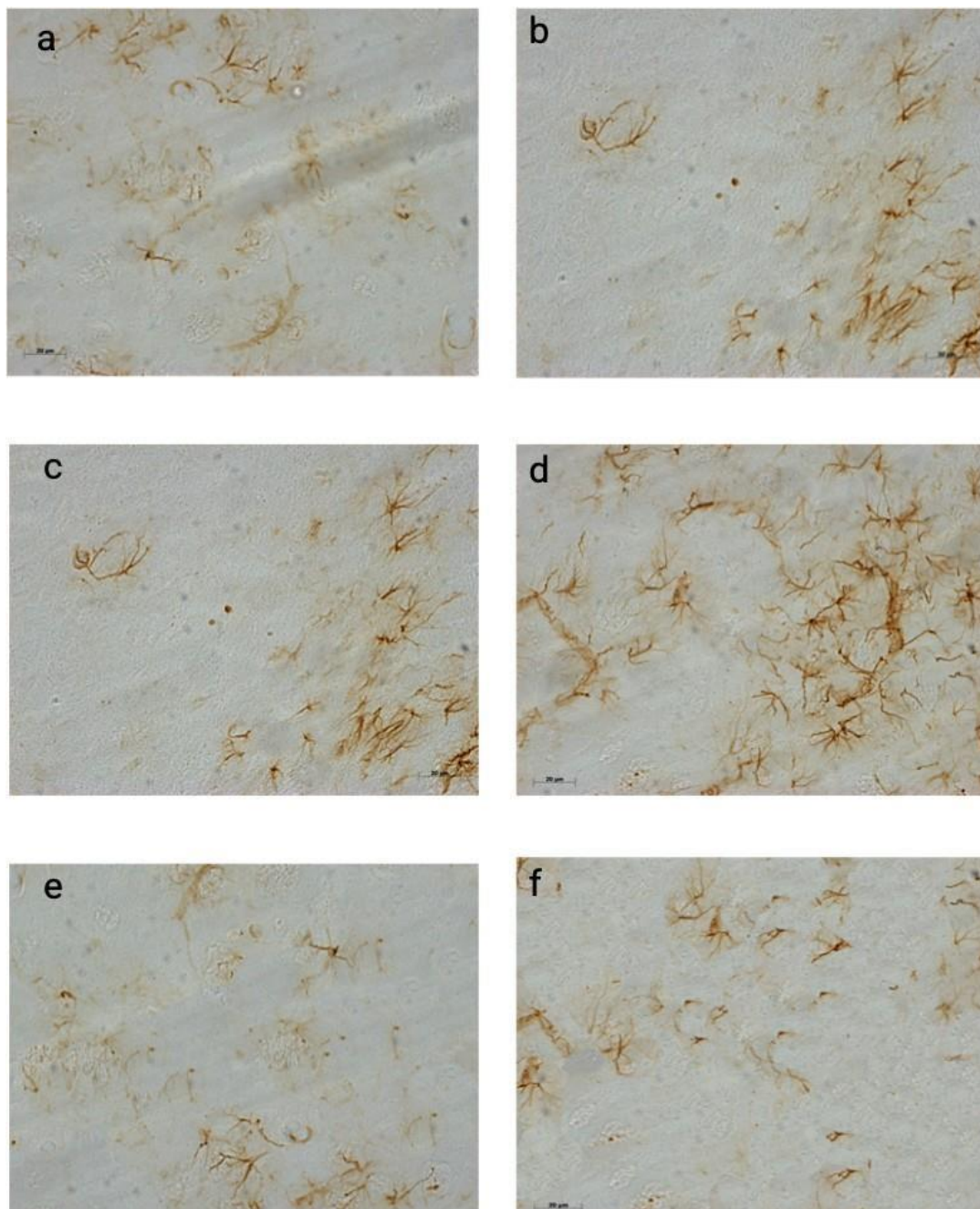


Figure 5. 54: GFAP immunostaining in the striatum of non-dyskinetic 6-OHDA treated animals, showing comparable astrocytic profiles between the 6-OHDA injected and intact hemispheres. GFAP positive fibre density remains low and symmetrical across both hemispheres, indicating absence of astrocytic activation or gliosis following saline administration. Images captured at 40× magnification. Scale bar = 20 μm .

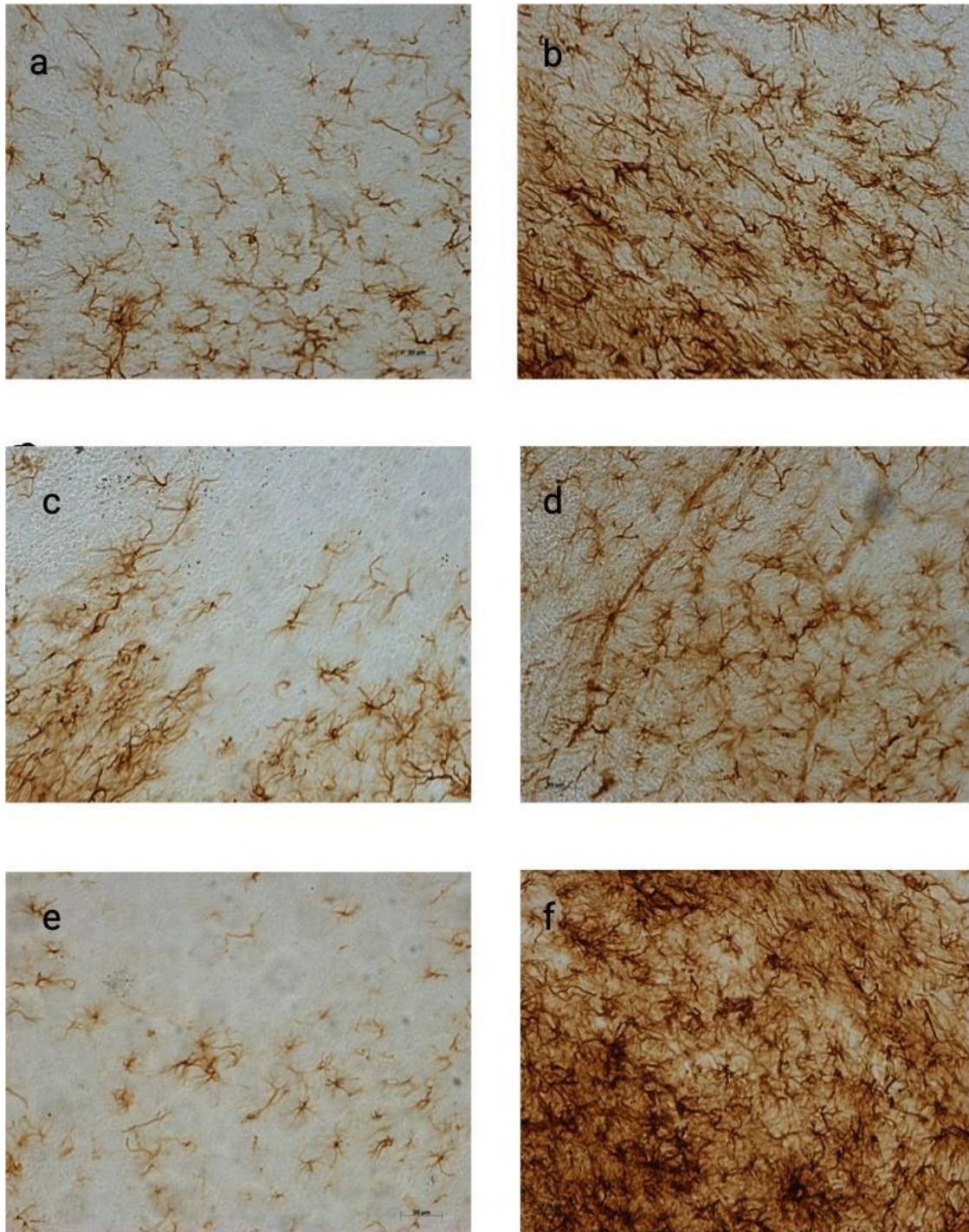


Figure 5. 55: GFAP immunostaining in the SNpc of 6-OHDA treated animals showing pronounced astrocytic activation. In the 12 dyskinetic animals, the lesioned hemisphere displays a marked increase in GFAP positive astrocytes compared to the intact hemisphere, indicating robust gliosis and neuroinflammatory response following 6-OHDA administration. Images captured at 40× magnification. Scale bar = 20 µm.

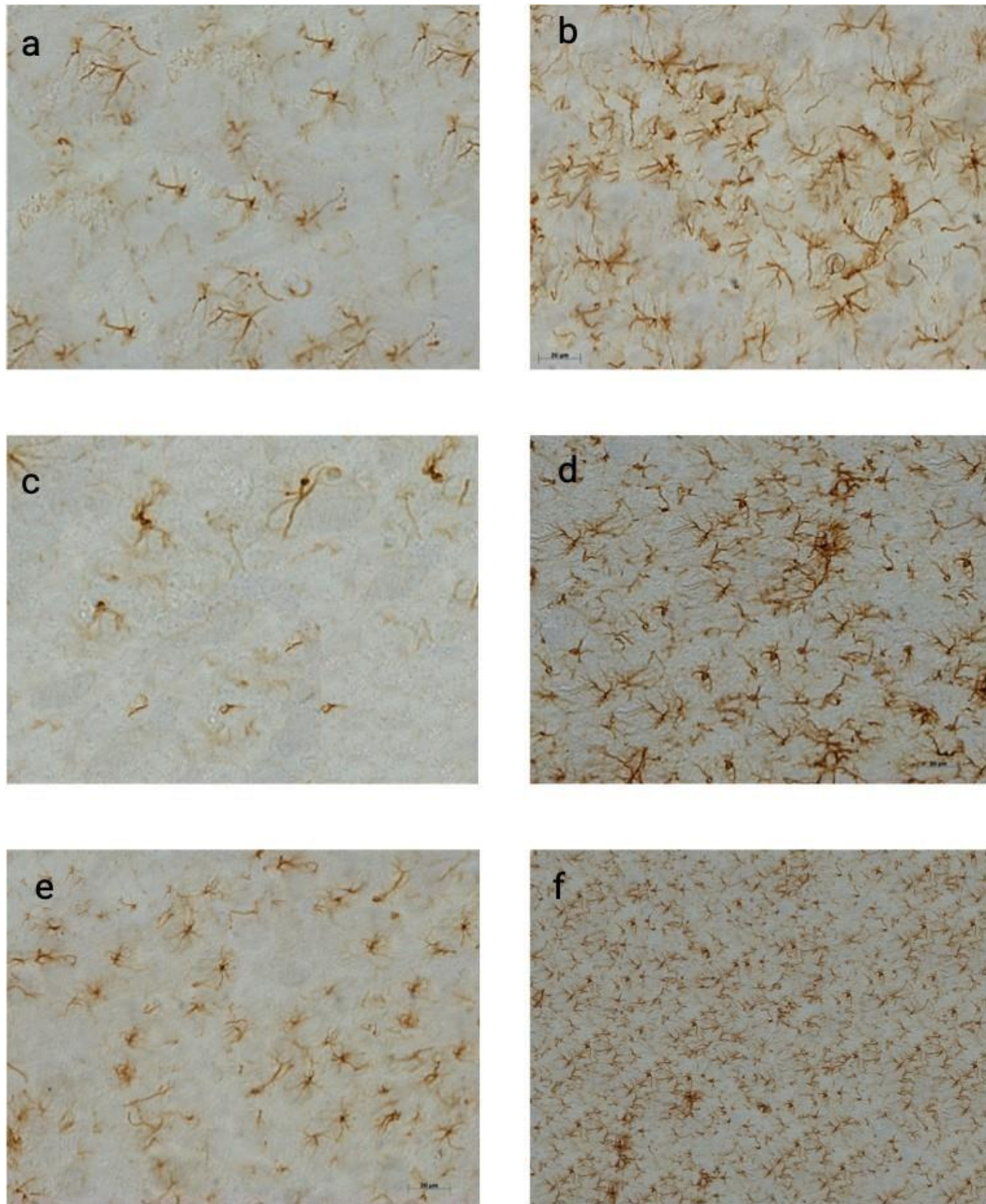


Figure 5. 56: GFAP immunostaining in the striatum of 6-OHDA treated dyskinetic animals showing pronounced astrocytic activation. In the 12 dyskinetic animals, the lesioned hemisphere exhibits a marked increase in GFAP-positive astrocytic fibres compared to the intact hemisphere, indicating robust striatal gliosis and neuroinflammatory response following 6-OHDA administration. Images captured at 40× magnification. Scale bar = 20 µm.

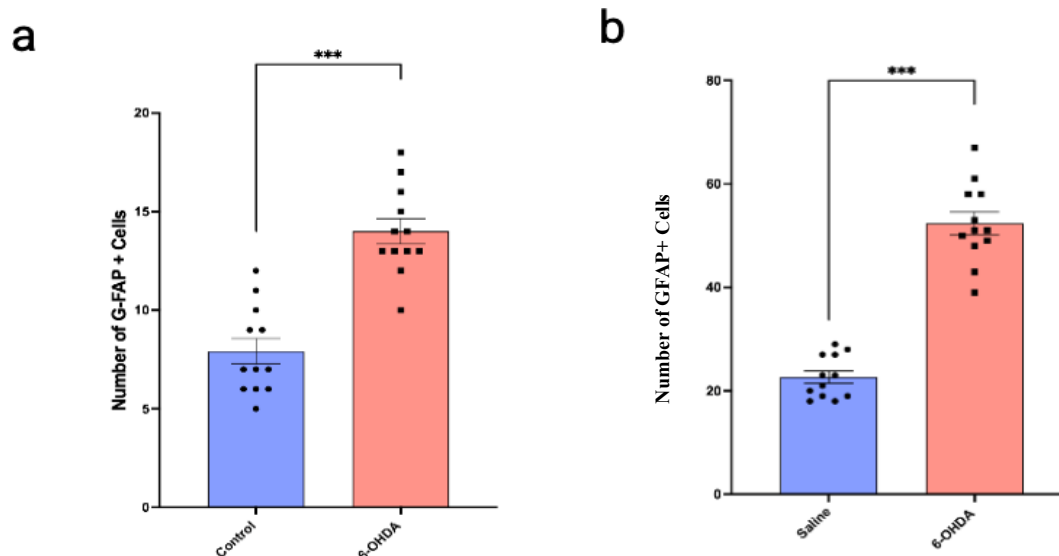


Figure 5.57: Quantification of GFAP expression in the SNpc and GFAP optical density, expressed in arbitrary units, in the striatum of the intact and 6-OHDA lesioned hemispheres. (a) Significant increase in GFAP-positive astrocytic profiles in the lesioned substantia nigra of dyskinetic rats, indicating elevated astrocytic activation and neuroinflammation. (b) Corresponding increase in GFAP optical density in the lesioned striatum compared to the intact hemisphere, consistent with gliosis and enhanced astrocytic reactivity. * $p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$; (two-way ANOVA followed by Tukey's *post hoc* test).

5.4.42 : 6-OHDA lesioning causes glial activation of levodopa induced dyskinetic rats in the striatum and substantia nigra (IBA-1)

Immunohistochemical analysis of IBA-1 expression was conducted to evaluate microglial activation in the substantia nigra pars compacta and striatum following unilateral 6-OHDA lesioning and subsequent levodopa treatment. IBA-1 immunoreactivity was assessed across three groups: saline treated controls, non-dyskinetic 6-OHDA lesioned animals (non-turners), and dyskinetic 6-OHDA lesioned animals (turners).

In the saline control group, IBA-1 expression was low and symmetrical between hemispheres in both the SNpc and striatum, indicating the absence of microglial activation or neuroinflammatory changes (Figure 5.58).

In non-dyskinetic 6-OHDA lesioned animals, a mild increase in IBA-1 immunoreactivity was observed in the lesioned hemisphere. Microglia displayed early activation characteristics in both the SNpc and striatum, suggesting a limited neuroinflammatory response in the absence of AIMs (Figures 5.60 and 5.61).

In dyskinetic (turner) animals, IBA-1 expression was markedly increased in the lesioned hemisphere. Pronounced microglial activation was evident in both the SNpc and striatum, characterised by dense IBA-1-positive staining, enlarged cell bodies, and retracted or thickened processes. This strong microglial response corresponded with the development of AIMs and more severe nigrostriatal neurodegeneration (Figures 5.62 and 5.63).

5.4.42.1 Saline treated group

In the saline treated control group ($n = 5$), which received saline during the lesioning phase, animals did not develop AIMs and exhibited no motor deficits indicative of parkinsonism. Both the SNpc and striatum appeared structurally intact, with no signs of neuroinflammatory changes.

IBA-1 immunostaining demonstrated low and symmetrical microglial presence across hemispheres. Microglia retained a resting, ramified morphology, with no increase in IBA-1-positive cell density or evidence of microglial activation in the SNpc (Figure 5.58, a–b). Likewise, in the striatum, IBA-1 expression remained minimal and comparable between the lesioned and contralateral hemispheres, indicating preserved tissue homeostasis and absence of 6-OHDA induced microgliosis (Figure 5.58, c–d).

