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EFEKTI REPETITIVNE TRANSKRANIJALNE MAGNETNE STIMULACIJE NA NEURODEGENERACIJU, NEUROINFLAMACIJU I KOMPONENTE PURINSKOG SIGNALNOG SISTEMA U MODELU PARKINSONOVE BOLESTI IZAZVANE 6-HIDROKSIDOPAMINOM KOD PACOVA

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EFFECTS OF REPETITIVE TRANSCRANIAL MAGNETIC STIMULATION ON NEURODEGENERATION, NEUROINFLAMMATION AND COMPONENTS OF THE PURINERGIC SIGNALING SYSTEM IN A 6-HYDROXYDOPAMINE RAT MODEL OF PARKINSON'S DISEASE

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Milica Zeljković Jovanović

Efekti repetitivne transkranijalne magnetne stimulacije na neurodegeneraciju, neuroinflamaciju i komponente purinskog signalnog sistema u modelu Parkinsonove bolesti izazvane 6-hidroksidopaminom kod pacova

Sažetak

Parkinsonova bolest (PB) predstavlja složeno, multisistemsko neurodegenerativno oboljenje koje beleži značajan rast globalne prevalence i opterećenja na zdravstveni sistem, dok terapijske strategije koje bi usporile ili zaustavile progresiju ove bolesti i dalje predstavljaju veliki izazov. Tačan uzrok koji pokreće kaskadu događaja koja dovodi do degeneracije dopaminskih (DA) neurona u regionu crne supstance i razvoja kliničke slike ove bolesti koja uključuje brojne motorne i nemotorne simptome i dalje nije jasno definisan, ali ono što je sigurno je postojanje složene interakcije genetičkih i sredinskih faktora. Mehanizmi patogeneze PB uključuju akumulaciju α-sinukleina, mitohondrijsku disfunkciju, oksidativni stres i neuroinflamaciju, koji dovode do degeneracije DA neurona i smanjenja nivoa dopamina, ali i drugih neurotransmitera. S obzirom na ograničenja trenutnih terapijskih strategija, današnja istraživanja se usmeravaju ka inovativnim pristupima kao što je repetitivna transkranijalna magnetska stimulacija (rTMS). rTMS je oblik neinvazivne stimulacije mozga koji pokazuje značajan potencijal u različitim patološkim stanjima. Predmet istraživanja ove doktorske disertacije bio je ispitivanje efekata 21-dnevnog tretmana rTMS-om na neurodegeneraciju u eksperimentalnom modelu PB izazavanom unilateralnom aplikacijom 6-hidroksidopamina (6-OHDA) u region crne supstance pacova, kao i uloga purinskog i glutamatnog signalnog sistema u tim efektima, sa ciljem da se proceni potencijalni terapijski efekat izabranog protokola u PB. Rezultati dobijeni u okviru ove disertacije ukazuju da je već nakon prve nedelje stimulacije tretman doveo do značajnih poboljšanja u pogledu motornih i nemotornih simptoma. Neuroprotektivni efekti rTMS-a su primećeni kroz povećanu ekspresiju tirozin-hidroksilaze, koja je uključena u sintezu dopamina, i povećanje nivoa dopamina i serotonina. Nadalje, rTMS je uzrokovao promene u ekspresiji ADP-zavisnih receptora u smeru koji ukazuje na potencijalnu ulogu u neuroprotekciji. Primećeno je smanjenje ekspresije i aktivnosti enzima eN/CD73 i ADA1, kao i povećanje u ekspresiji A_1R i smanjenje u ekspresiji A_{2A} receptora. Dodatno, rTMS je doveo do promena u komponentama glutamatnog signalnog sistema, uključujući povećanu ekspresiju GluN1 i GluN2A, kao i smanjenu ekspresiju GluN2B subjedinica NMDA receptora, što može pozitivno uticati na preživljavanje DA neurona. Uočeno je i povećanje antioksidativnih markera u tkivnim homogenatima ispitivanih struktura, ali i u serumu. Navedene promene ukazuju da primena iTBS-a utiče na regulaciju ključnih neurotransmitera, kao i na modulaciju komponenti purinskog i glutamatnog sistema, da ispoljava neuroprotektivni efekat, ali i povećani potencijal antioksidativnog kapaciteta što ukazuje da izabrani rTMS protokol može da utiče povoljno na ključne patofiziološke procese uključene u PB.

Ključne reči: Parkinsonova bolest, neurodegeneracija, 6-hidroksidopamin, repetitivna transkranijalna magnetna stimulacija (rTMS), stimulacija teta praskovima (iTBS), neuroprotekcija, neuromodulacija, NMDA receptor, purinska signalizacija.

Naučna oblast: Biološke nauke

Uža naučna oblast: Neurobiologija

Efects of repetitive transcranial magnetic stimulation on neurodegeneration, neuroinflamation and components of the purinergic signaling system in a 6-hydroxydopamine rat model of Parkinson's disease

Summary

Parkinson's disease (PD) is a complex, multisystemic neurodegenerative disorder whose global prevalence and burden on the healthcare system has increased significantly. At the same time therapeutic strategies to slow or halt the progression of the disease remain a major challenge. The exact cause that triggers the cascade of events leading to the degeneration of dopaminergic neurons in the substantia nigra and the development of the clinical picture of the disease, which includes numerous motor and non-motor symptoms, is still not clearly defined. However, a complex interplay of genetic and environmental factors is recognizable. Pathogenic mechanisms of PD include accumulation of α-synuclein, mitochondrial dysfunction, oxidative stress and neuroinflammation leading to degeneration of dopaminergic neurons and a decrease in dopamine levels as well as other neurotransmitters. Given the limitations of current therapeutic strategies, research has turned to innovative approaches such as repetitive transcranial magnetic stimulation (rTMS), a form of non-invasive brain stimulation that shows significant potential in various pathological conditions. This dissertation investigated the effects of 21days rTMS treatment on neurodegeneration in an experimental animal model of PD induced by unilateral administration of 6-hydroxydopamine (6-OHDA) in the substantia nigra, and the role of the purinergic and glutamatergic signaling systems in these effects, with the ultimate goal of evaluating the potential therapeutic effect of the chosen protocol in PD. The results of this dissertation show that rTMS led to significant improvements in motor and non-motor symptoms already after the first week of stimulation. The neuroprotective effects of iTBS were observed through increased expression of tyrosine hydroxylase, a marker for dopaminergic neurons, and increased dopamine and serotonin levels. In addition, rTMS decreased the expression of P2X7 receptors and led to changes in the expression of ADP-dependent receptors, also suggesting a possible role in neuroprotection. A reduction in the expression and activity of the enzymes eN/CD73 and ADA1 as well as changes in the expression of A1R and A2A receptors were observed. In addition, treatment with iTBS resulted in changes in the components of the glutamatergic signaling system, including increased expression of GluN1 and GluN2A and decreased expression of the GluN2B subunits of NMDA receptors, which may have a positive effect on the survival of dopaminergic neurons. An increase in antioxidant markers in tissue homogenates of the investigated structures as well as in serum was also observed. These changes indicate that rTMS influences the regulation of key neurotransmitters, as well as the components of the purinergic and glutamatergic signaling system, and has a neuroprotective effect and a potential to increase antioxidant capacity, leading to the conclusion that iTBS can positively influence important pathophysiological processes involved in PD.

Key words: Parkinson's disease, neurodegeneration, 6-hydroxydopamine, repetitive transcranial magnetic stimulation (rTMS), theta burst stimulation (iTBS), neuroprotection, neuromodulation, NMDA receptor, purinergic signaling

Research area: Biology

Research field: Neurobiology

LISTA SKRAĆENICA

ADP - Adenozin difosfat

AMP - Adenozin monofosfat

BBB – Krvno-moždana barijera (eng. *Blood-brain barrier*)

BDNF - Moždani neurotrofički faktor (eng. *Brain-Derived Neurotrophic Factor*)

CAT - Katalaza

CNS – Centralni nervni sistem

COMT - Katehol-O-metiltransferaza (eng. *Catechol-O-Methyltransferase*)

COX-2 - Ciklooksigenaza-2 (eng*. Cyclooxygenase-2*)

CSF - Cerebrospinalna tečnost

DAMP –Molekulski obrasci povezani sa oštećenjem (eng. *Danger Associated Molecular Pattern*)

DA – Dopaminski neuron (eng. *Dopaminergic Neuron*)

DAT - Dopaminski transporter (eng. *Dopamine Transporter*)

DBS - Duboka moždana stimulacija (eng. *Deep Brain Stimulation*)

DLPFC – Dorzolateralna prečeona kora (eng. *Dorsolateral Prefrontal Cortex*)

EAAT - Transporteri ekscitatornih amino-kiselina (eng. *Excitatory Amino Acid Transporters*)

EMG - Elektromiografija

FDA - Agencija za hranu i lekove (eng. *Food and Drug Administration*)

GABA - γ-aminobuterna kiselina (eng. *Gamma-Aminobutyric Acid*)

GFAP – Glijski kiseli fibrilarni protein (eng. *Glial fibrillary acidic protein*)

GPi - Unutrašnje bledo jedro (let. *Globus Pallidus Internal*)

GPe - Spoljno bledo jedro (lat. *Globus Pallidus External*)

GSH - Glutation-S-transferaza

HF-rTMS - Repetitivna transkranijalna magnetna stimulacija visoke frekvencije (eng. *High-Frequency repetitive Transcranial Magnetic Stimulation*)

iGluR - Jonotropni receptori za glutamat (eng. *Ionotropic Glutamate Receptors*)

iTBS - Intermitentna stimulacija teta praskovima (eng. *Intermittent Theta Burst Stimulation*)

L-DOPA - L-dihidroksifenilalanin (eng. *L-Dihydroxyphenylalanine*)

LF-rTMS - Repetitivna transkranijalna magnetna stimulacija niske frekvencije (eng. *Low-Frequency repetitive Transcranial Magnetic Stimulation*)

LTD - Dugotrajna depresija (eng. *Long-Term Depression*)

LTP - Dugotrajna potencijacija (eng. *Long-Term Potentiation*)

MAO - Monoamin-oksidaza

MDA - Malondialdehid

MEP – Motorni izazvani odgovori (eng. *Motor evoked potentials*)

MRI - Magnetna rezonanca

NMDA - N-metil-D-aspartat (eng. *N-Methyl-D-Aspartate*)

PB – Parkinsonova bolest

PDGF - Faktor rasta poreklom od trombocita (eng. *Platelet-Derived Growth Factor*)

PET - Pozitronska emisiona tomografija (eng. *Positron Emission Tomography*)

rTMS - Repetitivna transkranijalna magnetna stimulacija (eng. *Repetitive Transcranial Magnetic Stimulation*)

ROS - Reaktivne vrste kiseonika (eng. *Reactive Oxygen Species*)

SMA - Pomoćna motorna zona (eng. *Supplementary Motor Area*)

SN - Crna supstanca (lat. *Substantia Nigra*)

SNpc - (lat. *Substantia Nigra pars compacta*)

SOD - Superoksid-dizmutaza

STN - Subtalamičko jedro (lat. *Subthalamic Nucleus*)

TBS - Stimulacija teta praskovima (eng. *Theta Burst Stimulation*)

TH - Tirozin-hidroksilaza (eng. *Tyrosine Hydroxylase*)

TNF-α - Faktor nekroze tumora alfa (eng. *Tumor Necrosis Factor Alpha*)

tDCS - Transkranijalna stimulacija jednosmernom strujom (eng. *Transcranial Direct Current Stimulation*)

VEGF - Vaskularni endotelni faktor rasta (eng. *Vascular Endothelial Growth Factor*)

SADRŽAJ

1. UVOD

1.1. Parkinsonova bolest

1.1.1. Epidemiologija i istorijski aspekti Parkinsonove bolesti

Parkinsonova bolest (PB) kao najčešći uzrok parkinsonizma, motornog sindroma kojeg karakterišu tremor, bradikinezija, rigiditet i posturalna nestabilnost, predstavlja složeno neurodegenerativno oboljenje, koje se kao i druge neurodegenerativne bolesti odlikuje progresivnim propadanjem neurona koje dovodi do narušavanja brojnih neuroloških funkcija, smanjenog kvaliteta života i očekivanog životnog veka (Keener & Bordelon, 2016). Pored Alchajmerove bolesti predstavlja jedan od vodećih uzroka smrtnosti među neurološkim bolestima u razvijenim državama sveta, pogotovu među starijom populacijom (Tolosa et al., 2021). „Studije opterećenja populacije bolešću" na globalnom nivou procenjuju da trenutno između 7,0 i 8,5 miliona ljudi širom sveta boluje od PB (Dorsey et al., 2018). U razvijenim zemljama procenjena prevalenca PB u opštoj populaciji je 0,3%, pri čemu je 1,0% kod ljudi starijih od 60 godina i 3,0% kod osoba starijih od 80 godina (Zesiewicz, 2019). Danas je uobičajeno gledište da bolest, odnosno sam proces propadanja neurona počinje godinama pre pojave prvih simptoma, pri čemu se nemotorni simptomi javljaju i nekoliko godina pre pojave motornih simptoma, kada se najčešće pacijent obraća lekaru i kada se postavlja dijagnoza, što je najčešće u petoj ili šestoj deceniji života. Međutim, u 5-10% slučajeva dijagnoza se postavlja pre šezdesete godine života što se definiše kao rani početak PB (eng. *early-onset PD - EOPD*) koji se dalje deli na juvenilni oblik PB kada se pacijent javlja pre 21. godine života, i na PB mladih (eng. *young-onset PD - YOPD*), ako se dijagnoza postavi između 21. i 40. godine života (Anwar et al., 2019; Thomsen & Rodnitzky, 2010). Prevalencija bolesti kod muškaraca veća je 1,5 do 2 puta u odnosu na žene (Ascherio & Schwarzschild, 2016). Prema Institutu za javno zdravlje Srbije „Dr Milan Jovanović Batut" u 2020, 11.692 odraslih osoba ima dijagnozu PB, među kojima je 92 pacijenta mlađih od 19 godina. Imajući u vidu da se radi o bolesti koja značajno utiče na kvalitet života i koja može dovesti do invaliditeta nameće se potreba za boljim dijagnostičkim kriterijumima kako bi se što ranije započela terapija, ali i za razjašnjavanjem mehanizama koji leže u osnovi nastanka i razvoja bolesti koji bi doprineli razvoju strategija za prevenciju (Poewe et al., 2017).

Od početnog opisa engleskog lekara James Parkinson-a koji je nastao pre nešto više od 200 godina, koncept PB je značajno evoluirao. Naime, u monografiji iz 1817. godine *''An Essay on the Shaking Palsy''* predstavljena je tada nova klinička slika sa najistaknutijim motornim simptomima koji uključuju tremor prilikom mirovanja, usporeni hod, pognuto držanje, probleme sa spavanjem i konstipaciju (Toodayan, 2018). Od tog trenutka kada je zapravo postavljen temelj za sva buduća istraživanja, niz naučnika je doprineo da se opis kliničke slike, kao i histopatološka osnova ove bolesti, upotpuni. Jean–Martin Charcot je 1888. godine nizu simptoma dodao i bradikineziju/usporenost pokreta i rigidnost/ukočenost i preimenovao stanje koje je do tada bilo poznato pod nazivom *''Paralysis agitans''* u PB (Zesiewicz, 2019). Friedrich Heinrich Lewy je 1913. godine opisao sferična, citoplazmatska tela u histopatološkim preparatima tkiva mozga bolesnika sa simptomima PB (Luijeva tela), a pet godina kasnije Konstantin Tretiakoff je pokazao gubitak pigmentovanih neurona u regionu crne supstance (lat. *substantia nigra*, SN). Veza između dopamina i PB otkrivena je kasnijim istraživanjima tokom 1960-tih godina prošlog veka, kada je švedski farmakolog Carlsson pokazao da bazalne ganglije sadrže visoku koncentraciju dopamina i da je upravo ovaj neurotransmiter ključan za voljnu kontrolu pokreta (Carlsson et al., 1957). Svestan te regionalne lokalizacije dopamina u mozgu Oleh Hornykiewicz je odlučio da izmeri nivo dopamina obolelih od PB i ustanovio da je snižena koncentracija dopamina u strijatumu. Ova

saznanja su bila osnova za bolje razumevanje patofiziologije PB kao i za razvoj terapije u vidu primene prekursora dopamina, L-dihidroksifenilalanina (levodopa, L-DOPA) koji se koristikod pacijenata obolelih od PB (Cotzias et al., 1967). U drugoj polovini XX veka i u XXI veku napravljen je značajan napredak u pogledu rasvetljavanja patofizioloških mehanizama nastanka ove bolesti što je doprinelo i novim mogućnostima lečenja.

1.1.2. Etiologija Parkinsonove bolesti

Razumevanje etiologije PB ključno je za razvoj efikasnih lekova i preventivnih strategija, ali jasan odgovor na pitanje šta pokreće kaskadu događaja koja dovodi do degeneracije DA neurona i razvoja kliničke slike ove bolesti i dalje ostaje nerazjašnjen. Iako najvažniji etiopatogenetički činioci nisu jasno definisani, istraživanja ukazuju na složenu interakciju genetičkih i sredinskih faktora koja se dodatno komplikuje patološkim procesima koji se javljaju prilikom starenja, a koji uključuju niz promena u ekspresiji gena odgovornih za otpornost na stres i održavanje i regeneraciju ćelija, skraćivanje telomera, mitohondrijsku disfunkciju, oksidativni stres i hroničnu inflamaciju, kao i izmenjenu međućelijsku komunikaciju (Dong-Chen et al., 2023; Kalinderi et al., 2016; López-Otín et al., 2013). U tom kontekstu parkinsonizam kao klinički sindrom koji može biti izazvan različitim činiocima se može podeliti na primarni, idiopatski parkinsonizam odnosno PB, sekundarni, simptomatski parkinsonizam koji nastaje usled dejstva poznatog faktora, zatim atipični (parkinsonizam-plus sindromi) gde se pored parkinsonizma identifikuju i drugi neurološki poremećaji koji nisu karakteristični za PB (rani autonomni poremećaji, cerebelarni i piramidni znaci, poremećaj pokretljivosti bulbusa, rana demencija) ili pak parkinsonizam u okviru naslednih neurodegenerativnih bolesti. (Keener & Bordelon, 2016; Sveinbjornsdottir, 2016).

Sekundarni parkinsonizam kao što je pomenuto uzrokovan je specifičnim faktorima koji se mogu identifikovati, i najčešće je u pitanju uzimanje lekova poput antipsihotika koji blokiraju dopaminske receptore (Höllerhage, 2019). Takođe, uzroci mogu biti vaskularne lezije, tumori mozga kao i lezije izazvane imunološkim ili infektivnim procesima (virusni ili bakterijski encefalitis). Postoje i slučajevi sekundarnog parkinsonizma nakon hipoksije mozga ili izloženosti zračenju. Do sekundarnog parkinsonizma može doći i nakon izlaganja određenim toksinima poput pesticida, herbicida, različitih industrijskih hemikalija (rotenon, parakvat), zatim mangana, ugljen-monoksida ili narkotika kao što su različiti derivati amfetamina. Posebno je zanimljiv sekundarni parkinsonizam indukovan 1-metil-4-fenil-1,2,3,6-tetrahidropiridinom (MPTP), koji je korišćen kao "sintetički heroin" od strane zavisnika početkom 1980-ih godina, od kojih su skoro svi razvili simptome parkinsonizma (Langston et al., 1983). MPTP je u narednim godinama našao široku primenu u eksperimentalnim modelima PB zbog selektivnog uništavanja DA neurona, a ovaj događaj je pokrenuo brojna ispitivanja uticaja raznih hemijskih supstanci i nutrijenata kako bi se pokazao njihov štetan ili protektivan značaj u patogenezi PB.

Sa druge strane, brojna istraživanja upućuju na genske mutacije i polimorfizme koji ili direktno dovode do parkinsonizma sa različitim kliničkim manifestacijama ili povećavaju rizik od njegovog razvoja. Do danas identifikovani geni povezani sa klasičnim idiopatksim parkinsonizmom sa autozomno-dominantnim nasleđivanjem su PARK-*SNCA,* PARK-*LRK2,* PARK-*VPS35,* dok su mutacije u PARK-*Parkin,* PARK-*PINK1,* PARK-*DJ1,* PARK-*ATP13A2,* bilo u formi homozigota ili složenog heterozigota povezane sa autozomno-recesivnim nasleđivanjem PB ranog početka (Marras et al., 2016).

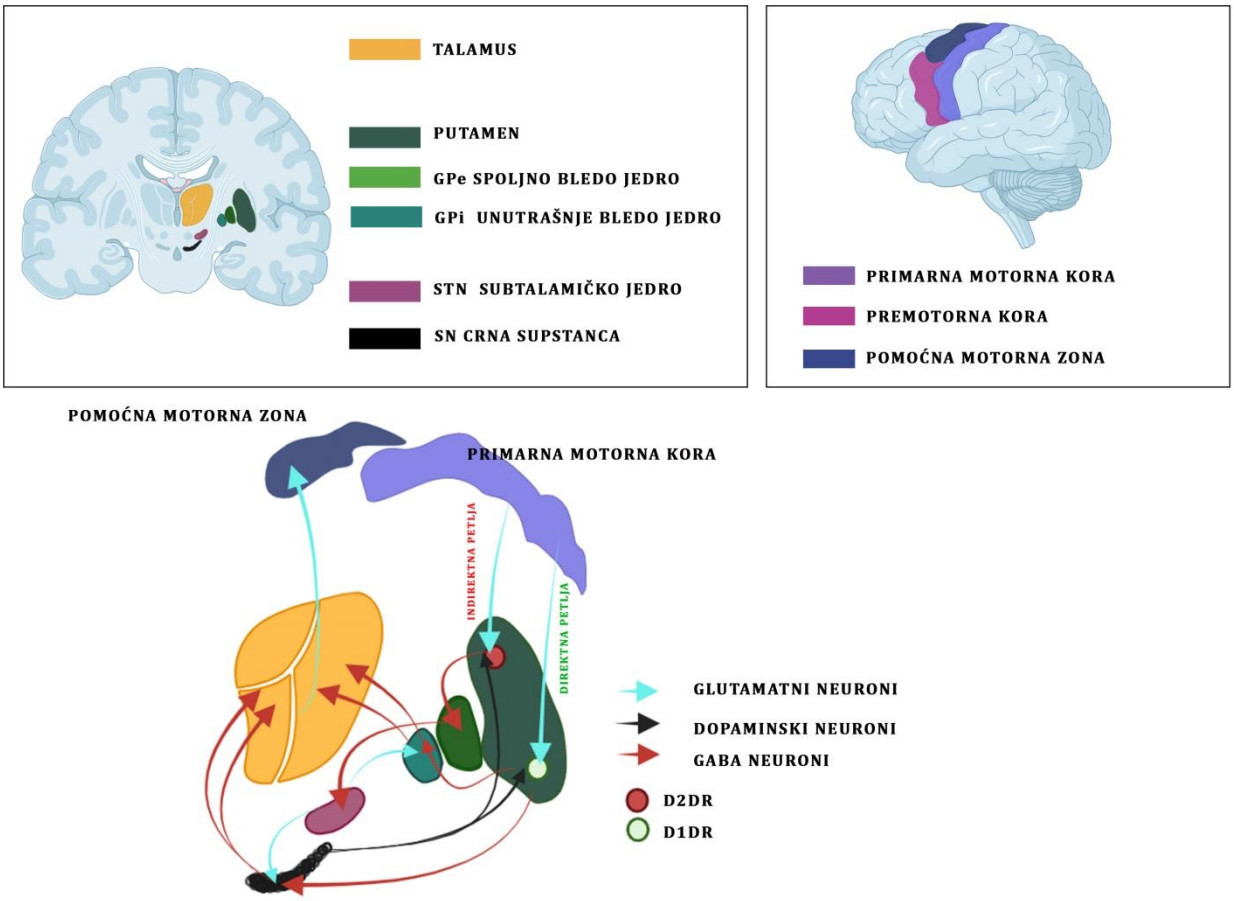
1.1.3. Hipoteze o patogenezi Parkinsonove bolesti

Razumevanje patofizioloških mehanizama PB je ključno za razjašnjenje etiologije i dinamike ovog kompleksnog neurodegenerativnog stanja, čime se otvara put ka inovacijama u

terapijskim pristupima i unapređenju kvaliteta života obolelih. Ključna patološka karakteristika PB je gubitak DA neurona u regionu crne supstance (lat. *substantia nigra pars compacta*, SNpc) (Kalia & Lang, 2015). Crna supstanca ili crno jedro (pored SNpc čini je i SNpr, lat. *substantia nigra pars reticulata*) je struktura srednjeg mozga koja zajedno sa crvenim jedrom srednjeg mozga (lat. *nucleus ruber*) i subtalamičkim jedrom (lat. *nucleus subthalamicus,* STN) međumozga predstavlja strukture odnosno jedra pridružena bazalnim ganglijama koja su funkcijski povezana sa njima i učestvuju u inicijaciji i odigravanju voljnih pokreta i istovremenom inhibiranju neželjenih pokreta koji bi mogli da ometaju pravilno izvođene isplaniranih pokreta (Lanciego et al., 2012). Pored pridruženih, postoje i glavna jedra koja sačinjavaju bazalne ganglije i tu spadaju strijatum (repato jedro (lat. *nucleus caudatus*) i ljuska (lat. *putamen*)) i bledo jedro (lat. *globus palidus external* i *globus palidus internal*; GPe i GPi). Bazalne ganglije i njima pridružene strukture su deo neuronske mreže (motorna petlja) koja je funkcijski podeljena na dva dela koja se označavaju kao direktni i indirektni put. Shematski prikaz pojednostavljene neuronske mreže uključene u patofiziologiju PB prikazan je na Slici 1. Ekscitatorna vlakna koja na svojim krajevima oslobađaju glutamat se projektuju iz talamusa do pomoćne zone motorne kore (eng. *supplementary motor area*; SMA), kao i iz motorne kore do putamena, i od STN do GPi i SN. Sa druge strane inhibitorna vlakna koja na svojim krajevima oslobađaju GABA se projektuju iz putamena do GPe i SN, kao i do GPi i dalje do talamusa. DA neuroni se projektuju iz SN do strijatuma, čime se formira nigrostrijatalni put. Suština je da talamokortikalne projekcije ove neuronske mreže moraju biti oslobođene inhibicije za inicijaciju pokreta (DeLong & Wichmann, 2007). U direktnom putu GABA neuroni strijatuma koji većinski eksprimiraju dopaminske D1 receptore (D1R), primaju ekscitatorne signale iz motorne kore i projektuju se na GPi i SN. GABA neuroni iz GPi inhibiraju GABA neurone koji se projektuju na talamus. Na taj način izostanak inhibicije glutamatnih neurona iz talamusa koji se projektuju na SMA omogućava voljne pokrete. Inače, mehanizam prenosa signala posredovan dopaminskim D1R koji je metabotropni receptor spregnut sa proteinom G_s se odvija putem aktivacije adenil-ciklaze, porasta cikličnog AMP-a (cAMP) i aktivacije protein kinaze A, koja dalje fosforiliše ciljne proteine. U indirektnom putu ekscitatorni stimulusi od kortikalnih glutamatnih neurona aktiviraju GABA neurone strijatuma koji eksprimiraju dopaminske D2 receptore (D2R). Ovi neuroni inhibiraju GABA neurone GPe koji se projektuju na STN, dezinhibirajući ovaj put. STN ima glutamatne projekcije na SN i GPi, što rezultira aktivacijom GABA neurona koji se projektuju na talamus. Inhibicija neurona talamusa rezultira inhibicijom glutamatnih neurona koji se projektuju na SMA, sprečavajući neželjene mišićne kontrakcije koje bi ometale voljne pokrete. Dopaminski D2R su takođe metabotropni receptori, ali spregnuti sa proteinom G_i, kod kojih se prilikom aktivacije dalji prenos signala odvija putem inhibicije adenil-ciklaze i smanjenom sintezom cAMP. U fiziološkim uslovima oslobađanje dopamina u strijatumu aktivira odnosno povećava verovatnoću aktivacije neurona koji eksprimiraju D1 receptore kroz posledičnu depolarizaciju, i nasuprot tome inhibira odnosno smanjuje verovatnoću aktivacije neurona koji eksprimiraju D2 receptore (Charles R Gerfen, 2022). U patološkim uslovima, manje dopamina dovodi do smanjenja aktivnosti direktnog puta i do povećane aktivnosti indirektnog puta. Dakle, neurodegeneracija SNpc pored posledične degeneracije nigrostrijatalnih projekcija dovodi i do smanjenja nivoa dopamina u strijatumu, čime se menja ravnoteža između ekscitatornih i inhibitornih puteva u bazalnim ganglijama što dalje dovodi do kompleksnih motornih poremećaja, rigidnosti, tremora, bradikinezije i posturalne nestabilnosti, koji su najprepoznatljivije kliničke manifestacije PB.

U trenutku pojave prvih motornih simptoma u regionu SNpc je već došlo do gubitka od 50% neurona i nivoi dopamina su redukovani za više od 80% u odnosu na fiziološke vrednosti (Dauer & Przedborski, 2003). Međutim, PB podrazumeva i patološke promene u drugim neurotransmiterskim sistema (acetil-holinskom, serotoninskom i noradrenalinskom) zbog

čega se danas opisuje kao multisistemska neurodegenerativna bolest. Danas je poznato da se u sklopu bolesti značajno pre motornih mogu javiti i brojni nemotorni fenomeni kojima se pridaje sve veći značaj i smatra se da je njihova pojava upravo uzrokovana disfunkcijom pomenutih neurotransmiterskih sistema. U tom kontekstu u okviru prodromalne faze bolesti pominju se autonomni poremećaji poput redukovane olfaktorne funkcije, konstipacije, poremećaji REM faze spavanja, prekomerna pospanost tokom dana, kao i poremećaji u ponašanju koji uključuju depresiju, anksioznost i apatiju. Kognitivni poremećaji koji uključuju poremećaje pažnje i koncentracije, poremećaje u učenju i pamćenju, demencija, urinarna inkontinencija, vizuelne halucinacije i deluzije javljaju se u kasnijim fazama bolesti, uz već ispoljene prepoznatljive motorne simptome (Armstrong & Okun, 2020; Kalia & Lang, 2015).