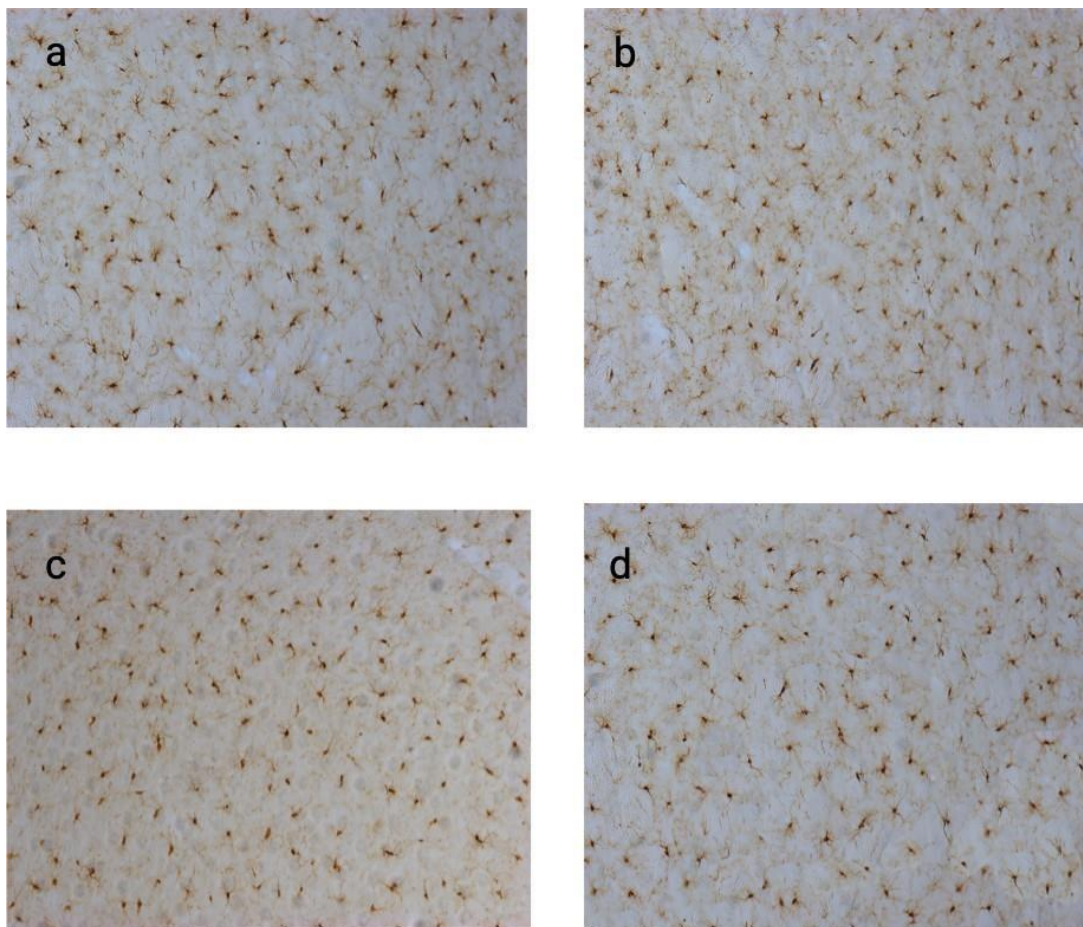


Figure 5. 58: IBA-1 immunostaining in the SNpc and striatum of the saline treated group, comparing the left and right hemispheres. (a–b) IBA-1 expression in the SNpc of the left (a) and right (b) hemispheres, showing a resting microglial profile with low and symmetrical staining, indicating absence of 6-OHDA induced microglial activation or AIMs associated neuroinflammation. (c–d) IBA-1 immunoreactivity in the striatum of the left (c) and right (d) hemispheres, demonstrating similarly low microglial density and preserved tissue morphology. Images captured at 10× magnification. Scale bar = 20 μ m.

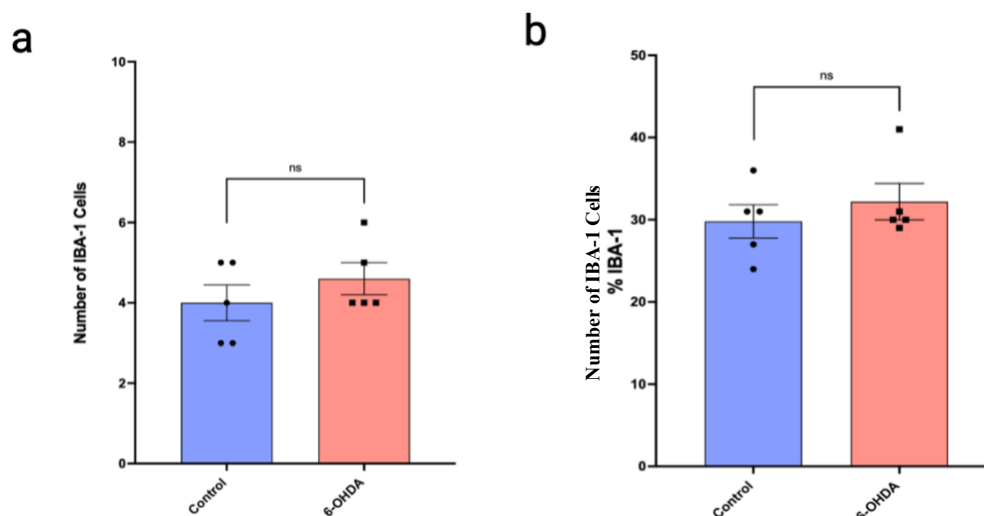


Figure 5.59: Quantification of IBA-1 expression in the SNpc and striatum of saline treated rats.

(a) No significant difference in IBA-1 positive microglial profiles between the left and right substantia nigra, indicating absence of microglial activation in saline controls. (b) Similarly, IBA-1 optical density, expressed in arbitrary units, in the striatum showed no significant difference between hemispheres, confirming the lack of microgliosis or neuroinflammatory response. Statistical analysis was performed using two-way ANOVA followed by Tukey's *post hoc* test.

5.4.42.2 6-OHDA treated group

In the 6-OHDA treated group ($n = 29$), which received levodopa and benserazide following unilateral 6-OHDA lesioning, seventeen animals did not develop AIMs and were classified as the non-dyskinetic group. In these animals, IBA-1 immunoreactivity in the lesioned substantia nigra pars compacta remained low and comparable to the contralateral hemisphere, indicating minimal microglial activation and a low neuroinflammatory response (Figure 5.60).

A similar pattern was seen in the striatum, where IBA-1-positive microglial density showed no significant difference between hemispheres, suggesting limited microgliosis in the absence of AIMs (Figure 5.61).

In contrast, twelve animals developed clear dyskinetic behaviours and were classified as the dyskinetic group. These animals demonstrated a significant increase ($P < 0.0001$) in IBA-

1 expression within the lesioned SNpc, characterised by activated microglia with enlarged cell bodies and thickened processes (Figure 5.62). In the striatum, dyskinetic animals also showed elevated IBA-1 positive cell density ($p = 0.0007$) in the lesioned hemisphere, consistent with pronounced microglial activation and heightened neuroinflammatory status following dopaminergic denervation (Figure 5.63).

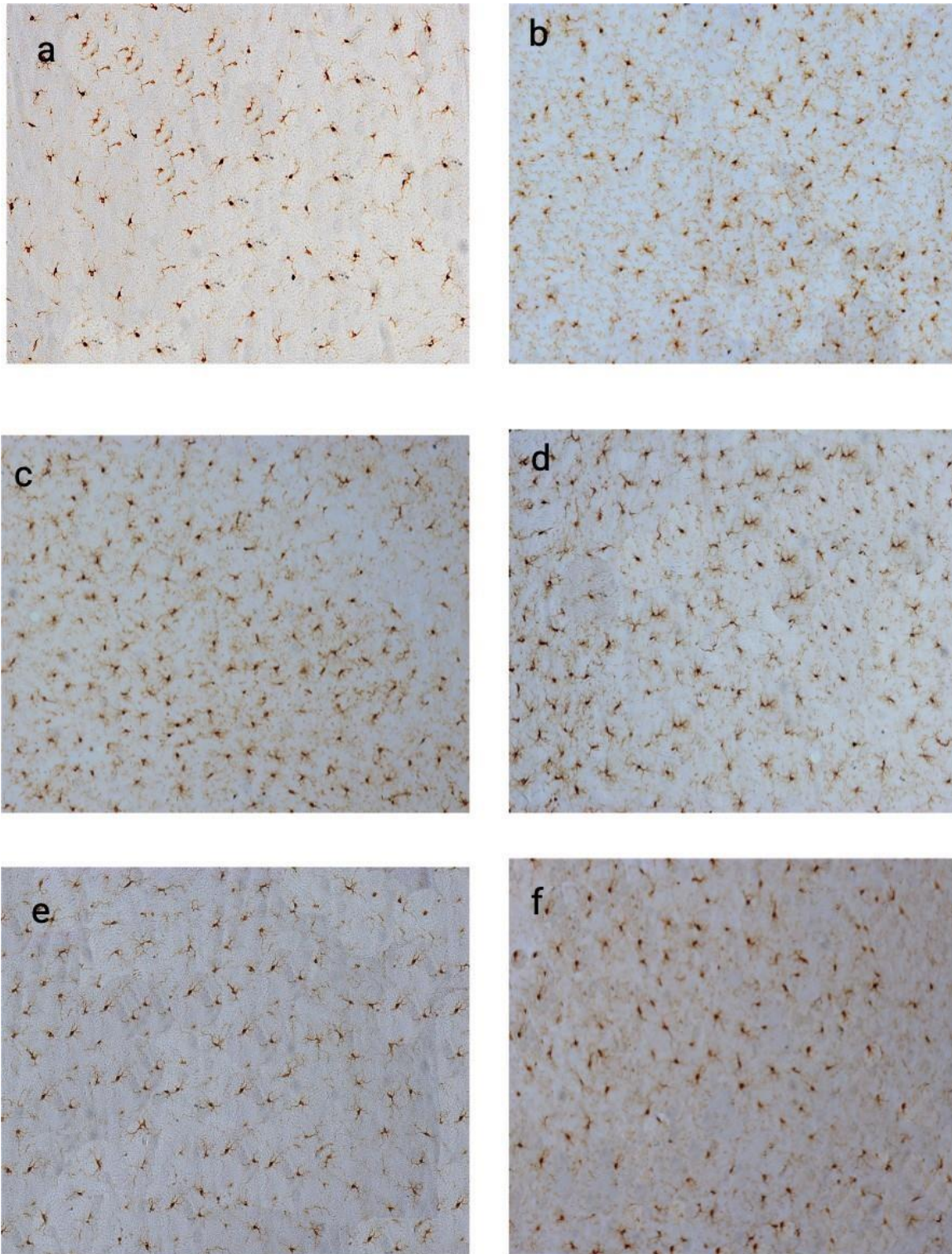


Figure 5. 60: IBA-1 immunostaining in the SNpc of non-dyskinetic 6-OHDA treated animals, showing comparable microglial profiles between the lesioned and intact hemispheres. IBA-1 expression remains low and largely symmetrical across both hemispheres, indicating minimal microglial activation and absence of substantial neuroinflammation despite 6-OHDA administration in animals that did not develop AIMs. Images captured at 10× magnification. Scale bar = 20 μ m.

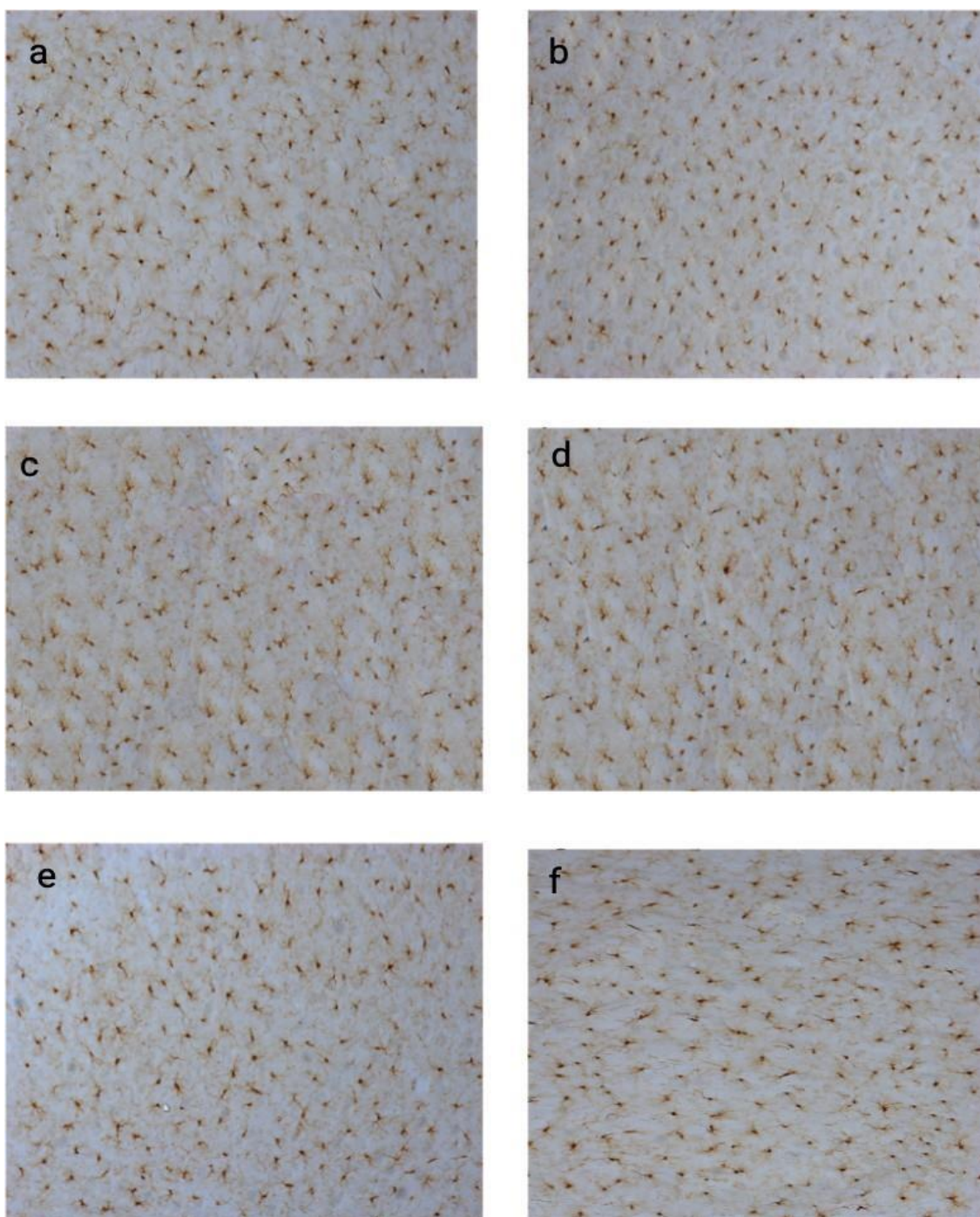


Figure 5. 61: IBA-1 immunostaining in the striatum of non-dyskinetic 6-OHDA treated animals, showing comparable microglial profiles between the lesioned and intact hemispheres. IBA-1 positive microglial density remains low and symmetrical across both hemispheres, with microglia predominantly exhibiting a resting morphology. This indicates an absence of significant microglial activation or lesion induced neuroinflammation in animals that did not develop AIMs. Images captured at 10 $\times$ magnification. Scale bar = 20 μ m.

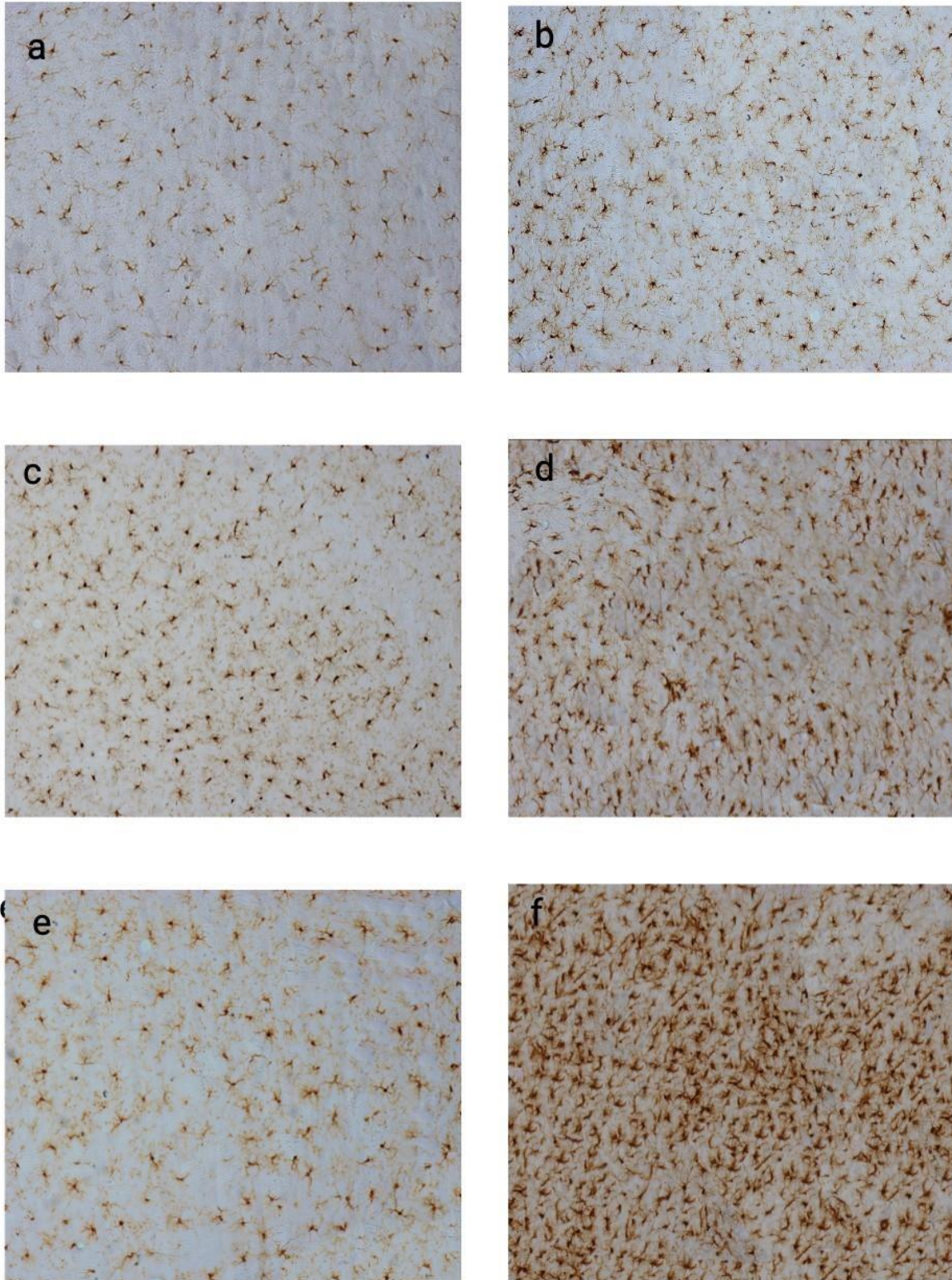


Figure 5.62: IBA-1 immunostaining in the SNpc of 6-OHDA treated animals showing pronounced microglial activation. In the 12 dyskinetic animals, the lesioned hemisphere exhibits a marked increase in IBA-1 positive microglia compared to the intact hemisphere, characterised by hypertrophic cell bodies and retracted processes, indicating a robust microglial response and neuroinflammation following 6-OHDA administration. Images captured at 10× magnification. Scale bar = 20 μ m.

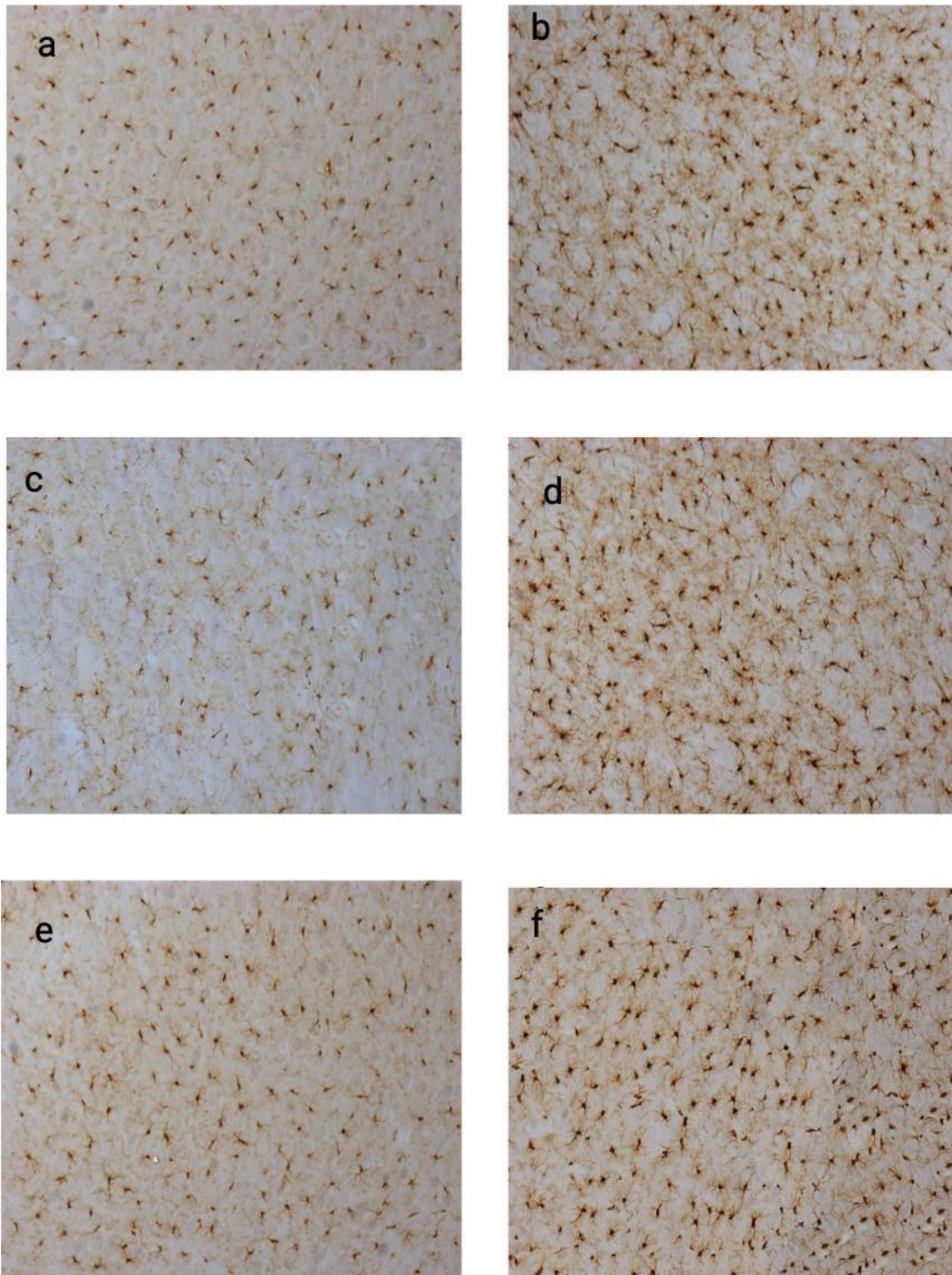


Figure 5. 63: IBA-1 immunostaining in the striatum of 6-OHDA treated dyskinetic animals showing pronounced microglial activation. In the 12 dyskinetic animals, the lesioned hemisphere exhibits a marked increase in IBA-1 positive microglial cells and processes compared to the intact hemisphere, indicating robust striatal microgliosis and neuroinflammatory response following 6-OHDA administration. Images captured at 10× magnification. Scale bar = 20 μm.

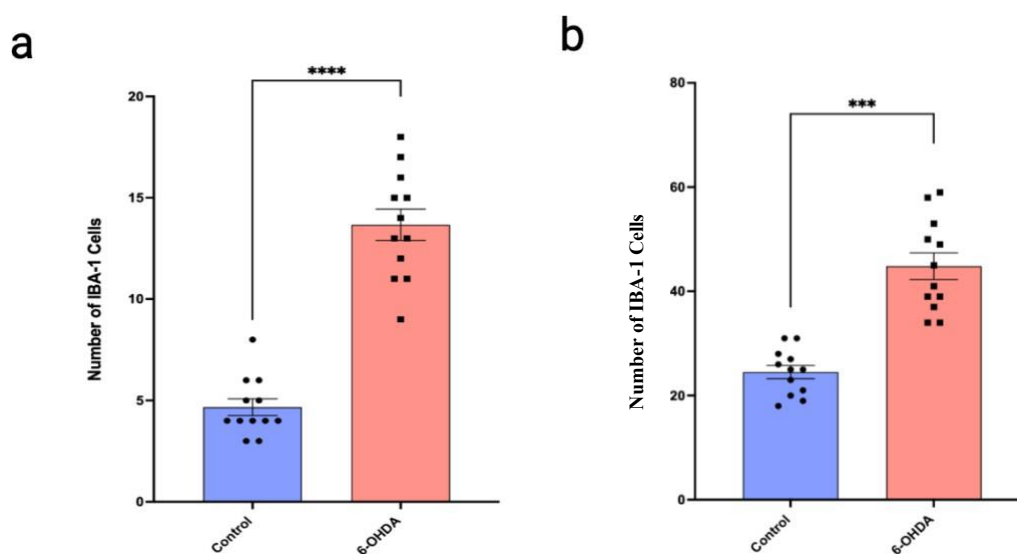


Figure 5.64: Quantification of IBA-1 expression in the SNpc and IBA-1 optical density, expressed in arbitrary units, in the striatum of the intact and 6-OHDA lesioned hemispheres. (a) Significant increase in IBA-1-positive microglial profiles in the lesioned substantia nigra of dyskinetic rats, indicating heightened microglial activation and neuroinflammation. (b) Corresponding increase in IBA-1 optical density in the lesioned striatum compared to the intact hemisphere, consistent with striatal microgliosis and enhanced microglial reactivity. *** $P < 0.001$, **** $P < 0.0001$ (two-way ANOVA followed by Tukey's *post hoc* test).

Table 5. 2: Summary of the effects of $\alpha 4\beta 2$ nicotinic receptor ligands on levodopa induced abnormal involuntary movements in the 6-OHDA rat model.

Drug	Action at receptor	Doses (mg/kg)	Effects on AIMs	Observations
A85380	Agonist	0.001, 0.003, 0.01, 0.03	Strong ↓	Robust reduction
DhβE	Antagonist	1.0, 3.0, 10.0	No effect	Blockade insufficient
NS9283	PAM	0.3, 1.0, 3.0	No effect	No desensitization
Sazetidine-A	Desensitizer	0, 1.0	Moderate ↓	Delayed reduction effect
A85380 + DhβE	Combo	0.01+1	No added effect	Competitive inhibition

5.4 Discussion

Levodopa remains the most effective therapy for Parkinson's disease, yet long-term administration frequently results in abnormal involuntary movements known as levodopa induced dyskinesia. These movements arise from maladaptive plasticity within the basal ganglia, driven by pulsatile dopaminergic stimulation and an imbalance between dopamine and acetylcholine signalling in the striatum. Nicotinic acetylcholine receptors, particularly those containing $\alpha 4\beta 2$ subunits, play a crucial role in regulating dopaminergic transmission. Pharmacological modulation of these receptors has previously been shown to lessen established LID (Quik et al., 2007; Bordia et al., 2008), but the receptor engagement state responsible for these effects, activation, desensitisation, antagonism, or potentiation remained unclear. The present study addressed this question by evaluating whether selective modulation of $\alpha 4\beta 2$ receptors could alter the expression of stable AIMs in the 6-OHDA rat model.

The results demonstrated that the $\alpha 4\beta 2$ agonist A85380 (0.01 mg/kg) produced the most robust antidyskinetic effect. This dose significantly reduced total ALO AIMs and specifically decreased limb and orolingual subtypes across basic, amplitude, and global scores. The reductions were consistent across both cumulative and time course analyses and occurred without impairing locomotive activity, indicating that A85380 restored dopaminergic balance rather than suppressing motor output.

Mechanistically, A85380 acts as a potent full agonist at $\alpha 4\beta 2$ nAChRs expressed on dopaminergic terminals in the striatum. Acute receptor activation transiently enhances dopamine release, but with continued occupancy, the receptors enter a desensitised state, reducing the amplitude and irregularity of dopamine transients. This stabilisation of dopaminergic tone limits overactivation of D1 receptor expressing medium spiny neurons and dampens the abnormal corticostriatal output that underlies dyskinesia (Quik et al., 2013; Bordia et al., 2010). In addition, $\alpha 4\beta 2$ receptor activation modulates glutamatergic and GABAergic

inputs within the basal ganglia (Livingstone & Wonnacott, 2009), further contributing to the restoration of network balance. These combined effects explain the broad and consistent suppression of AIMs observed following A85380 administration.

The partial agonist Sazetidine-A (1 mg/kg) also significantly reduced AIMs, though its pattern of efficacy was more selective. Reductions were observed in total ALO and limb AIMs, with amplitude and global analyses confirming comparable results. Notably, its antidyskinetic effect was strongest during the later stages of the session (60–120 minutes), consistent with its unique pharmacological profile. Sazetidine-A binds with high affinity to $\alpha 4\beta 2$ receptors, initiating only modest activation but promoting prolonged desensitisation (Xiao et al., 2006). This desensitised state suppresses hyperdopaminergic fluctuations and dampens corticostriatal overactivity, causing a stabilising influence similar to A85380 but via a slower, sustained mechanism. Beyond dopaminergic modulation, Sazetidine-A may also reduce excessive glutamatergic drive by desensitising presynaptic $\alpha 4\beta 2$ receptors on cortical terminals, thereby lowering excitatory input to the striatum and further limiting dyskinetic firing patterns.

In contrast, neither the positive allosteric modulator NS9283 nor the competitive antagonist Dh β E significantly reduced AIMs at the doses tested. NS9283 potentiates receptor responses to endogenous acetylcholine without directly inducing desensitisation, which likely explains its lack of efficacy in a state of heightened cholinergic tone. Similarly, Dh β E failed to produce measurable improvements, indicating that simple receptor blockade cannot restore functional balance once LID is established. The absence of effect with both agents reinforces that desensitisation, not potentiation or antagonism, is the key receptor state mediating the antidyskinetic action.

When A85380 was co-administered with Dh β E, no additive or synergistic benefit was observed. The most plausible explanation is that Dh β E competitively inhibited A85380 binding, thereby preventing the receptor from reaching or maintaining its desensitised conformation. Across all conditions, locomotive AIMs remained unaffected, confirming that the observed reductions in AIMs were not secondary to sedation or motor impairment.

An important consideration when interpreting these findings is the pharmacokinetic profile of the compounds used, particularly their half-life and potential peripheral actions. Nicotine, used in earlier chapters has a relatively short plasma half-life in rodents (approximately 45–60 minutes), although its primary metabolite cotinine persists substantially longer and may contribute to sustained nicotinic receptor modulation (Benowitz et al., 2009). In contrast, selective $\alpha 4\beta 2$ ligands such as A85380 exhibit a relatively short elimination half life in rodents, generally within the range of 1–2 hours consistent with the acute time course of the behavioural effects observed in this study (Baddick & Marks, 2011; Gotti et al., 2009). Sazetidine-A, while also cleared relatively rapidly, produces prolonged receptor occupancy and desensitisation which likely underlies the delayed yet sustained reduction in AIMs observed at later time points (Xiao et al., 2006; Marks et al., 2007). For the antagonist DH β E, its competitive mechanism and relatively short duration of receptor occupancy mean that its effects are closely tied to its plasma presence which may limit its ability to induce sustained changes in receptor state required to impact dyskinesia. Similarly, the positive allosteric modulator NS9283 does not directly activate or desensitise the receptor but instead enhances responses to endogenous acetylcholine; this indirect and transient mode of action is likely insufficient to overcome the established dyskinetic state. Together, these pharmacokinetic and pharmacodynamic differences support the interpretation that effective antidyskinetic action depends not simply on receptor engagement, but on the ability to induce a sustained desensitised receptor state within a relevant temporal window.

In addition, it is important to consider that all compounds were administered systemically and therefore have access to both central and peripheral nicotinic acetylcholine receptors. $\alpha 4\beta 2$ containing receptors are expressed not only within the striatum and other central structures, but also in peripheral autonomic ganglia, where their activation can influence cardiovascular and physiological states (Gotti et al., 2009). As such, peripheral receptor activation could indirectly contribute to behavioural outcomes, for example through modulation of arousal or autonomic tone. While the present findings are interpreted primarily in the context of central nicotinic modulation of striatal dopaminergic transmission, the contribution of peripheral mechanisms cannot be fully excluded. Future studies employing centrally restricted compounds or intracranial administration would be required to definitively dissociate central from peripheral effects.

Immunohistochemical analyses of the nigrostriatal system provided additional information for the behavioural findings. Tyrosine hydroxylase staining confirmed that unilateral 6-OHDA lesioning induced extensive dopaminergic denervation in dyskinetic animals. In saline treated control rats, TH immunoreactivity was robust and symmetric in both the SNpc and the striatum, indicating intact dopaminergic innervation with no motor deficits. Among 6-OHDA lesioned rats, those that did not develop severe AIMs (the non-dyskinetic group) showed relatively preserved TH in the lesioned hemisphere, with only minimal loss of TH-positive SNpc neurons and striatal fibers. In contrast, rats with pronounced dyskinesias exhibited >90% loss of TH-positive neurons in the SNpc and a near complete depletion of striatal TH fiber density. These results validate previous studies showing that the severity of nigrostriatal dopamine neuron loss is a strong predictor of LID severity (Carta et al., 2008; Johnson et al., 2010). In this study, only animals with near total dopamine denervation displayed AIMs, underscoring that severe dopaminergic denervation and the resulting destabilization of striatal signalling are required for AIMs expression.

A limitation of the present histological analysis is that tyrosine hydroxylase

immunoreactivity reflects the expression of the rate limiting enzyme in dopamine synthesis rather than the concentration of dopamine itself or its metabolites. As such, TH-ir provides a static endpoint measure at the time of tissue collection and cannot capture the dynamic fluctuations in dopaminergic transmission that occur during behavioural testing. In addition, the animals in this study were exposed to multiple treatments across sessions. Although appropriate washout periods were implemented, it remains possible that repeated drug exposure may have influenced enzyme expression or glial reactivity to some extent. However, this is unlikely to account for the overall pattern of results. Only animals with near complete unilateral lesions were included in the dyskinetic cohort and the marked loss of TH immunoreactivity observed is consistent with extensive nigrostriatal denervation. The behavioural differences are therefore unlikely to reflect residual differences in dopamine availability, but instead are more plausibly attributed to alterations in receptorlevel signalling and downstream circuit function.

This result was accompanied by a robust neuroinflammatory response. GFAP immunostaining revealed minimal astrocytic activation in saline controls and only a modest increase in the non-dyskinetic lesion group. However, turner (dyskinetic) rats showed pronounced astrogliosis: GFAP-positive astrocytes were hypertrophic and densely packed in both the SNpc and striatum of the lesioned side.