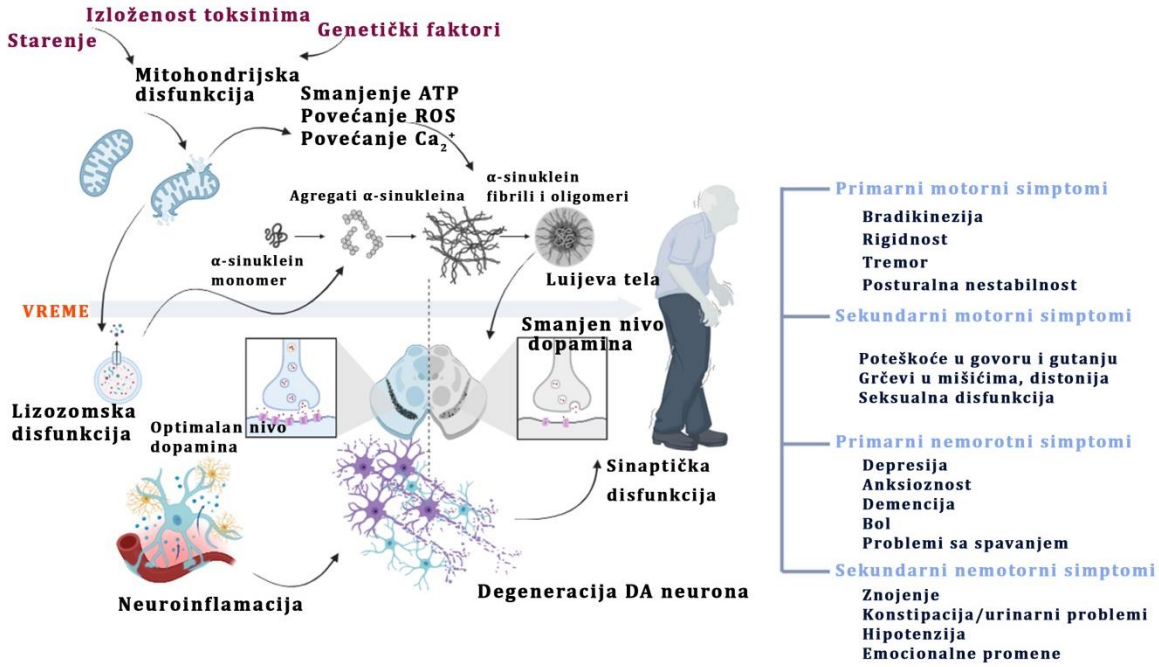


Slika 1. Strukture bazalnih ganglija uključene u patofiziologiju PB

Još jedno karakteristično obeležje PB je akumulacija proteina α-sinukleina u vidu citoplazmatskih inkluzija označenih kao Luijeva tela (eng. *Lewy bodies*, unutar tela neurona) i Luijevi neuriti (eng. *Lewy neurites*, unutar dendrita ili aksona) što ovu bolest svrstava i u grupu proteinopatija odnosno sinukleinopatija (Alafuzoff & Hartikainen, 2017). U fiziološkim uslovima uloga α-sinukleina nije u potpunosti razjašnjena, ali ono što je poznato je da je prisutan u presinaptičkim završecima neurona i igra ulogu u regulaciji oslobađanja neurotransmitera, sinaptičkoj plastičnosti, regeneraciji aksona i regulaciji biosinteze dopamina (Vasquez et al., 2020; Zhu et al., 2022). Proces patološke transformacije α-sinukleina, kojeg karakteriše nestabilna osnovna struktura te lako zadobija različite konformacije, počinje od neuvijenih monomera, koji prelaze u delimično savijene strukture, a zatim se oligomerizuju u male, rastvorljive agregate. Ovi prolazni intermedijarni oligomeri predstavljaju potencijalno najtoksičniji oblik α-sinukleina koji uzrokuje citotoksičnost,

uključujući mitohondrijsku disfunkciju, abnormalnu kalcijumsku signalizaciju i proizvodnju reaktivnih kiseoničnih vrsta (eng. *reactive oxygen species*; ROS) (Choi et al., 2022). Postepeno, oligomeri prelaze u strukturu β-nabrane ploče, formirajući stabilnije i nerastvorljive fibrile, koji se akumuliraju unutar neurona kao Luijeva tela. Proteomske studije pokazale su da Luijeva tela sadrže više od 300 proteina i da se pored kvantitativno najzastupljenijieg α-sinukleina u njihovom sastavu mogu naći i komponentne ubikvitin-proteazomne mašinerije za razgradnju proteina, neurofilamenti, šaperoni, alfa-tubulin, sinfilin-1, gliceraldehid 3-fosfat dehidrogenaza, kao i proteini povezani sa oštećenjem aksona (Wakabayashi et al., 2013). Međutim, pokazano je i da pomenuti fibrilarni agregati koji ulaze u sastav Luijevih tela nemaju direktan toksičan uticaj, te jos uvek nije razjašnjeno da li su Luijeva tela ono sto doprinosi oštecenju neurona, ili (verovatnije) formiranje ovih agregata predstavlja mehanizam da ćelija solubilne oligomere "upakuje" tako da njihova toksicnost bude manja (Shahmoradian et al., 2019). Do nakupljanja ovog proteina može da dođe usled pojave različitih mutacija u *SNCA* genu i/ili usled poremećene funkcije ćelijskih sistema za uklanjanje i razgradnju neadekvatno sintetisanih, oštećenih ili izmenjenih proteina (Nianping Zhang et al., 2024). Pojava inkluzija, kao i njihova progresija ima određenu prostornu i vremensku pravilnost prema Braak hipotezi i naizgled objašnjava klinički tok PB (Braak et al., 2003). Prema tom sistemu PB počinje u strukturama koje pripadaju olfaktornom sistemu i produženoj moždini (faza 1) odakle se ushodno širi ka strukturama moždanog stabla (faze 2-3), da bi u kasnijim fazama (faze 4-6) zahvatila i bazane ganglije, strukture limbičkog sistema kao i više regione kore velikog mozga. Veza između narušenog odnosa sinteze, modifikacije i razgradnje ovog proteina i hipoteze o neurodegeneraciji SNpc nije jasna u potpunosti, ali je važno napomenuti da patološko taloženje α-sinukleina počinje rano u životu i može pokrenuti nastajanje PB (Stefanis, 2012).

Degeneracija DA neurona SNpc-a i taloženje α-sinukleina su dva klasična i najvažnija patofiziološka obeležja PB međutim, svoju ulogu u patogenezi PB igraju i drugi složeni ćelijski procesi uključujući disfunkciju mitohondrija i lizozoma, ali i oksidativni stres i neuroinflamaciju, kao i i promene na genima (mutacije i polimorfizmi, koje mogu dovesti do bilo kog od pomenutih oštećenja). Ključnu ulogu u neuroinflamaciji ima aktivacija glijskih ćelija – astrocita i mikroglije, koje u zavisnosti od patofenotipa mogu da ostvaruju pro- ili antiinflamacijske efekte i time utiču na proces neurodegeneracije u PB. Jedan od presudnih faktora u modulaciji aktivnosti mikroglije i astrocita, a time i neuroinflamacije predstavlja purinska signalizacija. Detaljan prikaz razmatranih karakterističnih patoloških obeležja PB dat je na Slici 2.



Slika 2.Ključne patološke karakteristike PB

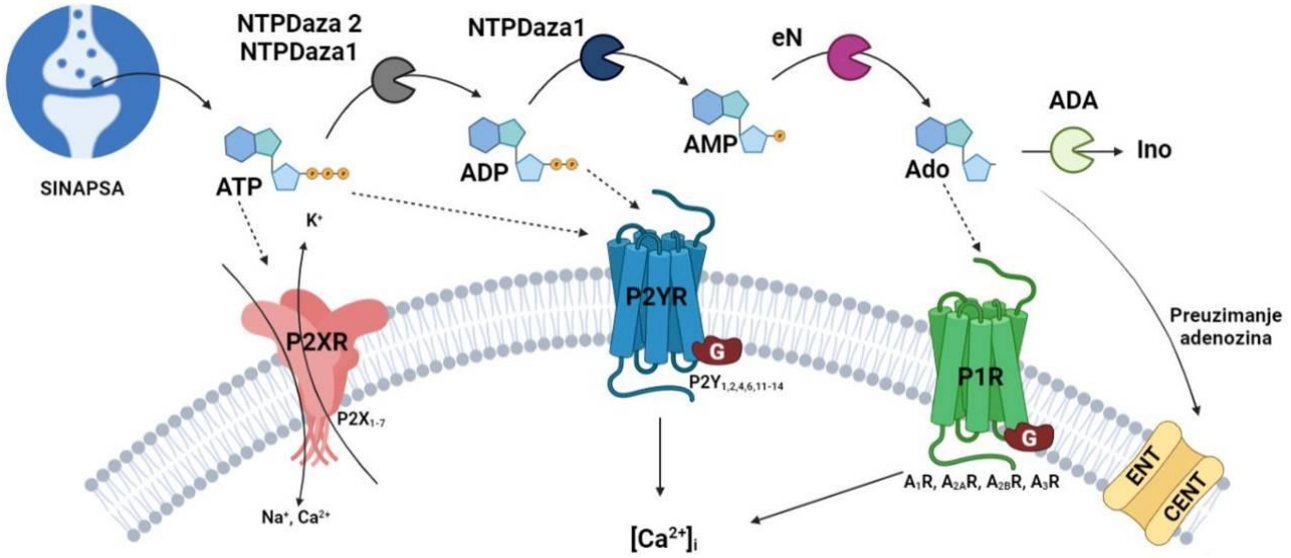
1.2. Uloga komponenti purinskog sistema u patogenezi Parkinsonove bolesti

1.2.1. Komponente purinskog sistema

Koncept purinske signalizacije, koji podrazumeva da adenozin 5'-trifosfat (ATP) ne služi samo kao univerzalna valuta energetskog metabolizma u ćelijama već kao i signalni molekul, predložen je početkom 1970-ih godina prošlog veka (Burnstock et al., 1970; Burnstock, 1972). Nekoliko godina kasnije, purini su opisani i kao ko-transmiteri i neuromodulatori u perifernom (PNS) i centralnom nervnom sistemu (CNS), budući da mogu da deluju zajedno sa drugim neurotransmiterima (acetilholinom, kateholaminima, γ-aminobuternom kiselinom (GABA) i glutamatom), kao i da utiču na njihovo oslobađanje, signalnu transmisiju i uopšteno na neuronsku aktivnost (G Burnstock, 1997). U fiziološkim uslovima, količina ATP u vanćelijskoj sredini regulisana je kontrolisanim oslobađanjem ATP putem vezikula, transportera ili jonskih kanala i njegovom razgradnjom posredstvom posebne klase enzima čije se aktivno mesto nalazi okrenuto ka vanćelijskoj sredini te se označavaju i kao ektonukleotidaze (Zimmermann, 2006). U patološkim uslovima poput neurodegenerativnih i inflamacijskih stanja ravnoteža između oslobađanja i razgradnje ATP u vanćelijskom prostoru je narušena i ATP iz fizioloških nanomolarnih koncentracija prelazi u mikromolarne koncentracije i deluje kao DAMP molekul (molekulski obrasci povezani sa oštećenjem, eng. *damage-associated molecular patterns* – DAMP) (Tanaka et al., 2014). ATP može da aktivira svoje P2 receptore, dok finalni produkt razgradnje ATP – adenozin aktivira adenozinske P1 receptore (Rodrigues et al., 2015).

Purinski membranski receptori obuhvataju dve podfamilije, P1 receptore koji vezuju nukleozide i P2 receptore koji vezuju nukleotide (Slika 3.). P1 receptori su metabotropni receptori spregnuti sa proteinom G i obuhvataju dva podtipa: inhibitorne A_1R i A_3R spregnute sa G_i proteinom i inhibicijom adenil-ciklaze i stimulatorne $A_{2A}R$ i $A_{2B}R$, spregnute sa G_s proteinom i stimulacijom adenil-ciklaze (Fredholm et al., 2011). P2 receptori obuhvataju dve klase: jonotropne ATP-zavisne P2X (P2X1-7R) receptore i metabotropne P2Y receptore, koje aktivira ATP ($P2Y_{1,2,4,11}$), ADP ($P2Y_{2,6,12,13}$), uridin di- i trifosfatom ($P2Y_{4,6,11}$) i UDP-glukoza ($P2Y_{14}$) (Burnstock, 2014). P1 receptori su distribuirani u različitim kortikalnim i

subkortikalnim strukturama mozga. Adenozinski A_1R pokazuje visoku ekspresiju u gotovo svim regionima mozga dok je $A_{2A}R$ visoko eksprimiran u strijatumu, zatim nešto manje u talamusu, ali i hipokampusu, što ih čini zanimljivim kandidatima u pogledu istraživanja PB. Za razliku od njih, $A_{2B}R$ i A_3R receptori se u fiziološkim uslovima slabo eksprimirani u CNS (Dixon et al., 1996; Sebastião & Ribeiro, 2009). Distribucija P2 purinskih receptora u CNS-u je takođe veoma heterogena i većina P2X i P2Y receptora je visoko eksprimirana u strijatumu i SN (Amadio et al., 2007).



Slika 3. Prikaz komponenti purinskog signalnog sistema

Pored pomenutih receptora važnu ulogu u purinskoj signalizaciji igraju i membranski nukleotid/nukleozidni transporteri i ektonukleotidaze. Dostupnost nukleotida kao vanćelijskih liganada purinskih receptora zavisi od enzimske aktivnosti ektonukleotidaza, koje katalizuju sekvencijalnu razgradnju ATP→ADP→AMP→adenozin. Četiri glavne porodice ektonukleotidaza razgrađuju nukleotide u vanćelijskom prostoru: ektonukleozidtrifosfat difosfohidrolaze (NTPDaze), ekto-5' nukleotidaza (eN, CD73), ektonukleotid pirofosfataze /fosfodiesteraze (NPP) i alkalne fosfataze (AP) (Yegutkin, 2008; Zimmermann et al., 2012). NTPDaze i NPP hidrolizuju ATP i ADP do AMP. Od ukupno osam izoformi NTPD-aza, tri se eksprimiraju u CNS-u i to NTPDaza1, 2 i 3. Ove NTPDaze imaju selektivnu tkivnu lokalizaciju i različit afinitet prema ATP i ADP (Zimmermann et al., 2012). AP katalizuje hidrolizu purin- i pirimidin 5'-tri-, di- i monofosfate (NMP) do odgovarajućeg nukleozida. CD73 hidrolizuje nukleozid monofosfate do nukleozida, i to u najvećoj meri AMP do adenozina. Adenozin se razgrađuje deaminacijom do inozina pomoću adenozin deaminaze ili se vraća u ćelije i uključuje u spasonosni put purina, a transport u ćelije se odvija olakšanom difuzijom posredstvom dvosmernih ekvilibrativnih (ENT) i/ili aktivnim transportom putem koncentrativnih (CNT) transportera (Frenguelli, 2019).

Zajedničkim delovanjem ove komponente purinskog sistema omogućavaju finu regulaciju različitih fizioloških procesa, od neurotransmisije, sinaptičke plastičnosti i neurogeneze, preko imunskog odgovora sve do ćelijske smrti i regeneracije. Razumevanje mehanizama ovog složenog sistema otvara vrata za nove terapijske pristupe u lečenju različitih patoloških stanja, uključujući i neurodegenerativne bolesti.

1.2.2. Uloga P1 receptora u Parkinsonovoj bolesti

Visoka ekspresija adenozinskih A_1R i $A_{2A}R$ u strijatumu ukazuje na njihovu važnu ulogu u kontroli pokreta. A_1R inhibiraju adenil-ciklazu i smanjuju nivo cAMP-a. U strijatumu A_1R i

dopaminski D1R se poglavito eksprimiraju na GABA neuronima direktnog puta, dok se $A_{2A}R$ i D2R eksprimiraju na neuronima indirektnog puta. A_1R i D1R formiraju heterodimere (A_1R-D1R), u kojima aktivacija A_1R negativno moduliše D1R smanjenjem afiniteta prema dopaminu, što rezultuje hipokinezijom (Cortés et al., 2019). Ovakav efekat aktivacije A_1R ukazuje da bi primena agonista ovog receptora mogla biti korisna u smanjenju komplikacija povezanih sa hroničnom upotrebom levodope (Mango et al., 2014). Značajnu pažnju privukla je uloga $A_{2A}R$ u patologiji PB. Aktivacija $A_{2A}R$ osim povećanja nivoa cAMP, aktivira fosfolipazu C (PLC) i posledično povećanje unutarćelijske koncentracije kalcijuma ($[Ca^{2+}]_i$). $A_{2A}R$ i D2R formiraju funkcionalne heterodimere ($A_{2A}R$-D2R) na GABA neuronima indirektnog puta. Aktivacija $A_{2A}R$ adenozinom, čija je koncentracija povećana kod pacijenata sa PB, povećava verovatnoću aktivacije neurona indirektne petlje i u odsustvu dopamina, te se prilikom izvođenja pokreta i ova petlja aktivira što dovodi do pojave tremora i neželjenih pokreta (Ferre et al., 1991). Povećano prisustvo $A_{2A}R$-D2R heterodimera detektovano je *post mortem* analizama u moždanom tkivu pacijenta sa PB u odnosu na kontrolne ispitanike (Fernández-Dueñas et al., 2019). Povećana ekspresija $A_{2A}R$ detektovana je i u eksperimentalnim modelima PB (Calon et al., 2004; Carmo et al., 2019; Morissette et al., 2006). Veliki broj studija pokazuje pozitivne efekte antagonista $A_{2A}R$ u pojačavanju terapijskog efekta levodope u tretmanu PB, kako u eksperimentalnim modelima tako i u kliničkim ispitivanjima. Istradefilin (Nouriast®) je trenutno prvi odobreni A_{2A} antagonista koji se primenjuje kao dopuna terapije uz levodopu u lečenju pacijenata sa PB (J.-F. Chen & Cunha, 2020; Dungo & Deeks, 2013).

1.2.3. Uloga P2 receptora u Parkinsonovoj bolesti

Topografska analiza pokazala je da su P2 jonotropni (P2X1–7) i metabotropni ($P2Y_{1,2,4,6,11–14}$) receptori veoma zastupljeni u bazalnim ganglijama, a posebno u strijatumu i SN (Amadio et al., 2007). Takođe, ista studija je pokazala da dolazi do značajnog menjanja ekspresije P2X i P2Y receptora na DA i GABA neuronima u eksperimentalnom modelu PB, što potvrđuje uključenost P2 receptora i eATP u patofiziologiji ove bolesti. Do sada najviše ispitivan u kontekstu PB je P2X7 receptor. Ovaj receptor se aktivira pri visokim koncentracijama eATP, što dovodi do aktivacije inflamazoma u mikrogliji i astrocitima, aktivacije kaspaze 1 i posledičnog oslobađanja IL-1β (Di Virgilio et al., 2017; Janks et al., 2018; Savio et al., 2018). Produžena aktivacija P2X7R i kaspaza može da započne apoptotsku kaskadu. Primena antagonista P2X7R (A-438059; Brilliant Blue G (BBG)) u neurotoksičnom modelu PB izaziva povoljne efekte, u vidu održavanja dopamina blizu koncentracije prisutne u homeostazi, povećanog preživljavanja DA neurona, smanjenja aktivacije mikroglije, kao i smanjenja amfetaminom indukovanog rotacionog kretanja koje je karakteristično za ovaj model PB (Carmo et al., 2014; Marcellino et al., 2010; Oliveira-Giacomelli et al., 2019).

U svetlu ovih podataka i *in vitro* studije su pokazale da antagonisti P2X7 receptora mogu da imaju neuroprotektivne efekte u PB. BV2 mikroglijske ćelije prethodno tretirane primenom BBG imaju manju produkciju ROS u prisustvu α-sinukleina (T. Jiang et al., 2015). S druge strane, ATP-zavisni P2X4 receptori povećavaju oslobađanje IL-6 i povećavaju degeneraciju DA neurona, dok supresija P2X4R rezultira povećanim oslobađanjem BDNF-a i povećanom fosforilacijom TrkB, što se dovodi u vezu sa povećanim preživljavanjem neurona (Jiangnan Ma et al., 2020; X. Zhang et al., 2021). Neki od P2Y receptora, poput $P2Y_6$, uključeni su u regulaciju fagocitoze mikroglije, što zavisno od konteksta i stadijuma bolesti može imati dvostruki uticaj na neurodegeneraciju. Opisana je i uloga $P2Y_{13}$ receptora u aktivaciji mikroglije i smanjenog oslobađanja IL-1β, što ovaj receptor čini zanimljivim za dalja istraživanja (Kyrargyri et al., 2020). Brojni literaturni podaci ukazuju na značaj purinske signalizacije, čije su komponente moguće mete farmakološkog delovanja u tretmanu PB. Blokada A_{2A}, P2X1, P2X7 i $P2Y_6$ receptora već predstavlja obećavajući prustup u lečenju PB na različite načine: smanjenjem diskinezije izazvane levodopom, uticajem na neuroinflamaciju,

sprečavanjem agregacije α-sinukleina i smanjenjem aktivacije mikroglije. Međutim, znanje o učešću ostalih P1/P2 receptora još uvek je u najvećoj meri nedovoljno, te su neophodna dalja istraživanja za razjašnjavanje njihove uloge u PB.

1.3. Uloga komponenti glutamatnog sistema u patogenezi Parkinsonove bolesti

1.3.1. Receptori i transporteri za glutamat

Glutaminska kiselina, neesencijalna aminokiselina čije su soli i karboksilati poznati kao glutamati, je najzastupljeniji ekscitatorni neurotransmiter u CNS-u. Iako je u patofiziologiju PB uključeno nekoliko neurotransmiterskih sistema, pre svega dopaminski, glutamat čini oko 80% ukupne količine svih oslobođenih neurotransmitera u nivou bazalnih ganglija, što ukazuje na izuzetno važnu ulogu u regulaciji motorne petlje (F Blandini et al., 1996). Glutamat ima ulogu u sinaptičkoj plastičnosti, učenju, memoriji, kao i u drugim kognitivnim funkcijama. Glutamat ostvaruje efekte posredstvom jonotropnih (iGluR) i metabotropnih receptora (mGluR). iGluR su glutamat-zavisni jonski kanali za katjone i odgovorni su za brzu ekscitatornu transmisiju, dok mGluR receptori izazivaju spore i dugotrajne promene u sinaptičkoj aktivnosti. iGluR obuhvataju NMDR receptore osetljive na N-metil-D-aspartatnu kiselinu, AMPA receptore osetljive na α-amino-3-hidroksi-5-metil-4-izoksazolpropionsku kiselinu i KA receptore osetljive na kainsku kiselinu. Identifikovano je osam podtipova mGluR koji se klasifikuju u tri grupe na osnovu strukture, funkcije i unutarćelijske signalizacije. Grupa I obuhvata mGluR1 i mGluR5, koja aktivira PLC-zavisno oslobađanje fosfatidil-inozitola i povećanje Ca^{2+}, dok grupa II obuhvata mGluR2 i mGluR3, koji deluju posredstvom inhibicije adeni-ciklaze (Neyman & Manahan-Vaughan, 2008). Glutamat oslobođen u sinapsi se preuzima kroz stopala sinaptičkih astrocita posredstvom EAAT transportera, dok se unutar nervnih ćelija transportuje u sinaptičke vezikule posredstvom Na^{+}-zavisnog transportera (vGLUT). Budući da je glutamat centralni posrednik između metabolizma glukoze i amino-kiselina, on se transportuje i u mitohondrije posredstvom H^{+}-zavisnog glutamatnog transportera ili glutamat/aspartat izmenom (Vandenberg & Ryan, 2013). Transporteri ekscitatornih amino-kiselina lokalizovani su na membrani i omogućavaju brzo preuzimanje glutamata iz sinaptičke pukotine što predstavlja značajan proces u sprečavanju efekata ekscitotoksičnosti, zaštiti ćelija od oksidativnog stresa, i regulisanju unutarćelijske koncentracije glutamata. Narušena funkcija EAAT je uočena u brojnim patološkim stanjima, uključujući PB (Zhang et al., 2016).

NMDA receptori bili su predmet intenzivnog proučavanja i najbolje su okarakterisani među jonotropnim receptorima. NMDA receptori su heterotetramerni kompleksi sačinjeni od dve konstitutivno ekspimirane NR1 subjedinice i najmanje jedne subjedinice tipa NR2 (A-D) ili NR3 (A-B) (Paoletti et al., 2013). Svaka subjedinica utiče na elektrofiziološka i farmakološka svojstva receptora i definiše prirodu signalne transdukcije posredovane NMDA receptorom (Götz et al., 1997). Sastav NMDAR subjedinica se menja tokom razvoja i u zavisnosti od aktivnosti neurona (Paoletti & Neyton, 2007). Brojna istraživanja ukazuju da su NMDA receptori sačinjeni od GluN1/GluN2A/GluN2B subjedinica uključeni u LTP mehanizam plastičnosti, mehanizam koji se odnosi na povećanje jačine i funkcije sinapse (eng. *long term potentiation*; LTP) (D. L. Hunt & Castillo, 2012; Ladagu et al., 2023; Shipton & Paulsen, 2014). Promene regulacije glutamatnih receptora se dešavaju u različitim neurodegenerativnim bolestima uključujući PB, pa je razumevanje mehanizama njihovog delovanja važno u kontekstu patofiziologije i neuroprotekcije.

1.3.2. Narušena glutamatna homeostaza u Parkinsonovoj bolesti

Veliki broj literaturnih podataka upućuje na važnu ulogu glutamatne signalizacije u razvoju PB, kao i levodopom-indukovanih diskinezija (Pagonabarraga et al., 2021; Z. Zhang et

al., 2019). Otkriveno je da je nivo glutamata u serumu pacijenata obolelih od PB značajno viši nego kod zdravih ispitanika (Figura et al., 2018; Iwasaki et al., 1992). Kliničke studije primenom magnetne rezonance (MRI) ili pozitronske emisione tomografije (PET) ukazuju na povećani sadržaj glutamata u mozgu PB pacijenata (Gröger et al., 2014; O'Gorman Tuura et al., 2018). Na koji način promene u nivou glutamata utiču na proces neurodegeneracije u PB još uvek nije jasno. Jedna od mogućnosti je da, sa napredovanjem bolesti, kontinuirana ekscitacija glutamatnih neurona u SNpc uzrokovana disinhibicijom STN može dodatno promovisati gubitak DA neurona kroz mehanizme glutamatne ekscitotoksičnosti, što su pokazale neke od studija sprovedenih na eksperimentalnim modelima PB. Pre svega, ranije studije su pokazale povećane vanćelijske koncentracije glutamata u strijatumu i SNpc-u u eksperimentalnim modelima PB čak i mesec dana od izazivanja lezije, odnosno pokretanja procesa degeneracije DA neurona (Ingham et al., 1998; G E Meredith et al., 2009; Meshul et al., 1999). Kvantitativan odnos subjedinica GluN2A/GluN2B NMDA receptora u strijatumu pacova i majmuna sa diskinezijom izmenjen je u odnosu na kontrolnu grupu, ali i u *post mortem* tkivu pacijenata sa PB koji su imali diskineziju (Mellone et al., 2015). Pokazano je i da pacovi u eksperimentalnom modelu PB ispoljavaju povećanu ekspresiju GluN2B, a da su nasuprot tome nivoi GluN1 smanjeni (Gan et al., 2014). Nedavna studija takođe je ukazala na uključenost i GluN2D podjedinice s obzirom na njeno značajno povećanje u strijatumu kod pacova sa PB tretiranih levodopom (Mellone et al., 2019). Na osnovu svega navedenog jasno je da ponovno uspostavljanje glutamatne homeostaze ima veliki potencijal u tretmanu PB, ali i motornih komplikacija koje nastaju kao posledica trenutno primenjivanih farmakoloških agenasa za nadoknadu dopamina.

U poslednjoj deceniji, nekoliko studija istraživalo je korisne efekte antagonista NMDA receptora u eksperimentalnim modelima PB (Löschmann et al., 2004; J E Nash et al., 1999; Wessell et al., 2004) i generalno postoji saglasnost da blokada NMDA receptora može da poboljša motorne simptome i indukovane diskinezije nakon primene levodope. Međutim, iako su pretkliničke studije pokazale neki nivo efikasnosti, klinička istraživanja su i dalje daleko od konačnog cilja zbog brojnih neželjenih efekata i činjenice da je nemoguće selektivno delovati samo na NMDA receptore u strukturama od interesa. Trenutno je jedino u upotrebi amantadin, umeren NMDA antagonista, odobren za tretman diskinezija (Wang et al., 2022). Amantadin povećava oslobađanje dopamina i/ili inhibira njegovo preuzimanje, a u velikoj retrospektivnoj studiji koja uključuje 836 pacijenata sa PB, amantadin je uticao na produženje životnog veka pacijenata, što sugeriše na njegova neuroprotektivna svojstva kroz antagonizam NMDA receptora, ali nedostaje mu dobro definisan mehanizam delovanja (Uitti et al., 1996). Jedan od razloga zašto je većina drugih NMDA antagonista sa obećavajućim pretkliničkim rezultatima nije dala rezultate u kliničkim ispitivanjima je zbog brojnih neželjenih efekata, pošto potpuni antagonizam glutamatnih receptora može izazvati neželjene kognitivne efekte. Istraživanja su se zatim fokusirala na selektivne antagoniste određenih subjedinica NMDA receptora kako bi se postigli anti-parkinsonski efekti sa smanjenjem neželjenih efekata. To bi olakšalo očuvanje ćelija tokom ekscitotoksičnih procesa ne uzrokujući potpunu inhibiciju receptora i time omogućavajući fiziološku neurotransmisiju. NR2B-selektivni antagonisti činili su se pogodnim za ovu svrhu. Međutim, nedavna studija o MK-0657, selektivnom antagonisti NR2B subjedinice sugerisala je da jedna doza MK-0657 nije poboljšala motorne simptome kod PD pacijenata, a ni levodopom indukovane diskinezije (Herring et al., 2017).

1.4. Uloga komponenti oksidativnog stresa u patogenezi Parkinsonove bolesti

Oksidativni stres se smatra jednim od ključnih faktora u patogenezi PB koji pokreće kaskadu događaja koja dovodi do oštećenja proteina, lipida, DNK i drugih biomakromolekula, ćelijske disfunkcije i na kraju do ćelijske smrti. Smatra se da su prekomerno generisane ROS,

narušen metabolizma kateholamina, narušena funkcija mitohondrijskog elektron-transportnog lanca i povećano taloženja gvožđa u SNpc glavni izvori oksidativnog stresa (Hwang, 2013). ROS se neprekidno proizvode u svim ćelijama tokom prenosa elektrona u mitohondrijalnom respiratornom ciklusu, ali i u reakcijama u kojima je molekuleski O_2 direktni učesnik (supstrat) u reakciji. Međutim, oksidativni stres nastaje kada dođe do neravnoteže između proizvodnje ROS i antioksidativne aktivnosti ćelije. U ROS spadaju slobodni radikali kao što su superoksid anjon radikal ($O_2^{\bullet-}$), hidroksil-radikal ($OH^{\bullet}$), hidroperoksil-radikal ($HO_2^{\bullet}$), kao i neradikalski metaboliti kao što su vodonik peroksid (H_2O_2), hipohlorna kiselina (HOCl), organski peroksidi (ROOH) i singlet kiseonik ($^1O_2$) (Ramalingam & Kim, 2012). Sa druge strane antioksidativni molekuli imaju ulogu da onemoguće ili kontrolišu formiranje slobodnih radikala ili reaktivnih vrsta u ćeliji. U njih spadaju tri ključna enzima: superoksid dizmutaza (SOD), katalaza (CAT) i glutation peroksidaza (GPx) koji katališu reakciju dismutacije $O_2^{\bullet-}$ i razgrađuju H_2O_2 do „bezopasnih" molekula. Ovde takođe spadaju i proteini koji vezuju metale, kao što su transferin i ceruloplazmin koji vezuju gvožđe ili bakar i posledično onemogućavaju formiranje slobodnih radikala. Postoji i klasa antioksidanasa nazvana „sakupljači", koja pretežno uklanjanja oksidanse i tu spada glutation-S-transferaza (GST), askorbinska kiselina, mokraćna kiselina, tiol jedinjenja i drugi (Zehiroglu & Ozturk Sarikaya, 2019).