Similarly, IBA-1 labelling showed that microglia remained inactive in controls and only mildly activated in non-dyskinetic, whereas dyskinetic rats displayed hypertrophic, intensely IBA-1-positive microglia with significantly higher cell densities in the lesioned hemisphere ($P < 0.0001$ in SNpc; $P = 0.0007$ in striatum). These findings align with an emerging view that chronic L-DOPA treatment after extensive dopamine loss provokes strong glial reactivity (Ryu et al., 2025). In particular, the marked astrogliosis and microgliosis in dyskinetic animals are consistent with reports that glial activation contributes to LID pathophysiology (Muñoz et al., 2025; others). By contrast, the relatively low GFAP and IBA-1 in non-dyskinetic rats suggests

that limited inflammation accompanies milder lesions without dyskinesia.

Taken together, these findings suggest that the antidyskinetic efficacy of $\alpha 4\beta 2$ receptor modulators depends critically on their ability to induce and maintain a desensitised receptor state. Both A85380 and Sazetidine-A, though pharmacologically distinct, exert similar effect on this mechanism: A85380 through initial activation followed by desensitisation, and Sazetidine-A through sustained partial agonism that stabilises the desensitised configuration. This receptor state appears to normalise dopaminergic and corticostriatal transmission, reducing excessive synaptic excitation without suppressing baseline motor function.

5.5 Conclusion

In summary, A85380 (0.01 mg/kg) and Sazetidine-A (1 mg/kg) effectively reduced the severity of levodopa induced AIMs in 6-OHDA lesioned rats, with both acting through receptor desensitisation mechanisms that stabilise striatal signalling. In contrast, NS9283 and Dh β E failed to modify AIMs, confirming that allosteric enhancement or competitive blockade alone does not achieve therapeutic modulation. These findings confirm $\alpha 4\beta 2$ receptor desensitisation as a key mechanism underlying antidyskinetic efficacy and support the development of compounds that selectively promote this receptor state.

By demonstrating that specific $\alpha 4\beta 2$ engagement can normalise dysregulated striatal circuits without impairing movement, this study refines the mechanistic understanding of nicotinic modulation in LID and identifies desensitiser type ligands such as Sazetidine-A and low dose A85380 as promising candidates for adjunctive therapy in Parkinson's disease.

Chapter 6

The Effects of an $\alpha 7$ Nicotinic Agonist
and a Selective $\alpha 7$ Nicotinic Positive
Allosteric Modulator on Levodopa
Induced AIMs

6.1 Introduction

Various neuropharmacological approaches have been tested in preclinical and clinical settings to reduce abnormal involuntary movements, including antagonists of the NMDA receptor, GABAergic agonists, and 5-HT_{1A} agonists (Iravani and Jenner, 2011). Among these, amantadine continues to be the most consistently effective compound, as supported by a recent meta-analysis of randomised controlled trials (Yan et al., 2025). Current clinical investigations, including phase II studies of NLX-112 (befiradol) (Svenigsson et al., 2025), are exploring new targets such as NMDA, mGluR4, and dopamine D₃ receptors for their potential to reduce LID (Al-Kassmy et al., 2025).

As shown in Chapter 3, beyond these established therapeutic strategies, extensive evidence from neurochemical and behavioural studies indicates that nicotine modulates dopaminergic neurotransmission within the basal ganglia and provides protection against nigrostriatal degeneration (Huang et al., 2009). Nicotine has been shown to reduce levodopa induced dyskinesia in both rodent and primate models, independently of its neuroprotective properties (Quik et al., 2013; Zhang et al., 2013). The basal ganglia contain multiple subtypes of nicotinic acetylcholine receptors, and identifying the specific receptor populations responsible for these effects has significant therapeutic implications. Selective activation of particular nAChR subtypes could provide anti-dyskinetic benefits without the undesirable side effects associated with nicotine itself.

Among the various subtypes, $\alpha 7$ nAChRs have emerged as key modulators in dyskinesia. Studies using $\alpha 7$ knockout mice have demonstrated that levodopa priming produces more severe AIMs in the absence of $\alpha 7$ receptors, suggesting an inhibitory role for these receptors in dyskinesia expression (Quik et al., 2013). Similarly, administration of the selective

$\alpha 7$ agonist ABT-107 reduced AIMs in animal models (Zhang et al., 2014; Bordia et al., 2015). Neuronal $\alpha 7$ nAChRs function as ligand gated ion channels that open upon acetylcholine binding to permit Ca^{2+} influx, thereby activating intracellular cascades involving ERK1/2 and PKA and influencing neurotransmitter release and synaptic plasticity (Sinclair and Kabbani, 2023). These mechanisms indicate that $\alpha 7$ receptor activity can modulate corticostriatal communication and thereby shape the motor responses associated with levodopa treatment.

The identification of $\alpha 7$ nAChR positive allosteric modulators has expanded the therapeutic possibilities for targeting this receptor. Unlike direct agonists, PAMs enhance receptor responsiveness to endogenous acetylcholine without initiating activation themselves, which can offer better control of receptor dynamics and reduce desensitisation (Williams et al., 2011; Uteshev, 2014; Echeverria et al., 2016). $\alpha 7$ receptors are highly expressed in the striatum on glutamatergic terminals, dopaminergic neurons, and interneurons (Zhou et al., 2002), where they help coordinate glutamate and dopamine signalling. This interaction maintains a balance between excitatory and inhibitory balance processes that are disrupted in PD and during chronic levodopa exposure. Activation of $\alpha 7$ receptors also influences intracellular Ca^{2+} homeostasis, a critical determinant of neuronal excitability and plasticity (Perez et al., 2017, 2018). In line with this, a recent study using a murine AIM model found that the $\alpha 7$ PAM PNU-120596 markedly reduced dyskinesia severity through modulation of Ca^{2+} signalling (Gomez-Paz et al., 2025).

Targeting nAChRs with PAMs presents several advantages over traditional nicotinic agonists. PAMs act as endogenous enhancers of receptor function, often displaying improved selectivity, reduced toxicity, and more favourable pharmacokinetics compared with nicotine or full $\alpha 7$ agonists (McLean et al., 2012; Uteshev, 2014; Perez de Vega et al., 2019). PNU-120596, a prototypical $\alpha 7$ PAM, has been extensively used to study $\alpha 7$ receptor activation in vitro and in vivo (Hurst et al., 2005; Uwada et al., 2020). As a type II PAM, PNU-120596 reduces

receptor desensitisation and prolongs channel open time in the presence of an orthosteric agonist such as acetylcholine, producing a more sustained nicotinic response (Hurst et al., 2005; King et al., 2018). Importantly, PNU-120596 crosses the blood brain barrier and is active through multiple routes of administration including oral, intraperitoneal, subcutaneous, and intravenous across both rodent and human studies (Hurst et al., 2005; Callahan et al., 2013).

Given the limited efficacy of current therapies for LID, this investigation aimed to compare the effects of a selective $\alpha 7$ nAChR PAM (PNU-120596), an $\alpha 7$ nAChR agonist (PHA-543613), nicotine, and the $\alpha 7$ nAChR antagonist Methylllycaconitine (MLA) in a unilaterally 6-OHDA lesioned rat model of abnormal involuntary movements. By examining both agonist and PAM mediated modulation of $\alpha 7$ receptors, the study sought to identify whether enhancing endogenous cholinergic tone through positive allosteric modulation could offer a more effective and physiologically grounded approach to reducing dyskinesia.

6.1.1 Aims and objectives

The aim of this chapter is to evaluate the role of $\alpha 7$ nicotinic acetylcholine receptors in the modulation of abnormal involuntary movements. This was based on the premise that $\alpha 7$ nAChR ligands regulate glutamatergic transmission (Howe et al., 2016), a key driver of dyskinesia expression (Morissette et al., 2016). By systematically testing a series of subtype selective ligands, this chapter sought to identify compounds with the greatest therapeutic potential for reducing L-DOPA induced dyskinesia.

A key objective, and central novelty of this work, was to move beyond receptor agonism and instead evaluate $\alpha 7$ positive allosteric modulation as a mechanistically distinct strategy. Specifically, the chapter investigated whether enhancing endogenous cholinergic signalling using the $\alpha 7$ PAM PNU-120596 could attenuate established dyskinesia while preserving normal

physiological function. In parallel, receptor blockade approaches were used to determine the contribution of endogenous $\alpha 7$ activity to dyskinesia regulation. Together, this approach aimed to define receptor specific mechanisms and establish $\alpha 7$ allosteric modulation as a more physiologically aligned and therapeutically relevant strategy for the treatment of L-DOPA-induced dyskinesia.

Table 6. 1: Range of orthostatic and allosteric nAChR ligands to be evaluated.

Drug	Action at receptor	Doses (mg/kg)
Nicotine	N/A	0.01, 0.03, 0.1, 0.3
PHA 543613	Agonist	3.0, 10.0
PNU 120596	Positive Allosteric Modulator	0.3, 1.0, 3.0
MLA	Antagonist	2.0, 6.0

6.2 Material and Methods

6.2.1 *Animals*

Female Sprague Dawley rats ($n = 30$; Charles River, UK, Harlow, Essex), each weighing between 250–275 g at the start of the experiment, were used in this study. Animals were housed in pairs within standard polypropylene cages under a controlled 12:12 hour light/dark cycle (lights on at 07:00, off at 19:00) in a temperature regulated environment (20 ± 1 °C). Food and water were provided *ad libitum* throughout the experimental period.

6.2.2 *Unilateral 6-OHDA Lesions*

All surgical procedures, including acclimation, anesthesia, 6-OHDA preparation, stereotaxic injections, and post-operative care, were carried out according to the methods described in Chapter 2 (see Section 2.4 for full details). All animals recovered without complications, and no adverse effects were noted. An overview of the experimental timeline is provided in Figure 6.1.

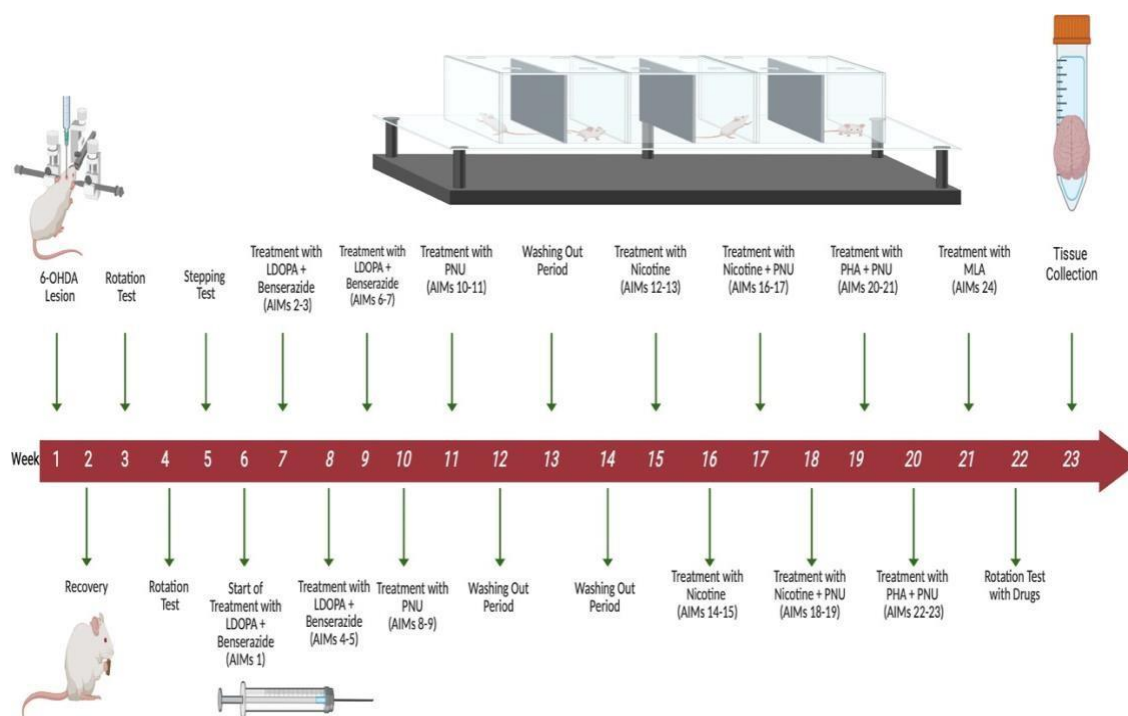


Figure 6. 1: Experimental timeline for the preparation of levodopa primed rats and assessment of abnormal involuntary movements. Rats were unilaterally lesioned with (6-OHDA; 8 μ g) and allowed to recover for two weeks. In weeks 3 and 4, lesion efficacy was verified using amphetamine induced (5.0 mg/kg) and apomorphine induced (0.5 mg/kg) rotational behaviour tests, followed by stepping tests in week 5. From week 6, fully lesioned animals received daily injections of levodopa (8 mg/kg) combined with benserazide (15 mg/kg) for three consecutive weeks. AIMs scoring began in the first week of levodopa administration and continued twice weekly until all animals were reliably primed. In week 10, animals were treated with the $\alpha 7$ positive allosteric modulator (PNU-120596) for two weeks (four AIMs sessions), followed by a three week washout period. From week 15, the same cohort was tested with nicotine for two weeks (four AIMs sessions, twice weekly). In week 17, animals received combined treatment with PNU-120596 and nicotine for four AIMs sessions (twice weekly). In week 19, rats were tested with the $\alpha 7$ nicotinic agonist PHA-543613 in combination with PNU-120596 for four AIMs sessions (twice weekly). Finally, in week 21, animals were treated with the $\alpha 7$ receptor antagonist (MLA) for one week (two AIMs sessions). At week 22, animals underwent amphetamine induced rotational testing in the presence of the experimental drugs. At week 23, all animals were terminally perfused.

6.2.3 Assessment of 6-OHDA induced Motor Asymmetry (rotational behaviour)

Assessment of lesion severity followed the procedures detailed in Chapter 2.6. Out of 30 rats, 12 rats were deemed fully lesioned and underwent levodopa priming as detailed in chapter 2. Those animals that failed these criteria were excluded from the study.

6.2.4 Assessment of 6-OHDA induced Motor Asymmetry – Stepping Test

All lesioned animals (n = 30) were assessed further for forelimb akinesia using the stepping test, as described in Chapter 2, Section 2.5.

6.2.5 Levodopa Priming for Inducing Abnormal Involuntary Movements

Fully lesioned animals (n = 12) received daily oral levodopa and benserazide for 21 days as described previously. AIMs were scored using the procedure outlined in Chapter 2.

6.2.6 Experimental Protocol

Experimental procedures for assessing drug effects on levodopa-induced dyskinesia followed the methods described in Chapter 3 (Section 3.2.5). The effects of nicotine alone are not presented in this chapter and are reported in Chapter 3, where they provide a reference for comparison with varenicline.

An exception to this protocol was made for PNU-120596, which required an escalating dosing regimen due to its high lipid solubility. In addition, a two-week washout period was incorporated to account for its lipophilic nature and potential prolonged interaction with $\alpha 7$ nicotinic receptors.

6.2.7 *Drugs*

Levodopa, benserazide and nicotine were prepared as previously described in Chapter 3, Section 3.2.7.

PNU-120596(N-(5-Chloro-2,4-dimethoxyphenyl)-N'-(5-methyl-3-isoxazolyl)-ure) purchased from Stratech (UK) was dissolved in 1% DMSO plus 30% polyethylene glycol plus 1% Tween 80 and administered subcutaneously at doses of 0.3, 1.0 and 3.0 mg/kg, 10 mins prior to levodopa/benserazide administration.

PHA-543613(N-(3R)-1-azabicyclo[2.2.2]oct-3-yl-furo[2,3-c]pyridine-5-carboxamide hydrochloride) was dissolved in deionised, sterile water and administered intraperitoneally at doses of 3.0 and 10.0 mg/kg, immediately after levodopa/benserazide administration.

Methyllycaconitine citrate (MLA) was dissolved in sterile saline and administered intraperitoneally at doses of 2.0 and 6.0 mg/kg, 20 mins prior to levodopa/benserazide.

6.2.8 *Statistical Analysis*

Statistical analyses followed the procedures outlined in Section 3.2.9.

6.3 Results

From the 30 animals, 24 received 6-OHDA and 6 received saline; of those receiving 6-OHDA, 12 fulfilled the rotation criteria and were deemed fully lesioned.

6.3.1 Amphetamine induced ipsiversive rotation test

Assessment of amphetamine induced rotational behaviour was conducted as described in Chapter 3 (Section 3.3.1; Figure 3.2). As previously reported, administration of (+)-amphetamine (5 mg/kg, i.p.) produced a significant increase in ipsilateral rotations [$F(1,23) = 7.77$, $p = 0.0105$], confirming dopaminergic lesioning. A comparable pattern was observed in the present cohort, consistent with the established time dependent increase in rotational behaviour.

6.3.2 Apomorphine induced contravasive rotation test

Apomorphine induced rotational behaviour was assessed as described in Chapter 3 (Section 3.3.2; Figure 3.4). Consistent with these findings, apomorphine (0.5 mg/kg, s.c.) elicited a significant contralateral rotational response [$F(1,17) = 5.19$, $p = 0.0359$], with lesioned animals showing greater rotations than controls.

6.3.3 Adjusted step test

Stepping test assessment was performed as described in Chapter 3 (Section 3.3.3; Figure 3.6). As previously reported, a subset of lesioned and primed animals displayed a robust contralateral forelimb deficit. In the present cohort, 12 animals met this criterion, corresponding to those classified as turners based on amphetamine and apomorphine induced rotational behaviour. Consistent with earlier findings, a highly significant impairment in contralateral stepping was observed in this group (**** $p \leq 0.0001$).