Dopamin sam po sebi može biti izvor reaktivnih vrsta (Segura-Aguilar et al., 2014). Za sintezu dopamina kao i drugih kateholamina (adrenalina i noradrenalina) neophodna je aminokiselina tirozin koja može biti transportovana u neurone ili pak sintetisana iz esencijalne aminokiseline fenilalanin. Enzim tirozin-hidroksilaza (TH) prevodi tirozin u dihidroksfenilalanin (DOPA), a dalju konverziju DOPA u dopamin katalizuje DOPA-dekarboksilaza (DDC), poznata i kao dekarboksilaza aromatičnih amino kiselina (AADC) (Wise, 2004). Sintetisan dopamin se transportuje i skladišti u sinaptičkim vezikulama posredtsvom vezikularnog transportera monoamina 2 (eng. *vesicular monoamine transporter 2*; VMAT2). Dopamin kao finalni produkt u DA neuronima može biti ponovo iskorišćen ili razgrađen nakon preuzimanja pomoću DAT-a, kako bi se minimizovala sinteza novih molekula dopamina radi uštede energije. Put za degradaciju dopamina sastoji se od dve enzimske reakcije katalizovane monoamin oksidazom (MAO-A/MAO-B) i katehol-O-metil transferazom (COMT), a krajnji produkt metabolizma dopamina je homovanilinska kiselina (HVA), jedinjenje koje se može identifikovati u urinu zdravih osoba (Franco et al., 2021). Primećeno je da nivo HVA u urinu opada kod pacijenata sa PB koji nisu na terapiji, dok raste kod pacijenata koji primaju levodopu (Marín-Valencia et al., 2008).

Dopamin podleže i procesima autooksidacije, pri čemu s uz prateće formiranje amonijaka i H_2O_2 prevodi do citotoksičnih hinona/semihinona (Sulzer & Zecca, 2000). Nastali hinoni mogu modifikovati niz proteina povezanih sa PB, kao što su α-sinuklein, parkin, DJ-1 i SOD2 (Belluzzi et al., 2012; da Silva et al., 2013; Girotto et al., 2012). Pokazano je i da uzrokuju inaktivaciju dopaminskog transportera (DAT) koji ponovo preuzima dopamin iz sinaptičke pukotine nazad u citosol, kao i inaktivaciju TH (Kuhn et al., 1999) i disfunkciju mitohondrijalnog kompleksa I (Jana et al., 2011). Konačni proizvod neenzimske degradacije citosolnog dopamina, aminohrom, je prekursora neuromelanina, pigmenta koji daje tamnu boju SN (Muñoz et al., 2012). Neuromelanin se akumulira u SN tokom procesa starenja. Unutarćelijski neuromelanin može imati neuroprotektivnu ulogu, štiteći ćelije od toksičnih efekata redoks aktivnih metala, posebno gvožđa, toksina i viška citosolnih kateholamina. Nasuprot tome, u patološkim uslovima akumulacija neuromelanina još je veća usled vezivanja neurotoksičnih agenasa što povećava rizik od oštećenja i smrti neurona, a neuromelanin koji oslobađaju umirući neuroni može doprineti aktivaciji glijskih ćelija koja pokreće neuroinflamaciju (Zucca et al., 2014).

Brojna istraživanja ukazuju na povećanje indikatora oksidativnog stresa u mozgu i cerebrospinalnoj tečnosti (CSF) kod osoba sa PB. *Post mortem* analize otkrile su značajno povećanje nivoa malondialdehida (MDA), sporednog proizvoda lipidne peroksidacije, u SN kod pacijenata sa PB (Dexter et al., 1989). Takođe, pokazano je i značajno smanjenje aktivnosti enzima ključnh za odbranu od štetnih efekata ROS (SOD, CAT i GST), kod pacijenata sa PB (Khan & Ali, 2018). Zbog svega navedenog jasan je razlog postojanja velikog broja istraživanja usmerenih na obnavljanje ravnoteže između ROS i antioksidativnih mehanizama kako bi se ublažio napredak i težina ove bolesti.

1.5. Neurodegeneracija izazvana 6-hidroksidopaminom

Upotreba eksperimentalnih modela je od velike važnosti u biomedicinskim istraživanjima, naročito u istraživanjima mehanizama nastanaka humanih bolesti. Ovi modeli omogućavaju istraživanja koja nisu moguća na pacijentima, doprinose razjašnjavanju i boljem razumevanju patofizioloških mehanizama bolesti, pružaju mogućnost za razvoj efikasnijih terapija i bolju prevenciju. Komplementarnost eksperimentalnih modela sa humanim bolestima, uključujući etiologiju, simptome, patofiziološke mehanizme i odgovor na terapiju, ključna je za njihovu validnost. Nažalost, većina *''in vivo''* i *''in vitro''* eksperimentalnih modela u najboljem slučaju zadovoljava samo deo ovih uslova. I pored svih napora da se pronađu idealni model organizmi i dalje ostaje pitanje do koje mere je moguće da se kompleksne humane bolesti predstave eksperimentalnim modelom.

Kada je u pitanju PB, eksperimentalni modeli se najčešće zasnivaju na dva metodološka pristupa: sistemska ili lokalna primena neurotoksina koji izaziva neurodegeneraciju specifične populacije neurona u određenom regionu ili upotrebu genetički modifikovanih životinja (transgene životinje). Oba pristupa imaju svoje specifične karakteristike, prednosti i ograničenja, a krajnji izbor zavisi od ciljeva istraživača.

Modeli neurotoksičnih lezija predstavljaju ujedno i najstarije i najčešće korišćene modele za izazivanje neuropatologije slične PB i mogu biti izazvani 6-hidroksidopaminom (6-OHDA) (Breit et al., 2001; J. Hunt et al., 2022; Rodríguez Díaz et al., 2001; Schwarting & Huston, 1996; Ungerstedt, 1968), 1-metil-4-fenil-1,2,3,6-tetrahidropiridinom (MPTP) (Blesa & Przedborski, 2014), kao i nekim pesticidima poput rotenona i parakvata (Grandi et al., 2018).

1.5.1. 6-hidroksidopamin

Prva demonstracija toksičnih efekata 6-hidroksidopamina (6-OHDA) zabeležena je pre više od 50 godina, kada je grupa naučnika pokazala da ovaj toksin kada se injektuje u SNpc može da izazove efikasnu i dugotrajnu degeneraciju nigrostrijatalnog dopaminskog sistema (Ungerstedt, 1968). 6-OHDA je strukturni analog dopamina koji se lako oksiduje i može biti preuzet putem DAT, NAT i SSRI (Chotibut et al., 2012). Budući da 6-OHDA ne prolazi krvno-moždanu barijeru (KMB), u modelima 6-OHDA-izazvane bolesti se aplicira intracerebralno u željenu strukturu. Strukture od interesa pri modelovanju PB su SNpc, medijalni snop prednjeg mozga (MBF), strijatum (Blesa & Przedborski, 2014) ili ređe komore (Rodríguez Díaz et al., 2001). Injekcija u SNpc (Jeon et al., 1995) ili MBF rezultira masivnom smrću DA neurona tokom nekoliko dana, dok injekcija u strijatum dovodi do uniformne degeneracije koja se razvija znatno sporije, tokom 1-3 nedelje (Kirik et al., 1998). Stoga je primena toksina u SNpc i MBF bolja sa stanovišta kasnijih faza PB, dok bi efekti primene toksina u strijatum mogli biti bliži događajima u početku bolesti.

Životinje sa unilateralnom lezijom koja je izazvana primenom 6-OHDA pokazuju prisustvo različitih motornih simptoma kao što su asimetrični poremećaji kretanja, uključujući akineziju i bradikineziju, kao i kontralateralne rotacije (izraženo kružno kretanje

prema strani suprotnoj od lezije). Takođe, životinje mogu pokazati i rigidnost i tremore pri većim dozama ili pri bilateralnoj mikroinjekciji (Glajch et al., 2012). Nemotorni simptomi koji se uočavaju nakon unilateralne 6-OHDA lezije, odnose se na učenje i pamćenje, ponašanje nalik depresivnom i anksioznom, kao i poremećaj olfaktorne funkcije i cirkadijalnog ritma (Masini et al., 2021; Meredith & Kang, 2006). Takođe, kao što je u ranijim poglavljima navedeno, 6-OHDA dovodi do promene u ekspresiji NMDA/AMPA receptora i sastava subjedinica kao i transportera za glutamat. Degeneracija izazvana 6-OHDA menja relativne nivoe GluN1 i GluN2B, bez uticaja na GluN2A (Dunah et al., 2000; Gan et al., 2014). Injekcija 6-OHDA u strijatum pacova dovodi do smanjenja ekspresije EAAT 1/2 u strijatumu, ali ne i u SNpc (Chung et al., 2008). U ovom modelu je i prenos signala posredovan AMPA receptorima bio pojačan, što je povećalo oslobađanje glutamata (Y. Chang et al., 2019). Povećano oslobađanje glutamata koje dalje pospešuje prenos signala posredovan GluN1/GluN2B, u kombinaciji sa smanjenim nivoom dopamina, dovodi do motornih deficita (Joanne E Nash & Brotchie, 2002). Ćelije koje su izložene 6-OHDA povećavaju oslobađanje vanćelijskog ATP, što dovodi do iscrpljivanja unutarćelijskog ATP što pojačava signalizaciju posredovanu P2X7R koja doprinosi razvoju PB patologije (M. Carmo et al., 2019). 6-OHDA model takođe rekapitulira glavne promene u $A_{2A}R$ posredovanoj signalizaciji (Gonçalves et al., 2023). U 6-OHDA modelu je zabeleženo povećanje nivoa adenozina kao i povećana ekspresija $A_{2A}R$ u strijatumu (Tomiyama et al., 2004). Takođe, pri intoksikaciji pacova sa 6-OHDA, oslobađanje GABA je povećano u GP, a sistemska primena istradefilina, antagoniste $A_{2A}R$, neutrališe ovo povećanje (Ochi et al., 2000). Zanimljiv je i zaključak jedne studije, u kojoj su životinje istovremeno tretirane levodopom i selektivnim antagonistom NR1A/2B subjedinica NMDA receptora, koji kaže da bi zapravo blokada NMDA receptora mogla da spreči razvoj diskinezija posredstvom $A_{2A}R$ jer dolazi do smanjenja njegove ekspresije (Morissette et al., 2006). 6-OHDA model verno reprezentuje određene aspekte patoloških promena karakterističnih za PB na nivou sinapsi – sinaptopatija, ali bez nakupljanja α-sinukleina i progresivne neuroinflamacije.

1.5.2. Mehanizam neurotoksičnog delovanja 6-OHDA

Oštećenja neurona izazvana delovanjem 6-OHDA uglavnom su posledica masivnog oksidativnog stresa koji toksin izaziva kroz nekoliko dobro okarakterisanih mehanizama. Jedan od predloženih mehanizama koji objašnjava neurotoksično delovanje 6-OHDA je autooksidacija unutar i van ćelije koja favorizuje proizvodnju H_2O_2, $O_2^{\bullet-}$ i $OH^{\bullet-}$(Soto-Otero et al., 2000). Takođe, 6-OHDA je poput dopamina, supstrat za MAO. Ova enzimska reakcija dovodi do stvaranja H_2O_2 koji se dalje u prisustvu gvožđa (Fentonova reakcija) redukuje do izuzetno reaktivnog $OH^{\bullet-}$. Ovi mehanizmi nezavisno ili u kombinaciji generišu ROS koje dalje oštećuju ćelijske komponente lipide, proteine i DNK. Dodatno, 6-OHDA može direktno da inhibira mitohondrijski kompleks I i IV, što takođe dovodi do proizvodnje ROS i poremećaja ćelijskog energetskog metabolizma (Blum et al., 2001). Ovaj niz događaja pokreće puteve apoptotske smrti ćelija, i posledično rezultira degeneracijom DA neurona. ROS takođe narušava funkciju ubikvitin-proteazomskog sistema, utičući time na patološku akumulaciju proteina (Dantuma & Bott, 2014).

Na osnovu svega navedenog jasno je da 6-OHDA predstavlja pogodan neurotoksin za brzo i specifično izazivanje neurodegeneracije nigrostrijatalnog sistema i proučavanje PB. Jedna od glavnih prednosti ovog modela je ta što stepen neurodegeneracije zavisi od mesta lezije i date količine toksina, što znači da se stepen neurodegeneracije može modelovati sa relativno visokom preciznošću i ponovljivošću. Još jedna prednost je ta što ovaj model nudi mogućnost praćenja i motornih i nemotornih simptoma PB (Ciric et al., 2019; J. Hunt et al., 2022; Petrovic et al., 2021). Osim toga, 6-OHDA model se često koristi i validiran je za ispitivanje diskinezija izazvanih levodopom (Tronci & Francardo, 2018). Takođe, prednost

unilateralnog modela 6-OHDA je ta što suprotna strana može služiti kao unutrašnja kontrola. Međutim, treba napomenuti da može doći do među-hemisferne kompenzacije, a pokazano je i da nivoi dopamina na suprotnoj strani mogu porasti nakon lezije 6-OHDA (Del-Bel et al., 2014). Iako model 6-OHDA ima mnoge prednosti, glavni nedostatak je relativno brz razvoj i odsustvo stvarne progresije patologije (Kuter et al., 2018; Oliynyk et al., 2023). Još jedan nedostatak je činjenica da ovaj model nije pogodan za ispitivanje uloge imunskih ćelija u razvoju i napretku PB, s obzirom da se procesi inflamacije završavaju vrlo brzo nakon intoksikacije. Konačno, akumulacija α-sinukleina, koja je jedan od glavnih patoloških markera PB, nije prisutna u ovom modelu (Hernandez-Baltazar et al., 2017), ali i pored ovih ograničenja ostaje odličan izbor za proučavanje različitih terapijskih pristupa u PB.

1.6. Osnovni terapijski pristupi u Parkinsonovoj bolesti

Trenutno dostupne farmakološke i nefarmakološke terapijske opcije ograničene su na motorne i nemotorne simptome bolesti, te iako doprinose unapređenju kvaliteta života pacijenata nisu u mogućnosti da suštinski menjaju tok bolesti i uspore ili zaustave neurodegenerativni proces. Primena supstitucione terapije ima za cilj da pacijentima omogući što efikasniju kontrolu simptoma uz minimalne neželjene efekte. Međutim, levodopa koja je i dalje zlatni standard u terapiji PB, nakon duže upotrebe dovodi do neželjenih efekata u vidu diskinezija (Pandey & Srivanitchapoom, 2017). Levodopa se nakon peroralne primene u telu veoma brzo dekarboksiliše i pre nego što dospe do mozga, pod dejstvom DOPA, te se kombinuje sa inhibitorima DOPA-dekarboksilaze (karbidopom i benzerazidom) kako bi se sprečila ovako intenzivna razgradnja levodope na periferiji, a i da bi se smanjili akutna neželjena dejstva poput mučnine (Hauser, 2009). Kada je u pitanju farmakološka terapija u upotrebi su još i dopaminski agonisti poput ropinirola i rotigotina, inhibitori katehol-O-metiltransferaze (COMT), koji se uzimaju isključivo uz levodopu i produžavaju njen učinak i inhibitori MAO-B koji usporavaju razgradnju dopamina (Stoker et al., 2018). U nekim zemljama u upotrebi su kao ko-terapija i već pomenuti antagonisti A_{2A} i NMDA receptora, istradefilin i amantadin. Međutim, nemotorni simptomi koji su za većinu pacijenata sa PB u velikoj meri onesposobljujući, slabo reaguju na dopaminsku terapiju i zahtevaju primenu lekova iz redova inhibitora acetilholinesteraze, a nekada i antipsihotika, antidepresiva i benzodiazepina (J. J. Ferreira et al., 2013).

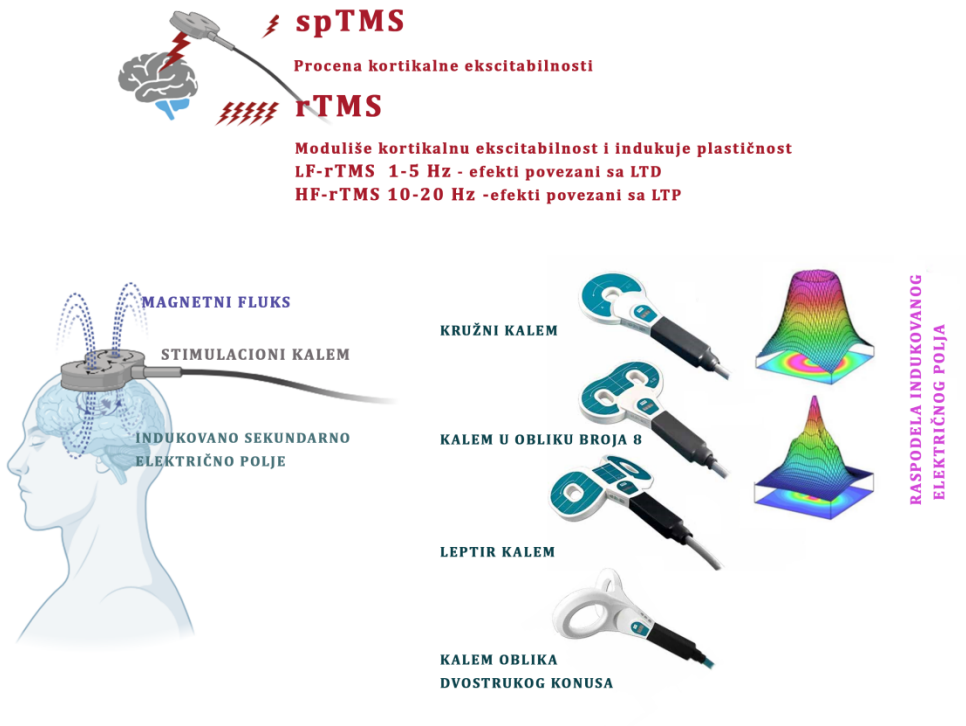
Pored farmakološke terapije, važnu ulogu igraju nefarmakološki tretmani kao što su fizikalna terapija i psihološka podrška. U tom kontekstu treba spomenuti i duboku moždanu stimulaciju (eng. *deep brain stimulation*, DBS) koja podrazumeva implantaciju električnih stimulatora (poput pejsmejkera) sa ciljem pružanja kontinuirane visokofrekventne električne stimulacije unutar određenih moždanih regiona, najčešće STN i GPi (Groiss et al., 2009; Y. Smith et al., 2012). Iako je u poređenju sa ranijim hirurškim procedurama u ovoj oblasti DBS metoda manje rizična, i dalje je poprilično invazivna i predstavlja efikasan tretman samo za manji broj pacijenata koji ne reaguju na dostupnu farmakološku terapiju. Relativan uspeh DBS i napredak u razumevanju patofiziologije PB povećali su interesovanje za neinvazivne tehnike moždane stimulacije koje danas obuhvataju transkranijalnu magnetnu stimulaciju (eng. *transcranial magnetic stimulation*, TMS) i transkranijalnu stimulaciju jednosmernom/ naizmeničnom strujom (eng. t*ranscranial direct/alternating current stimulation*, tDCS, tACS) koje svojim neuromodulatornim efektima ublažavaju simptome bolesti (Schulz et al., 2013). Iako ove tehnike neinvazivne stimulacije mozga imaju bogatu istorijsku pozadinu, njihova široka primena je tek nedavno zabeležila značajan rast.

1.6.1. Transkranijalna magnetna stimulacija (TMS)

Razvoj neinvazivne stimulacije mozga koji uključuje upotrebu električnih ili magnetnih pulseva bez prethodnih hirurških postavki elektroda direktno na mozak, započeo je

eksperimentima koji datiraju još iz XIX veka. Svakako je za sva buduća istraživanja na temu tehnika neinvazivne stimulacije ključan princip elektromagnetne indukcije, otkriven od strane Majkla Faradeja 1831. godine. Eksperimenti d'Arsonval-a krajem XIX veka prvi su pružili uvid u moguću primenu ovog principa u medicinske svrhe, prikazujući efekte magnetnih polja na ljudsko telo u vidu pojave fosfena i sinkopa (d'Arsonval, 1893). Slične efekte, desetak godina kasnije, u svom istraživanju zabeležio je i Tomson (Thompson, 1910) Iako su elektromagneti korišćeni za kontrakciju perifernih mišića žabe (Kolin et al., 1959) nisu ličili na moderne kaleme za magnetnu stimulaciju sve do 1985. godine, kada je grupa naučnika razvila jedan za stimulaciju motorne kore čoveka. Međutim, ovoj važnoj prekretnici prethodila je demonstracija električne stimulacije motorne kore indukovana visokonaponskim stimulatorom od strane Mertona i Mortona 1980. godine (Merton & Morton, 1980). Zbog bolnosti postupka i visokog nivoa električnog otpora koji lobanja pruža, potreba za manje bolnom alternativom postala je očigledna. To je rezultiralo time da 1985. godine, Antoni Barker i njegov tim sa Šefild Univerziteta u Engleskoj predstave redizajniran model magnetnog stimulatora sa kojim je moguće stimulisati na bezbolan i bezbedan način humanu motornu koru (Barker et al., 1985). Ovaj događaj je predstavljao kulminaciju prethodnih teorijskih i eksperimentalnih napora, omogućavajući dalji razvoj visoko sofisticiranih, komercijalno dostupnih neinvazivnih moždanih stimulatora, primenjivanih najpre u dijagnostičke svrhe u cilju provere integriteta nervog sistema, a zatim i u terapijske svrhe.

Savremeni magnetni stimulatori rade na istom principu kao i oni razvijeni 1980-ih godina i sastoje se iz kondenzatora i stimulacionog kalema. Napon kondenzatora se podiže na 4 kV, a potom dolazi do njegovog pražnjenja preko kalema. Struja koja se javlja u tom procesu dostiže 5000 A (Weber & Eisen, 2002). Kalem za stimulaciju je napravljen od bakarnih žičanih navoja koji su obloženi izolacionom plastikom. Prolaskom struje kroz kalem po principu elektromagnetne indukcije generiše se magnetno polje čije se linije fluksa pružaju vertikalno u odnosu na ravan kalema. Ovako nastalo magnetno polje dostiže relativno visok intenzitet (1,5 – 2,5 T) za veoma kratko vreme (100 - 200 μs) (Vincenzo Di Lazzaro et al., 2008; Hallett, 2007). Magnetni fluks nesmetano prolazi kroz visoko rezistentna tkiva poglavine i kosti lobanje do moždanog tkiva stvarajući sekundarno električno polje koje rezultuje strujom paralelnom onoj u kalemu, ali suprotnog smera, i to bez aktivacije nociceptora u koži glave, pa je samim tim primena ovog tipa stimulacije praktično bezbolna (Rossini et al., 2015). Stimulacija mozga po ovom principu zapravo predstavlja pomenutu TMS (Slika 4.).



Slika 4.Transkranijalna magnetna stimulacija

Električno polje koje TMS stvara može menjati aktivnost neurona u stimulisanoj regiji mozga. TMS može ili povećati ili smanjiti nadražljivost neurona. Smatra se da se ova modulacija neuronske aktivnosti dešava kroz depolarizaciju ili hiperpolarizaciju neurona, utičući time na obrasce prostiranja akcionih potencijala i na ukupno funkcionisanje neuronskih kola (Terao & Ugawa, 2002). Više parametara utiče na geometriju indukovanog električnog polja i, posledično, na način na koji TMS utiče na neuronsku aktivnost. Ovi parametri uključuju oblik, veličinu i orijentaciju kalema, kao i oblik magnetnog pulsa, koji može biti monofazan ili bifazan (Kammer et al., 2001). Takođe, sama orijentacija neurona koji se razlikuju po veličini, funkciji i stepenu mijelinizacije može uticati na odgovor nakon TMS. Prvobitno su korišćeni kružni kalemovi velikog i malog prečnika koji generišu široko i difuzno magnetno polje. Zatim su dizajnirani kalemovi u obliku broja osam (eng. *figure-of-eight coil*) kako bi se postigla preciznija stimulacija na mestu neposredno ispod ukrštanja krakova osmice do dubine od 1.5-2 cm (Groppa et al., 2012). Da bi se dosegle dublje moždane strukture konstruisani su specifični kalemovi oblika dvostrukog konusa (kombinacija dve velike kružne zavojnice pod uglom od 120 stepeni) ili "H-kalemovi" (Zangen et al., 2005). Dakle, tip kalema, kao što je navedeno određuje područje stimulacije, dok je orijentacija neurona u odnosu na indukovano električno polje ključna za stepen njihove aktivacije (Abdeen & Stuchly, 1994). Smatra se da se indukcija električne aktivnosti najpre javlja na neuronima u okviru girusa zbog ograničene dubine prodiranja TMS stimulusa, tačnije na aksonskom brežuljku, mestu gde je najveća koncentracija jonskih kanala pa na mestima savijanja ili grananja aksona (Siebner et al., 2022). Ukoliko stimulus izazove pražnu depolarizaciju, nastali akcioni potencijali koji se nishodno šire ka presinaptičkim završecima, pokreću oslobađanje neurotransmitera i dalje nastanak i prostiranje akcionih potencijala u postsinaptičkom neuronu. Glutamat, kao glavni neurotransmiter većine kortikalnih neurona, definiše ih kao ekscitacijske, dok se neuroni koji oslobađaju GABA kategorizuju kao inhibitorni (Huerta & Volpe, 2009). Električna struja indukovana TMS stimulusom neselektivno deluje i na jedne i na druge neurone zbog čega čak i slab TMS stimulus uvek izaziva mešavinu složenih ekscitatornih i inhibitornih odgovora. TMS takođe izaziva značajnu

direktnu perifernu somatosenzornu i auditivnu ko-stimulaciju (Koponen, Goetz, et al., 2020; Schmid et al., 1995).

TMS se široko koristi u kliničkoj neurofiziologiji za procenu provodljivosti nervnog impulsa duž kortikospinalnog trakta merenjem motornih izazvanih odgovora (eng. *motor evoked potentials*, MEP). U tom slučaju, pojedinačni TMS (eng. *single-pulse TMS*; spTMS) stimulus dovodi do depolarizacije određenih neurona primarne motorne kore koji izazivaju merljive efekte. Reakcija odgovarajućeg mišićnog efektora je na kontralateralnoj strani od mesta stimulacije, a registrovanje MEP-a je u tehničkom smislu istovetno klasičnom elektromiografskom (EMG) registrovanju pomoću površinskih elektroda (Kobayashi & Pascual-Leone, 2003). Takođe, pojedinačni TMS stimulusi se koriste za utvrđivanje pasivnog i aktivnog motornog praga, odnosno služi za individualno prilagođavanje minimalnog intenziteta TMS stimulusa koji će izazvati MEP minimalne amplitude (< 50mV) ciljnog mišića u mirovanju (Hallett, 2007; Rossini et al., 1994).

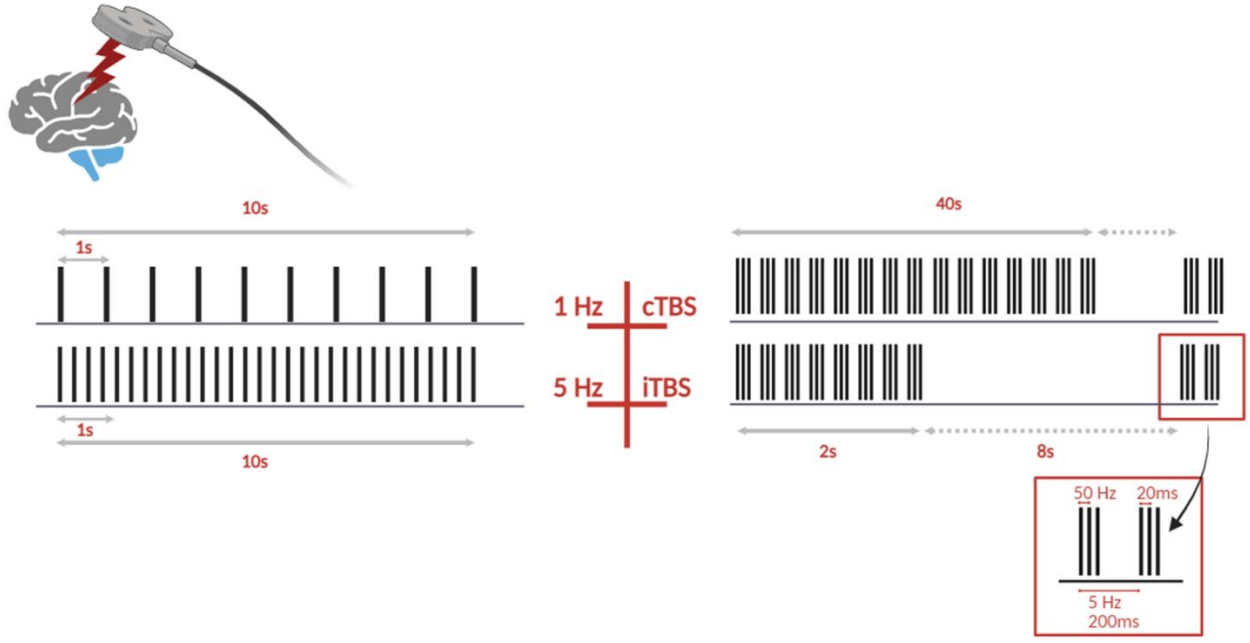
Tokom poslednjih 20 godina razvijeni su brojni protokoli TMS-a. Pored pomenute stimulacije pojedinačnim pulsevima postoji i uparena stimulacija koja podrazumeva da se dva stimulusa aplikuju jedan za drugim u određenom vremenskom razmaku i repetitivna transkranijalna magnetna stimulacija koja podrazumeva nizove stimulusa na određenim frekvencijama (rTMS). Intenzivno se ispituju efekti svih navedenih TMS protokola kako bi se došlo do najefikasnijih i najpouzdanijih koji bi se dalje primenili u kliničke svrhe za lečenje različitih patoloških stanja (Rossi et al., 2009).

1.6.2. Repetitivna transkranijalna magnetna stimulacija (rTMS)

Brojni literaturni podaci upućuju na to da korišćenje višestruko ponovljenih TMS pulseva, organizovanih u specifične sekvence, može indukovati promene u nadražljivosti moždane kore koje se zadržavaju znatno duže nakon završetka stimulacije (Pascual-Leone et al., 1994; Rossini et al., 2015). Ove promene mogu podstaći plastičnost u kortikalnim sinapsama i u neuronskim kolima povezanim sa njima (King & Tang, 2024). Dakle, kada se višestruka stimulacija dešava u serijama (eng. ''*trains*'') sa kratkim pauzama bez stimulacije, govorimo o rTMS. Postoje dve klasične paradigme rTMS-a: rTMS niske frekvencije (eng. *low-frequency rTMS*; LF rTMS) koji se sastoji od neprekidnih nizova pojedinačnih impulsa frekvencije manje od 1 Hz, i rTMS visoke frekvencije (eng. *high-frequency rTMS*; HF rTMS), 5 Hz i više, koji obuhvata serije stimulacija koje obično traju 5 do 10 sekundi i razdvojene su pauzama od 20 do 50 sekundi (Chen et al., 1997; Speer et al., 2000). Fiziološki efekti koji se javljaju pri ovom vidu stimulacije nisu ni približno jednostavni kao pri primeni pojedinačnih TMS pulseva. Višestruka, ponovljena stimulacija osim što izaziva mešavinu ekscitatornih i inhibitornih efekata, dodatno generiše efekte koje sežu mnogo dalje od početne tačke stimulacije jer se neki od aktiviranih neuralnih elemenata projektuju ka drugim, kako kortikalnim, tako i subkortikalnim strukturama. Osim toga fiziološki efekti su dugotrajni i mogu se meriti nekoliko minuta, pa čak i do nekoliko sati po završetku stumulacije, i verovatno su povezani sa fenomenima sinaptičke plastičnosti, dugotrajnom potencijacijom (eng. *long-term potentiation*; LTP) i dugotrajnom depresijom (eng. *long-term depression*; LTD) (PMID: 19859782). LTP je dugotrajno „pojačanje“ u komunikaciji između dva neurona koja rezultuje iz njihove simultane aktivacije, a LTD je dugotrajno „smanjenje“ u komunikaciji koja nastaje zbog asinhrone aktivacije dva neurona (Abbott & Nelson, 2000). LTP i LTD se smatraju glavnim ćelijskim mehanizmima koji obezbeđuju uspešno učenje i pamćenje (Abraham et al., 2019). Postoji relativni konsenzus da su stimulacije frekvencijom ispod 1Hz uglavnom inhibitorne i dovode do LTD, dok su stimulacije frekvencijom od 5 Hz i više uglavnom ekscitatorne, i dovode do LTP (Hoogendam et al., 2010).

Jedan od izazova u primeni rTMS je vreme stimulacije koje može trajati i do 30 minuta, zbog čega se javila potreba za razvojem nove metode koja bi omogućila brzu aplikaciju većeg broja stimulusa u kratkom vremenskom periodu. Stimulacija teta praskovima (eng. *theta burst stimulation*; TBS) predstavlja obrazac ponovljenih magnetnih pulseva kojim se oponaša oscilatorna aktivnost mozga. Tačnije, brze gama oscilacije (50Hz) se javljaju tokom periode sporotalasnih teta (5Hz) oscilacija (odnosno unutar jednog šireg, sporijeg ciklusa teta oscilacija se odvijajaju mnogi brži ciklusi - gama oscilacije) na čemu se zapravo baziraju postojeći mehanizmi plastičnosti koji se javljaju pri procesima učenja i pamćenja (Canolty et al., 2006; Herweg et al., 2020; Y.-Z. Huang & Rothwell, 2004).

Postoje dva različita TBS protokola, intermitentna (iTBS) i kontinuirana stimulacija teta prskovima (cTBS). iTBS protokol povećava nadražljivost motorne kore i generiše ekscitatorni odgovor, slično kao LTP. U ovom protokolu se tokom 2s, na svakih 200 ms, emituje serija od 3 visokofrekventna pulsa od 50 Hz koji se ponavljaju na 5 Hz, odnosno u teta ritmu. Posle 8 sekundi pauze slede naredne 2s sa stimulacijom. Ovaj obrazac koji traje ukupno 10s se ponavlja 20 puta, tako da se tokom protokola emituje ukupno 600 pulseva. Suprotno tome, drugi tip protokola, cTBS, proizvodi "inhibitorne" efekte na koru, slično kao LTD i sastoji se od 300 do 600 pulseva koji se kontinuirano ponavljanju na svakih 200ms tokom 40 sekundi (Y.-Z. Huang et al., 2005; Solomon et al., 2021). Studije koje su koristile ove specijalizovane podtipove rTMS protokola označile su ih kao atraktivniji i superiorniji izbor u pogledu izazivanja neuromodulatornih efekata koji prevazilaze one viđene kod standardnih rTMS protokola (Di Lazzaro et al., 2011; Iezzi et al., 2011). Takođe, usled kratkog trajanja TBS sesija, sve je veći broj studija koje primenjuju više TBS sesija sa određenim vremenskim razmakom kako bi se postigli bolji efekti (Slika 5.).



Slika 5.Različiti protokoli rTMS-a

S obzirom da je navedenim metodama moguće modulisati stepen ekscitacije i metaboličke aktivacije pojedinih regiona mozga, različiti TMS protokoli su opsežno proučavani kao mogući terapijski pristup za različite neurodegenerativne, psihijatrijske i druge neurološke poremećaje. HF-rTMS je odobrena od strane Agencije za hranu i lekove (eng. *Food and Drug Administration*; FDA) kao opcija za terapijski rezistentnu depresiju još 2008. godine (Holtzheimer et al., 2010), a od 2018. odobren je i iTBS protokol (Caulfield, 2020; Yulug et al., 2016). Sa druge strane, LF-rTMS pokazuje obećavajuće rezultate u

tretmanu auditivnih halucinacija i deluzija kod shizofrenije (Cordes et al., 2006). Kada su u pitanju bolesti zavisnosti HF-rTMS smanjuje žudnju i poboljšava kognitivne funkcije kod zavisnika od metamfetamina (Su et al., 2017). Nekoliko studija istraživalo je upotrebu rTMS-a za lečenje pojedinaca zavisnih od alkohola i nikotina, međutim, još uvek ne postoji pouzdan dokaz o efikasnosti tretmana (Del Felice et al., 2016; Dieler et al., 2014). TMS se efikasno koristi za lečenje migrena, hroničnog bola, tinitusa (Lan et al., 2017), a pokazano je i da LF-rTMS poboljšava oporavak motornih funkcija nakon moždanog udara (Du et al., 2019). Takođe značajan potencijal rTMS se pokazao i kada su u pitanju neurodegenerativna oboljenja uključujući Alchajmerovu, Parkinsonovu, Hantingtonovu bolest kao i multiplu i amiotrofičnu lateralnu sklerozu (Antczak et al., 2021; Chou et al., 2020; Dileone et al., 2010; Zhou et al., 2022). Međutim, iako su brojne studije pokazale pozitivne efekte TMS-a, koji mogu da traju i do 6 meseci nakon završetka tretmana, ostaje nejasno koji su to mehanizmi koji omogućavaju terapijski efekat u ovako širokom spektru stanja.

Kada su u pitanju ćelijski i molekulski mehanizmi koji leže u osnovi ovih pozitivnih terapijskih efekata do sada je pokazano da TMS može da utiče na oslobađanje ključnih neurotransmitera poput glutamata, GABA, dopamina, serotonina i noradrenalina (Dubin, 2017). PET dijagnostikom je kod zdravih ispitanika utvrđeno da TBS povećava sintezu i oslobađanje dopamina (Ko et al., 2008), kao i da rTMS od 10 Hz utiče na oslobađanje serotonina u celom limbičkom sistemu kod zdravih dobrovoljaca (Sibon et al., 2007). TMS može da utiče i na nivoe neurotrofnih faktora. Većina rTMS studija se do sada fokusirala na promene u neurotrofnom moždanom faktoru, BDNF-u (eng. *Brain Derived Neurotrofic Factor*; BDNF) imajući u vidu njegove brojne funkcije kao što su: poboljšanje preživljavanja neurona nakon oštećenja CNS-a, neurogeneza, migracija i diferencijacija neurona, rast dendrita i aksona i formiranje sinapsi (Baquet et al., 2004). Pored toga, HF-rTMS povećava nivoe BDNF u serumu i afinitet BDNF za TrkB receptore (eng. *tropomyosin receptor kinase B*, TrkB) kod zdravih dobrovoljaca, dok LF-TMS smanjuje BDNF nivoe (Wang et al., 2011). Nekoliko studija pokazalo je povećanu ekspresiju BDNF-a u tkivima oko zone infarkta (Liu et al., 2020). LF-rTMS efikasno povećava i ekspresiju NGF (eng. *nerve growth factor*) pored toga što povećava nivo BDNF, ali i ekspresiju NMDA receptora (Tan et al., 2013). Kao što je već pomenuto, TMS promoviše i sinaptičku plastičnost kroz LTP i LTD mehanizme (Huerta & Volpe, 2009).

Nadalje, TMS ispoljava neuroprotektivne efekte kroz aktivaciju signalnih puteva relevantnih za preživljavanje i diferencijaciju ćelija kao i kroz inhibiciju apoptoze putem regulisanja ekspresije Bcl-2 (eng. *B-cell lymphoma 2*) i Bax (eng. *Bcl-2-associated X protein*) regulatornih proteina. Pokazano je da u tkivu hipokampusa nakon ishemijske lezije rTMS povećava ekspresiju Bcl-2 i smanjuje Bax, što ukazuje da rTMS suprimira apoptotske puteve (Guo et al., 2017). Da rTMS doprinosi i procesima neurogeneze potvrđuje povećana ekspresija VEGF-a (eng. *vascular endothelial growth factors;* VEGF)(Fukuda et al., 2020; Zhang et al., 2015).

Značajan je i efekat TMS-a na glijske ćelije, uključujući astrocite, mikrogliju i oligodendrocite. Primena HF-rTMS rezultira smanjenjem reaktivnosti astroglije i mikroglije, a dovodi posledično i do smanjenog oslobađanja proinflamacijskih citokina kod životinja sa povredom kičmene moždine (Kim et al., 2013). Ovaj trend smanjenja reaktivnosti pokazan je i nakon upotrebe iTBS-a, a primećena je i proliferacija oligodendrocita (Ferreira et al., 2024). Dakle TMS dovodi do smanjenja neuroinflamacijskih procesa i oksidativnog stresa. Takođe, postoje podaci da TMS reguliše signalizaciju posredovanu kalcijumom, čime promoviše neuroplastičnost i neuroregenerativne efekte. Nedavna studija je pokazala da su se nivoi kalcijuma u plazmi značajno povećali nakon sesija rTMS-a (Stateman et al., 2014), kao i da HF-rTMS značajno povećava unutarćelijsku koncentraciju kalcijuma u kortikalnim neuronima što podstiče mehanizme LTP-a (Banerjee et al., 2017). Kompleksnost uticaja TMS-a se ogleda i u

modifikovanju propustljivosti KMB, čime se otvaraju novi putevi za terapijske intervencije (Vazana et al., 2020). Brojne studije su nedvosmisleno pokazale da TMS modifikuje ekspresiju gena i povećava proizvodnju niza enzima. Iz svega navedenog vidimo da je primena TMS-a široko zastupljena i da zaista obećava kao terapijska opcija za različita klinička stanja, međutim potrebna su dodatna istraživanje da bi se optimizovali protokoli stimulacije i razumeli svi mehanizmi koji stoje iza terapijskih efekata.

1.6.3. Primena rTMS u Parkinsonovoj bolesti

PB karakteriše širok spektar kliničkih simptoma što je dovelo do objavljivanja više stotina referenci koje su koristile različite TMS protokole u tretmanu mnogobrojnih aspekata ove bolesti. Do danas je najviše ispitivan efekat stimulacije motorne/premotorne kore i SMA u kontekstu poboljšanja motornih simptoma, kao i stimulacija dorzolateralne prečeone kore (eng. *dorsolateral prefrontal cortex*; DLPFC) u kontekstu poboljšanja nemotornih simptoma, a u okviru toga najviše simptoma depresije. Dosadašnje objavljene studije o smanjenju motornih oštećenja u PB pomoću rTMS-a su brojne i obuhvataju široku lepezu protokola i vremena stimulacije, što zajedno sa varijabilnošću profila pacijenata (različiti farmakološki tretmani, dužina trajanja bolesti, ozbiljnost i tip motornih simptoma), čini postizanje konsenzusa o bilo kom skupu procedura stimulacije izuzetno teškim (Edwards et al., 2008; Elahi et al., 2009; Wu et al., 2008). Iako je motorna kora bila najčešće proučavano ciljno mesto za stimulaciju, klinička efikasnost bila je značajnija pri stimulaciji SMA (Hamada et al., 2008; Shirota et al., 2013). Jedna od najranijih studija na pacijentima sa PB pokazala je da primena rTMS od 5 Hz na motornu koru može poboljšati bradikineziju (Siebner et al., 1999). Takođe, primena ove HF-rTMS nad SMA kod pacijenata sa PB jednom nedeljno tokom osam nedelja, može uticati na poboljšanje hoda i brzine hoda (Hamada et al., 2008). U prilog ovim rezultatima, meta-analiza 12 studija podržava ideju da rTMS poboljšava motorne funkcije kod pacijenata sa PB (Fregni et al., 2005). Pokazano je i da je nivo dopamina u serumu pacijenata sa PB nakon šestodnevne primene HF-rTMS značajno porastao (Khedr et al., 2007). rTMS kao i tDCS primenjen nad motornom korom gde se nalaze reprezentacije za donje udove mogu biti efikasni u smanjenju zamrzavanja hoda (Chang et al., 2016; Dagan et al., 2018). Pokazano je da LF-rTMS primenjen nad motornom korom 4 uzastopna dana utiče na poboljšanje motorne spretnosti kod pacijenta sa PB koji su imali diskinezije indukovane levodopom (Filipović et al., 2009). Sa druge strane, TBS, konkretno iTBS, povećava kortikalnu nadražljivost i nudi slična poboljšanja, ali i poboljšanje simptoma depresije i anksioznosti kada se primeni nad DLPFC (Zhang et al., 2022). Nekoliko studija je obradilo i druge aspekte PB nakon primene rTMS, kao što su funkcije govora (Dias et al., 2006), funkcija bešike (Brusa et al., 2009), san (van Dijk et al., 2009) ili kognitivne funkcije (Jiang et al., 2020). TMS metode su u većini slučajeva dobro tolerisane, sa minimalnim neželjenim efektima što ih čini privlačnim opcijama za pacijente. Teorijski, postoji rizik od izazivanja epileptičnog napada, ali samo pod uslovom da se TMS upotrebi najvećim intenzitetima, dugog trajanja i bez pauza. Takođe, postoji upitnik o bezbednosnim i etičkim smernicama za korišćenje TMS-a koji je razvila Međunarodna federacija za kliničku neurofiziologiju, koji pacijenti popunjavaju pre bilo kakvih intervencija (Rossi et al., 2009).

Uprkos velikom broju objavljenih radova, koji su izuzetno ohrabrujući, još uvek nema pouzdanih rezultata koji nude jedinstvene parametre stimulacije sa precizno određenim vremenskim okvirima i identičnim mestom i jačinom stimulacije. Zbog toga, je traganje za najefikasnijim rTMS protokolom još uvek u toku, i do sada nema zvanično prihvaćenih smernica za terapijsku primenu rTMS-a u lečenju PB.

1.6.4. Primena rTMS u eksperimentalnim modelima Parkinsonove bolesti

U poređenju sa rastućim brojem rTMS kliničkih istraživanja, broj istraživanja na eksperimentalnim modelima koja se bave bazičnim mehanizmima dejstva rTMS još uvek je iznenađujuće mali. U tom kontekstu su se glodari, uključujući miševe i pacove, prvi put našli u centru pažnje istraživača tokom 1990. godine. Iako njihova upotreba pruža brojne prednosti, neophodno je razmotriti i njihova ograničenja. Jedan od značajnih nedostataka korišćenja glodara kao modela je neproporcionalna veličina njihovih glava u odnosu na veličinu stimulacionog kalema (Koponen, Stenroos, et al., 2020). Trenutno dostupni kalemovi omogućavaju fokalnu stimulaciju različitih područja ljudskog mozga, ali kalemovi manje veličine razvijeni za glodare i dalje nisu u odgovarajućem odnosu kao oni namenjeni za upotrebu kod ljudi pa stimulišu mozak u celini. Promena veličine kalema implicira prilagođavanje drugih parametara, što dodatno komplikuje interpretaciju rezultata, a dovodi i do problema poput pregrevanja kalema, što ograničava intenzitet i dužinu stimulacije (Boonzaier et al., 2018). Takođe, stres koji pacovi doživljavaju zbog imobilizacije, zvuka koji proizvodi magnetni stimulator, kao i efekata TMS-a na mišiće predstavlja još jedan aspekt koji zahteva pažnju.

Kada su u pitanju eksperimentalni modeli PB većina studija istraživala je promene u sintezi i oslobađanju dopamina nakon tretmana TMS-om. Studija na 6-OHDA izazvanom PB modelu pokazala je da tretman rTMS-om od 0,5 Hz proizvodi neuroprotektivni efekat na dopaminske neurone, što je rezultiralo značajno povećanim nivoima dopamina (X. Yang et al., 2010). Pored toga, ustanovljeno je da su ciklooksigenaza-2 (COX-2) i faktor nekroze tumora-alfa (TNF-α) bili niži u regionu SN nakon primene rTMS-a. Druga studija zaključila je da akutna primena HF-rTMS tokom 3 dana rezultira poboljšanjem motornih simptoma indukovanih 6-OHDA, povećavajući vanćelijsku koncentraciju dopamina u dorzolateralnom strijatumu (Kanno et al., 2004). Pored toga pokazano je da LF-rTMS povećava preživljavanje TH-pozitivnih neurona, nivo ekstracelularnog dopamina, kao i njegovih metabolita (3,4-dihidroksifenilacetičnu kiselinu i homovanilinsku kiselinu), takođe na 6-OHDA PB modelu, ali i da smanjuje rotaciono ponašanje izazvano apomorfinom, kao i nivo apoptotskog proteina kaspaze-3 (Ba et al., 2017). Nivoi ekspresije BDNF, GDNF, PDGF i VEGF su bili povećani u SNpc kod 6-OHDA pacova tretiranih rTMS-om što upućuje na njegov neuroprotektivni efekat (J. Y. Lee et al., 2013). rTMS može imati značajan neuroprotektivni efekat delujući na parametre oksidativnog stresa i antioksidativne zaštite (Túnez et al., 2006). Takođe, pokazano je i da akutna primena iTBS u 6-OHDA indukovanom PB modelu može da dovede do poboljšanja motornih simptoma, a identifikovana je i uključenost određenih subjedinica NMDA receptora čime je ukazano na mehanizam putem kojeg bi iTBS mogao da ostvaruje svoje efekte (Natale et al., 2021).

Zaključci svih dosadasnjih istraživanja ističu važnost eksperimentalnih modela PB za proučavanje i razumevanje mehanizama koji čine osnovu terapijskog potencijala TMS-a, a nas dovode do glavne ideje ove teze, odnosno ispitivanja iTBS protokola koji predstavlja specijalizovani rTMS protokola u 6-OHDA modelu PB.

2. CILJEVI ISTRAŽIVANJA

Iako su brojne kliničke studije na pacijentima obolelim od PB, kao i eksperimentalne studije u životinjskim modelima PB demonstrirale povoljne efekte delovanja rTMS u ublažavanju motornih i nemotornih simptoma PB i dalje ne postoji dovoljno podataka koji bi ukazali na konkretne molekulske mehanizme delovanja ovog tipa stimulacije. Stoga je osnovni cilj ove doktorske disertacije je da se rasvetli efekat primene rTMS-a, konkretno iTBS protokola na neurodegeneraciju i neuroinflamaciju u modelu PB izazavane primenom 6-OHDA, sa posebnim osvrtom na promene u nivou purinskog i glutamatnog signalnog sistema. Krajnji cilj studije da se proceni potencijalna klinička korist primene iTBS protokola u terapiji PB.

U skladu sa opštim naučnim ciljem postavljeni su sledeći specifični ciljevi:

1) Opisivanje histoloških promena, stepena neurodegeneracije i odgovora astrocita i mikroglije nakon unilateralne aplikacije 6-OHDA u desni SNpc, u odnosu na levi SNpc koji će poslužiti kao šam;
2) Razvijanje 3D modela za procenu prostorne raspodele jačine električnog i magnetnog polja primenom kalema 25 mm u mozgu eksperimentalnih pacova;
3) Ispitati efekte rTMS-a na motorne i nemotorne simptome izazvane unilateralnom lezijom desnog SNpc;
4) Ispitati efekat rTMS-a na preživljavanje DA neurona, ekspresiju TH i nivo dopamine i serotonina u desnom SNpc;
5) Ispitati efekte rTMS-a na aspekte reaktivne astroglioze i mikroglioze u kontekstu neuroinflamacije u SNpc;
6) Ispitati uticaj rTMS-a na ekspresiju komponenti glutamatnog signalnog sistema;
7) Ispitati uticaj rTMS-a na ekspresiju i aktovnost komponenti purinskog signalnog sistema (NTPDaze1/2 i eN/CD73, P1, P2);
8) Ispitati uticaj rTMS-a na nivo oksidativnog stresa i aktivnosti enzimskih i ne-enzimskih komponenti antioksidativne zaštite;

3. RADOVI PROIZAŠLI IZ DOKTORSKE DISERTACIJE

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Intermittent Theta Burst Stimulation Improves Motor and Behavioral Dysfunction through Modulation of NMDA Receptor Subunit Composition in Experimental Model of Parkinson's Disease

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disorder characterized by the progressive degeneration of the dopaminergic system, leading to a variety of motor and nonmotor symptoms. The currently available symptomatic therapy loses efficacy over time, indicating the need for new therapeutic approaches. Repetitive transcranial magnetic stimulation (rTMS) has emerged as one of the potential candidates for PD therapy. Intermittent theta burst stimulation (iTBS), an excitatory protocol of rTMS, has been shown to be beneficial in several animal models of neurodegeneration, including PD. The aim of this study was to investigate the effects of prolonged iTBS on motor performance and behavior and the possible association with changes in the NMDAR subunit composition in the 6-hydroxydopamine (6-OHDA)-induced experimental model of PD. Two-month-old male Wistar rats were divided into four groups: controls, 6-OHDA rats, 6-OHDA + iTBS protocol (two times/day/three weeks) and the sham group. The therapeutic effect of iTBS was evaluated by examining motor coordination, balance, spontaneous forelimb use, exploratory behavior, anxiety-like, depressive/anhedonic-like behavior and short-term memory, histopathological changes and changes at the molecular level. We demonstrated the positive effects of iTBS at both motor and behavioral levels. In addition, the beneficial effects were reflected in reduced degeneration of dopaminergic neurons and a subsequent increase in the level of DA in the caudoputamen. Finally, iTBS altered protein expression and NMDAR subunit composition, suggesting a sustained effect. Applied early in the disease course, the iTBS protocol may be a promising candidate for early-stage PD therapy, affecting motor and nonmotor deficits.

Keywords: Parkinson's disease; 6-OHDA; rTMS; iTBS; NMDA receptor; neuroprotection

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder, affecting ~8 million people worldwide, with a prevalence increasing 2.5-fold over the past three decades [1]. The main characteristic of PD is the progressive degeneration of dopaminergic (DA) neurons in the *substantia nigra pars compacta* (SNpc) and the resulting

decrease in striatal DA levels. The degeneration of the complex nigrostriatal networks and the resulting predominance of corticostriatal glutamatergic inputs seem to be crucial for the pathogenesis of PD [2–4] and responsible for most of the observed motor and behavioral dysfunction (i.e., cognitive impairment, anxiety, depression, sleep disorder) [5]. The currently available symptomatic therapy, a combination of levodopa/carbidopa, after an initial period of significant benefit, usually leads to drug-related motor complications (i.e., "wearing-off" symptoms, dyskinesia, "on-off" phenomenon), autonomic dysfunction and mood swings, as well as drug-related side effects (psychosis), all of which severely reduce quality of life [6]. Therefore, the development of new therapeutic approaches targeting all aspects of the disease remains the highest priority and an unmet need. Intracerebral injection of the neurotoxin 6-hydroxydopamine (6-OHDA) provides a valuable experimental rodent model that selectively targets DA neurons and induces neurodegeneration with synaptic rearrangements and motor and behavioral deficits consistent with those seen in humans [7–9]. The model reproduces the changes in the postsynaptic *N*-methyl-*D*-aspartate receptors (NMDAR) subunit composition and the resulting changes in basal ganglia connectivity typical of PD patients [3]. Notably, 6-OHDA–induced denervation alters relative GluN1 and GluN2B levels without affecting GluN2A [10–12], suggesting that variations in NMDAR subunit composition play a central role in deregulating corticostriatal plasticity [3,4]. Overall, the 6-OHDA-induced PD model is a reliable tool for testing the clinical efficacy of novel therapeutic approaches in PD.

Repetitive transcranial magnetic stimulation (rTMS) is a safe and noninvasive method of brain stimulation that has emerged as one of the potential candidates for PD therapy. In rodents, rTMS has been shown to be capable of eliciting complex neurobiochemical effects affecting early gene expression, changes in Ca^{2+} dynamics, changes in neurotransmitter release and glutamate receptor expression, reduction of oxidative stress and inflammation and activation of neurotrophic factors [13]. Previous research by several groups shows that intermittent theta burst stimulation (iTBS), a highly efficient excitatory protocol of rTMS, has beneficial effects on neuroinflammation, anxiety-like behavior, depressive-like behavior and learning and memory in several animal models of neurodegeneration [14–16]. To date, a few studies have been conducted to evaluate the effects of various rTMS protocols on the rodent model of PD [14,17]. Most studies reported beneficial effects of rTMS, but focused primarily on motor symptoms and survival of DA neurons (for a review please see [17]), neglecting behavioral aspects of the disease and underlying mechanisms. Given the complexity of PD pathology and the presence of nonmotor dysfunction that often precedes motor symptoms, further research is needed to provide a clear rationale for whether and how an rTMS-specific protocol, such as iTBS, can influence pathological processes underlying both motor and nonmotor aspects of PD. Therefore, the aim of this study was to examine the effects of prolonged iTBS on motor performance and behavior and the potential crosstalk with alterations in synaptic plasticity and NMDAR subunit composition. Our findings contribute to the understanding of the mechanisms underlying iTBS effects in experimental PD and provide new insights into how iTBS might be used as a therapy in PD patients.

2. Materials and Methods

2.1. Animals and Housing Conditions

A total of 90 two-month-old male Wistar rats (280 ± 20 g), bred at the Center of Veterinary Services animal facility, Ministry of Defense, were used for this study. Animals (3–4/cage) were maintained under constant conditions (23 ± 2 °C, 12 h light/dark cycle), a standard diet and tap water ad libitum. All experimental procedures were performed in accordance with the EU Directive 2010/63/EU and approved by the Ethics Committee for Animal Experiments of the College University of Belgrade—Faculty of Biology (No. 323-07-08250/2021-05). The experiments were repeated twice to avoid a litter effect (n = 50 per experiment).

2.2. Unilateral 6-Hydroxydopamine Lesion of the Right Substantia Nigra Pars Compacta

The animals were positioned in a stereotaxic frame (Stoetling Co., Wood Dale, IL, USA) under ketamine (100 mg/kg) and xylazine (10 mg/kg) anesthesia. Two microliters of 6-OHDA (6 µg/µL, Sigma Aldrich, St. Louis, MO, USA) in sterile saline supplemented with 0.2% ascorbic acid were injected into the region of rSNpc (right SNpc), whereas the same volume of vehicle was injected into the lSNpc (left SNpc) (+5.40 mm AP; ±2.10 mm ML and +7.40 mm DV, according to the stereotaxic Atlas of Paxinos and Watson). The neurotoxin was administered through a 50-µL Hamilton syringe at a constant flow rate of 0.4 µL/min (Harvard Apparatus, Holliston, MA, USA) [18]. The needle was left in place for an additional 5 min to allow diffusion of the solution in the SNpc and then slowly retracted. Another group of animals (control) received the vehicle solution bilaterally in the SNpc using the same procedure. Seven days postsurgery, a tail suspension test was performed to functionally assess the motor asymmetry induced by unilateral administration of 6-OHDA and performance in this test was used as a selection criterion [19]. In contrast, animals without motor asymmetry were excluded from the experiment (n = 4).

2.3. Experimental Design

Figure 1 summarizes the experimental design. Animals injected unilaterally with 6-OHDA were randomly divided into three experimental groups: 6-OHDA (n = 22), 6-OHDA + iTBS (n = 22) and 6-OHDA + iTBSsh (sham; n = 20). The fourth group of animals received the vehicle solution bilaterally and was used as controls (n = 20). The animals were subjected to the iTBS protocol or sham stimulation for 21 days and sacrificed after 30-dpi by decapitation (Harvard Apparatus, Holliston, MA, USA).

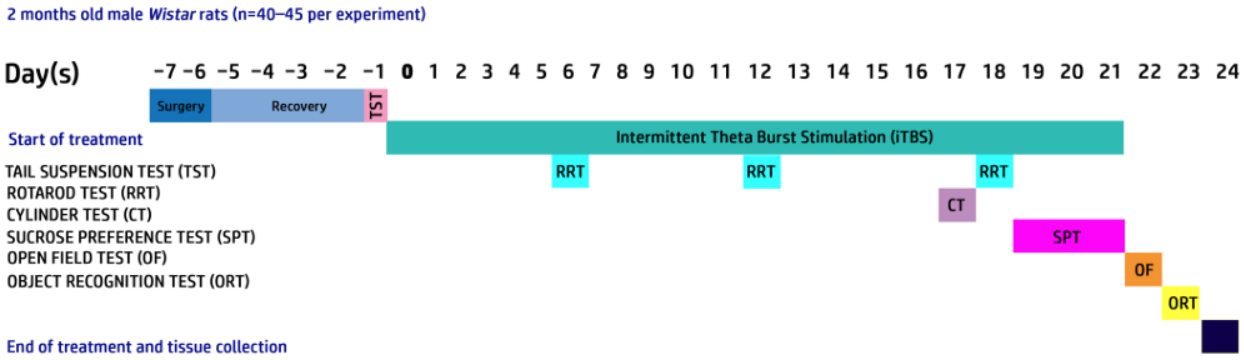

Figure 1. Outline of the experimental paradigm.

2.4. Theta Burst Stimulation Protocol

Seven days after surgery, animals in the 6-OHDA + iTBS group underwent the intermittent theta burst stimulation (iTBS) protocol, as previously reported [16]. Because DA neuronal cell death peaks approximately 7 days after surgery, coinciding with the onset of motor symptoms [20,21] the iTBS treatment began at this time point. Stimulation was performed with the MagStim Rapid^2 device and a 25-mm figure-of-eight coil (MagStim Company, Whitland, UK). Twenty trains of ten bursts each (three pulses at a frequency of 50 Hz), repeated at 5 Hz (10 s intervals between trains, with a total duration of the stimulation of 192 s, with the magnetic stimulation intensity set at 35%) were delivered to each animal. The animals were gently held during stimulation while allowed to move freely during the 10 s intervals between trains. The sham group (6-OHDA+ iTBS sham) was exposed to the noise artifact by placing a cage containing two animals next to the stimulation device. The same treatment protocol was repeated for 21 consecutive days. The 6-OHDA and control groups did not receive iTBS treatment.