6.3.4 Levodopa + Benserazide AIMs Sessions (ALO development)

The induction and assessment of levodopa induced dyskinesia were performed as described in Chapter 3 (Section 3.3.4; Figures 3.8 and 3.9). As previously reported, animals exhibiting >6 rotations per minute following lesioning were selected for chronic treatment with levodopa (8 mg/kg) and benserazide (15 mg/kg). Dyskinetic movements were assessed across seven sessions using a standardised AIMs rating scale, with observations conducted by three blinded observers.

Consistent with earlier findings, chronic levodopa and benserazide treatment reliably induced AIMs across all sessions, with peak dyskinesia observed between 40 and 80 minutes post-administration (Figure 3.8). Statistical analysis revealed a significant effect of time [$F(3.803, 295.0) = 45.49, p < 0.0001$], with AIMs scores remaining elevated throughout the 120-minute observation period.

Analysis of AIMs subtypes further demonstrated a progressive increase in dyskinetic behaviour across sessions, as reflected by AUC measurements (Figure 3.9), consistent with the development of stable LID.

6.3.5 The effects of $\alpha 7$ PAM, PNU-120596

When the effects of the $\alpha 7$ PAM, PNU-120597 at 0.0 (vehicle), 0.3, 1.0 and 3.0 mg/kg were examined on ALO AIMs against time, there was a significant effect of time [$F(3.517, 154.7) = 29.90, P < 0.0001$] and dose [$F(3, 44) = 5.195; P = 0.0037$] and dose x time interaction [$F(35, 385) = 1.724, P = 0.0078$] (fig 6.2). The most prominent effects of PNU-120596 were observed 60 min after administration of levodopa. At the higher doses, 1.0 and 3.0 mg/kg of PNU-12597 caused significant reductions of peak AIMs at 100 (1.0 mg/kg: $P = 0.001$; 3.0mg/kg: $P = 0.0047$) and 120 min (1.0 mg/kg: $P = 0.0058$; 3.0mg/kg: $P = 0.0163$); (Figure 6.2).

Figure 6.3 (panels a–d) presents the effects of PNU-120596 on individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$). The total ALO scores shown in panel (a) represent the combined measure of axial, limb, and orolingual movements. Statistical analysis using repeated measures one-way ANOVA revealed a significant reduction in total levodopa induced AIMs following administration of PNU-120596 at 1.0 and 3.0 mg/kg. Panel (b) illustrates the effects on axial AIMs, where both 1.0 mg/kg ($p = 0.0207$) and 3.0 mg/kg ($p = 0.0175$) significantly reduced scores. Panel (c) shows the effects on limb AIMs, with significant reductions observed at 1.0 mg/kg ($p = 0.0381$) and 3.0 mg/kg ($p = 0.0181$). Panel (d) depicts the effects on orolingual AIMs, which were significantly decreased at 1.0 mg/kg ($p = 0.0027$) and 3.0 mg/kg ($p = 0.0004$). All data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

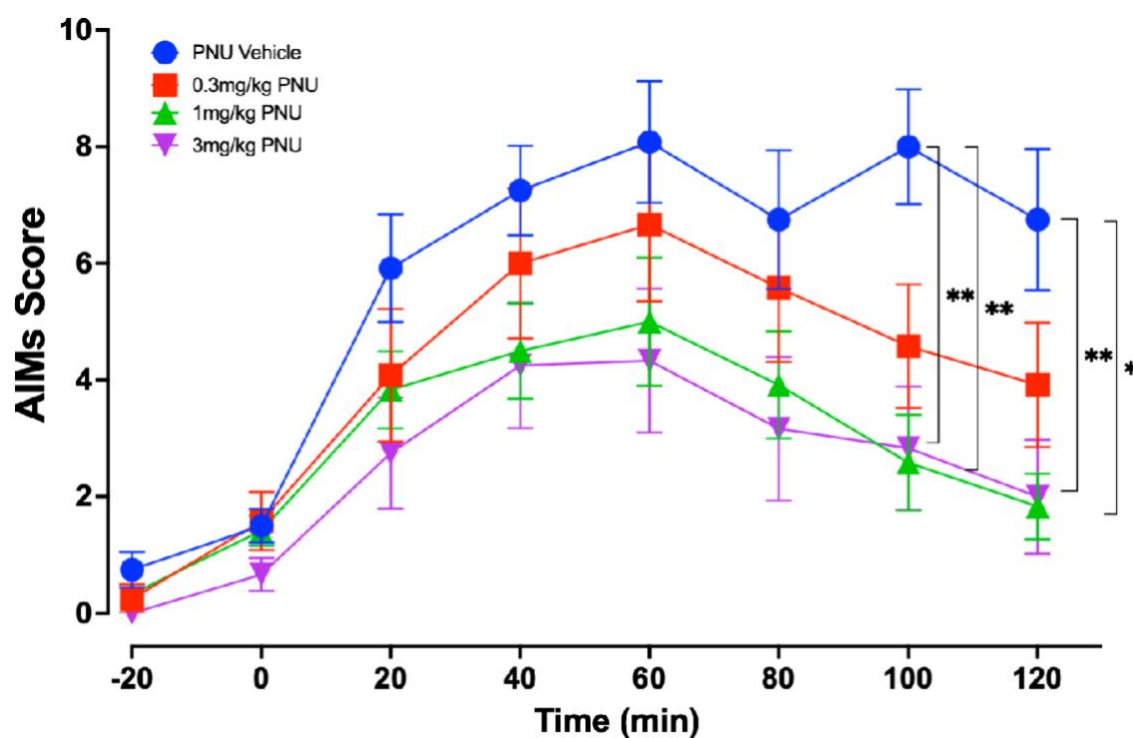


Figure 6. 2: Effect of Acute PNU-120596 Administration on AIMS Scores. The effects of de novo subcutaneous administration of an $\alpha 7$ positive allosteric modulator, PNU-120596, on unilaterally 6-OHDA lesioned AIMS in levodopa primed rats (n=12). PNU-120596 dose-dependently reduced peak total ALO AIMS 40 minutes post-subcutaneous administration. Data are presented as mean $\pm$ standard error, and significance is considered when $p < 0.05$.

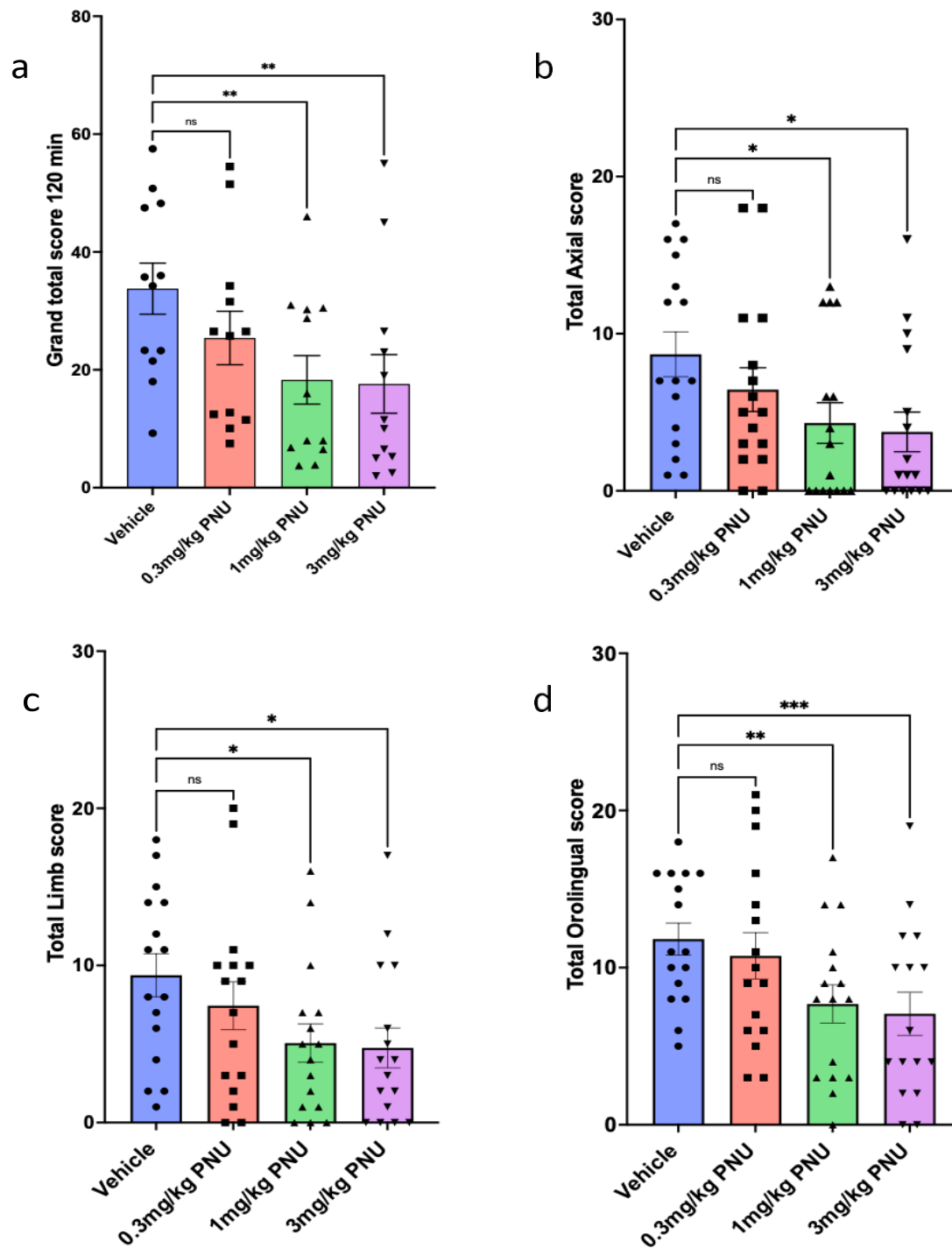


Figure 6. 3: Effects of PNU-120596 on ALO scores, Axial, Limb, and Orolingual AIMs. The bar graphs represent the effects of different doses of PNU-120596 on abnormal involuntary movement scores in rats with L-DOPA induced dyskinesia. Panel (a) shows the total of ALO AIMs score across all subtypes. Panel (b) illustrates the effects of treatment on total axial AIMs, panel (c) on total limb AIMs, and panel (d) on total orolingual AIMs. Administration of PNU-120596 at 1 mg/kg and 3 mg/kg significantly reduced AIMs scores across all subtypes of AIMs compared to the vehicle control, while the 0.3 mg/kg dose produced no significant effect. Data are presented as mean $\pm$ SEM for n=12 animals per treatment group. *P<0.05, **P<0.01, ***P<0.001.

6.3.6 *The combined effects of nicotine and PNU-120596*

To investigate whether the $\alpha 7$ positive allosteric modulator PNU-120596 enhances the anti-dyskinetic effects of nicotine, a medium dose of PNU-120596 (1.0 mg/kg) was co-administered with low doses of nicotine (0.01 and 0.03 mg/kg) (Figures 6.9 and 6.10).

A two-way ANOVA revealed that the combination of nicotine and PNU-120596 significantly affected treatment outcome [$F(7, 77) = 21.6$; $p < 0.0001$], time [$F(5, 55) = 5.25$; $p = 0.0005$], and the treatment $\times$ time interaction [$F(35, 385) = 1.724$; $p = 0.0078$] across the 120 minute observation period (Figure 6.4).

Figure 6.5 (panels a–d) presents the effects of nicotine and PNU-120596 on individual AIM subtypes over a 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$). Statistical analysis showed a significant reduction in total ALO (axial, limb, and orolingual) AIMs at 0.03 mg/kg nicotine alone ($p = 0.0283$), 0.01 mg/kg nicotine combined with 1 mg/kg PNU-120596 ($p = 0.0062$), and 0.03 mg/kg nicotine combined with 1 mg/kg PNU-120596 ($p = 0.0026$), compared with the vehicle control (panel a).

Panel (b) illustrates the effects on axial AIMs. A significant reduction was observed at 0.03 mg/kg nicotine alone ($p = 0.0075$), 0.01 mg/kg nicotine plus 1 mg/kg PNU-120596 ($p = 0.0353$), and 0.03 mg/kg nicotine plus 1 mg/kg PNU-120596 ($p = 0.0052$). Panel (c) shows the effect on limb AIMs, where co-administration of nicotine and PNU-120596 produced significant reductions at 0.01 mg/kg nicotine plus 1 mg/kg PNU-120596 ($p = 0.0413$) and 0.03 mg/kg nicotine plus 1 mg/kg PNU-120596 ($p = 0.0111$). Panel (d) depicts the effects on orolingual AIMs. A clear dose related reduction was observed across all nicotine and nicotine/PNU-120596 treatments: 0.01 mg/kg nicotine ($p = 0.0228$), 0.03 mg/kg nicotine ($p = 0.0030$), 1 mg/kg PNU-120596 alone ($p = 0.0022$), 0.01 mg/kg nicotine plus 1 mg/kg PNU-120596 ($p < 0.0001$), and 0.03 mg/kg nicotine plus 1 mg/kg PNU-120596 ($p < 0.0001$). All

data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

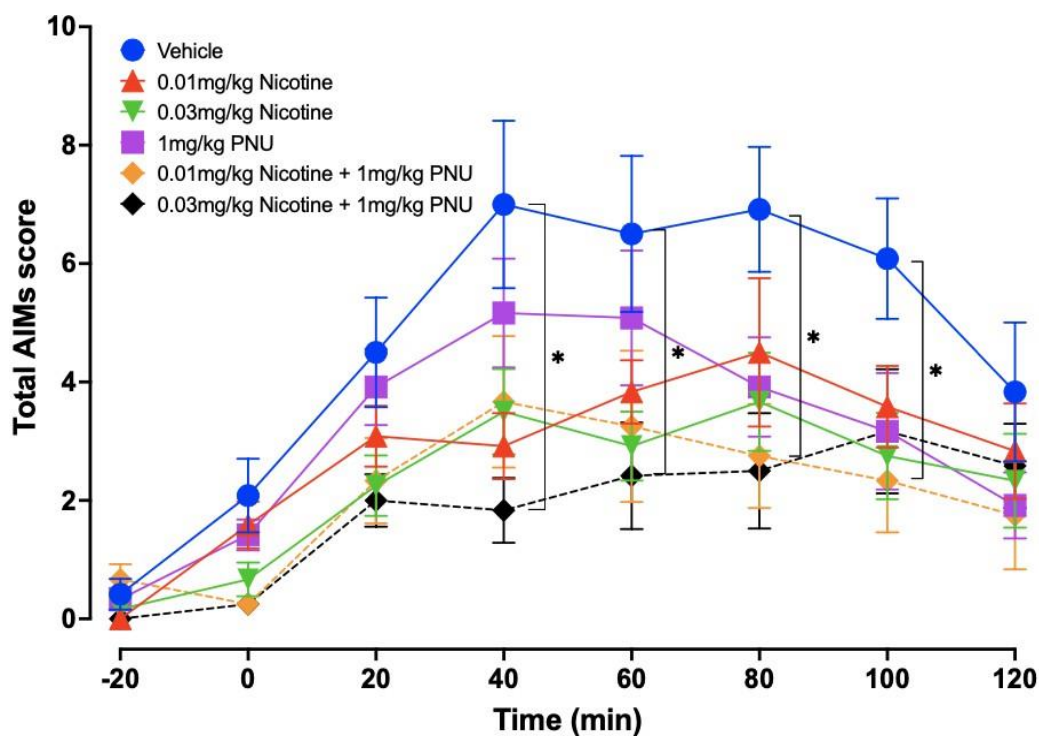


Figure 6. 4: Combined effects of Nicotine and PNU-120596 on L-DOPA Induced AIMS Across 120 Minutes. The effects of combined administration of nicotine and $\alpha 7$ PAM, PNU-120596 on AIMS. Following 20 min post-subcutaneous administration, nicotine dose dependently reduced peak total ALO AIMS between 20-80 min. While 1.0 mg/kg PNU-120596 on its own had no significant effects, it enhanced the anti-dyskinetic effects of 0.01 and 0.03 mg/kg nicotine. Data are presented as mean $\pm$ standard error for n=12 animals per treatment group and *P<0.05.