2.5. FEM Modeling Methodology

To investigate the TMS parameters and induced electric field (E) in this study, a 3D finite element method (FEM) was used to create a rat head and TMS model. The head model was derived from high-resolution anatomical T2-weighted images with isotropic voxels of an ex vivo rat's head (weighing 280 ± 20 g) acquired with a 9.4T Bruker Biospec 94/30 small animal MRI machine. MRI images were processed and high-resolution images [22] were registered using the FMRIB software library [23] and the original rat brain was extracted using the Brain Extraction Tool (BET) (Figure 2). The 3D surfaces for each tissue type were segmented using ITK-SNAP [24] based on the image signal intensity values and imported into COMSOL Multiphysics version 6.0 for optimization, meshing and TMS simulation (Supplementary Figure S1). The final rat head and coil model consisted of ~1.13 million domain elements. The brain was 26.85 mm long (excluding the spinal trigeminal tract and spinal cord). The MagStim Rapid2 TMS device and 25-mm eight-coil models were created using two homogenized multiturn coils (inner diameter 18 mm, outer diameter 42 mm), each with 14 turns of apartment copper (0.75 mm × 6 mm) [25,26]. The model boundary contained an infinite domain unaffected by boundary conditions. All model domains were meshed with at least one "extra fine" element size and the coil and its core were meshed with the swept function. rTMS was simulated by driving the coil with a 584.5 V (V_c) pulse generated by the MagStim system, with the current change (dI/dt) inducing the E-field peaking at 50.7 A/μs ($dI/dt|_{Max} = V_c/L$). This simulation was performed in the frequency domain using the biphasic pulse rise time similar to the method used by Tang et al. (2016) [27]. The coil was aligned centrally over the rat brain so that the current direction of the coil would induce an E-field in the brain running anterior to posterior. Dielectric properties such as isotropic conductances (σ) were set for each tissue layer as soft tissue = 0.465 S/m, skull = 0.02 S/m, cerebrospinal fluid = 1.654 S/m, gray matter = 0.106/m and white matter = 0.126 S/m; other dielectric properties were set to values used in previous rodent TMS studies [28,29]. The E-field and magnetic flux density (B) induced by rTMS were calculated using the magnetic and electric field equations in the AC /DC module of COMSOL, which solves Maxwell's equations by determining the magnetic vector potential field (A) in the frequency domain. The rate of change of the coil voltage determines the electric field induced in the brain and the magnetic flux density is given by the expressions $E = -\nabla V - j\omega A$ and $B = \nabla \times A$, respectively, where ∇ is the curl, ω is the frequency and j is the free current density.

2.6. Behavioral Tests

Behavioral tests were performed in a secluded room to provide acoustic and visual isolation. Before each test, animals were allowed at least 60-min habituation period. A single researcher conducted all behavioral tests to ensure consistency in treatment of the animals. Odors were removed by cleaning the apparatus with 70% alcohol between test sessions. Control and treated animals were treated in parallel on the same day to keep conditions as constant as possible.

2.7. Rota-Rod Test

A rota-rod device (Elunit, Belgrade, Serbia) was used to assess motor coordination and balance. Seven days before surgery, the animals underwent three conditioning sessions on a rota-rod device. The animals were placed on a stationary suspended cylinder (rod) for 30 s to stimulate fall avoidance behavior. Animals were then placed on the rotating cylinder at a constant speed of 10 rpm for 90 s. Animals that did not withstand the challenge in all training sessions were removed from the experiment. The experimental animals were subjected to 3 rota-bar test sessions, accelerating from 4 to 20 rpm in 200 s [30]. Each test session consisted of three trials on the rota-rod, with a maximum duration of 200 s per trial and a 30 min inter-trial interval. For each animal, the latency to fall and traveled distance were recorded and the best performance from the trial was used for further analysis.

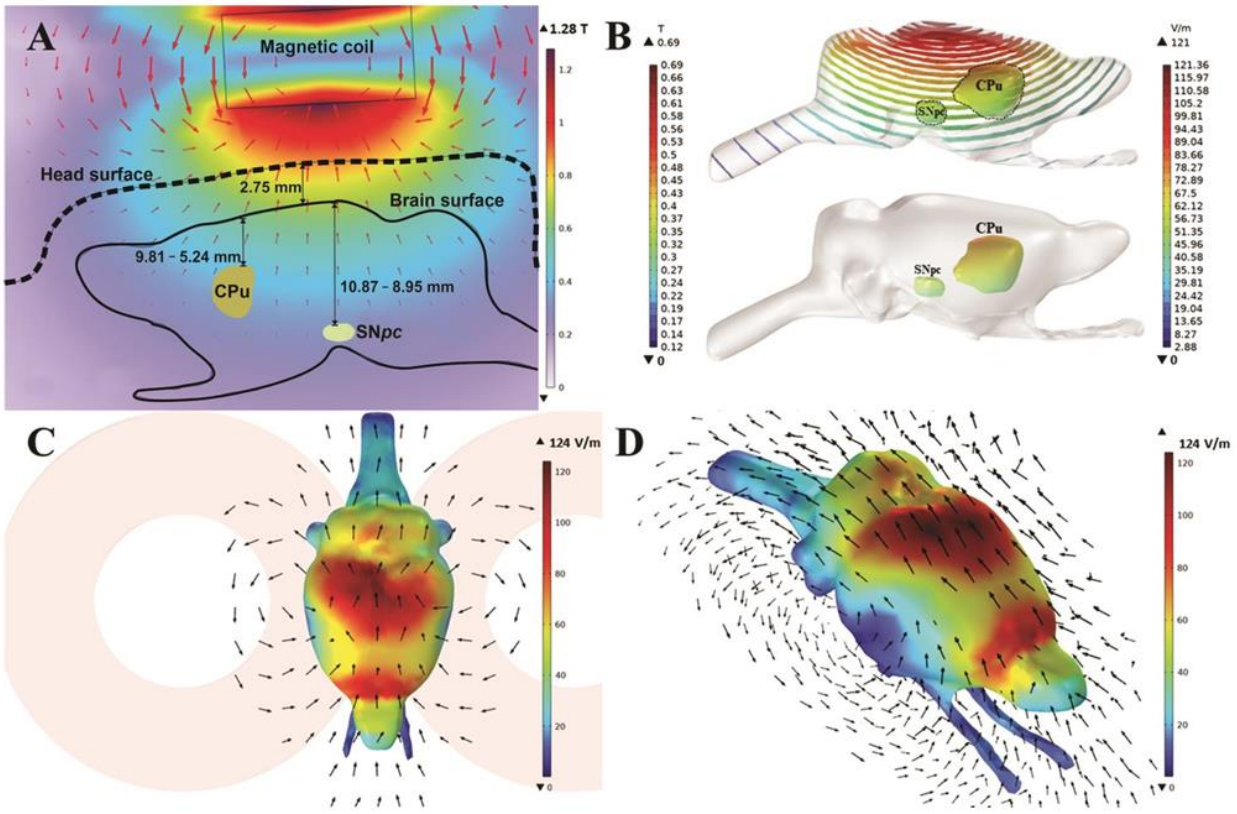

Figure 2. Heat maps of magnetic field (**A**,**B**) and electric field (**C**,**D**) measured in the stimulated iTBS FEM model. Representative FEM models of iTBS stimulation depicting 2D (**A**) and 3D (**B**) heat maps of magnetic field and electric fields (**C**,**D**). Arrows show the direction of the generated electric field (**C**,**D**). Scale bars = 10 mm.

2.8. Limb Use Asymmetry (Cylinder Test)

The cylinder test is used to study spontaneous use of the forelimbs [31]. An animal was placed in a glass cylinder (H: 40 cm × D: 20 cm) and the number of times it reared up and touched the cylinder wall was recorded for 5 min. Wall contacts were sorted as contralateral forelimb (CF), ipsilateral forelimb (IF) or both forelimb (BF) contacts. The number of impaired forelimb contacts relative to the total number of contacts was calculated using [(CF × 0.5 BF)/(IF + CF + BF)] and was used as an index of asymmetry [32]. Unaware of the experimental groups, two researchers independently analyzed the data and averaged the results.

2.9. Open Field

After completion of iTBS treatment, an open field test (OFT) was performed to assess anxiety-like behavior [16,33]. The animals were placed in the upper right corner of a black arena (100 × 100 × 50 cm) divided into 25 × 25 cm squares and their activity was recorded for 5 min. The total number of movements and the time spent in the center fields (in seconds) were analyzed using ANY-maze Video Tracking System 7.11.

2.10. Object Recognition Test

Object recognition was applied to test the learning and memory performance of the 6-OHDA animals subjected to iTBS. The animals were placed in the center of the arena, equidistant from two identical rectangular objects. They were allowed to freely explore the arena and the objects for 5 min (sampling phase) before returning to their home cages. After

one hour, the animals returned to the arena, where a new conical object was introduced in place of a rectangular one. Their activity was recorded for an additional 5 min (test phase), provided that sniffing, climbing and exploring the object for more than 2 s was classified as active exploration. Performance was analyzed by ANY-maze Video Tracking System 7.11. The time spent with the novel object relative to the total time spent with both objects is expressed as the recognition index (RI) [34].

2.11. Sucrose Preference Test

The sucrose preference test was used to assess anhedonia and/or depressive-like behavior [35]. Briefly, animals were deprived of food and water for 18 h, beginning at 3 pm the day before the test. At 9 am, the animals were placed in a cage for one hour and given two preweighed bottles, one containing tap water and the other containing 2% sucrose. The procedure was repeated for three consecutive days, switching the position of the bottles. The volume of ingested liquids was measured after each session and the mean volume was used to evaluate the sucrose preference (%) = [(sucrose intake/(sucrose intake + water intake)) × 100].

2.12. Brain Tissue Preparation and Immunohistochemical Staining

The brains were rapidly removed from the skull after decapitation (n = 3–4/group) and fixed in 4% paraformaldehyde (PFA) for 24 h, cryoprotected and dehydrated in graded sucrose solution (10–30% in 0.2 M PBS, pH 7.4). The 25 µm-thick coronal sections at the level of the caudoputamen and the midbrain were mounted on supefrost glass slides, air-dried for 1–2 h at RT and stored at −20 °C until use.

After rehydration in PBS, the sections were treated with 0.3% hydrogen peroxide for 20 min and washed with PBS for 3 × 5 min. Subsequently, sections were blocked with 5% normal donkey serum at room temperature for 1 h, followed by incubation with primary antibodies overnight at 4 °C (Table 1). The slides were then probed with appropriate secondary antibodies (Table 1) for 2 h at room temperature. The signal was visualized using the 3,3′-S-diaminobenzidine-tetrahydrochloride kit (DAB, Abcam, Cambridge, UK) as a chromogen for HRP-conjugated secondary antibodies. After dehydration in graded ethanol (70–100%) and clearance in xylene, the sections were mounted with the DPX-mounting medium (Sigma Aldrich, USA). The sections were examined under a LEITZ DM RB light microscope (Leica Mikroskopie and Systems GmbH, Wetzlar, Germany) equipped with a LEICA DFC320 CCD camera (Leica Microsystems Ltd., Heerbrugg, Switzerland) and analyzed using LEICA DFC Twain Software (Leica, Wetzlar, Germany).

Table 1. List of used primary and secondary antibodies.

Antibody	Source and Type	Used Dilution	Manufacturer
DAT	Rabbit, polyclonal	1:500 ^{WB}	Abcam, #ab184451, RRID:AB_2890225
TH	Rabbit, polyclonal	1:2000 ^{WB}, 1:500 ^{IHC}	Millipore #AB152, RRID:AB_390204
GluN1	Rabbit, polyclonal	1:4000 ^{WB}	Cell Signaling Technology, #5704, RRID:AB_1904067
GluN2A	Rabbit, polyclonal	1:4000 ^{WB}	Millipore, #07-632, RRID:AB_310837
GluN2B	Mouse, monoclonal	1:3000 ^{WB}	Abcam #ab93610, RRID:AB_10561972
BDNF	Goat, polyclonal	1:1000 ^{WB}	Santa Cruz Biotechnology, #sc-33904, RRID:AB_2259044
EAAT1	Rabbit, polyclonal	1:1000 ^{WB}	Cell Signaling Technology, #5684T, RRID:AB_10695722
EAAT2	Rabbit, polyclonal	1:1000 ^{WB}	Abcam, #ab69098, RRID:AB_2190732
GluR1	Mouse, monoclonal	1:1000 ^{WB}	Santa Cruz Biotechnology, #sc-55509, RRID:AB_629532

Table 1. *Cont.*

Antibody	Source and Type	Used Dilution	Manufacturer
Synaptophysin	Rabbit, polyclonal	1:5000 WB	Santa Cruz Biotechnology, #sc-9116, RRID:AB_2199007
PSD-95	Mouse, monoclonal	1:1000 WB	Millipore, #MAB1598, RRID:AB_94278
GAPDH	Rabbit, polyclonal	1:2000 WB	Thermo Fisher Scientific, #PA1-987, RRID:AB_2107311
Goat anti-rabbit IgG, HRP-conjugated	Goat, polyclonal	1:30,000 WB	Abcam, #ab6721, RRID: AB_955447
Goat anti-mouse IgG, HRP-conjugated	Goat, polyclonal	1:30,000 WB	Abcam, #ab97240, RRID:AB_10695944
Rabbit anti-goat IgG, HRP-conjugated	Rabbit, polyclonal	1:10,000 WB	R and D Systems, #HAF017, RRID:AB_562588

WB—western blot; IHC—immunohistochemistry.

2.13. Measurements of Dopamine (DA) Content in Striatum by HPLC Assay

For the HPLC analysis, brains (5–6 animals/experimental group) were isolated from the skull and the midbrain and striatum were dissected for subsequent determination of dopamine and serotonin concentrations [36]. Tissue samples were homogenized in DEPROT (1 mg/10 µL) using an Ultra-Turrax homogenizer, sonicated (3 × 10 s), centrifuged (30 min, 15,000 rpm, 4 °C) and the supernatants were transferred to separate tubes. An aliquot of each sample (40 µL) was injected into the UltiMate3000 HPLC system (Thermo Scientific, Waltham, MA, USA) and applied to C18 HPLC column (Thermo Scientific, Waltham, MA, USA) with 100 mM ammonium formate buffer, pH 3.6 (A) and methanol (B), as a mobile phase. The mobile phase was pumped at a flow rate of 500 µL/min, with an initial A:B ratio of 98:2%. Under these conditions, serotonin and dopamine were readily separated and detected by an electrochemical detector (850 mV, 25 °C). Data were analyzed using the Chromeleon7 Chromatography Data System (Thermo Scientific). The catecholamine concentrations were expressed as µg/mg tissue.

2.14. Tissue Isolation and Western Blot Analysis

Animals in the Sham group and the iTBS group (5–7 animals per group) were decapitated and the brains were rinsed in ice-cold saline. The right caudoputamen (rCPu) and left caudoputamen (lCPu) and right midbrain (rMB) and left midbrain (lMB) were dissected and separately frozen in liquid nitrogen and stored at −80 °C. For Western blot analysis, the expression level of target proteins in the lCPu and lMB of each animal was used as an internal control for the expression level in the rCPu and rMB, respectively. Tissue samples were homogenized in the isolation buffer (0.32 M sucrose, 10 mM HEPES, pH 7.4) at 4 °C, the resulting homogenates were centrifuged at 3000× *g* for 10 min at 4 °C and the supernatants obtained were collected for Western blot analysis. The protein content in each sample was determined using the Pierce™ BCA Protein Assay Kit (Thermo Fisher Scientific, USA). Equal sample aliquots (20 µg of the sample proteins) were resolved using SDS-PAGE and transferred to PVDF membrane using semidry transfer, as described previously [37]. The supporting membrane was incubated with primary antibodies (Table 1), rinsed in TBST and incubated with appropriate horseradish-peroxidase (HRP)-conjugated secondary antibodies using SmartBlot apparatus. Chemiluminescent signals were detected by the ECL solution (Bio-Rad, Hercules, CA, USA) in ChemiDocIt Imager (UltraViolet Products Ltd., Cambridge, UK). The optical densities (OD) of the target band and GAPDH band (loading control) in each lane were determined in the ImageJ program (https://imagej.nih.gov/ij/, accessed on 1 October 2020) and the ratio in each lane was expressed relative to the same ratio in Sham-L. The results are expressed as mean ± SD, from n = 2–4 independent replicates.

2.15. Statistical Analysis

All data were analyzed for normality using the Shapiro—Wilk test and appropriate parametric or nonparametric tests were used. The results of the behavioral tests were analyzed using one-way ANOVA, followed by Tukey's *post hoc* test for multiple comparisons. The results obtained by HPLC and Western blot analysis were analyzed using Student's *t* test or the Mann—Whitney test if the normality condition was not met. The values represent mean ± SD as indicated in Figure legends. The values of $p < 0.05$ were considered statistically significant. Analysis and graphical presentation were performed in the GraphPad Prism 9.0 (San Diego, CA, USA) software package.

3. Results

3.1. FEM Modeling Results

FEM modeling of the MagStim Rapid^2 apparatus estimated the generated B-field peak to be 1.28 T at the base of each coil forming the figure of eight loop. At the superior surface of the brain (closest to the coil), the value was estimated to be 698.5 mT, whereas in the CPu it ranged from 480.4 mT to 302.2 mT and in the SN from 358.8 mT to 303.4 mT (Figure 2B). Accordingly, the estimated E-field value at the cortical surface of the brain was 124.05 V/m, decreasing down to a minimum of ~21 V/m at the base of the brain (Figure 2B–D). The CPu was located between 5.24 mm and 9.81 mm below the scalp surface and experienced simulated E-field values between 57.92 V/m and ~33 V/m at these depths, respectively. The SN was located 8.95 mm to 10.87 mm below the scalp surface and exhibited E-field values between ~34 V/m and 37.98 V/m, respectively, (Figure 2A,B).

3.2. Intermittent Theta Burst Stimulation Improves 6-OHDA-Induced Motor Dysfunction

The effects of 6-OHDA injection into the rSNpc and the iTBS protocol on the extent of motor impairment were determined by measuring latency to fall and traveled distance in the rota-rod test (Figure 3). In each of the three consecutive sessions, the latency to fall was significantly lower in the 6-OHDA group compared to the control animals, whereas the animals receiving iTBS stimulation showed a dramatic improvement in motor performance, reflected as significantly prolonged latency to fall, which was comparable to the control (Figure 3A—$F_{(3, 76)} = 21.10$, $p < 0.0001$; Figure 3B—$F_{(3, 76)} = 17.52$, $p < 0.0001$; Figure 3C—$F_{(3, 76)} = 13.89$, $p < 0.0001$). The same holds for travelled distance measures, which were significantly reduced in 6-OHDA animals and comparable to the control level in the iTBS group (Figure 3D—$F_{(3, 76)} = 19.01$, $p < 0.0001$; Figure 3E—$F_{(3, 76)} = 17.26$, $p < 0.0001$; Figure 3F—$F_{(3, 76)} = 14.84$, $p < 0.0001$). The effect of iTBS on the motor performance was further tested in the cylinder test. The use of contralateral forelimb was severely impaired in the 6-OHDA animals compared to the control. The animals subjected to iTBS showed a significant improvement, reflected in the percentage of contralateral forelimb contacts comparable to the control (Figure 3G, $F_{(3, 76)} = 17.36$, $p < 0.0001$).

3.3. Intermittent Theta Burst Stimulation Improves 6-OHDA-Induced Non-Motor Symptoms and Neurochemical Imbalance

The efficacy of iTBS in attenuating 6-OHDA-induced anxiety-like behavior was examined in the open-field test. Measured parameters, including the number of entries (Figure 4A, $F_{(3, 75)} = 11.20$, $p < 0.0001$) and time spent in central fields (Figure 4B, $F_{(3, 73)} = 21.05$, $p < 0.0001$), show significant improvement in iTBS-treated animals compared to the 6-OHDA group (Figure 4A,B). Similarly, iTBS attenuated depressive-like behavior in the sucrose preference test ($F_{(3, 40)} = 11.49$, $p < 0.0001$). The sham and 6-OHDA animals showed a moderate reduction in sucrose intake compared to the control, whereas sucrose intake in iTBS animals was comparable to the control (Figure 4C). The efficacy of iTBS stimulation on learning and memory was tested through an object recognition test (Figure 4D). A significant difference was observed in the recognition index between the iTBS, 6-OHDA and sham groups ($F_{(3, 62)} = 16.97$, $p < 0.0006$), which implies a beneficial influence of iTBS on short-term memory performance.

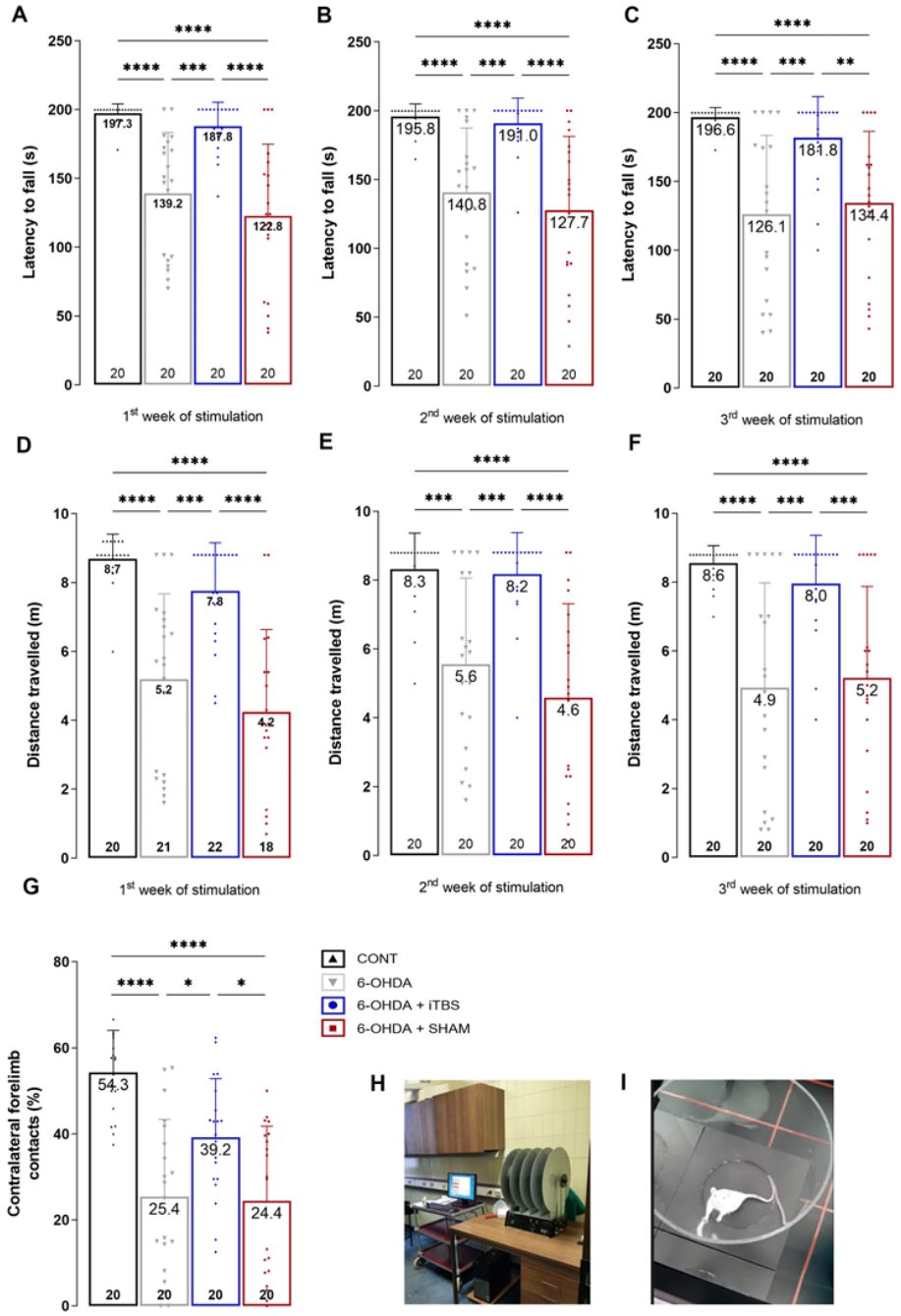

Figure 3. Intermittent theta burst stimulation improves 6-OHDA-induced motor dysfunction. Motor performance in control and 6-OHDA-lesioned rats following iTBS treatment was evaluated using rota-rod (**A**–**F**) and cylinder test. Rota-rod measured the latency to fall (s) and travelled distance (m) after first, (**A**,**D**) second (**B**,**E**) and third (**C**,**F**) week of stimulation. Cylinder test measured the contralateral forelimb contacts with the wall (**G**). Values are expressed as mean ± SD. Results of *post hoc* Tukey's test and significance are shown inside the graphs: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$. Dots in the graphs represent the values of individual animals. (**H**) Rota-rod setting. (**I**) Cylinder arena.

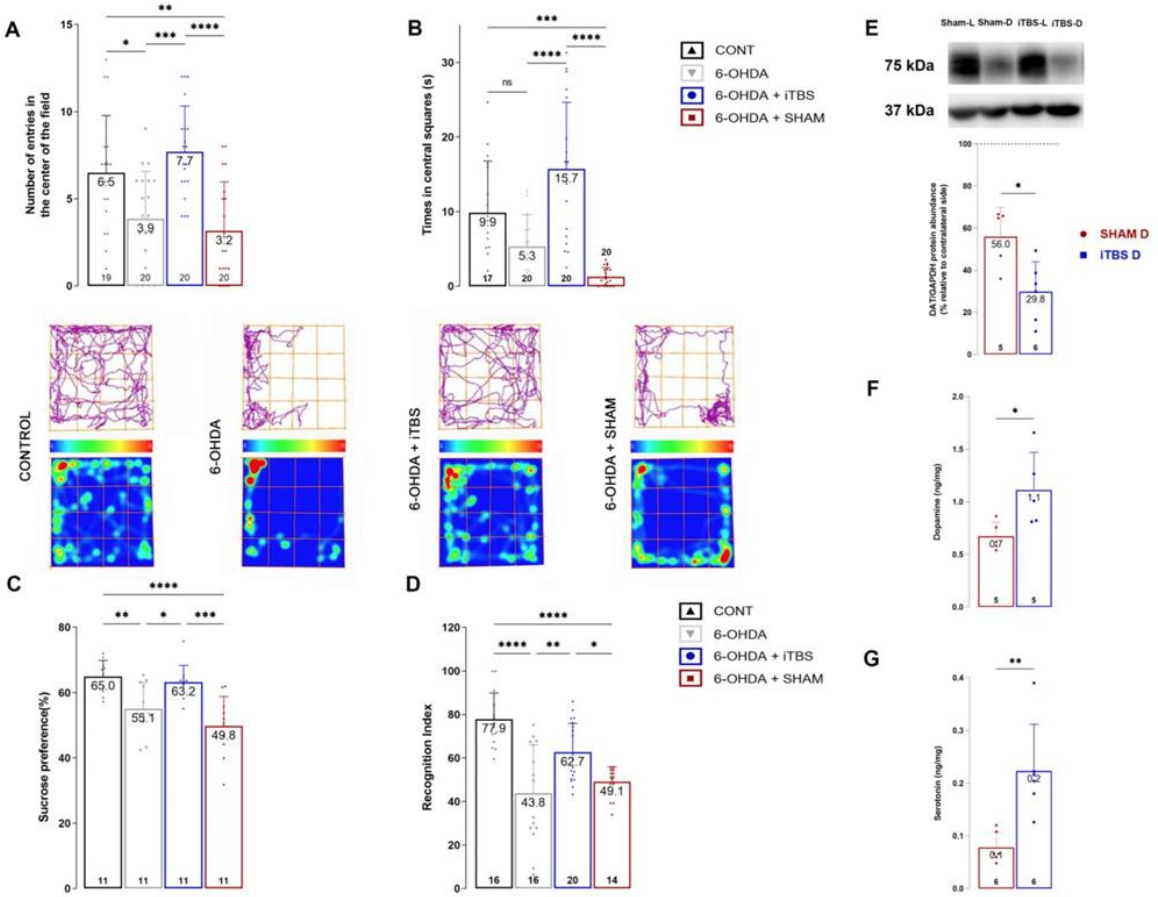

Figure 4. Intermittent theta burst stimulation improves 6-OHDA-induced nonmotor symptoms and neurochemical imbalance. Quantitative analysis of open field behavioral test represented as number of center entries (**A**) and time in central squares (**B**). Track plots and heat maps of animals from each group are shown below the graphs. Sucrose preference test was used to assess anhedonia (**C**), while short-term memory was assessed with novel object recognition test expressed as recognition index (**D**). Results of *post hoc* Tukey's test and significance are shown in graphs, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$, ns—not significant. Representative immunoblot membrane and quantitative data analysis showing relative DAT protein abundance in caudoputamen (**E**). Bars represent the mean value of target protein normalized to GAPDH abundance ± SD (from n = 5–6 individual animals, in 2–4 technical replicates), expressed relative to left caudoputamen, which serves as internal control, arbitrarily defined as 100%. Results of HPLC analysis of dopamine (**F**) and serotonin (**G**) concentrations in lesioned caudoputamen expressed as ng/mg tissue. Data are expressed as mean ± SD (n = 5–6/group). * $p < 0.05$ different from sham (two-tailed unpaired Student's *t*-test, **E**–**G**). Dots in the graphs represent values of individual animals.

The neurochemical changes induced by 6-OHDA and iTBS stimulation were assessed by determining the difference between the rCPu and lCPu in respect of protein abundance of dopamine transporter (DAT) (Figure 4E) and dopamine and serotonin levels (Figure 4F,G). The protein abundance of DAT protein expression in the iTBS group was lower than in the sham group ($t = 3.082$, $p < 0.01$). The ratio of dopamine (Figure 4F, $t = 2.590$, $p < 0.05$) and serotonin (Figure 4G, $t = 3.798$, $p < 0.01$) in the rCPu and lCPu increased significantly in the iTBS animals compared to the sham group.

3.4. Intermittent Theta Burst Stimulation Reduces 6-OHDA-Induced Neuronal Death of the Lesioned SNpc and Loss of TH Positive Fibers Density in the Caudoputamen

The neuroprotective effects of iTBS treatment on dopaminergic neurons in the SNpc and their projections in the CPu were assessed using tyrosine hydroxylase (TH) immunohistochemistry (Figure 5A,B) as well as TH protein quantification with Western blot analysis (Figure 5C,D). Unilateral injection of 6-OHDA in a sham group resulted in a marked decrease in TH-immunoreactivity in rSNpc (Figure 5A) and TH-positive projections in the rCPu (Figure 5B) compared to the contralateral sides. Analysis of relative protein expression of TH-positive neurons (Figure 5C, $t = 5.471$, $p < 0.001$) in MB and TH-positive fibers (Figure 5D, $t = 3.147$, $p < 0.01$) in the CPu confirmed the higher survival of the iTBS group compared to the sham group.

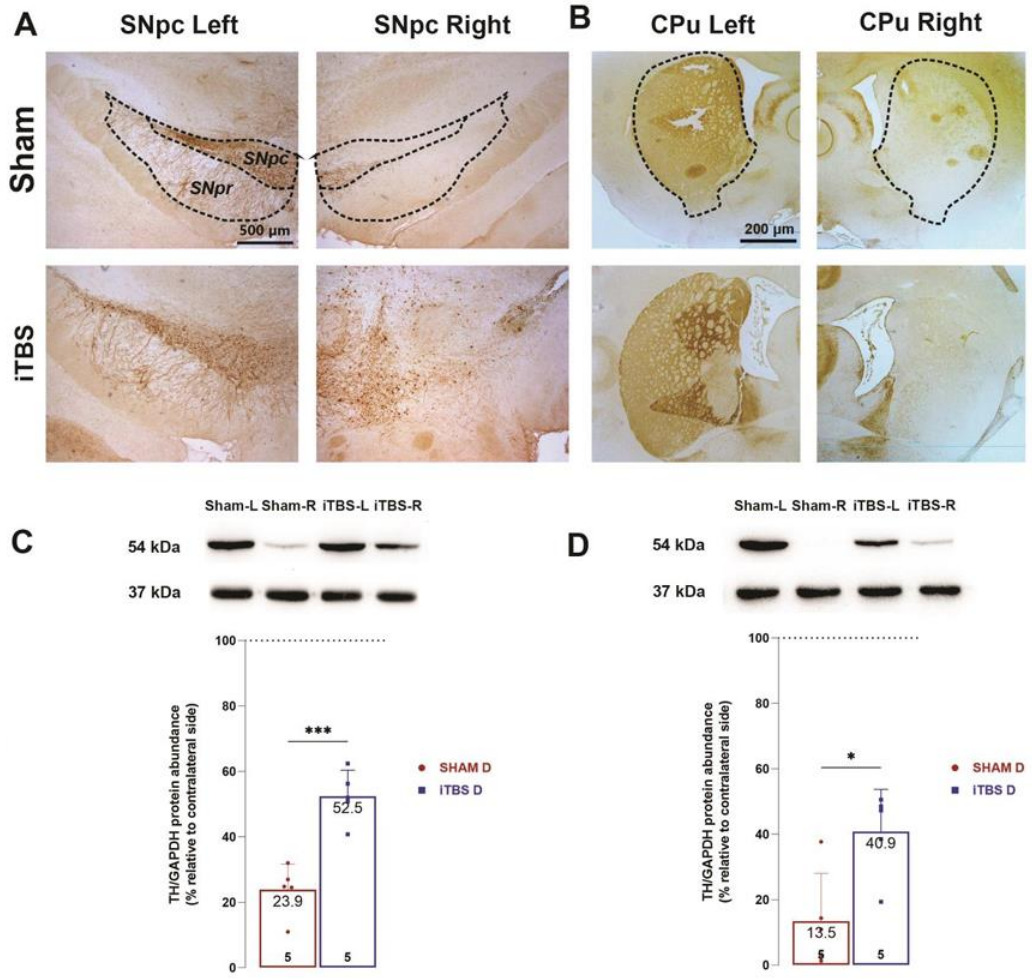

Figure 5. Intermittent theta burst stimulation reduces 6-OHDA-induced neuronal death of the lesioned SNpc and loss of TH positive fibers density in the caudoputamen. Representative coronal sections of TH-positive neurons in the SNpc (**A**) and TH-positive fibers in striatum (**B**) from sham-treated and iTBS-treated rats after 4 weeks. Scale bar: 500 µm. Representative immunoblot membrane and quantitative data analysis showing relative TH protein abundance in SNpc (**C**) and caudoputamen (**D**). Bars represent the mean value of target protein normalized to GAPDH abundance ± SEM (from n = 5 individual animals, in 2–4 technical replicates), expressed relative to left midbrain or caudoputamen, which serves as internal control, arbitrarily defined as 100%. Results of two-tailed unpaired Student's *t*-test and significance are shown in graphs: * $p < 0.05$, *** $p < 0.001$.

3.5. Intermittent Theta Burst Stimulation Modulates NMDA Receptor Subunit Composition

The expression levels of the NMDA receptor subunits GluN1, GluN2A and GluN2B, the AMPA receptor (GluR1) and the glutamate transporters GLAST (EAAT1) and GLT (EAAT2) were further examined in the CPu after 6-OHDA and iTBS treatment. The expression levels of BDNF, SIN and PSD-95, which are involved in synaptic plasticity and functional recovery were also examined (Figure 6). As presented in Figure 5, protein abundance of GluN1 (Figure 6A, $t = 5.040$, $p < 0.001$), GluN2A (Figure 6B, $t = 5.537$, $p < 0.001$), GLAST (Figure 6D, $t = 4.212$, $p < 0.001$), GLT1 (Figure 6E, $t = 2.783$, $p < 0.05$) and BDNF (Figure 6F, $t = 3.672$, $p < 0.006$) in rSNpc compared to lSNpc significantly increased in animals receiving iTBS treatment relative to the sham group. The iTBS treatment resulted in an increase in SYN/PSD-95 ratio relative to the sham group (Figure 6H, $t = 4.768$, $p < 0.001$).

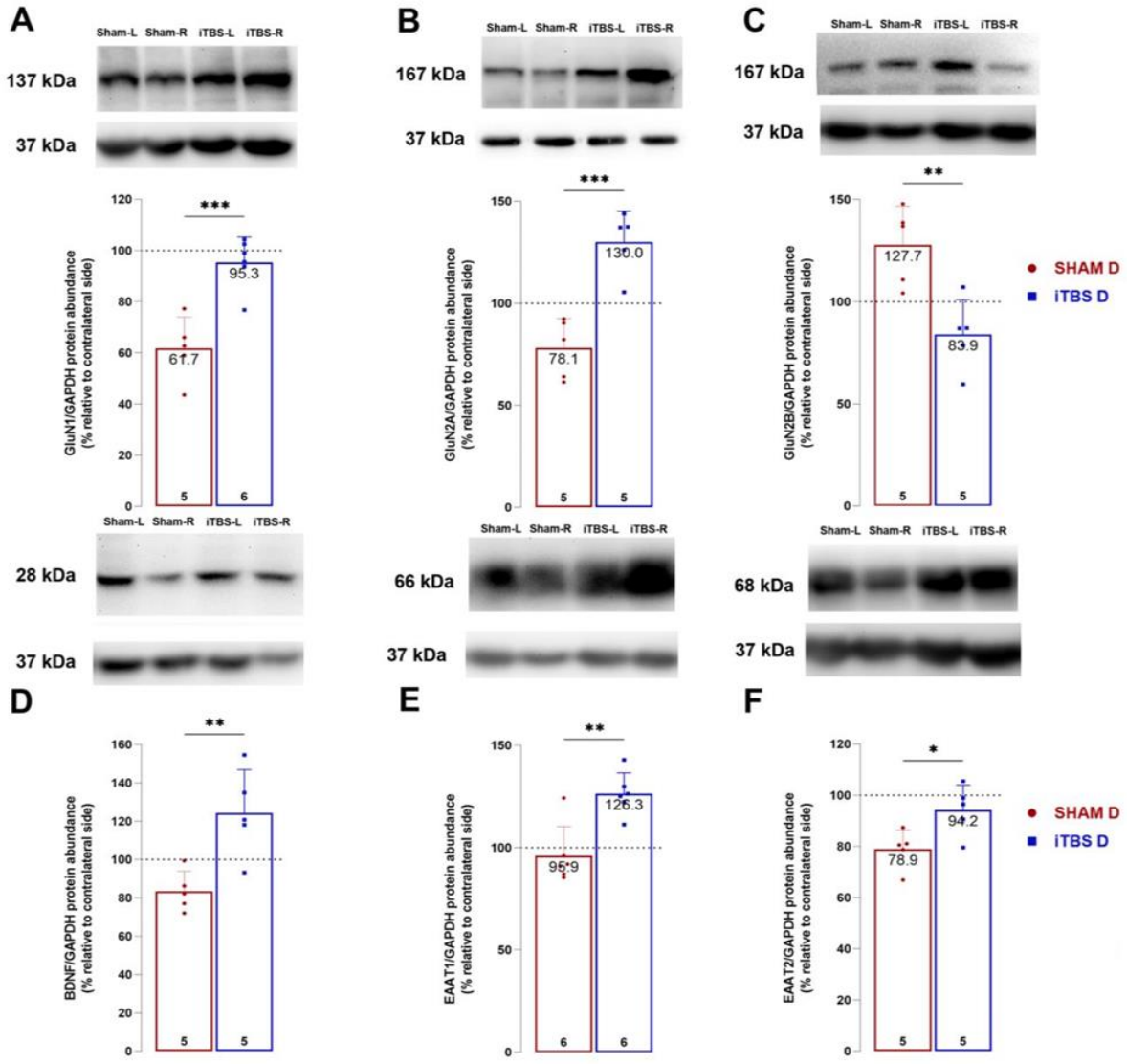

Figure 6. *Cont.*

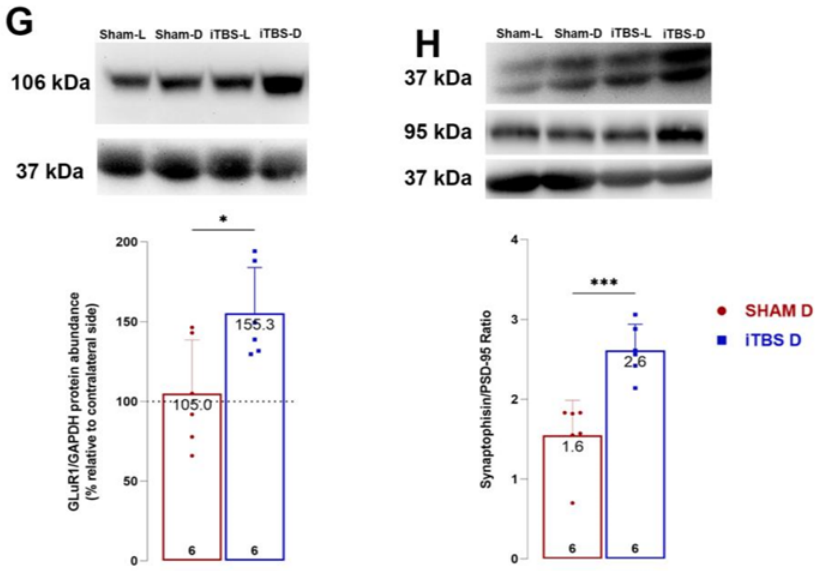

Figure 6. Intermittent theta burst stimulation modulates NMDA receptor subunit composition. Representative immunoblot membrane and quantitative data analysis showing relative subunit protein abundance of GluN1 (**A**), GluN2A (**B**), GluN2B (**C**), BDNF (**D**), EAAT1 (**E**), EAAT2 (**F**) and GluR1 (**G**). Relative abundance of the ratio of synaptic proteins Synaptophysin and PSD-95 ratio in caudoputamen of sham and iTBS animals (**H**). Bars represent the mean value of target protein normalized to GAPDH abundance ± SD (from n = 5–6 individual animals, in 2–4 technical replicates), expressed relative to left caudoputamen, which serves as internal control, arbitrarily defined as 100%. Results of two-tailed unpaired Student's *t*-test and significance are shown in graphs: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

4. Discussion

The aim of the present study was to investigate the effect of a prolonged iTBS stimulation protocol on gross motor performance and nonmotor symptoms in the 6-OHDA model of Parkinson's disease. Neurodegeneration was induced by unilateral injection of 6-OHDA into the right SNpc, resulting in selective DA neuronal cell loss and anterograde degeneration of the nigrostriatal neural pathway. We would like to emphasize that the experimental paradigm used in this work may be more appropriate than the other commonly used when the toxin is infused in the CPu. Although both experimental paradigms lead to similar motor impairments, the underlying cause might be different at the molecular level. Because 6-OHDA enters the cell via DAT, when used in CPu it unselectively destroys all synaptic terminals and possibly glial cells expressing DAT [38,39] as well as other elements expressing serotonin and norepinephrine transporters, since 6-OHDA can also enter the cell via these transporters [38]. On the other hand, if it is injected into the SNpc, it destroys the cell bodies of the dopaminergic neurons and consequently all projections to the CPu, but also to other regions [40] leading to a more suitable model of PD. The main focus of this paper was to evaluate the specific iTBS protocol that started 7 dpi with the onset of motor and behavioral deficits [19,20,41] and lasted for three consecutive weeks. The effects of iTBS were assessed in terms of motor and nonmotor symptoms and underlying neurochemical and biochemical responses.

To analyze which parts of the brain are affected by the iTBS protocol, we created the FEM model. The FEM model describes that E-field levels in most parts of the brain, including the caudoputamen and substantia nigra, are above the value of ~28 V/m, sufficient to generate action potentials in the affected neuronal tissue [42], predicting that the iTBS protocol would have been effective in eliciting electrical changes in the structures studied. It should be noted that the electrical field generated also affects glial cells and their

physiology, which may contribute to the overall response seen after iTBS [43]. The FEM model gives us the opportunity and rationale to hypothesize that the iTBS protocol used in this study may have a direct effect by targeting the CPu and SNpc, apart from the likely indirect effects resulting from stimulation of other brain regions.

Previous modeling attempts have described that TMS-induced maximal E-field values depend strongly on the species and brain size. However, rodent and human values are similar for the 25-mm coil used in our study, supporting the translational potential of this protocol [44].

The study confirmed that the iTBS protocol was able to improve the motor deficits of 6-OHDA-affected animals, as evidenced by significantly improved performance on the rota-rod and cylinder tests. Motor improvements were observed as early as one week after initiation of iTBS stimulation, in contrast to other rTMS protocols that required two or more weeks of application to produce measurable motor progress [45,46]. Furthermore, the iTBS protocol resulted in sustained motor improvement that lasted for at least three weeks after intoxication; that is, for the entire duration of the stimulation. At the cellular level, motor improvement may be associated with a significant reduction in DA neuronal cell loss and preservation of nigrostriatal projections [14]. Several TMS protocols have been shown to reduce neuronal cell death by interfering with pro-apoptotic signaling pathways [14,16] and reducing the production of reactive oxygen species [47] involved in 6-OHDA-induced neurotoxicity [48]. In addition to direct neuroprotective effects, iTBS also attenuates glial cell-mediated secondary damage [15,49], all of which may be responsible for the increased TH expression observed after treatment.

Our study demonstrated that the iTBS protocol improves nonmotor symptoms, including anxiety- and depressive/anhedonic-like behavior and cognitive deficits induced by 6-OHDA. The neurotoxin directly induces loss of dopaminergic neurons and subsequent dopamine depletion in the striatum, which contributes to both motor impairments and anxiety- and depressive/anhedonic-like behavior. Several studies have shown that 6-OHDA-induced loss of dopaminergic terminals, as well as other catecholaminergic projections, leads to a neurochemical imbalance that partially underlies the observed behavioral deficits [50]. Accordingly, iTBS protocol restored dopamine levels in the striatum, confirming previous data obtained with other rTMS protocols in animal models of neurodegenerative [20,46] and psychiatric disorders [51]. The recovery of dopamine levels after iTBS [20,43,46] is likely due to reduced loss of DA neuronal projections and striatal DAT expression. The beneficial effects of iTBS on behavioral and motor deficits in 6-OHDA are probably due, in part, to increased serotonin levels as well, which is critically involved in cognitive and motor functions and in the pathophysiology of depressive-like behavior in animals and humans [50,52]. Serotonin in the striatum originates from the dense terminal projections arising from the dorsal *raphe* nucleus. Its interactions with the striatal dopaminergic system [53] decrease corticostriatal [54] and thalamostriatal [53] glutamatergic input and modulated reward-mediated learning [55], thus the observed increase may influence aforementioned processes. The behavioral deficits resulting from 6-OHDA, such as anxiety- and depressive/anhedonic behavior, are a complex phenomenon with an elusive molecular background, so that increases in dopamine and serotonin, although beneficial, are not solely responsible for the deficits or improvements after iTBS. It is also worth noting that due to the size of the coil, the stimulation affects virtually the entire brain, so it is possible that some of the improvement in motor and nonmotor symptoms is also due to stimulation of other brain regions and increased hemispheric compensation [56]; however, this is beyond the scope of this study and requires further investigation.

In this regard, our study is the first to show changes in NMDAR subunit composition in a model of 6-OHDA-induced SNpc degeneration. In 6-OHDA animals, increased extracellular glutamate from corticostriatal inputs potentiates GluN1/GluN2B-mediated signaling, which, together with depletion of DA, can lead to motor and behavioral deficits [57]. The decreased expression of GluN1 and GluN2A and upregulation of GluN2B suggest that 6-OHDA alters NMDA receptor subunit composition in favor of GluN1 and GluN2B.

Therefore, iTBS treatment reversed these effects and resulted in an increase in GluN1 and GluN2A and a decrease in GluN2B subunit expression. Interestingly, selective GluN2B receptor antagonism was found to alleviate symptoms in rodent and primate models of PD [58–62], suggesting an important role of GluN2B-mediated signaling in PD. Moreover, the iTBS-induced increase in GLAST and GLT1 expression may offset the increased extracellular glutamate levels. Several studies have shown that extracellular glutamate levels and/or binding of glutamate to its receptors are increased in both the 6-OHDA model and PD pathology [63–65]. Because chronic rTMS has been shown to induce the release of neurotransmitters in vivo, including glutamate, the increase in astrocytic GLAST and GLT1 may be a compensatory mechanism that can regulate extracellular glutamate levels, which is also relevant to the pathology.

Overall, our data suggest that iTBS enhances GluN1/GluN2A-mediated signaling and DA-glutamate crosstalk in the striatum, which is essential for motor and nonmotor behavior. Moreover, it has been shown that induction of LTP by high-frequency stimulation in the dorsolateral striatum requires GluN2A-, but not GluN2B-containing NMDAR [66]. Additional evidence comes from our findings that prolonged iTBS increased expression of both presynaptic and postsynaptic markers, which may indicate enhanced synaptic contacts and which may point to improved structural plasticity and improved motor/behavioral function [12,13]. This phenomenon was demonstrated by other groups in the PD model after acute iTBS [67,68]; however, it would require additional electrophysiological evidence to examine synaptic plasticity in our conditions. Dopaminergic terminals converge with cortical glutamatergic inputs on striatal spiny GABAergic neurons, which control many behavioral outputs [3]. NMDARs containing different subunits play distinct roles in the striatum, i.e., GluN2A-containing NMDARs regulate glutamatergic synaptic transmission and evoked dopamine release in the striatum [69]. Moreover, altered NMDAR subunit composition in the striatum has been shown to be closely associated with the pathophysiology and progression of both PD and experimental parkinsonism [4,10]. Finally, iTBS as well as other rTMS protocols have been shown to affect the expression of NMDAR subunits [70] and that their effects are mediated by NDMA signaling [71–73], further supporting the data we obtained.

In summary, although the results of the present study clearly demonstrate the beneficial effects of iTBS and address some of the potential underlying mechanisms, several limitations of the study should be noted. The first relates to the technical limitations associated with the size and manual placement of the coil, which do not allow focal stimulation of specific areas but can be considered as whole-brain stimulation. Therefore, the observed effects of iTBS may be the result of both cortical and subcortical stimulation and their interconnectivity. The second and a very important limitation concerns the nature of the tissue component. More specifically, it is impossible to precisely determine the changes in a particular cellular compartment (i.e., extrasynaptic vs. synaptic, membrane vs. cytoplasm) in the fraction used, so we can only discuss the overall changes in the striatal region. Finally, more in-depth analyses are required to connect the changes in NMDAR components to the behavioral deficits to strengthen and elucidate the observed benefits following iTBS.

5. Conclusions

To our knowledge, this is the first study to show positive effects of prolonged iTBS on motor and especially, on emotional behavior as well as on learning and memory in the 6-OHDA-induced SNpc degeneration experimental paradigm of PD. This study is also the first to report molecular changes that may contribute to the understanding of the action of iTBS in this model. Overall, the results suggest that prolonged iTBS rescues dopaminergic cells and increases striatal levels of DA, serotonin and glutamate transporter expression and alters NMDAR subunit composition, leading to predominant GluN1/GluN2A-mediated signaling. In conclusion, iTBS protocol, if applied at the onset of early symptoms, may be a promising candidate for the early-stage therapy of PD targeting motor and nonmotor deficits.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/cells12111525/s1, Figure S1: Representative images of MRI rat's brain scan.

Author Contributions: M.Z.J.: Methodology, Validation, Formal Analysis, Investigation, Interpretation of results, Writing—Original Draft; J.S.: Methodology, Formal Analysis, Writing—Review & Editing; I.S.: Methodology, Formal Analysis, Resources, Writing—Review & Editing; A.S.: Methodology, Formal Analysis, Writing—Review & Editing; S.J.B.: Methodology, Formal Analysis, Writing—Review & Editing; N.J.: Methodology, Formal Analysis, Writing—Review & Editing; M.N.: Resources, Writing—Review & Editing; M.Z.K.: Resources, Writing—Review & Editing; T.V.I.: Supervision, Resources, Writing—Review & Editing; J.R.: Interpretation of results, Methodology, Supervision, Writing—Review & Editing; N.N.: Interpretation of results, Supervision, Funding Acquisition, Resources, Writing—Review & Editing; M.D.: Conceptualization, Interpretation of results, Methodology, Validation, Visualization, Formal Analysis, Investigation, Funding Acquisition, Writing—Original Draft. All authors have read and agreed to the published version of the manuscript.

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Institutional Review Board Statement: The animal study protocol was approved by Ethics Committee for Animal Experiments of the College University of Belgrade—Faculty of Biology (No. 323-07-08250/2021-05).

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to policy of our Institute.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tolosa, E.; Garrido, A.; Scholz, S.W.; Poewe, W. Challenges in the diagnosis of Parkinson's disease. *Lancet Neurol.* **2021**, *20*, 385–397. [CrossRef] [PubMed]
2. Schirinzi, T.; Madeo, G.; Martella, G.; Maltese, M.; Picconi, B.; Calabresi, P.; Pisani, A. Early synaptic dysfunction in Parkinson's disease: Insights from animal models. *Mov. Disord.* **2016**, *31*, 802–813. [CrossRef] [PubMed]
3. Gardoni, F.; Ghiglieri, V.; Di Luca, M.; Calabresi, P. Assemblies of glutamate receptor subunits with post-synaptic density proteins and their alterations in Parkinson's disease. *Prog. Brain Res.* **2010**, *183*, 169–182. [CrossRef] [PubMed]
4. Campanelli, F.; Natale, G.; Marino, G.; Ghiglieri, V.; Calabresi, P. Striatal glutamatergic hyperactivity in Parkinson's disease. *Neurobiol. Dis.* **2022**, *168*, 105697. [CrossRef]
5. Obeso, J.A.; Rodriguez-Oroz, M.C.; Goetz, C.G.; Marin, C.; Kordower, J.H.; Rodriguez, M.; Hirsch, E.C.; Farrer, M.; Schapira, A.H.; Halliday, G. Missing pieces in the Parkinson's disease puzzle. *Nat. Med.* **2010**, *16*, 653–661. [CrossRef]
6. Schapira, A.H.V.; Olanow, C.W.; Greenamyre, J.T.; Bezard, E. Slowing of neurodegeneration in Parkinson's disease and Huntington's disease: Future therapeutic perspectives. *Lancet* **2014**, *384*, 545–555. [CrossRef]
7. Iancu, R.; Mohapel, P.; Brundin, P.; Paul, G. Behavioral characterization of a unilateral 6-OHDA-lesion model of Parkinson's disease in mice. *Behav. Brain Res.* **2005**, *162*, 1–10. [CrossRef]
8. Paillé, V.; Picconi, B.; Bagetta, V.; Ghiglieri, V.; Sgobio, C.; Di Filippo, M.; Viscomi, M.T.; Giampà, C.; Fusco, F.R.; Gardoni, F.; et al. Distinct levels of dopamine denervation differentially alter striatal synaptic plasticity and NMDA receptor subunit composition. *J. Neurosci. Off. J. Soc. Neurosci.* **2010**, *30*, 14182–14193. [CrossRef]
9. Kin, K.; Yasuhara, T.; Kameda, M.; Date, I. Animal Models for Parkinson's Disease Research: Trends in the 2000s. *Int. J. Mol. Sci.* **2019**, *20*, 5402. [CrossRef]
10. Dunah, A.W.; Wang, Y.; Yasuda, R.P.; Kameyama, K.; Huganir, R.L.; Wolfe, B.B.; Standaert, D.G. Alterations in subunit expression, composition, and phosphorylation of striatal N-methyl-D-aspartate glutamate receptors in a rat 6-hydroxydopamine model of Parkinson's disease. *Mol. Pharmacol.* **2000**, *57*, 342–352.
11. Gan, J.; Qi, C.; Mao, L.-M.; Liu, Z. Changes in surface expression of N-methyl-D-aspartate receptors in the striatum in a rat model of Parkinson's disease. *Drug Des. Devel. Ther.* **2014**, *8*, 165–173. [CrossRef]
12. Zhang, Z.; Zhang, S.; Fu, P.; Zhang, Z.; Lin, K.; Ko, J.K.-S.; Yung, K.K.-L. Roles of Glutamate Receptors in Parkinson's Disease. *Int. J. Mol. Sci.* **2019**, *20*, 4391. [CrossRef]
13. Cirillo, G.; Di Pino, G.; Capone, F.; Ranieri, F.; Florio, L.; Todisco, V.; Tedeschi, G.; Funke, K.; Di Lazzaro, V. Neurobiological after-effects of non-invasive brain stimulation. *Brain Stimul.* **2017**, *10*, 1–18. [CrossRef] [PubMed]
14. Uzair, M.; Abualait, T.; Arshad, M.; Yoo, W.-K.; Mir, A.; Bunyan, R.F.; Bashir, S. Transcranial magnetic stimulation in animal models of neurodegeneration. *Neural Regen. Res.* **2022**, *17*, 251–265. [CrossRef] [PubMed]

15. Dragic, M.; Zeljkovic, M.; Stevanovic, I.; Ilic, T.; Ilic, N.; Nedeljkovic, N.; Ninkovic, M. Theta burst stimulation ameliorates symptoms of experimental autoimmune encephalomyelitis and attenuates reactive gliosis. *Brain Res. Bull.* **2020**, *162*, 208–217. [CrossRef] [PubMed]
16. Stekic, A.; Zeljkovic, M.; Kontic, M.Z.; Mihajlovic, K.; Adzic, M.; Stevanovic, I.; Ninkovic, M.; Grkovic, I.; Ilic, T.V.; Nedeljkovic, N.; et al. Intermittent Theta Burst Stimulation Ameliorates Cognitive Deficit and Attenuates Neuroinflammation via PI3K/Akt/mTOR Signaling Pathway in Alzheimer's-Like Disease Model. *Front. Aging Neurosci.* **2022**, *14*, 889983. [CrossRef]
17. Zong, X.; Gu, J.; Geng, D.; Gao, D. Repetitive transcranial magnetic stimulation (rTMS) for multiple neurological conditions in rodent animal models: A systematic review. *Neurochem. Int.* **2022**, *157*, 105356. [CrossRef]
18. George, M.S.; Wassermann, E.M.; Williams, W.A.; Callahan, A.; Ketter, T.A.; Basser, P.; Hallett, M.; Post, R.M. Daily repetitive transcranial magnetic stimulation (rTMS) improves mood in depression. *Neuroreport* **1995**, *6*, 1853–1856. [CrossRef]
19. Rosa, I.; Di Censo, D.; Ranieri, B.; Di Giovanni, G.; Scarnati, E.; Alecci, M.; Galante, A.; Florio, T.M. Comparison between Tail Suspension Swing Test and Standard Rotation Test in Revealing Early Motor Behavioral Changes and Neurodegeneration in 6-OHDA Hemiparkinsonian Rats. *Int. J. Mol. Sci.* **2020**, *21*, 2874. [CrossRef]
20. Jeon, B.S.; Jackson-Lewis, V.; Burke, R.E. 6-Hydroxydopamine lesion of the rat substantia nigra: Time course and morphology of cell death. *Neurodegener. A J. Neurodegener. Disord. Neuroprot. Neuroregener.* **1995**, *4*, 131–137. [CrossRef]
21. Esmaeili-Mahani, S.; Haghparast, E.; Nezhadi, A.; Abbasnejad, M.; Sheibani, V. Apelin-13 prevents hippocampal synaptic plasticity impairment in Parkinsonism rats. *J. Chem. Neuroanat.* **2021**, *111*, 101884. [CrossRef] [PubMed]
22. Papp, E.A.; Leergaard, T.B.; Calabrese, E.; Johnson, G.A.; Bjaalie, J.G. Waxholm Space atlas of the Sprague Dawley rat brain. *Neuroimage* **2014**, *97*, 374–386. [CrossRef]
23. Jenkinson, M.; Beckmann, C.F.; Behrens, T.E.J.; Woolrich, M.W.; Smith, S.M. FSL. *Neuroimage* **2012**, *62*, 782–790. [CrossRef]
24. Yushkevich, P.A.; Gao, Y.; Gerig, G. ITK-SNAP: An interactive tool for semi-automatic segmentation of multi-modality biomedical images. In Proceedings of the 2016 38th Annual International Conference of the IEEE Engineering in Medicine and Biology Society (EMBC), Orlando, FL, USA, 16–20 August 2016; pp. 3342–3345. [CrossRef]
25. Deng, Z.-D.; Lisanby, S.H.; Peterchev, A.V. Electric field depth-focality tradeoff in transcranial magnetic stimulation: Simulation comparison of 50 coil designs. *Brain Stimul.* **2013**, *6*, 1–13. [CrossRef] [PubMed]
26. Salvador, R.; Miranda, P.C. Transcranial magnetic stimulation of small animals: A modeling study of the influence of coil geometry, size and orientation. In Proceedings of the 2009 Annual International Conference of the IEEE Engineering in Medicine and Biology Society, Minneapolis, MN, USA, 3–6 September 2009; pp. 674–677. [CrossRef]
27. Tang, A.D.; Lowe, A.S.; Garrett, A.R.; Woodward, R.; Bennett, W.; Canty, A.J.; Garry, M.I.; Hinder, M.R.; Summers, J.J.; Gersner, R.; et al. Construction and Evaluation of Rodent-Specific rTMS Coils. *Front. Neural Circuits* **2016**, *10*, 47. [CrossRef]
28. Nowak, K.; Mix, E.; Gimsa, J.; Strauss, U.; Sriperumbudur, K.K.; Benecke, R.; Gimsa, U. Optimizing a rodent model of Parkinson's disease for exploring the effects and mechanisms of deep brain stimulation. *Parkinsons. Dis.* **2011**, *2011*, 414682. [CrossRef]
29. Wagner, T.A.; Zahn, M.; Grodzinsky, A.J.; Pascual-Leone, A. Three-dimensional head model simulation of transcranial magnetic stimulation. *IEEE Trans. Biomed. Eng.* **2004**, *51*, 1586–1598. [CrossRef]
30. Lattanzi, W.; Corvino, V.; Di Maria, V.; Michetti, F.; Geloso, M.C. Gene expression profiling as a tool to investigate the molecular machinery activated during hippocampal neurodegeneration induced by trimethyltin (TMT) administration. *Int. J. Mol. Sci.* **2013**, *14*, 16817–16835. [CrossRef]
31. Schallert, T.; Fleming, S.M.; Leasure, J.L.; Tillerson, J.L.; Bland, S.T. CNS plasticity and assessment of forelimb sensorimotor outcome in unilateral rat models of stroke, cortical ablation, parkinsonism and spinal cord injury. *Neuropharmacology* **2000**, *39*, 777–787. [CrossRef]
32. Miyanishi, K.; Choudhury, M.E.; Watanabe, M.; Kubo, M.; Nomoto, M.; Yano, H.; Tanaka, J. Behavioral tests predicting striatal dopamine level in a rat hemi-Parkinson's disease model. *Neurochem. Int.* **2019**, *122*, 38–46. [CrossRef] [PubMed]
33. De Butte-Smith, M.; Gulinello, M.; Zukin, R.S.; Etgen, A.M. Chronic estradiol treatment increases CA1 cell survival but does not improve visual or spatial recognition memory after global ischemia in middle-aged female rats. *Horm. Behav.* **2009**, *55*, 442–453. [CrossRef]
34. Antunes, M.; Biala, G. The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cogn. Process.* **2012**, *13*, 93–110. [CrossRef] [PubMed]
35. Perić, I.; Stanisavljević, A.; Gass, P.; Filipović, D. Fluoxetine exerts subregion/layer specific effects on parvalbumin/GAD67 protein expression in the dorsal hippocampus of male rats showing social isolation-induced depressive-like behaviour. *Brain Res. Bull.* **2021**, *173*, 174–183. [CrossRef]
36. Stefanovic, B.; Spasojevic, N.; Jovanovic, P.; Jasnic, N.; Djordjevic, J.; Dronjak, S. Melatonin mediated antidepressant-like effect in the hippocampus of chronic stress-induced depression rats: Regulating vesicular monoamine transporter 2 and monoamine oxidase A levels. *Eur. Neuropsychopharmacol. J. Eur. Coll. Neuropsychopharmacol.* **2016**, *26*, 1629–1637. [CrossRef]
37. Dragic, M.; Stekic, A.; Zeljkovic, M.; Zaric Kontic, M.; Mihajlovic, K.; Adzic, M.; Grkovic, I.; Nedeljkovic, N. Altered Topographic Distribution and Enhanced Neuronal Expression of Adenosine-Metabolizing Enzymes in Rat Hippocampus and Cortex from Early to late Adulthood. *Neurochem. Res.* **2022**, *47*, 1637–1650. [CrossRef]
38. Blum, D.; Torch, S.; Lambeng, N.; Nissou, M.; Benabid, A.L.; Sadoul, R.; Verna, J.M. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: Contribution to the apoptotic theory in Parkinson's disease. *Prog. Neurobiol.* **2001**, *65*, 135–172. [CrossRef] [PubMed]