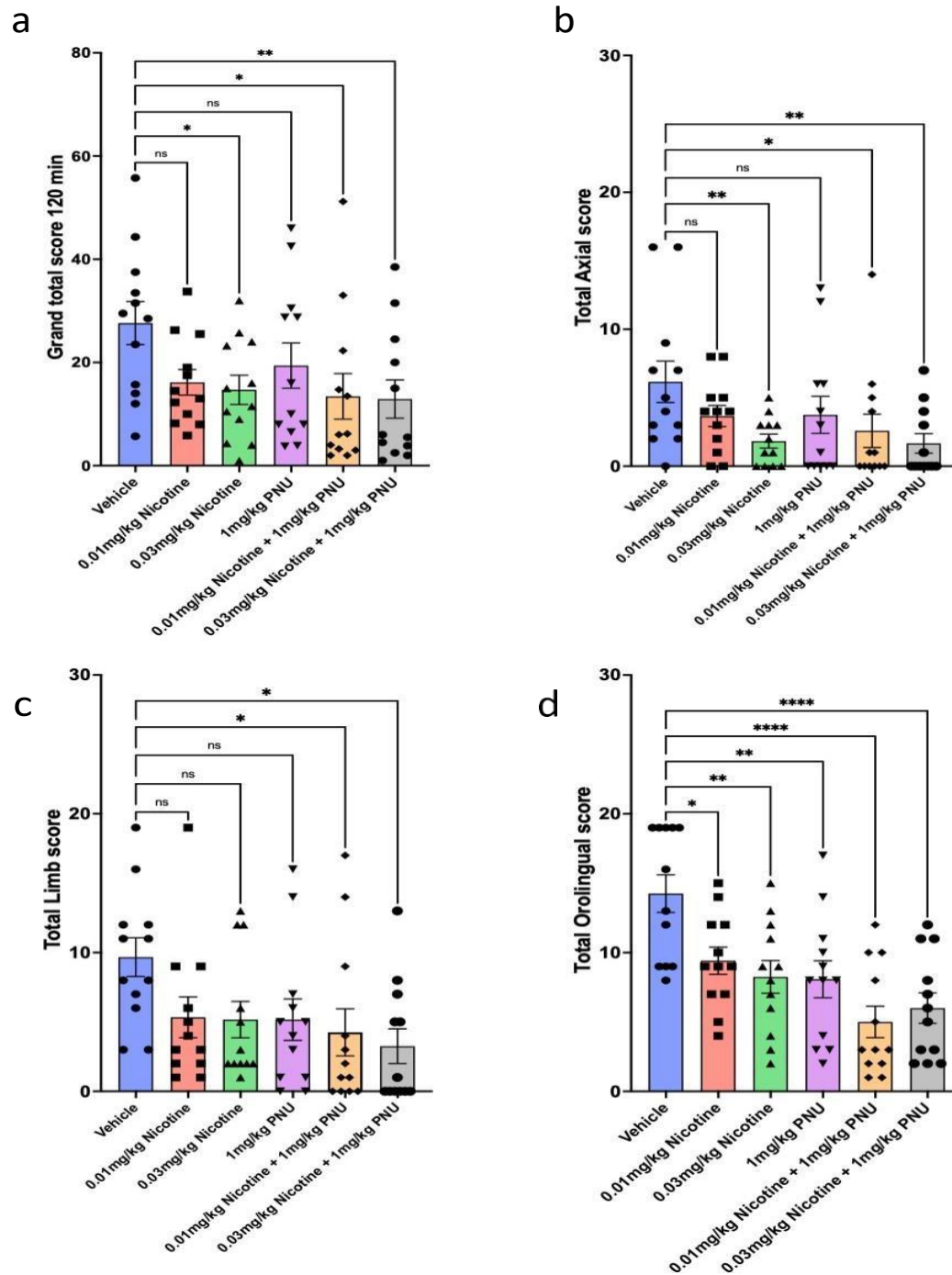


Figure 6. 5: Combined Effects of Nicotine and PNU-120596 on Total ALO, Axial, Limb, and Orolingual AIMs. The bar graphs illustrate the effects of combined nicotine and PNU-120596 treatment on AIM scores in rats with L-DOPA induced dyskinesia. Co-administration of nicotine (0.01 or 0.03 mg/kg) with 1 mg/kg PNU-120596 produced a significant reduction in AIMs across all subtypes of AIMs compared to the vehicle control. Panel (a) shows the total ALO AIM score, panel (b) total axial AIMs, panel (c) total limb AIMs, and panel (d) total orolingual AIMs. The combined treatments were particularly effective in reducing axial and orolingual AIMs, with 0.03 mg/kg nicotine plus 1 mg/kg PNU-120596 producing the greatest overall reduction. Data are expressed as mean $\pm$ SEM, with $n=12$ animals per treatment group. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, **** $P\leq 0.0001$.

6.3.7 The effects of $\alpha 7$ agonist, PHA-543613 and its co-administration with PNU-120596

To examine whether the $\alpha 7$ nicotinic agonist PHA-543613 exerts anti-dyskinetic effects alone or in combination with the $\alpha 7$ positive allosteric, PNU-120596, two doses of PHA-543613 (3.0 and 10.0 mg/kg) were investigated both in the absence and presence of 1.0 mg/kg PNU-120596 (Figures 6. and 6.7).

A two-way ANOVA revealed that PHA-543613 treatment significantly affected AIMs scores across the 120 minute observation period [dose: $F(2.584, 28.43) = 3.484$; $p = 0.0341$]; time: [$F(1.988, 21.87) = 17.63$; $p < 0.0001$]; dose $\times$ time interaction: [$F(4.537, 49.91) = 4.572$; $p = 0.0022$] (Figure 6.6).

Figure 6.7 (panels a–d) presents the effects of PHA-543613 and its combination with PNU-120596 on individual AIM subtypes over a 120 minute period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$). Statistical analysis showed that administration of 10.0 mg/kg PHA-543613 alone significantly reduced total ALO (axial, limb, and orolingual) AIMs compared with the vehicle control ($p = 0.0485$; panel a). Panel (b) illustrates the effects on axial AIMs, where 10.0 mg/kg PHA-543613 produced a significant reduction ($p = 0.0253$). Panel (c) shows a similar effect on limb AIMs, with 10.0 mg/kg PHA-543613 significantly reducing scores ($p = 0.0247$). Panel (d) depicts orolingual AIMs, which were also reduced following administration of 10.0 mg/kg PHA-543613 ($p = 0.0337$).

When the combined effects of 3.0 mg/kg PHA-543613 and 1.0 mg/kg PNU-120596 were examined, the results contrasted with those observed for the nicotine and PNU-120596 combinations. Unlike low dose nicotine combined with PNU-120596 which produced a significant reduction in total and subtype AIMs, co-administration of PHA-543613 with PNU-120596 had no significant effect on levodopa induced AIMs or any of the ALO AIMs

components. All data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

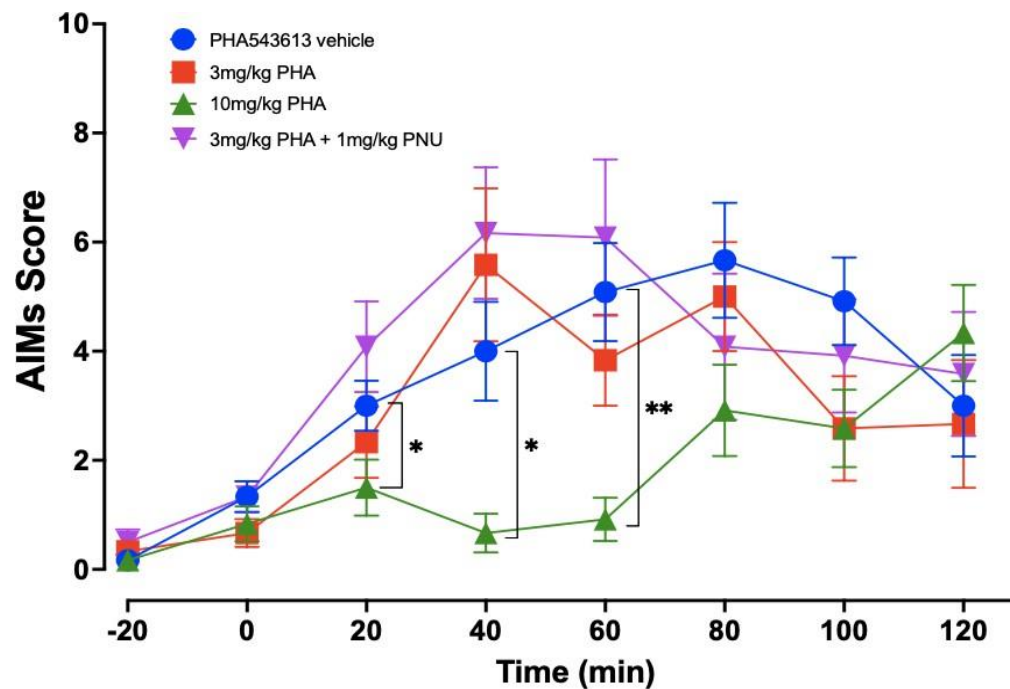


Figure 6. 6: Effect of PHA-543613 on AIMs Scores. Across 120 Minutes. This figure illustrates the significant effects of subcutaneous administration of a selective $\alpha 7$ agonist PHA-543613 (PHA), and PHA-543613 + PNU-120596 (PNU) on unilaterally 6-OHDA lesioned AIMs in levodopa primed rats (n=12). After 40 min post-subcutaneous administration, PNU-120596 dose dependently reduced peak total ALO AIMs. Data are presented as mean $\pm$ standard error and *P<0.05, **P<0.01.

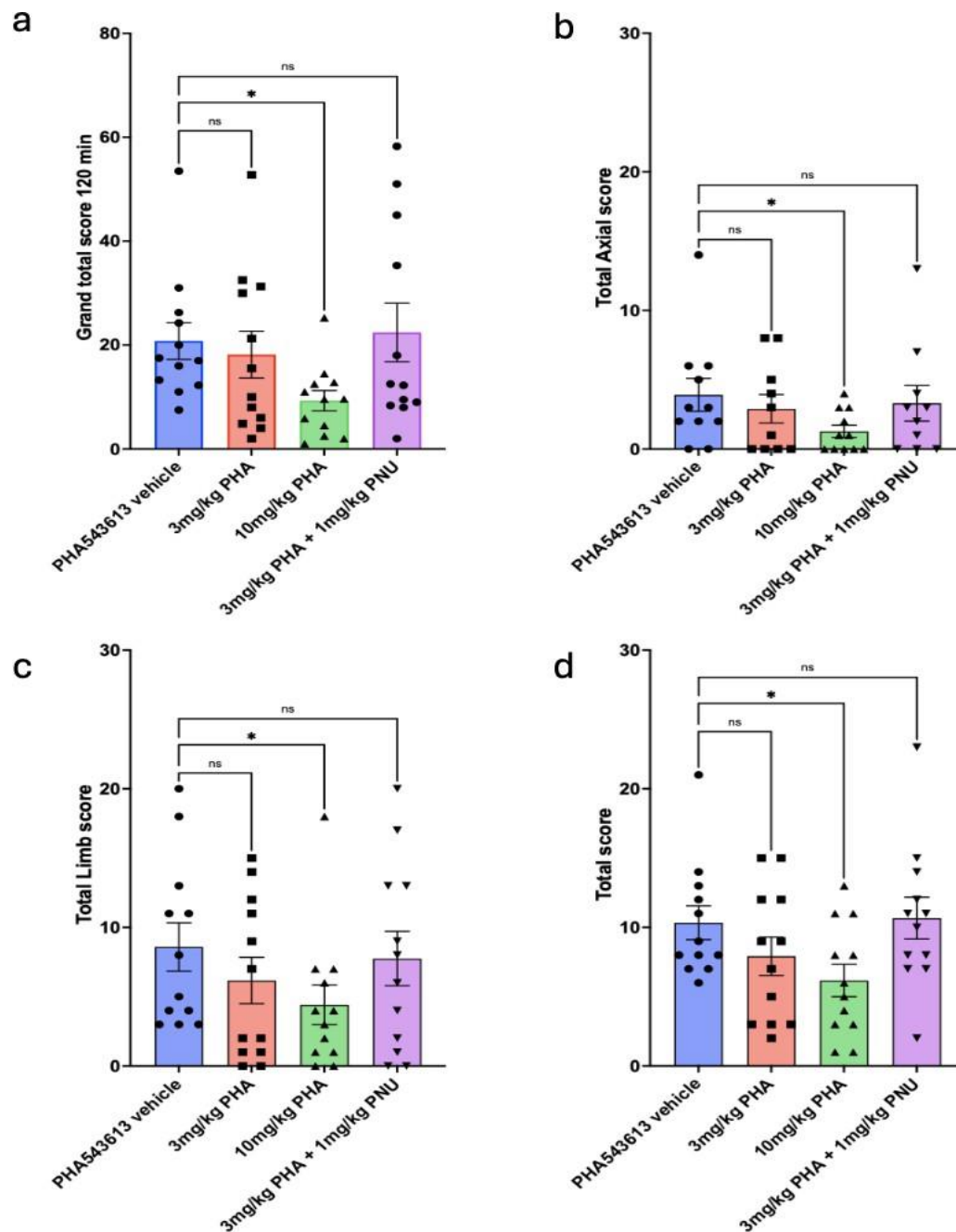


Figure 6. 7: Effects of PHA-543613 Alone and in Combination with PNU-120596 on Total ALO, Axial, Limb, and Orolingual AIMs. The bar graphs illustrate the effects of the selective $\alpha 7$ nicotinic agonist PHA-543613, administered alone or in combination with the $\alpha 7$ positive allosteric modulator PNU-120596, AIM scores in rats with L-DOPA induced dyskinesia. Administration of 10 mg/kg PHA-543613 significantly reduced AIMs across all components compared to the vehicle control, while the lower dose (3 mg/kg) produced no significant effect. Co-administration of 3 mg/kg PHA-543613 with 1 mg/kg PNU-120596 did not further reduce AIMs. Panel (a) shows the total ALO AIMs score, panel (b) total axial AIMs, panel (c) total limb AIMs, and panel (d) total orolingual AIMs. Data are expressed as mean $\pm$ SEM, with $n=12$ animals per treatment group. * $P<0.05$.

6.3.8 The effects $\alpha 7$ antagonist, MLA on AIMs

To further assess the role of $\alpha 7$ nicotinic acetylcholine receptors, the effects of the selective $\alpha 7$ receptor antagonist methyllycaconitine citrate (MLA) on levodopa induced abnormal involuntary movements were examined.

MLA was tested at doses of 2.0 and 6.0 mg/kg, and its effects on ALO AIMs were analysed over a 120 minute observation period. A two-way ANOVA revealed significant main effects of time [$F(4.480, 147.8) = 13.19$; $p < 0.0001$] and dose [$F(2, 33) = 4.204$; $p = 0.0236$], whereas the dose $\times$ time interaction was not significant [$F(4.372, 48.09) = 2.478$; $p = 0.0516$]. Interestingly, during the 20–40 minute time window, both MLA doses (2.0 and 6.0 mg/kg) significantly reduced peak ALO AIMs compared to the control. However, the effect of 2.0 mg/kg MLA was short lived, with AIMs scores returning to control levels by 60 minutes (Figure 6.8).

Figure 6.9 (panels a–d) presents the effects of MLA on individual AIM subtypes over the 120 minute observation period in unilaterally 6-OHDA lesioned, levodopa primed rats ($n = 12$). Administration of 6.0 mg/kg MLA significantly reduced total ALO (axial, limb, and orolingual) AIMs compared to the vehicle control ($p = 0.0034$; panel a). Panel (b) illustrates the effects on axial AIMs, where 6.0 mg/kg MLA produced a significant reduction ($p = 0.0051$). Panel (c) shows the effects on limb AIMs, with a significant decrease following 6.0 mg/kg MLA treatment ($p = 0.0011$). Panel (d) depicts orolingual AIMs, which were also significantly reduced after administration of 6.0 mg/kg MLA ($p = 0.0046$). All data are expressed as mean $\pm$ SEM and were analysed using one-way ANOVA followed by Dunnett's *post hoc* test.

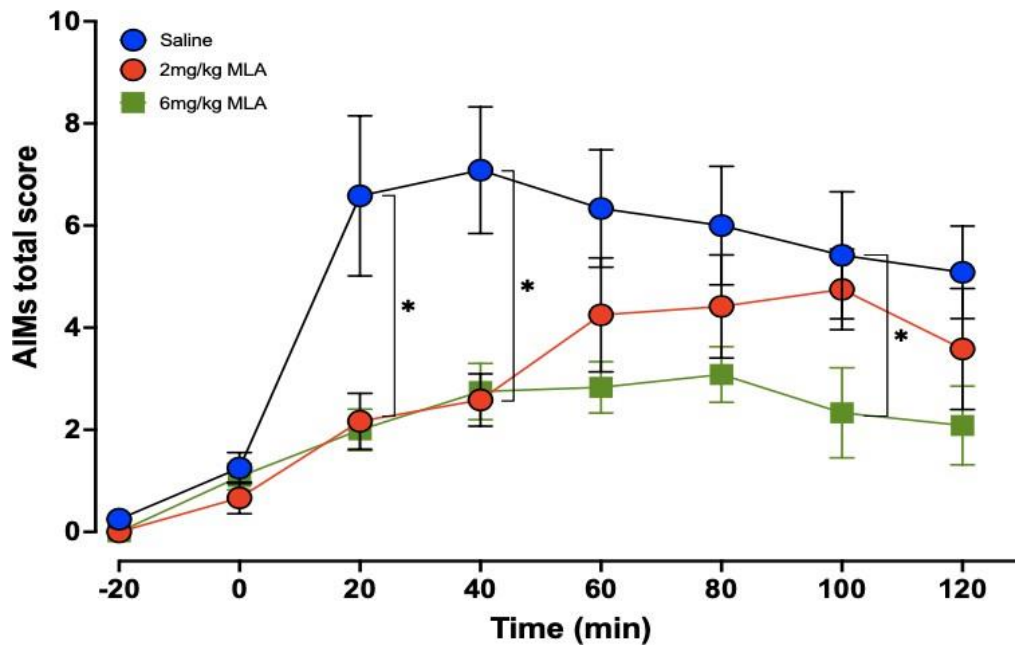


Figure 6. 8: Effect of MLA on LID and AIMs Across 120 Minutes. The effects of subcutaneous administration of an $\alpha 7$ antagonist, Methyllcaconitine (MLA), on unilaterally 6-OHDA lesioned AIMs in levodopa primed rats. After 20 min post subcutaneous administration, MLA dose dependently reduced peak total ALO AIMs. Data are presented as mean $\pm$ standard error (n=12; *p $\leq$ 0.05).

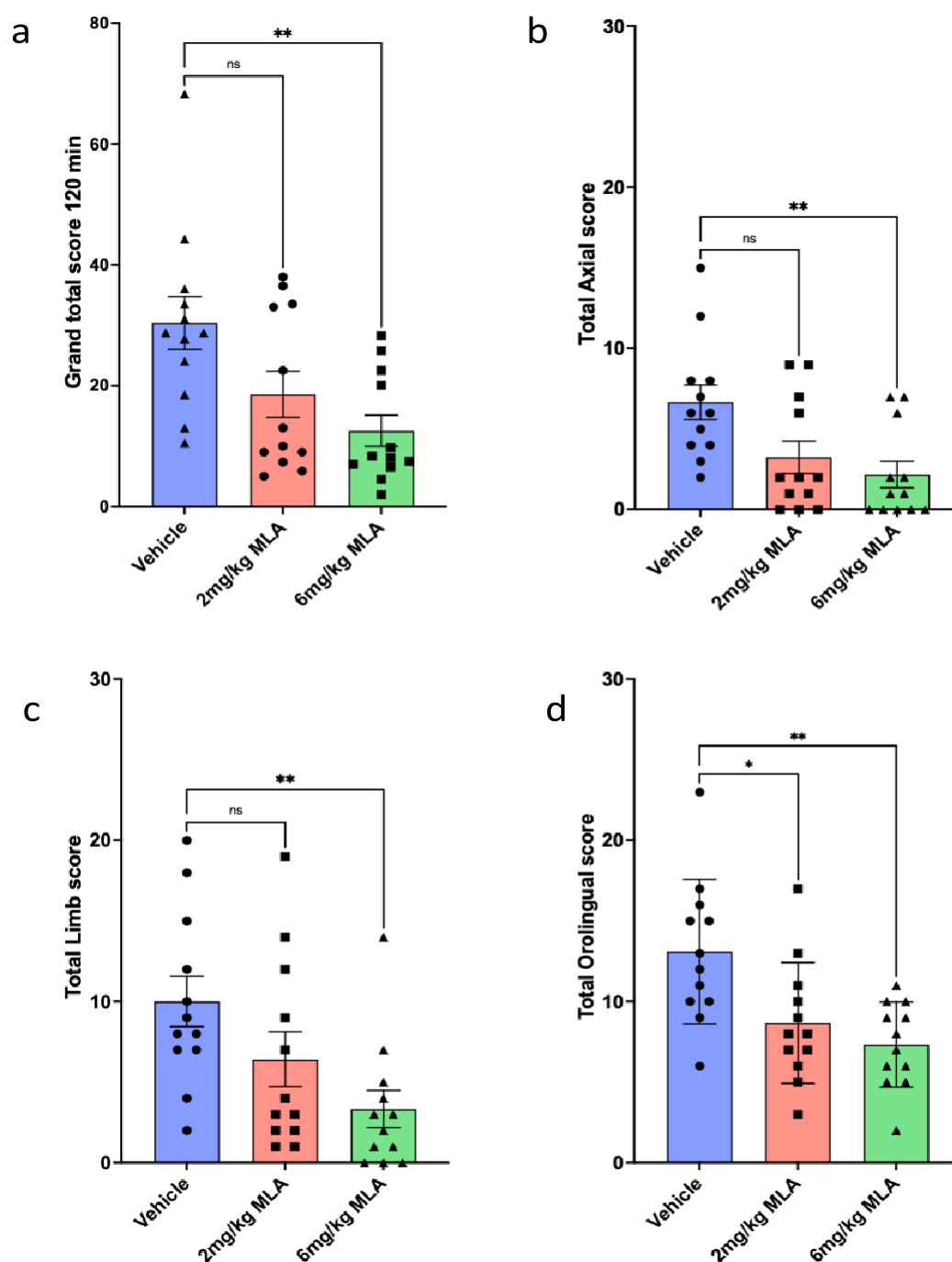


Figure 6. 9: Effects of MLA on Total ALO, Axial, Limb, and Orolingual AIMs. The bar graphs illustrate the effects of the selective $\alpha 7$ nicotinic receptor antagonist MLA on AIM scores in rats with L-DOPA induced dyskinesia. Acute administration of MLA produced a dose dependent reduction in AIMs, with the higher dose (6 mg/kg) significantly decreasing scores across all components compared to the vehicle control, while the lower dose (2 mg/kg) showed partial or no effect. Panel (a) shows the total ALO AIMs, panel (b) total axial AIMs, panel (c) total limb AIMs, and panel (d) total orolingual AIMs. Data are expressed as mean $\pm$ SEM, with $n=12$ animals per treatment group. * $P<0.05$, ** $P<0.01$.

6.3.9 The effects of nicotine and $\alpha 7$ PAM and agonist on ipsiversive rotation

To rule out any direct effects of $\alpha 7$ agonists on the ipsilateral intact dopaminergic systems, the effects of the maximum dose of nicotine, PNU-120596 and PHA-543613 used in this study were compared against 5 mg/kg (+)-amphetamine. While (+)-amphetamine produced a robust ipsilateral rotation of 1278 ± 145 , PNU-120596 and PHA-543613 produced 44 ± 7.8 , 26 ± 11.5 and 22 ± 5.2 turns in 90 minutes of the test period respectively (Figure 6.10). There was a significant difference between the effects of nicotine and $\alpha 7$ agents [$F(2, 36) = 2.521$; $P = 0.045$].

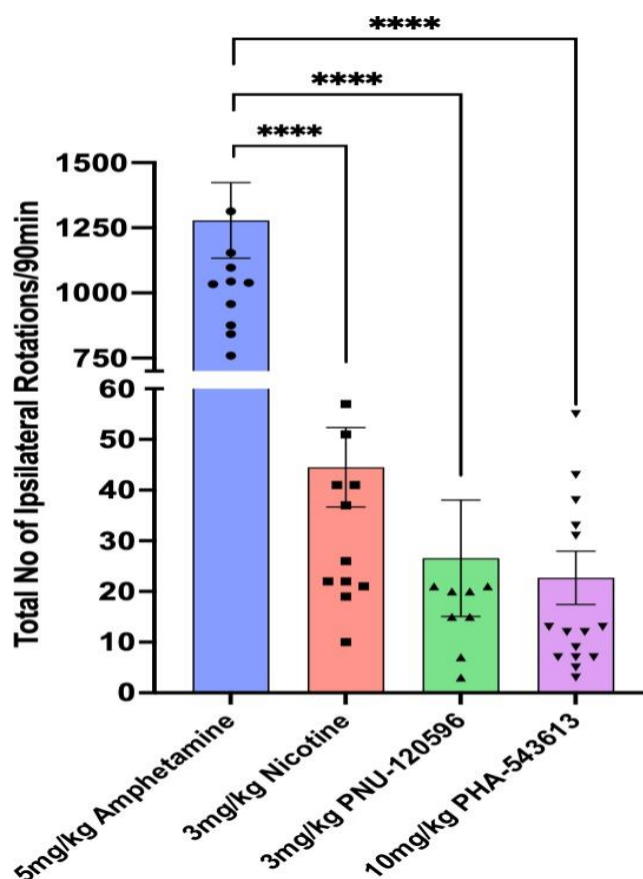


Figure 6. 10: Effects of nicotine, PNU-120596, and PHA-543613 in comparison to 5 mg/kg (+)-amphetamine on ipsiversive rotations in 6-OHDA lesioned, primed rats. Data are expressed as mean $\pm$ standard error ($n = 12$ per treatment group). Statistical significance is denoted as **** $P < 0.0001$ compared to the control group.

6.4 Discussion

Unlike $\beta 2$ nAChRs on dopaminergic terminals, $\alpha 7$ nAChRs in the striatum are largely expressed on corticostriatal glutamatergic afferents (Gowayed et al., 2022). This positions $\alpha 7$ receptors at the interface of excitatory transmission and maladaptive plasticity in LID. In support, the results show that positive allosteric modulation of $\alpha 7$ nAChRs via PNU-120596 markedly reduced axial, limb and orolingual (ALO) abnormal involuntary movements, even without prior nicotine exposure. This finding aligns with epidemiological and preclinical evidence that enhanced cholinergic tone reduces LID but now highlights $\alpha 7$ PAMs as effective in the absence of exogenous agonists, likely by amplifying endogenous ACh signalling with high spatial and temporal precision.

Several mechanisms may underlie the anti-dyskinetic action of $\alpha 7$ activation. Dysregulated corticostriatal glutamatergic activity is a central contributor to LID, as evidenced by the efficacy of glutamate antagonists (amantadine), (Shen et al., 2020). Notably, it was shown that amantadine's beneficial effect derives from blocking striatal Kir2 potassium channels rather than NMDA receptors (Shen et al., 2020). Intriguingly, nicotine similarly inhibits Kir2 currents in heterologous systems (Wang et al., 2000). It was therefore proposed that $\alpha 7$ nAChR activation may engage intracellular cascades converging on Kir2 channel inhibition. By stabilizing spiny projection neuron (SPN) membrane potentials and firing patterns, this could normalize dopamine dependent signalling and pacemaker activity. In fact, enhancing cholinergic tone via $\alpha 7$ PAMs might mimic amantadine's effect on Kir2, thereby reducing aberrant glutamatergic drive and AIMs (Wang et al., 2000).

An additional, distinct mechanism involves neuroinflammation. Chapter 4 demonstrated that acute nicotine or varenicline during levodopa priming significantly suppressed astrogliosis and microgliosis in the nigrostriatal pathway. There seems to be an

interplay between NMDA receptor activation, neuroinflammation and dyskinesia (Zhang et al., 2023). Several recent studies have shown that striatal inflammation causes levodopa-induced dyskinesia (dos Santos Pereira et al., 2021; Pinna et al., 2021; Lane and Lelos, 2022; Rentsch et al., 2023). A recent study reported the beneficial effects of PNU-120596 by halting striatal neuroinflammation which was only partially reversed following MLA treatment (Gowayed et al., 2022). There is also localisation of $\alpha 7$ nAChRs on the immune cells of the CNS, the microglia (Carnevale et al., 2007; Egea et al., 2015) In accord with this, PHA-543613 has been shown to exert neuroprotective effects by reducing neuroinflammation and partially preserving the condition of striatal dopaminergic nerve ends in 6-OHDA lesioned rats (S  rri  re et al., 2015), further confirming that selective $\alpha 7$ nAChRs agonists may reduce dyskinesia by reducing neuroinflammation. So, it seems that the anti-dyskinetic property of the $\alpha 7$ PAM and $\alpha 7$ receptor effects are mediated through inhibition of glutamate release from astrocytes and microglia (Stuckenholtz et al., 2013; Gowayed et al., 2022).

The anti-inflammatory role of $\alpha 7$ nAChRs is well established: $\alpha 7$ receptors on microglia and astrocytes mediate a “cholinergic anti-inflammatory pathway,” inhibiting cytokine release via JAK2/STAT3 and NF- κ B signalling (Shytle et al., 2004). In line with this, PNU-120596 treatment suppressed JAK2/NF- κ B/GSK3 β signalling and TNF- α expression in a parkinsonism model (Gowayed et al., 2022). Thus, $\alpha 7$ PAMs may reduce LID not only by modulating neurons, but also by tempering neuroinflammation. The data suggest these effects are synergistic: $\alpha 7$ PAM enhanced cholinergic tone could reduce glutamate release from cortical terminals while simultaneously shifting glial cells toward an anti-inflammatory state, together stabilizing the striatal environment.

Neuronal $\alpha 7$ nAChRs operate via both ionotropic and metabotropic modes (Kabbani & Nichols, 2018). Upon ACh binding, ionotropic $\alpha 7$ channels allow fast Ca²⁺ influx, activating kinases (ERK1/2, PKA) that modulate synaptic strength and plasticity. In parallel,

metabotropic $\alpha 7$ signalling ($\alpha 7^m$) recruits G-proteins to sustain IP_3 -mediated Ca^{2+} release from intracellular stores (Kabbani & Nichols, 2018). This dual signalling prolongs the influence of transient cholinergic inputs. Type II PAMs like PNU-120596 further extend the $\alpha 7$ open state, enhancing both ionotropic and metabotropic output (Kabbani & Nichols, 2018). In this chapter,

it was shown that, acute PNU-120596 likely amplified endogenous ACh effects in the dopaminergic denervated striatum, driving sufficient Ca^{2+} signalling to confer anti-dyskinetic benefit. The fact that PNU-120596 alone, in drug naïve LID rats, reduced AIMs support a model in which basal cholinergic tone is “boosted” to therapeutically effective levels. It is also

notable that the selective $\alpha 7$ agonist PHA-543613 reduced AIMs only at the high dose (10 mg/kg), consistent with a relatively low potency. The absence of an additive effect when combining nicotine with PNU-120596 or PNU-120596 with PHA-543613 suggests overlapping mechanisms, possibly receptor desensitization which needs further investigation.

The critical role of $\alpha 7$ receptors in LID is supported by genetic studies: mice lacking $\alpha 7$ subunits fail to develop L-DOPA induced AIMs (Wang et al., 2002). This implies that $\alpha 7$ activation sits at a key juncture between dopaminergic and glutamatergic systems. Paradoxically, $\alpha 7$ agonism is typically associated with increased glutamate release (Campos et al., 2010; Ryu et al., 2017). The anti-dyskinetic results observed here may therefore result from receptor desensitization or from nuanced modulation of presynaptic glutamate release patterns. In fact, the dose dependent efficacy of the $\alpha 7$ antagonist methyllycaconitine (MLA) in inhibiting AIMs suggests that reducing $\alpha 7$ activity can also reduce dyskinesia, perhaps by curtailing excess glutamate or by shifting network excitability. Thus, both agonists/PAMs and antagonists ultimately appear to mitigate LID, likely via convergent effects on striatal circuit stability.

Several key implications emerge from these findings. First, $\alpha 7$ PAMs offer a physiological means to engage cholinergic tone: they enhance the response to endogenously

released ACh (or choline) only when and where it is present (Kabbani & Nichols, 2018). This confers spatiotemporal precision and avoids the full-blown receptor desensitization and side effects seen with high-dose agonists (Kabbani & Nichols, 2018). Second, by acting at the nexus of excitatory transmission and immune signalling, $\alpha 7$ PAMs provide a multifaceted therapeutic effect, combining motor symptom relief with potential neuroprotection. Third, dose optimization will be crucial. Excessive $\alpha 7$ activation can lead to Ca^{2+} overload; the data indicate that both too little (low-dose agonist) and too much (combined high-dose nicotinic stimulation) may be suboptimal. Careful titration of PAM activity should maximize benefit while minimizing excitotoxic risk.

6.5 Conclusion

This chapter demonstrates that positive allosteric modulation of $\alpha 7$ nAChRs, exemplified by PNU-120596, significantly reduces established L-DOPA induced dyskinesia. Importantly, $\alpha 7$ PAMs do so without exogenous agonist administration, effectively amplifying intrinsic cholinergic signalling in the striatum. These agents appear to restore synaptic homeostasis by suppressing aberrant glutamatergic output and by reducing neuroinflammation, two processes that have been shown to underlie LID in previous chapters and validated by recent literature (Pinna et al., 2021). The effect is a stabilization of motor circuits without impairing baseline function. Future work should translate these findings toward clinical application: exploring $\alpha 7$ PAM safety and efficacy in human LID, optimizing dosing to apply their dual anti-dyskinetic and neuroprotective actions, and investigating potential cognitive benefits from enhanced cholinergic transmission (given $\alpha 7$'s role in learning and memory (Kabbani & Nichols, 2018)). In sum, targeting $\alpha 7$ nAChRs, especially via PAMs emerges as a promising strategy to both alleviate dyskinesia and promote neuronal resilience in Parkinson's disease.

Chapter 7

General Discussion

7.1 Limitations of Levodopa Therapy

Levodopa remains the most effective symptomatic treatment for Parkinson's disease, reliably improving bradykinesia, tremor and rigidity by restoring striatal dopamine. As disease progresses, however, the diminishing capacity of dopaminergic terminals to store and buffer dopamine gives rise to motor fluctuations and L-DOPA induced dyskinesia (LID). Long-term cohort studies show that a substantial proportion of patients develop troublesome dyskinesia after several years of treatment, with risk increasing alongside advancing nigrostriatal degeneration (Ahlskog & Muentner, 2001; Fabbrini et al., 2007).

Therapeutic options for LID remain limited. Amantadine, an NMDA receptor antagonist, is the only widely used oral antidyskinetic therapy. Randomised trials report moderate reductions in dyskinesia severity, typically in the range of 30–50 percent, but many patients show incomplete or transient responses, and adverse effects often limit long term use, particularly in older adults (Thomas et al., 2004; Pahwa et al., 2017). Adjusting levodopa dosing schedules or adding adjunctive dopaminergic drugs may reduce fluctuations, yet these strategies do not reliably prevent or reverse established dyskinesia.

Together these limitations demonstrate the urgent need for new adjunctive approaches that can reduce dyskinesia without diminishing levodopa's antiparkinsonian efficacy. This therapeutic gap provides the rationale for investigating nicotinic acetylcholine receptors, which influence dopaminergic, glutamatergic and GABAergic signalling and may offer a mechanistically distinct route for modulating the maladaptive plasticity underlying LID.

7.2 Cholinergic Interneurons and Nicotinic Receptors as Therapeutic Targets

Loss of nigrostriatal dopamine produces a characteristic shift in striatal microcircuitry, including heightened activity of cholinergic interneurons and an altered balance between acetylcholine and dopamine tone (Pisani et al., 2005; Perez-Lloret & Barrantes, 2016). Historically, this motivated the use of anticholinergic drugs in PD, but their non-selective muscarinic blockade frequently causes cognitive and psychiatric adverse effects, limiting their applicability. More recently, attention has turned to nicotinic acetylcholine receptors, which offer a more selective means of modulating striatal signalling.