39. Winner, B.M.; Zhang, H.; Farthing, M.M.; Karchalla, L.M.; Lookingland, K.J.; Goudreau, J.L. Metabolism of Dopamine in Nucleus Accumbens Astrocytes Is Preserved in Aged Mice Exposed to MPTP. *Front. Aging Neurosci.* **2017**, *9*, 410. [CrossRef]
40. Suzuki, K.; Okada, K.; Wakuda, T.; Shinmura, C.; Kameno, Y.; Iwata, K.; Takahashi, T.; Suda, S.; Matsuzaki, H.; Iwata, Y.; et al. Destruction of dopaminergic neurons in the midbrain by 6-hydroxydopamine decreases hippocampal cell proliferation in rats: Reversal by fluoxetine. *PLoS ONE* **2010**, *5*, e9260. [CrossRef] [PubMed]
41. Prasad, E.M.; Hung, S.-Y. Behavioral Tests in Neurotoxin-Induced Animal Models of Parkinson's Disease. *Antioxidants* **2020**, *9*, 1007. [CrossRef]
42. Radman, T.; Ramos, R.L.; Brumberg, J.C.; Bikson, M. Role of cortical cell type and morphology in subthreshold and suprathreshold uniform electric field stimulation in vitro. *Brain Stimul.* **2009**, *2*, 215–228, 228.e1–3. [CrossRef]
43. Cullen, C.L.; Young, K.M. How Does Transcranial Magnetic Stimulation Influence Glial Cells in the Central Nervous System? *Front. Neural Circuits* **2016**, *10*, 26. [CrossRef] [PubMed]
44. Alekseichuk, I.; Mantell, K.; Shirinpour, S.; Opitz, A. Comparative modeling of transcranial magnetic and electric stimulation in mouse, monkey, and human. *Neuroimage* **2019**, *194*, 136–148. [CrossRef]
45. Lee, J.Y.; Kim, H.S.; Kim, S.H.; Kim, H.-S.; Cho, B.P. Combination of Human Mesenchymal Stem Cells and Repetitive Transcranial Magnetic Stimulation Enhances Neurological Recovery of 6-Hydroxydopamine Model of Parkinsonian's Disease. *Tissue Eng. Regen. Med.* **2020**, *17*, 67–80. [CrossRef] [PubMed]
46. Lee, J.Y.; Kim, S.H.; Ko, A.-R.; Lee, J.S.; Yu, J.H.; Seo, J.H.; Cho, B.P.; Cho, S.-R. Therapeutic effects of repetitive transcranial magnetic stimulation in an animal model of Parkinson's disease. *Brain Res.* **2013**, *1537*, 290–302. [CrossRef] [PubMed]
47. Stevanovic, I.; Ninkovic, M.; Mancic, B.; Milivojevic, M.; Stojanovic, I.; Ilic, T.; Vujovic, M.; Djukic, M. Compensatory Neuroprotective Response of Thioredoxin Reductase against Oxidative-Nitrosative Stress Induced by Experimental Autoimmune Encephalomyelitis in Rats: Modulation by Theta Burst Stimulation. *Molecules* **2020**, *25*, 3922. [CrossRef]
48. Schober, A. Classic toxin-induced animal models of Parkinson's disease: 6-OHDA and MPTP. *Cell Tissue Res.* **2004**, *318*, 215–224. [CrossRef]
49. Stanojevic, J.; Dragic, M.; Stevanovic, I.; Ilic, T.; Stojanovic, I.; Zeljkovic, M.; Ninkovic, M. Intermittent theta burst stimulation ameliorates cognitive impairment and hippocampal gliosis in the Streptozotocin-induced model of Alzheimer's disease. *Behav. Brain Res.* **2022**, *433*, 113984. [CrossRef]
50. Vieira, J.C.F.; Bassani, T.B.; Santiago, R.M.; Guaita, G.D.O.; Zanoveli, J.M.; da Cunha, C.; Vital, M.A. Anxiety-like behavior induced by 6-OHDA animal model of Parkinson's disease may be related to a dysregulation of neurotransmitter systems in brain areas related to anxiety. *Behav. Brain Res.* **2019**, *371*, 111981. [CrossRef]
51. Lee, C.-W.; Wu, H.-F.; Chu, M.-C.; Chung, Y.-J.; Mao, W.-C.; Li, C.-T.; Lin, H.-C. Mechanism of Intermittent Theta-Burst Stimulation in Synaptic Pathology in the Prefrontal Cortex in an Antidepressant-Resistant Depression Rat Model. *Cereb. Cortex* **2021**, *31*, 575–590. [CrossRef]
52. Prinz, A.; Selesnew, L.-M.; Liss, B.; Roeper, J.; Carlsson, T. Increased excitability in serotonin neurons in the dorsal raphe nucleus in the 6-OHDA mouse model of Parkinson's disease. *Exp. Neurol.* **2013**, *248*, 236–245. [CrossRef]
53. Cavaccini, A.; Gritti, M.; Giorgi, A.; Locarno, A.; Heck, N.; Migliarini, S.; Bertero, A.; Mereu, M.; Margiani, G.; Trusel, M.; et al. Serotonergic Signaling Controls Input-Specific Synaptic Plasticity at Striatal Circuits. *Neuron* **2018**, *98*, 801–816.e7. [CrossRef] [PubMed]
54. Mathur, B.N.; Capik, N.A.; Alvarez, V.A.; Lovinger, D.M. Serotonin induces long-term depression at corticostriatal synapses. *J. Neurosci. Off. J. Soc. Neurosci.* **2011**, *31*, 7402–7411. [CrossRef] [PubMed]
55. Blomeley, C.P.; Bracci, E. Serotonin excites fast-spiking interneurons in the striatum. *Eur. J. Neurosci.* **2009**, *29*, 1604–1614. [CrossRef]
56. Blesa, J.; Juri, C.; García-Cabezas, M.Á.; Adánez, R.; Sánchez-González, M.Á.; Cavada, C.; Obeso, J.A. Inter-hemispheric asymmetry of nigrostriatal dopaminergic lesion: A possible compensatory mechanism in Parkinson's disease. *Front. Syst. Neurosci.* **2011**, *5*, 92. [CrossRef]
57. Nash, J.E.; Brotchie, J.M. Characterisation of striatal NMDA receptors involved in the generation of parkinsonian symptoms: Intrastriatal microinjection studies in the 6-OHDA-lesioned rat. *Mov. Disord.* **2002**, *17*, 455–466. [CrossRef]
58. Löschmann, P.-A.; De Groote, C.; Smith, L.; Wüllner, U.; Fischer, G.; Kemp, J.A.; Jenner, P.; Klockgether, T. Antiparkinsonian activity of Ro 25-6981, a NR2B subunit specific NMDA receptor antagonist, in animal models of Parkinson's disease. *Exp. Neurol.* **2004**, *187*, 86–93. [CrossRef]
59. Steece-Collier, K.; Chambers, L.K.; Jaw-Tsai, S.S.; Menniti, F.S.; Greenamyre, J.T. Antiparkinsonian actions of CP-101,606, an antagonist of NR2B subunit-containing N-methyl-d-aspartate receptors. *Exp. Neurol.* **2000**, *163*, 239–243. [CrossRef]
60. Nash, J.E.; Hill, M.P.; Brotchie, J.M. Antiparkinsonian actions of blockade of NR2B-containing NMDA receptors in the reserpine-treated rat. *Exp. Neurol.* **1999**, *155*, 42–48. [CrossRef]
61. Blanchet, P.J.; Konitsiotis, S.; Whittemore, E.R.; Zhou, Z.L.; Woodward, R.M.; Chase, T.N. Differing effects of N-methyl-D-aspartate receptor subtype selective antagonists on dyskinesias in levodopa-treated 1-methyl-4-phenyl-tetrahydropyridine monkeys. *J. Pharmacol. Exp. Ther.* **1999**, *290*, 1034–1040.
62. Rylander, D.; Recchia, A.; Mela, F.; Dekundy, A.; Danysz, W.; Cenci, M.A. Pharmacological modulation of glutamate transmission in a rat model of L-DOPA-induced dyskinesia: Effects on motor behavior and striatal nuclear signaling. *J. Pharmacol. Exp. Ther.* **2009**, *330*, 227–235. [CrossRef] [PubMed]

63. Calon, F.; Rajput, A.H.; Hornykiewicz, O.; Bédard, P.J.; Di Paolo, T. Levodopa-induced motor complications are associated with alterations of glutamate receptors in Parkinson's disease. *Neurobiol. Dis.* **2003**, *14*, 404–416. [CrossRef] [PubMed]
64. Gao, H.-C.; Zhu, H.; Song, C.-Y.; Lin, L.; Xiang, Y.; Yan, Z.-H.; Bai, G.-H.; Ye, F.-Q.; Li, X.-K. Metabolic changes detected by ex vivo high resolution 1H NMR spectroscopy in the striatum of 6-OHDA-induced Parkinson's rat. *Mol. Neurobiol.* **2013**, *47*, 123–130. [CrossRef] [PubMed]
65. Caudle, W.M.; Zhang, J. Glutamate, excitotoxicity, and programmed cell death in Parkinson disease. *Exp. Neurol.* **2009**, *220*, 230–233. [CrossRef]
66. Li, P.; Li, Y.-H.; Han, T.-Z. NR2A-containing NMDA receptors are required for LTP induction in rat dorsolateral striatum in vitro. *Brain Res.* **2009**, *1274*, 40–46. [CrossRef] [PubMed]
67. Natale, G.; Pignataro, A.; Marino, G.; Campanelli, F.; Calabrese, V.; Cardinale, A.; Pelucchi, S.; Marcello, E.; Gardoni, F.; Viscomi, M.T.; et al. Transcranial Magnetic Stimulation Exerts "Rejuvenation" Effects on Corticostriatal Synapses after Partial Dopamine Depletion. *Mov. Disord.* **2021**, *36*, 2254–2263. [CrossRef]
68. Cacace, F.; Mineo, D.; Viscomi, M.T.; Latagliata, E.C.; Mancini, M.; Sasso, V.; Vannelli, A.; Pascucci, T.; Pendolino, V.; Marcello, E.; et al. Intermittent theta-burst stimulation rescues dopamine-dependent corticostriatal synaptic plasticity and motor behavior in experimental parkinsonism: Possible role of glial activity. *Mov. Disord.* **2017**, *32*, 1035–1046. [CrossRef]
69. Schotanus, S.M.; Chergui, K. NR2A-containing NMDA receptors depress glutamatergic synaptic transmission and evoked-dopamine release in the mouse striatum. *J. Neurochem.* **2008**, *106*, 1758–1765. [CrossRef]
70. Ma, J.; Zhang, Z.; Kang, L.; Geng, D.; Wang, Y.; Wang, M.; Cui, H. Repetitive transcranial magnetic stimulation (rTMS) influences spatial cognition and modulates hippocampal structural synaptic plasticity in aging mice. *Exp. Gerontol.* **2014**, *58*, 256–268. [CrossRef]
71. Vlachos, A.; Müller-Dahlhaus, F.; Rosskopp, J.; Lenz, M.; Ziemann, U.; Deller, T. Repetitive magnetic stimulation induces functional and structural plasticity of excitatory postsynapses in mouse organotypic hippocampal slice cultures. *J. Neurosci. Off. J. Soc. Neurosci.* **2012**, *32*, 17514–17523. [CrossRef]
72. Labedi, A.; Benali, A.; Mix, A.; Neubacher, U.; Funke, K. Modulation of inhibitory activity markers by intermittent theta-burst stimulation in rat cortex is NMDA-receptor dependent. *Brain Stimul.* **2014**, *7*, 394–400. [CrossRef]
73. Brown, J.C.; DeVries, W.H.; Korte, J.E.; Sahlem, G.L.; Bonilha, L.; Short, E.B.; George, M.S. NMDA receptor partial agonist, d-cycloserine, enhances 10 Hz rTMS-induced motor plasticity, suggesting long-term potentiation (LTP) as underlying mechanism. *Brain Stimul.* **2020**, *13*, 530–532. [CrossRef] [PubMed]

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Sustained Systemic Antioxidative Effects of Intermittent Theta Burst Stimulation beyond Neurodegeneration: Implications in Therapy in 6-Hydroxydopamine Model of Parkinson's Disease

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Abstract: Parkinson's disease (PD) is manifested by the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and caudoputamen (Cp), leading to the development of motor and non-motor symptoms. The contribution of oxidative stress to the development and progression of PD is increasingly recognized. Experimental models show that strengthening antioxidant defenses and reducing pro-oxidant status may have beneficial effects on disease progression. In this study, the neuroprotective potential of intermittent theta burst stimulation (iTBS) is investigated in a 6-hydroxydopamine (6-OHDA)-induced PD model in rats seven days after intoxication which corresponds to the occurrence of first motor symptoms. Two-month-old male Wistar rats were unilaterally injected with 6-OHDA to mimic PD pathology and were subsequently divided into two groups to receive either iTBS or sham stimulation for 21 days. The main oxidative parameters were analyzed in the caudoputamen, substantia nigra pars compacta, and serum. iTBS treatment notably mitigated oxidative stress indicators, simultaneously increasing antioxidative parameters in the caudoputamen and substantia nigra pars compacta well after 6-OHDA-induced neurodegeneration process was over. Serum analysis confirmed the systemic effect of iTBS with a decrease in oxidative markers and an increase in antioxidants. Prolonged iTBS exerts a modulatory effect on oxidative/antioxidant parameters in the 6-OHDA-induced PD model, suggesting a potential neuroprotective benefit, even though at this specific time point 6-OHDA-induced oxidative status was unaltered. These results emphasize the need to further explore the mechanisms of iTBS and argue in favor of considering it as a therapeutic intervention in PD and related neurodegenerative diseases.

Keywords: Parkinson's disease; 6-hydroxidopamine; rTMS; intermittent theta burst stimulation; oxidative stress; neuroprotection

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1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized primarily by the degeneration of dopaminergic (DA) neurons in the substantia nigra pars compacta (SNpc), which often correlates with the presence of α-synuclein-containing Lewy bodies [1]. This pathological process culminates in a discernible decline in dopamine levels in the striatal region and is closely associated with the onset of a variety of motor and non-motor symptoms that severely affect the lives of millions of people around the world [2]. Although the exact etiology of PD remains elusive, there is growing consensus

that oxidative stress is one of the key factors in the development of the disease, including both idiopathic and genetic PD. This concept finds substantiation in the identification of oxidized lipids and proteins within the *post mortem* SNpc tissue of individuals affected by PD [3,4]. The neurotoxin 6-hydroxydopamine (6-OHDA), a dopamine analogue, is known to induce significant oxidative stress, damaging DA neurons [5]. This selective catecholaminergic neurotoxin was identified more than 60 years ago [6] and remains one of the most commonly used toxins to produce lesions of nigrostriatal dopaminergic neurons in rats [7]. 6-OHDA exerts its cytotoxic effects via several well-described mechanisms, i.e., the intracellular or extracellular auto-oxidation of 6-OHDA and the direct inhibition of mitochondrial respiratory chain complex I and IV [8]. These actions can generate reactive oxygen species (ROS), leading to cellular damage, oxidation of cellular macromolecules, mutations in mitochondrial DNA, and the initiation of apoptosis through mitochondrial pathways, indicated by the release of cytochrome c and other proteins involved in the apoptosis [9]. ROS also impairs the ubiquitin–proteasome system, causing an accumulation of defective proteins, a pathology mirrored in PD [10]. The vulnerability of dopaminergic neurons is further heightened by factors like ROS-generating enzymes, essential for dopamine synthesis, and elevated intracellular iron levels, facilitating oxidative reactions [11]. Against this background, understanding the role of oxidative stress is crucial for the development of effective PD treatments and prevention strategies. While recent years have seen advancements, existing therapies, which have centered on restoring dopamine, are proving insufficient to halt neuronal loss or mitigate the side effects associated with current treatments for PD. Repetitive transcranial magnetic stimulation (rTMS) is a form of non-invasive and painless brain stimulation that has shown therapeutic potential in many neurodegenerative disorders, including PD [12]. The exact mechanism of action remains elusive, but there is evidence that it can bring long-lasting benefits, that may last weeks or even months after the last stimulation [13]. rTMS can exert significant neuroprotective effects by acting on neuronal metabolism, neuroinflammation, and excitotoxicity, and by acting as a potent antioxidant and neuromodulator [14,15]. Intermittent theta burst stimulation (iTBS) is a specialized rTMS protocol that elicits an LTP-like increase in cortical excitability, and is proving to be an attractive and superior choice for neuromodulatory treatments in clinical disorders, mainly due to the rapid onset of modulatory effects compared to conventional rTMS [16]. In addition, our team's research has demonstrated the marked efficacy of iTBS in attenuating inflammation and oxidative stress in various models of neurodegenerative disease [17–21]. However, the precise effects of iTBS on oxidative stress parameters in the context of PD remain largely unexplored. Therefore, the aim of the present study was to investigate the effects of iTBS protocol on the modulation of oxidative/antioxidative parameters and to determine the extent to which this modulation might be beneficial in a 6-OHDA-induced PD model.

2. Materials and Methods

2.1. Animals and Housing Conditions

A total of 16 two-month-old male Wistar rats (n = 16, 250 ± 30 g) housed at the Center of Veterinary Services animal facility, University of Defense, were used in this study. Animals (3–4/cage) were kept in constant environmental conditions (temperature of 23 ± 2 °C, a 12 h light–dark cycle) and ad libitum access to a standard diet and tap water. All animal care and experimental procedures in this study were performed in accordance with the "3Rs" principles and procedures as in the European Union Directive (2010/63/EU) and were approved by the Ethics Committee for Animal Experiments of the University of Belgrade—Faculty of Biology (No. 323-07-08250/2021-05).

2.2. Unilateral 6-Hydroxydopamine Lesion of the Right Substantia Nigra Pars Compacta

Before initiating the lesioning process, rats were sedated with a mixture of ketamine (100 mg/kg) and xylazine (10 mg/kg) to ensure their well-being and minimize discomfort, and then fixed in a stereotaxic instrument (Stoelting Co., Wood Dale, IL, USA). A dose of

2 µL of 6-OHDA (6 µg/µL, Catalog No. 28094-15-7) dissolved in sterile saline containing 0.2% ascorbic acid was administered into the right SNpc (rSNpc) area. Conversely, a similar volume of saline was injected into the left SNpc (lSNpc) as a control. Injection coordinates were set at −5.40 mm AP, ± 2.10 mm ML, and + 7.40 mm DV, as indicated in the stereotaxic atlas of Paxinos and Watson. Because the stereotaxic references were derived for Wistar rats weighing 290 g, we applied a correction to each stereotaxic coordinate for all animals weighing more or less than 290 g, as described in [22]. The neurotoxin was infused through a 50-µL Hamilton syringe at a constant flow rate of 0.4 µL/min (Microinjector; Harvard Apparatus, Holliston, MA, USA) and the needle remained in place for an additional 5 min post-injection to ensure proper diffusion within the SNpc and was then carefully withdrawn [23]. The anesthesia administered during the injection procedure would have been sufficient to relieve pain for a reasonable duration following surgery. Immediately after the surgery, each animal received subcutaneously 1 mL of sterile saline to hydrate them through the anesthesia. To further minimize discomfort to the animals, buprenorphine (0.05 mg/kg) was administered subcutaneously every 24 h for three days, and the animals were monitored daily by a veterinarian in charge. Following the 6-OHDA injections, the rats were divided into two groups for further investigation: those that received iTBS sham stimulation (iTBSsh; $n = 7$) and those that underwent actual iTBS treatment (iTBS; $n = 7$). These animals were exposed to the respective treatments for 21 days before being humanely euthanized by decapitation (Harvard Apparatus, Holliston, MA, USA).

2.3. Rotarod Performance Test

To evaluate the impact of surgical interventions on motor coordination and balance, a Rotarod test was conducted. To accustom the animals to the Rotarod apparatus, they were first given three training sessions. During these sessions, they were first placed on an immobile cylinder for 30 s to encourage them to avoid falling. Subsequently, they were then placed on a rotating cylinder for 90 s, which moved at a constant speed of 10 revolutions per minute (rpm). Animals were tested one day before the operation to obtain baseline values and one day before stimulation began to assess changes in motor coordination caused by surgery. Test performance before stimulation served as a criterion for animal selection, i.e., to exclude animals that did not exhibit motor dysfunction ($n = 2$ animals). Animals underwent three test sessions, each session included three trials with an acceleration of 4 to 20 rpm and a maximum duration of 200 s per trial and a 30-min interval between trials [23]. Latency to fall and distance traveled were recorded for each animal, and the best performance from the trial was used for analyses.

2.4. Theta Burst Stimulation Protocol

The iTBS was administered using a MagStim Rapid2 system equipped with a 25 mm figure-of-eight coil (MagStim Company, Whitland, UK). The protocol consisted of twenty sequences of ten bursts (each burst contained 3 pulses at 50 Hz) and a repetition rate of 5 Hz. There was a 10 s rest period between each sequence, culminating in a total stimulation time of 192 s per session. The intensity of the magnetic stimulation was maintained at 35% of the device's maximum output, generating a magnetic field strength of 690 mT [17,23]. Animals were gently held during stimulation while they were allowed to move freely during the 10 s interval between restraints. The sham group (iTBSsh) was exposed to the noise artifact by placing the cage containing two animals next to the stimulation device while held gently to reproduce the mild restraint stress. All animals started the treatment 7 days after intoxication with the occurrence of the first motor symptoms. The same treatment protocol was repeated for 21 consecutive days. The iTBS protocol used in the present study is a whole-brain stimulation that affects the CPu and SNpc, among other brain regions, with E-field strengths above 28 V/m, which is sufficient to generate action potentials. Our previous research provides a 3D FEM model showing the geometry and gradient of magnetic and electric field density throughout the brain [23].

2.5. Blood Serum and Brain Tissue Collection

After decapitation, whole blood was collected in anticoagulant-free containers. For serum analysis, the collected samples were allowed to coagulate for 30 min, followed by centrifugation at 1000× *g* for 10 min. The resulting supernatant was designated as serum. Multiple aliquots of serum were stored and frozen for subsequent analysis. In addition, following decapitation, the brains (n = 7/group) were swiftly extracted from the skull and rinsed with ice-cold saline. The right and left substantia nigra pars compacta and caudoputamen (rSNpc, lSNpc, rCPu, and lCPu) were dissected, frozen in liquid nitrogen, and stored at −80 °C. The samples were manually homogenized using a Teflon/glass homogenizer with 0.32 M sucrose in 5 mM Tris-HCl buffer at pH 7.4 (1 g wet tissue/10 mL buffer). The resulting homogenates were then centrifuged at 3000× *g* for 10 min at 4 °C, and the obtained supernatants were collected for further analysis [24]. Protein concentration was determined after isolation using the Pierce™ BCA Protein Assay Kit (Cat. No. 23225; Thermo Scientific, Waltham, MA, USA), following the manufacturer's instructions.

2.6. Malondialdehyde Determination

Reactive oxygen species can initiate a chain reaction in polyunsaturated lipids that leads to the formation of products such as malondialdehyde (MDA). To measure the MDA concentration, we followed the spectrophotometric method outlined by Girotti et al. [25]. In this method, samples were combined with a mixture of thiobarbituric acid (TBA) and Tris-HCl (pH 7.4) and then heated at 100 °C for 60 min. The reaction between MDA and TBA resulted in a red supernatant whose absorbance was measured spectrophotometrically at 535 nm. The variations in MDA levels were quantified as µmol of MDA per milligram of protein. The assay was performed in duplicate, and the mean values were reported along with the standard deviation.

2.7. Superoxide Anion Radical Determination

Superoxide anion radical ($O_2^{\bullet -}$) quantification involved a spectrophotometric approach wherein nitro blue-tetrazolium (NBT; Merck, Darmstadt, Germany) reduction occurred in an alkaline, nitrogen-saturated medium [26]. The resulting yellow-colored reduced product, which was proportional to the superoxide radical concentration, was measured at 550 nm using an Ultrospec 2000 spectrophotometer. The results were expressed as nmol of reduced NBT per minute per milligram of protein.

2.8. Nitric Oxide Determination

Nitrosative stress was assessed by measuring nitrite and nitrate (NO_2 + NO_3) concentrations in the deproteinized samples. The combined concentration of NO_2 + NO_3 was determined using a spectrophotometric method at 492 nm. Nitrites were directly assayed using the Griess colorimetric method, which involved the use of 1.5% sulfanilamide in 1 M HCl and 0.15% N-(1-naphthyl) ethylenediamine dihydrochloride in distilled water. Conversely, nitrates were converted into nitrites through cadmium reduction prior to analysis [27,28]. The concentrations of nitrites in the samples were determined based on a standard curve generated using known nitrite concentrations and expressed as µmol/mg protein.

2.9. SOD Assay

The determination of the total superoxide dismutase activity (tSOD) was performed using a spectrophotometric approach based on the measurement of the decrease in the rate of spontaneous epinephrine auto-oxidation at 480 nm. The kinetic activity was monitored in a carbonate buffer, after the addition of 10 mM of epinephrine (Sigma, St. Louis, MO, USA) [29,30]. The obtained results were expressed as units per milligram of total protein (U/mg protein), where one unit represents the amount of enzyme required to inhibit epinephrine auto-oxidation by 50%.

2.10. Catalase Assay

The determination of catalase (CAT) activity involved a spectrophotometric method, where the formation of a yellow complex between ammonium molybdate (Serva, Feinbiochemica, Heidelberg, Germany) and H_2O_2 was monitored at 405 nm [31]. Data were expressed as mU of CAT per mg of protein. One unit of CAT activity is defined as μM H_2O_2/min/mg protein.

2.11. GSH Content Determination

The spectrophotometric assay for glutathione (GSH) utilizes the oxidation of GSH by the sulfhydryl reagent 5,5′-dithio-bis(2-nitrobenzoic acid) (DTNB), resulting in the formation of the yellow derivative 5′-thio-2-nitrobenzoic acid (TNBA), which is measurable at 412 nm. The formed glutathione disulfide (GSSG) could be regenerated to GSH by glutathione reductase in the presence of NADPH. The quantified results were expressed in nmol per mg of protein [32].

2.12. Total Sulfhydryl Groups (SH) Determination

The concentration of total sulfhydryl (SH) groups in tissue homogenates was measured spectrophotometrically at 412 nm in a phosphate buffer (0.2 mol + 2 mmol EDTA, pH 9) using 5,5-dithiobis-2-nitrobenzoic acid (DTNB, 0.01 M) [33]. The results were expressed as nanomoles of SH per milligram of protein.

2.13. Statistical Analyses

Normality of all data was assessed using the Shapiro–Wilk test, and the appropriate parametric or nonparametric tests were applied accordingly. Results of tests from serum were evaluated with an unpaired, two-tailed Student's *t*-test, whereas tests from tissue samples were evaluated with a two-way ANOVA with treatment and hemisphere as the two factors. All ANOVA results are represented in Table 1, while the post hoc data are in the Section 3. We compared the left vs. right hemisphere in both the sham and iTBS group, and only right hemispheres between two groups. Values are presented as mean ± SD, as indicated in the figure captions. A significance level of $p < 0.05$ was considered statistically significant. Data analysis and graphical representation were performed using the GraphPad Prism 9.0 software package (San Diego, CA, USA).

Table 1. Results of the two-way ANOVA test.