Epidemiological studies consistently observe a reduced incidence of PD among smokers, supporting the long-standing hypothesis that chronic nicotine exposure (or downstream nAChR modulation) may exert neuroprotective or neuromodulatory effects (Li et al., 2015; Ritz et al., 2014). Experimental work provides a direct link to dyskinesia: nicotine reduces established LID by approximately 40–60 percent in both rodent and non-human primate models, without diminishing the antiparkinsonian benefit of levodopa (Quik et al., 2007; Bordia et al., 2008; Huang et al., 2011). This selective effect on dyskinesia suggests that nAChR activation alters the abnormal corticostriatal signalling that mediates LID rather than impairing dopaminergic function globally.

Genetic evidence supports a critical role for $\beta 2$ containing nAChRs. Mice lacking the $\beta 2$ subunit (eliminating $\alpha 4\beta 2$ and $\alpha 6\beta 2$ receptors) show markedly reduced dyskinesia and fail to respond to nicotine (Huang et al., 2011). These receptors are densely expressed on dopaminergic terminals, where they influence dopamine release dynamics and desensitize with sustained activation. In contrast, $\alpha 7$ nAChRs, located on glutamatergic terminals and interneurons, appear to act through a different mechanism: their activation reduces excessive

glutamatergic drive and engages anti-inflammatory pathways that may contribute to limiting dyskinesia and its associated neuroinflammatory changes (Gotti & Clementi, 2004; Papke, 2014). Selective $\alpha 7$ agonists such as ABT-126 and ABT-107 have produced substantial reductions in LID in MPTP lesioned monkeys, even in the setting of severe dopaminergic denervation (Zhang et al., 2014; Zhang et al., 2015).

Taken together, these findings indicate that specific nAChR subtypes, particularly $\alpha 4\beta 2$, $\alpha 6\beta 2$ and $\alpha 7$ receptors, modulate striatal output in ways that counteract the sensitised, plasticity driven circuits responsible for LID. Building on this rationale, a series of experiments (Chapters 3–6) was designed to test whether specific nAChR modulators could ameliorate LID. The central hypothesis was that activating or desensitizing key nAChR subtypes would reduce dyskinetic motor circuits without compromising the therapeutic action of levodopa. The following sections describe the findings and their implications.

7.3 Acute Nicotinic Stimulation Reduces Established Dyskinesia

In parkinsonian rats with fully developed LID, it was first tested whether acute activation of nAChRs could immediately suppress involuntary movements. Consistent with prior primate and rodent work (Perez-Lloret & Barrantes, 2016), systemic nicotine produced a clear, dose dependent reduction in dyskinesia severity. Low doses (0.01–0.3 mg/kg, s.c.) lowered AIM scores across axial, limb and orolingual subtypes, with the maximal effect appearing 20–80 minutes after dosing, during the same period in which levodopa typically provokes its strongest dyskinesia. Varenicline (0.1–1 mg/kg), acting as a partial $\alpha 4\beta 2$ agonist and full $\alpha 7$ agonist, also reduced dyskinesia, though its effect was less pronounced than that of nicotine (Huang et al., 2011).

Neither nicotine nor varenicline impaired baseline motor function, as treated rats showed no sedation or worsening of parkinsonian signs. This parallels previous reports

showing that nicotinic drugs can reduce LID without diminishing levodopa's antiparkinsonian benefit (Patel et al., 2017). By comparison, many standard antidyskinetic agents, including amantadine and high dose anticholinergics, tend to suppress overall movement (Thomas et al., 2004).

The receptor dependence of this effect aligns with earlier mechanistic evidence. Nicotine's antidyskinetic action is absent in $\beta 2$ knockout mice, which lack $\alpha 4\beta 2$ and $\alpha 6\beta 2$ receptors (Huang et al., 2011), indicating that $\beta 2$ containing nAChRs are essential for the response. Varenicline's partial efficacy suggests that $\alpha 7$ receptors also contribute, consistent with the substantial reductions in LID reported with selective $\alpha 7$ agonists such as ABT-126 in MPTP lesioned primates (Perez-Lloret & Barrantes, 2016).

Taken together, these findings suggest that acute nAChR stimulation can rapidly rebalance striatal output and suppress dyskinetic activity while preserving levodopa's therapeutic effect. The timing, receptor specificity and preserved motor function all support the model that nicotinic modulation acts against the maladaptive corticostriatal plasticity that underlies LID (Huang et al., 2011; Patel et al., 2017).

7.4 Nicotinic Co-Therapy During Levodopa Priming Prevents AIMs

Since nicotinic agents can reduce existing dyskinesia, it was next examined whether early nAChR stimulation could prevent LID from emerging as levodopa treatment begins. In this experiment, nicotine or varenicline was given with each initial L-DOPA dose during the priming period. Rats treated with levodopa alone developed the expected rise in dyskinesia across sessions, reflected in steadily increasing AIM scores. In contrast, animals receiving nicotine or varenicline alongside L-DOPA showed a markedly slower escalation in AIMs. Both compounds lowered cumulative LID scores by roughly 40-60% across global and subtype measures, despite an identical levodopa regimen. The antiparkinsonian effect of L-DOPA was

preserved, as nicotinic treated rats displayed normal forelimb use and mobility during “on” periods.

Post-mortem tissue analysis added important detail. Although all groups exhibited severe nigrostriatal lesions typical of the 6-OHDA model, nicotine and varenicline treated rats retained a greater number of TH-positive fibres compared with levodopa only controls. This partial preservation of dopaminergic terminals was accompanied by lower dyskinesia severity, consistent with the recognised relationship between degree of denervation and susceptibility to LID. A similar pattern appeared in the glial markers. Saline treated dyskinetic rats showed pronounced astrocytic (GFAP) and microglial (IBA-1) activation in the striatum and nigra, whereas animals receiving nicotinic co-therapy displayed noticeably reduced glial reactivity.

These findings fit with growing evidence that nicotinic signalling shapes inflammatory responses in the basal ganglia. Astrocytes and microglia express $\alpha 7$ nAChRs that regulate cytokine production through NF- κ B suppression and activation of JAK2/STAT3 and antioxidant pathways (Egea et al., 2015; Patel et al., 2017). Activation of these receptors during priming could limit the inflammatory environment that promotes maladaptive plasticity. At the same time, engagement or desensitisation of $\alpha 4\beta 2/\alpha 6\beta 2$ receptors on dopaminergic terminals may dampen the dopamine fluctuations that drive sensitisation early in levodopa treatment.

Taken together, Chapter 4 shows that nicotinic co-therapy initiated from the first L-DOPA dose can markedly reduce the development of dyskinesia while maintaining levodopa’s clinical benefit (Huang et al., 2011). The combined behavioural and histological patterns point to a protective shift in striatal function involving both neuronal and glial mechanisms. They also suggest $\alpha 4\beta 2$ containing receptors (and possibly $\alpha 7$ receptors on glia) as the most likely contributors to this preventive effect, providing the rationale for the subtype focused experiments described in the following chapter.

7.5 $\alpha 4\beta 2$ Selective Agonists Reduce AIMs by Receptor Desensitization

Given the importance of $\beta 2$ containing receptors, it was next examined whether selective engagement of the high affinity $\alpha 4\beta 2$ subtype could account for the antidyskinetic effects observed with nicotine. In LID rats, the $\alpha 4\beta 2$ selective agonist A-85380 produced a marked reduction in AIM scores at an optimal dose (0.01 mg/kg), with the clearest improvements in limb and orolingual subtypes. Importantly, normal movement was preserved, consistent with earlier work showing that $\alpha 4\beta 2$ activation reduces dyskinesia without compromising levodopa's benefit (Gowayed et al., 2022). The dose dependence of the effect argues against nonspecific sedation, supporting a receptor mediated mechanism. A coherent interpretation is that A-85380 initially increases dopamine release but then desensitizes $\alpha 4\beta 2$ receptors, placing them in a long lasting inactive state. This would limit the large dopamine transients that normally drive D1 linked overactivation and the downstream signalling changes characteristic of LID.

Sazetidine-A, which preferentially desensitises $\alpha 4\beta 2$ receptors, produced reductions in AIMs of similar magnitude, though with a slower onset that reflects its pharmacokinetic profile (Gowayed et al., 2022). Because Sazetidine-A binds tightly but only weakly activates the receptor, it effectively suppresses $\alpha 4\beta 2$ driven dopamine and glutamate release. Both compounds therefore converge on the same mechanistic point, sustained $\alpha 4\beta 2$ desensitisation, yet do so through complementary pharmacological routes.

By contrast, drugs that do not promote desensitisation showed little impact on AIMs. NS9283 (a positive allosteric modulator) and Dh β E (a competitive antagonist) failed to alter AIM scores at the doses tested, and Dh β E prevented A-85380 from reducing AIMs when co-administered. Together, these findings demonstrate that simply potentiating or blocking $\alpha 4\beta 2$ activity is insufficient once dyskinetic circuits are established; it is the transition of the receptor into a desensitised state that disrupts the pathological signalling.

The histological findings in Chapter 5 further strengthen this mechanistic interpretation. Dyskinetic animals showed the expected profound nigrostriatal degeneration (>90% loss of TH-positive neurons), accompanied by strong astrocytic and microglial activation in the striatum. In animals with reduced AIMs, both the degree of dopaminergic loss and the glial response were reduced, suggesting that $\alpha 4\beta 2$ desensitisation tempers not only synaptic activity but also the broader neuroinflammatory environment. This relationship between AIMs and glial activation aligns with existing evidence that LID is accompanied by persistent astrocytic and microglial reactivity (Cardinale et al., 2024). Interventions that block AIMs (such as inhibition of nitric oxide synthesis) also prevent the associated increase in GFAP and IBA-1 (Bortolanza et al., 2014), supporting the idea that neuroinflammation is tightly coupled to the dyskinetic state.

The reduced glial activation seen with A-85380 and Sazetidine-A is consistent with prior findings that nicotine's antidyskinetic effect is accompanied by lower astrocytic and microglial reactivity (Fares et al., 2023). These convergent patterns suggest that $\alpha 4\beta 2$ desensitisation modifies both neuronal and glial contributors to dyskinesia, placing the receptor at a key point in the network that links dopamine dynamics, corticostriatal plasticity and neuroinflammatory signalling. In this regard, $\alpha 4\beta 2$ desensitisation represents not simply a pharmacological effect but a functional shift in striatal state, one that aligns with a broader model in which nAChRs exert a modulatory influence across multiple levels of the LID pathway.

7.6 $\alpha 7$ nAChR Modulation Reduces AIMs via Endogenous Cholinergic Tone

With $\beta 2$ containing receptors implicated, it was next examined whether the $\alpha 7$ subtype, which remains expressed on corticostriatal terminals and interneuron synapses even in advanced PD, also played a role. Several features made $\alpha 7$ a compelling target. Its activation engages calcium dependent signalling that influences glutamate release and plasticity, and $\alpha 7$ PAMs provide a way to enhance physiological cholinergic tone without persistent receptor activation. In this chapter, an $\alpha 7$ PAM (PNU-120596), a selective $\alpha 7$ agonist (PHA-543613), and an $\alpha 7$ antagonist (methyllycaconitine, MLA) were tested.

Enhancing $\alpha 7$ activity reduced AIMs. Acute administration of PNU-120596 lowered AIM scores in a dose dependent manner, even in the absence of exogenous nicotine, showing that potentiating endogenous cholinergic input at $\alpha 7$ receptors is sufficient to suppress abnormal movements. In contrast, the direct $\alpha 7$ agonist PHA-543613 required a high dose (10 mg/kg) to produce a modest effect, and lower doses were ineffective. This pattern suggests that full agonists may have a narrow therapeutic window, whereas PAMs amplify signalling only when endogenous ACh is released during behaviour, resulting in greater selectivity and fewer off-target actions.

Interestingly, MLA also reduced AIMs. Although this may seem unexpected at first, but this aligns with what is known about $\alpha 7$ physiology. $\alpha 7$ receptors facilitate glutamate release, and their prolonged inhibition or desensitisation can reduce the excessive glutamatergic drive that fuels the overactive direct pathway in LID. A similar outcome is seen with chronic nicotine (shown in chapter 3), which progressively desensitises $\alpha 7$ receptors. Notably, the clinical trial of the $\alpha 7$ agonist AQW051 in PD patients did not show improvement in LID (Trenkwalder et al., 2016), supporting the idea that simple $\alpha 7$ agonism may be

insufficient because it fails to preserve the receptor state needed to influence AIMs circuits. Overall, our findings indicate that adjusting $\alpha 7$ signalling, through PAM driven amplification of endogenous activation or through functional desensitisation, can re-establish a more stable corticostriatal pattern. These effects may converge on intracellular pathways previously associated with nicotinic modulation, including AMPK/GSK3 β and Kir channel regulation (Patel et al., 2017).

PNU-120596 has also been shown in other studies to ameliorate parkinsonian motor deficits and reduce striatal inflammation via JAK2/NF- κ B/GSK3 β pathways (Gowayed et al., 2022). This external evidence aligns with the present findings and supports the view that $\alpha 7$ modulation offers benefits across several dimensions, including synaptic stability, inflammatory control and possibly neuroprotection. In practice, $\alpha 7$ PAMs may provide a targeted means of enhancing both the cholinergic anti-inflammatory pathway and corticostriatal homeostasis, complementing the $\beta 2$ dependent mechanisms identified earlier in the chapter.

7.7 Summary, Limitations, and Future Directions

Across these studies, nicotinic acetylcholine receptor modulation consistently reduced levodopa induced dyskinesia in parkinsonian models. Acute nicotine and varenicline suppressed established AIMs without diminishing levodopa's therapeutic benefit (Gowayed et al., 2022). When administered during the priming phase, both compounds markedly limited the emergence of LID, preserved a greater proportion of dopaminergic terminals and substantially reduced astrocytic and microglial reactivity. Selective engagement of $\alpha 4\beta 2$ receptors through desensitising agonists produced similar effects, demonstrating that stabilising this receptor population in a desensitised state is a key determinant of antidyskinetic efficacy. Modulation of $\alpha 7$ receptors, whether by amplifying endogenous acetylcholine activity or by reducing

excessive receptor drive, also alleviated dyskinesia, likely through effects on glutamatergic signalling and neuroinflammation. Together, these findings show that targeting defined nAChR states can recalibrate corticostriatal activity and dampen the sensitised plasticity that sustains LID.

When viewed collectively, these results outline a coherent mechanistic framework. Dyskinesia reflects an unstable striatal environment characterised by exaggerated dopamine fluctuations, heightened glutamatergic input and persistent glial activation. Nicotinic agents intervene at several points along this pathway. $\beta 2$ containing receptors regulate dopamine release dynamics and become therapeutically effective when shifted into a desensitised state, preventing the rapid dopaminergic oscillations that trigger D1 driven maladaptation. $\alpha 7$ receptors influence glutamate release and inflammatory signalling, enabling PAMs or functional inhibition to restore a more balanced excitatory tone. This multi-level modulation distinguishes nAChR based approaches from traditional dopaminergic or glutamatergic therapies, offering a means of regulating the broader neuromodulatory environment without the cognitive or peripheral side effects associated with non-selective anticholinergics. The tolerability of varenicline, already licensed for long-term human use, further underscores the translational promise of this strategy.

Several limitations should be acknowledged. All experiments were conducted in rodent models, and although these models capture core elements of LID, they cannot fully replicate the complexity of human PD, including heterogeneous patterns of nigrostriatal loss, serotonergic pathology and widespread cortical involvement. Pharmacokinetic and pharmacodynamic properties of nicotinic drugs differ across species, and receptor expression profiles in human PD brains may introduce variability not reflected in this work. Clinical translation has also proven challenging; for example, the $\alpha 7$ agonist AQW051 did not improve dyskinesia in a phase 2 trial (Trenkwalder et al., 2016), indicating that receptor activation alone

may not be sufficient and highlighting the importance of receptor state. Tolerability remains another consideration. Nicotine can cause gastrointestinal and autonomic side effects, and varenicline may produce vivid dreams or rare neuropsychiatric symptoms. The long-term consequences of sustained nAChR desensitisation, including potential receptor upregulation or compensatory network changes, remain unknown. Finally, while this thesis focuses on $\alpha 4\beta 2$ and $\alpha 7$ receptors, other subtypes, particularly $\alpha 6$ -containing receptors located on dopaminergic terminals, may contribute to LID and warrant systematic study.

Future research should refine and extend these findings. Testing nAChR based therapies in non-human primates would provide more robust translational insight, particularly regarding long-term safety and receptor regulation. Given varenicline's established safety profile, early phase clinical trials in dyskinetic PD patients may be justified, especially using dosing regimens tailored to minimise side effects while maintaining receptor engagement. Combining nAChR modulators with existing therapies, such as low-dose amantadine or targeted NMDA modulators, could reveal additive or synergistic effects. Mechanistically, it will be important to define how $\alpha 4\beta 2$ and $\alpha 7$ signalling intersects with intracellular pathways (including AMPK, GSK3 β , Kir channel modulation and cytokine cascades) to influence dyskinesia expression. Determining the optimal timing, dosing schedule and receptor state specificity for each drug class will be crucial for translating these findings into therapy.

In conclusion, the work presented here shows that nicotinic receptor modulation can address a major unmet need in Parkinson's disease by reducing levodopa's most debilitating motor complication while maintaining its symptomatic benefit (Huang, Grady et al., 2011). By influencing dopaminergic, glutamatergic and glial pathways, nAChR based interventions have the potential to stabilise the striatal circuitry that becomes dysregulated in LID. This thesis provides a strong foundation for advancing nicotinic approaches toward clinical application,

offering a credible route to improving long-term motor stability and quality of life for individuals living with PD.

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