Parameters	SNpc	CPu
MDA	Interaction: $F_{(1, 21)} = 1.22$; $p = 0.2814$ Hemisphere: $F_{(1, 21)} = 2.13$; $p = 0.1593$ Treatment: $F_{(1, 21)} = 42.51$; $p < 0.0001$	Interaction: $F_{(1, 22)} = 3.44$; $p = 0.0769$ Hemisphere: $F_{(1, 22)} = 3.30$; $p = 0.0826$ Treatment: $F_{(1, 22)} = 211.0$; $p < 0.0001$
$O_2^{\bullet -}$	Interaction: $F_{(1, 20)} = 4.02$; $p = 0.0585$ Hemisphere: $F_{(1, 20)} = 13.62$; $p = 0.0015$ Treatment: $F_{(1, 20)} = 83.32$; $p < 0.0001$	Interaction: $F_{(1, 18)} = 0.83$; $p = 0.3730$ Hemisphere: $F_{(1, 18)} = 0.83$; $p = 0.3729$ Treatment: $F_{(1, 18)} = 220.1$; $p < 0.0001$
NO	Interaction: $F_{(1, 20)} = 0.66$; $p = 0.4235$ Hemisphere: $F_{(1, 20)} = 0.003$; $p = 0.9563$ Treatment: $F_{(1, 20)} = 22.00$; $p = 0.0001$	Interaction: $F_{(1, 24)} = 0.79$; $p = 0.3805$ Hemisphere: $F_{(1, 24)} = 0.79$; $p = 0.3805$ Treatment: $F_{(1, 24)} = 39.77$; $p < 0.0001$
tSOD	Interaction: $F_{(1, 21)} = 0.66$; $p = 0.8945$ Hemisphere: $F_{(1, 21)} = 0.003$; $p = 0.1376$ Treatment: $F_{(1, 21)} = 22.00$; $p < 0.0001$	Interaction: $F_{(1, 18)} = 0.83$; $p = 0.3730$ Hemisphere: $F_{(1, 18)} = 0.83$; $p = 0.3729$ Treatment: $F_{(1, 18)} = 220.1$; $p < 0.0001$
CAT	Interaction: $F_{(1, 20)} = 2.27$; $p = 0.1471$ Hemisphere: $F_{(1, 20)} = 1.31$; $p = 0.2656$ Treatment: $F_{(1, 20)} = 22.32$; $p = 0.0001$	Interaction: $F_{(1, 21)} = 1.42$; $p = 0.2463$ Hemisphere: $F_{(1, 21)} = 0.68$; $p = 0.4158$ Treatment: $F_{(1, 21)} = 49.55$; $p < 0.0001$
GSH	Interaction: $F_{(1, 20)} = 0.21$; $p = 0.6476$ Hemisphere: $F_{(1, 20)} = 3.41$; $p = 0.0795$ Treatment: $F_{(1, 20)} = 35.64$; $p = 0.0001$	Interaction: $F_{(1, 18)} = 10.02$; $p = 0.0054$ Hemisphere: $F_{(1, 18)} = 0.04$; $p = 0.8333$ Treatment: $F_{(1, 18)} = 60.78$; $p < 0.0001$
SH^-	Interaction: $F_{(1, 21)} = 5.80$; $p = 0.0252$ Hemisphere: $F_{(1, 21)} = 7.35$; $p = 0.0130$ Treatment: $F_{(1, 21)} = 13.32$; $p = 0.0015$	Interaction: $F_{(1, 22)} = 0.39$; $p = 0.5342$ Hemisphere: $F_{(1, 22)} = 2.80$; $p = 0.1081$ Treatment: $F_{(1, 22)} = 27.97$; $p < 0.0001$

3. Results

3.1. Behavioral Outcomes after Unilateral 6-OHDA Injection

When 6-OHDA is injected into the SNpc, it induces specific selective damage to dopaminergic neurons and a subsequent loss of terminals of dopaminergic neurons in their projection areas (CPu), leading to a reduction in dopamine production, similar to that observed in PD patients. After animals underwent unilateral injection of 6-OHDA into the right SNpc, the precision of the stereotaxic injection was confirmed by motor behavior and histological assessments (Figure 1). The animals showed marked impairment of motor skills. Because of the unilateral rSNpc lesion, the animals had difficulty using the contralateral left limbs. The animals also showed a significant reduction in latency to fall (Figure 1A, $t = 7.917$, $d_f = 13$, $p < 0.0001$) and distance traveled (Figure 1B, $t = 6.604$, $d_f = 13$, $p < 0.0001$) compared to their baseline performance in the rotarod test assessed one day before the lesion (Figure 1A,B).

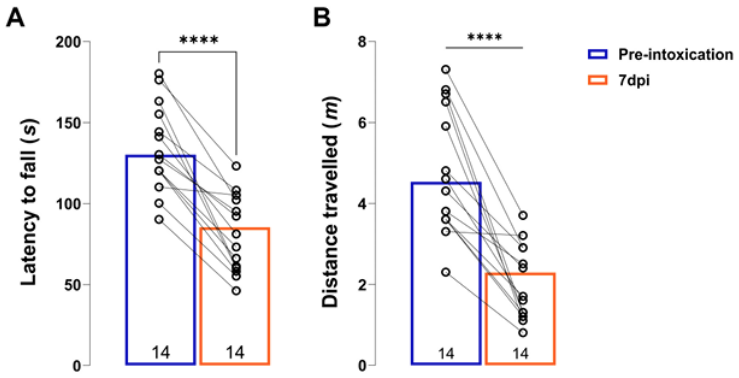

Figure 1. Unilateral 6-OHDA lesion induces motor impairment. Histograms showing latency to fall (**A**) and distance travelled (**B**) on a rotarod test of animals before (pre-intoxication) and 7-days after 6-OHDA unilateral lesion (7 dpi). All data are represented as mean ± SD. Dots in the graphs represent individual values. Number in the bottom of the graphs represents number of individual animals included in analysis. Results of post hoc Tukey's test and significance are shown inside graphs: **** $p < 0.0001$.

3.2. Effects of Prolonged iTBS Treatment on Oxidative Balance in the Caudoputamen of 6-OHDA-Induced Model of PD

Studies involving 6-OHDA have shown that the primary cause of specific dopaminergic neuron damage in models of PD is cell death triggered by oxidative stress [34]. To determine the changes in the caudoputamen homogenates (CPu) of iTBSsh and iTBS animals three weeks after the start of the treatment, we performed measurements of oxidative stress markers and nonenzymatic/enzymatic components of antioxidative protection (Figure 2). When we compare the right CPu hemispheres of sham (iTBSsh) and iTBS animals, we observed a significant reduction in MDA levels ($p < 0.0001$) as well as $O_2^{\bullet-}$ ($p < 0.01$) and NO levels ($p < 0.001$) (Figure 2A). No changes between left and right hemisphere within the group iTBSsh or iTBS were observed for all three parameters. The effects of iTBS on antioxidative capacity were evaluated through enzymatic (tSOD, CAT; Figure 2B) and nonenzymatic (GSH, SH^-; Figure 2C) components of an antioxidative system. We observed an almost 2-fold increase in tSOD activity ($p < 0.0001$), in catalase activity ($p < 0.0001$) as well as in GSH ($p < 0.05$) and SH^- ($p < 0.05$) levels when we compared the right CPu hemispheres of sham and iTBS animals. Similarly, as for other parameters, no changes between the left and right hemisphere within the group iTBSsh or iTBS for all antioxidative parameters were observed.

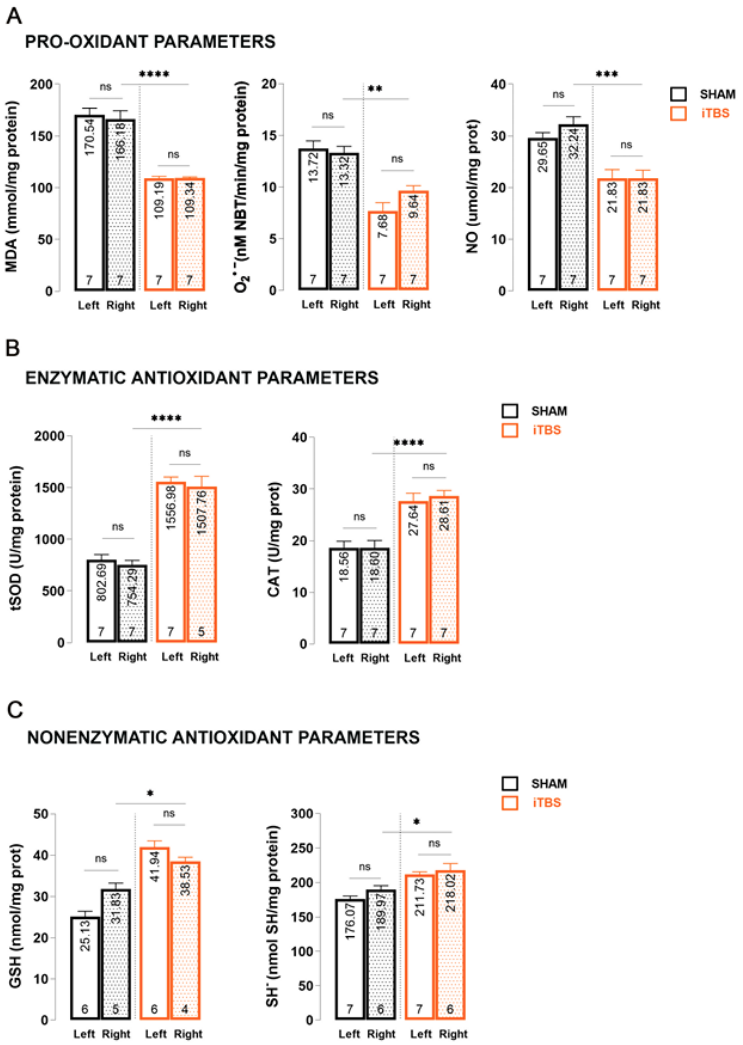

Figure 2. Effects of prolonged iTBS treatment on oxidative balance in the caudoputamen of 6-OHDA-induced model of PD. Spectrophotometric analysis of pro-oxidative and enzymatic/non-enzymatic antioxidative parameters: (**A**) MDA, $O_2^{\bullet-}$, NO; (**B**) total SOD, CAT; (**C**) GSH and SH^- levels measured in caudoputamen homogenates (left and right hemisphere) from sham and iTBS animals after three weeks of stimulation. Bars shows mean activity expressed as U/mg protein or mol/mg protein. All data are presented as mean ± SD. Number in the bottom of the graphs represent number of individual animals included in analysis. Results of post hoc Tukey's test and significance are shown inside graphs: ns–not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

3.3. Effects of Prolonged iTBS Treatment on Oxidative Balance in the Substantia Nigra Pars Compacta of 6-OHDA-Induced Model of PD

The same set of measurements were performed to analyze changes in the SNpc homogenates of iTBSsh and iTBS animals three weeks after we started the treatment (Figure 3). When we compared the right CPu hemispheres of sham (iTBSsh) and iTBS animals, we observed a significant reduction in pro-oxidant parameters—MDA levels ($p < 0.001$) as well as $O_2^{\bullet-}$ ($p < 0.0001$) and NO levels ($p < 0.01$) (Figure 3A).

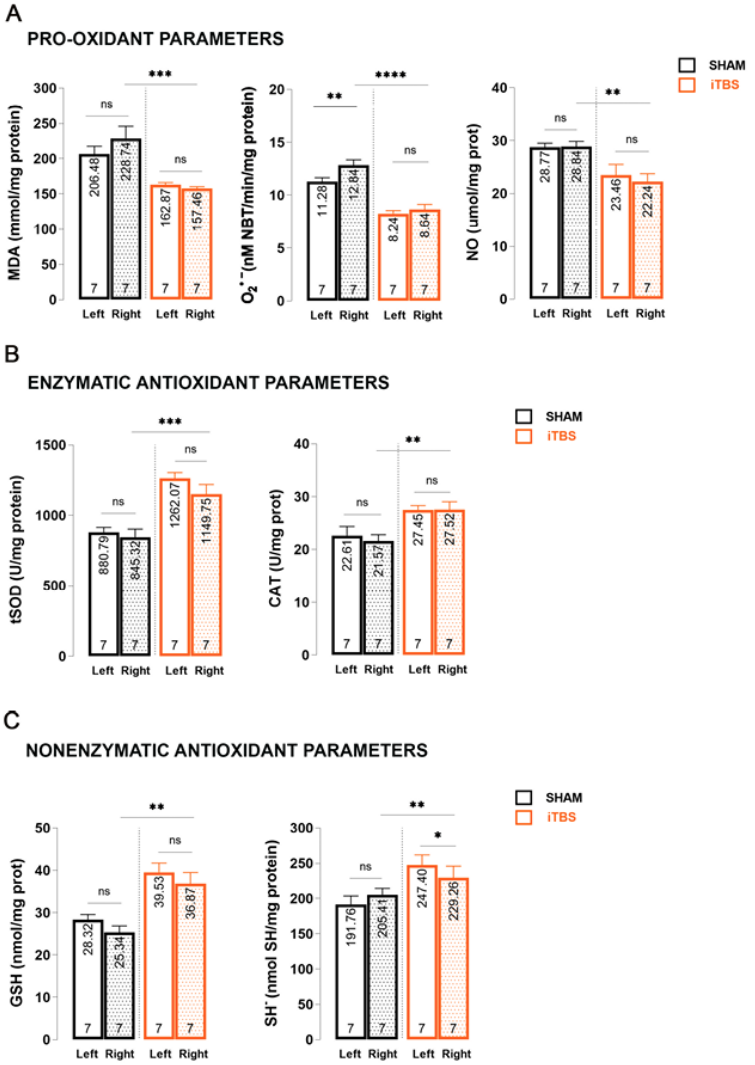

Figure 3. Effects of prolonged iTBS treatment on oxidative balance in the substantia nigra pars compacta of 6-OHDA-induced model of PD. Spectrophotometric analysis of pro-oxidative and enzymatic/non-enzymatic antioxidative parameters: (**A**) MDA, $O_2^{\bullet-}$, NO; (**B**) total SOD, CAT, (**C**) GSH and SH^- levels measured in midbrain homogenates (left and right hemisphere) from sham and iTBS animals after three weeks of stimulation. Bars shows mean activity expressed as U/mg protein or mol/mg protein. All data are presented as mean ± SD. The numbers at the bottom of the graphs indicate the number of individual animals included in the analysis. Results of post hoc Tukey's test and significance are shown inside graphs: ns—not significant, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

There were no changes when we compared the left and right hemispheres within the groups for MDA and NO, but there was a slight but significant increase in $O_2^{\bullet-}$ levels ($p < 0.01$) when we compared the left and right hemispheres of iTBSsh. The effects of iTBS on antioxidative capacity were evaluated through enzymatic (tSOD, CAT; Figure 3B) and nonenzymatic (GSH, SH^-; Figure 3C) components of an antioxidative system and we observed a significant increase in tSOD ($p < 0.001$), CAT ($p < 0.01$) as well as in GSH ($p < 0.01$) and SH^- ($p < 0.01$) levels when we compared the right CPu hemispheres of sham (iTBSsh) and iTBS animals. There were no changes/interactions when we compared the left and

right hemispheres within the group iTBSsh or iTBS for all antioxidative parameters except SH^- levels where we observed a slight, but significant decrease in the right hemisphere of iTBS animals when we compared it with left iTBS ($p < 0.05$).

3.4. Effects of Prolonged iTBS Treatment on Oxidative Balance in the Serum of 6-OHDA-Induced Model of PD

We found that iTBS-treated animals had lower serum MDA levels than iTBSsh animals (Figure 4A; $t = 4.264$, $d_f = 8$; $p = 0.0027$). Another pro-oxidant parameter was also significantly reduced in iTBS-treated animals, NO (Figure 4B, $t = 3.646$, $d_f = 8$; $p = 0.0065$). Finally, we examined a non-enzymatic antioxidant parameter, SH, whose serum level was significantly increased in iTBS-treated animals (Figure 4C; $t = 2.713$, $d_f = 8$: $p = 0.0265$).

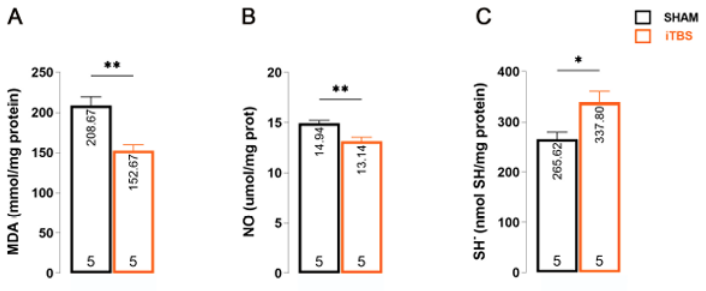

Figure 4. Effects of prolonged iTBS treatment on oxidative balance in the serum of 6-OHDA-induced model of PD. Spectrophotometric analysis of pro-oxidative and antioxidative parameters: (**A**) MDA-, (**B**) NO-, (**C**) SH^- levels measured in blood serum of sham and iTBS animals after three weeks of stimulation. The bars show the mean activity expressed as U/mg protein or mol/mg protein. All data are presented as mean ± SD. The numbers at the bottom of the graphs indicate the number of individual animals included in the analysis. Results of post hoc Tukey's test and significance are shown inside graphs: * $p < 0.05$, ** $p < 0.01$.

4. Discussion

Within this study, we evaluated the potential therapeutic benefits of the iTBS protocol at the molecular and systemic levels in the context of oxidative balance in a 6-OHDA-induced Parkinson's disease model. The unilateral injection of 6-OHDA into the right SNpc is a widely recognized method for modeling PD. This approach leads to the targeted destruction of dopaminergic (DA) neurons and progressive degeneration of the nigrostriatal pathway [23]. Although 6-OHDA-induced degeneration does not mimic the best-known feature of PD, the formation of Lewy bodies, it does produce robust and relatively stable lesions without spontaneous recovery, effectively mimicking the primary behavioral and histopathological aspects of human PD [35]. In addition, once 6-OHDA enters the cell via the dopamine transporter, it is involved in auto-oxidation, intraneuronal generation of ROS, and ultimately apoptosis [36]. Many of these effects are thought to reflect processes in the PD brain, so the 6-OHDA model has a high degree of construct validity, making the model a perfect tool for studying different neuroprotective strategies. Some research indicates an increase in oxidative stress and its indicators in the brain and cerebrospinal fluid (CSF) of individuals with PD. Postmortem examinations revealed significantly increased levels of MDA, a by-product of lipid peroxidation, in the substantia nigra of PD sufferers—levels up to ten times higher than those in other brain regions and in individuals of the same age without PD [37]. Also, several studies have shown that injection of 6-OHDA into the SNpc causes a significant increase in MDA and TBARS levels [38,39]. Moreover, after injection of 6-OHDA into the striatum, one day after neurotoxin administration, an increase in the parameters of HNE, PC, and 3-NT, which are also products of oxidative damage, was observed, and this increase returned to baseline levels by the seventh day [40]. Several different studies using 6-OHDA have consistently shown a significant reduction in the activity of essential enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione

S-transferase (GST), which are critical for defense against the damaging effects of reactive oxygen species (ROS) in brain regions such as the striatum and substantia nigra [41–45]. Given the close link between oxidative stress and the etiology of PD, extensive efforts have been made to develop strategies aimed at restoring the balance between ROS/RNS and antioxidant mechanisms to mitigate the progression and severity of the disease. Due to its auto-oxidant properties, levodopa, the primary treatment for Parkinson's disease, may exacerbate the disease progression rather than halting it by increasing oxidative stress and stimulating proinflammatory cytokine secretion, leading to neuroinflammation and subsequent dopaminergic neuron death [46]. In light of these challenges, our study explored the possibility of iTBS acting as an antioxidant, where an antioxidant could be considered in a broader sense as something that is able to stop the harmful effects of oxidative stress by either scavenging/decreasing ROS or stimulating the natural antioxidant system by targeting both neuronal and glial cells [47,48].

We demonstrated that after 21 days of iTBS stimulation, significant changes in antioxidant and pro-oxidant parameters occurred in the right hemisphere of the SNpc and CPu in animals subjected to iTBS treatment. Enzymatic antioxidants, including total superoxide dismutase (tSOD) and catalase (CAT), as well as non-enzymatic antioxidants such as reduced glutathione (GSH) and sulfhydryl groups (SH^-) showed a significant increase. Conversely, the pro-oxidative markers malondialdehyde (MDA), nitric oxide (NO), and superoxide anion ($O_2^{\bullet -}$) decreased. These changes were consistent in both the SNpc and CPu of iTBS animals. However, the absence of differences between left and right hemispheres within each group suggests that neurotoxic events associated with neurodegeneration and oxidative stress likely subside well before the 21-day sham/stimulation period ends. It has been shown that the peak of neuronal death is reached around the 7th day after intoxication, while oxidative stress occurs within the first week of use and then returns to baseline levels [49]. This highlights the need to interpret the results considering the broader effects of iTBS beyond localized brain regions. Accordingly, serum analysis revealed a decrease in MDA and NO levels together with an increase in the antioxidant defense parameter SH^-, confirming the positive effects of iTBS and indicating its systemic effect. Building on our previous results showing recovery of motor performance and positive effects on emotional behavior, learning, and memory as well as decreased histopathological signs and neuroinflammation in a 6-OHDA-induced SNpc degeneration paradigm of PD [23], our present study extends the therapeutic benefits of iTBS. Furthermore, iTBS treatment in other neurodegeneration models, such as streptozotocin (STZ)-induced Alzheimer's-like disease and trimethyltin (TMT)-induced Alzheimer's-like disease, demonstrated a significant reduction in oxidative stress markers and increased antioxidant capacity [17,21]. One possible explanation for the observed improvement in the general oxidation status after iTBS lies in the nuclear transcription factor E2-related factor 2 (Nrf2), which is recognized as a central player in managing the oxidative stress response. Nrf2 associates with antioxidant response elements (ARE) upon nuclear entry, triggering the activation of genes related to this response pathway and promoting the expression of various antioxidant genes, thereby enhancing cellular defenses against oxidative damage [49]. The critical involvement of Nrf2-mediated signaling pathways in PD has been demonstrated by microchip analysis of different tissues from PD patients. This revealed a reduction in the expression of 31 genes with ARE sequences to which NRF2 binds [48]. The activation of the Nrf2 signaling pathway by rTMS has the potential to modulate the expression of antioxidant proteins like HO-1 and SOD1 [50]. This activation could reduce the damage caused by oxidative stress and protect brain tissue. Consistent with these findings, our previous studies have shown that Nrf2 is increased after iTBS treatment [21]. Furthermore, brain-derived neurotrophic factor (BDNF), which is known for its role as a modulator of synaptic plasticity, has been shown to induce the nuclear translocation of Nrf2. In our previous study, we found an increased protein expression of BDNF after 21 days of iTBS stimulation in 6-OHDA-induced PD [21,23]. Collectively, these factors could contribute to the observed enhancement in the general state of oxidative balance.

The considerable differences in the predefined settings of various rTMS protocols, such as the intensity of the device, the duration of the sessions, and the timing of application, represent a major hurdle to the standardization and comparison of the results of different studies. However, the iTBS paradigm proves to be a powerful excitatory protocol in the field of rTMS, characterized by consistent parameters as documented in the literature. In particular, it shows the same or even better efficacy at a shorter exposure time, making it a compelling approach for both human and animal studies. Nevertheless, there are some limitations to our study, one concerning a disadvantage of this technique due to the size and manual placement of the coil, which prevents focal stimulation of specific areas and categorizes the technique as a form of whole-brain stimulation. Consequently, the observed effects of iTBS may be due to a combination of cortical and subcortical stimulation, which is emphasized by their complex interconnectivity. The second limitation relates to the use of the 6-OHDA model. This model, although most commonly used, recapitulates only one main feature of the human pathology and the effects caused by this feature, namely dopamine deficiency. This model lacks the neuroinflammatory component and progressivity, meaning that it often produces changes at the synaptic level, i.e., synaptopathy, which is probably why we did not observe oxidative stress four weeks after intoxication. Finally, an important question and limitation that our manuscript does not address due to its primary objective is whether the observed effects are due to a primary pathology, i.e., 6-OHDA intoxication, or whether the same effects would also be observed in healthy, untreated animals, which requires further research. Nevertheless, the results obtained so far suggest that prolonged iTBS stimulation contributes to a better balance of ROS/RNS, possibly through its antioxidant properties. This effect is likely achieved by increasing the activity of enzymes that scavenge harmful oxidative molecules, thus protecting neurons from oxidative stress and contributing to their maintenance. In addition, iTBS may have overall systemic benefits that contribute to its therapeutic potential. Furthermore, it appears that iTBS has a cumulative effect and that the reduction in pro-oxidant parameters and the increase in antioxidant capacity is a result of whole brain stimulation and the effect persists even after 6-OHDA-induced degeneration and oxidative damage. The promising results of this study underscore the importance of further investigation of the specific signaling pathways and molecular cascades involved in the observed modulation of oxidative/antioxidant parameters. Future research efforts should focus on elucidating these precise mechanisms to solidify the understanding of how iTBS exerts its neuroprotective effects, thereby facilitating the development of targeted and effective therapeutic interventions for PD and related neurodegenerative diseases.

5. Conclusions

The study demonstrates that 21 days of iTBS treatment significantly bolsters antioxidative defenses in a rat model of PD induced by 6-OHDA, particularly in the critical brain regions SNpc and CPu. Additionally, serum analysis confirms iTBS's systemic antioxidative impact, highlighting its potential in combating oxidative stress and neurodegeneration.

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Institutional Review Board Statement: The animal study protocol was approved by the Ethics Committee for Animal Experiments of the College University of Belgrade—Faculty of Biology (No. 323-07-08250/2021-05).

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author. The data are not publicly available due to the policy of our Institute.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Balestrino, R.; Schapira, A.H.V. Parkinson disease. *Eur. J. Neurol.* **2020**, *27*, 27–42. [CrossRef] [PubMed]
2. Tolosa, E.; Garrido, A.; Scholz, S.W.; Poewe, W. Challenges in the diagnosis of Parkinson;s disease. *Lancet Neurol.* **2021**, *20*, 385–397. [CrossRef]
3. Nakabeppu, Y.; Tsuchimoto, D.; Yamaguchi, H.; Sakumi, K. Oxidative damage in nucleic acids and Parkinson's disease. *J. Neurosci. Res.* **2007**, *85*, 919–934. [CrossRef]
4. Zeevalk, G.D.; Razmpour, R.; Bernard, L.P. Glutathione and Parkinson's disease: Is this the elephant in the room? *Biomed. Pharmacother.* **2008**, *62*, 236–249. [CrossRef]
5. Hernandez-Baltazar, D.; Zavala-Flores, L.M.; Villanueva-Olivo, A. The 6-hydroxydopamine model and parkinsonian pathophysiology: Novel findings in an older model. El modelo de 6-hidroxidopamina y la fisiopatología parkinsoniana: Nuevos hallazgos en un viejo modelo. *Neurologia* **2017**, *32*, 533–539. [CrossRef] [PubMed]
6. Masini, D.; Plewnia, C.; Bertho, M.; Scalbert, N.; Caggiano, V.; Fisone, G. A Guide to the Generation of a 6-Hydroxydopamine Mouse Model of Parkinson's Disease for the Study of Non-Motor Symptoms. *Biomedicines* **2021**, *9*, 598. [CrossRef]
7. Kin, K.; Yasuhara, T.; Kameda, M.; Date, I. Animal Models for Parkinson's Disease Research: Trends in the 2000s. *Int. J. Mol. Sci.* **2019**, *20*, 5402. [CrossRef] [PubMed]
8. Soto-Otero, R.; Méndez-Alvarez, E.; Hermida-Ameijeiras, A.; Muñoz-Patiño, A.M.; Labandeira-Garcia, J.L. Autoxidation and neurotoxicity of 6-hydroxydopamine in the presence of some antioxidants: Potential implication in relation to the pathogenesis of Parkinson's disease. *J. Neurochem.* **2000**, *74*, 1605–1612. [CrossRef]
9. Ott, M.; Gogvadze, V.; Orrenius, S.; Zhivotovsky, B. Mitochondria, oxidative stress and cell death. *Apoptosis Int. J. Program. Cell Death* **2007**, *12*, 913–922. [CrossRef]
10. Jenner, P. Oxidative stress in Parkinson's disease. *Ann. Neurol.* **2003**, *53* (Suppl. S3), S26–S38. [CrossRef]
11. Hwang, O. Role of oxidative stress in Parkinson's disease. *Exp. Neurobiol.* **2013**, *22*, 11–17. [CrossRef] [PubMed]
12. Somaa, F.A.; de Graaf, T.A.; Sack, A.T. Transcranial Magnetic Stimulation in the Treatment of Neurological Diseases. *Front. Neurol.* **2022**, *13*, 793253. [CrossRef] [PubMed]
13. Chail, A.; Saini, R.K.; Bhat, P.S.; Srivastava, K.; Chauhan, V. Transcranial magnetic stimulation: A review of its evolution and current applications. *Ind. Psychiatry J.* **2018**, *27*, 172–180. [CrossRef] [PubMed]
14. Yulug, B.; Hanoglu, L.; Kilic, E.; Polat, B.; Schabitz, W.R. The Neuroprotective Role of Repetitive Transcranial Magnetic Stimulation (rTMS) for Neurodegenerative Diseases: A Short Review on Experimental Studies. *Mini Rev. Med. Chem.* **2016**, *16*, 1269–1273. [CrossRef] [PubMed]
15. Bashir, S.; Uzair, M.; Abualait, T.; Arshad, M.; Khallaf, R.A.; Niaz, A.; Thani, Z.; Yoo, W.K.; Túnez, I.; Demirtas-Tatlidede, A.; et al. Effects of transcranial magnetic stimulation on neurobiological changes in Alzheimer's disease (Review). *Mol. Med. Rep.* **2022**, *25*, 109. [CrossRef] [PubMed]
16. Chung, S.W.; Rogasch, N.C.; Hoy, K.E.; Sullivan, C.M.; Cash, R.F.H.; Fitzgerald, P.B. Impact of different intensities of intermittent theta burst stimulation on the cortical properties during TMS-EEG and working memory performance. *Hum. Brain Mapp.* **2018**, *39*, 783–802. [CrossRef] [PubMed]
17. Stekic, A.; Zeljkovic, M.; Zaric Kontic, M.; Mihajlovic, K.; Adzic, M.; Stevanovic, I.; Ninkovic, M.; Grkovic, I.; Ilic, T.V.; Nedeljkovic, N.; et al. Intermittent Theta Burst Stimulation Ameliorates Cognitive Deficit and Attenuates Neuroinflammation via PI3K/Akt/mTOR Signaling Pathway in Alzheimer's-Like Disease Model. *Front. Aging Neurosci.* **2022**, *14*, 889983. [CrossRef]
18. Dragic, M.; Zeljkovic, M.; Stevanovic, I.; Ilic, T.; Ilic, N.; Nedeljkovic, N.; Ninkovic, M. Theta burst stimulation ameliorates symptoms of experimental autoimmune encephalomyelitis and attenuates reactive gliosis. *Brain Res. Bull.* **2020**, *162*, 208–217. [CrossRef]
19. Stanojevic, J.; Dragic, M.; Stevanovic, I.; Ilic, T.; Stojanovic, I.; Zeljkovic, M.; Ninkovic, M. Intermittent theta burst stimulation ameliorates cognitive impairment and hippocampal gliosis in the Streptozotocin-induced model of Alzheimer's disease. *Behav. Brain Res.* **2022**, *433*, 113984. [CrossRef]
20. Dragić, M.; Zeljković, M.; Stevanović, I.; Adžić, M.; Stekić, A.; Mihajlović, K.; Grković, I.; Ilić, N.; Ilić, T.V.; Nedeljković, N.; et al. Downregulation of CD73/A2AR-Mediated Adenosine Signaling as a Potential Mechanism of Neuroprotective Effects of Theta-Burst Transcranial Magnetic Stimulation in Acute Experimental Autoimmune Encephalomyelitis. *Brain Sci.* **2021**, *11*, 736. [CrossRef]
21. Stanojevic, J.B.; Zeljkovic, M.; Dragic, M.; Stojanovic, I.R.; Ilic, T.V.; Stevanovic, I.D.; Ninkovic, M.B. Intermittent theta burst stimulation attenuates oxidative stress and reactive astrogliosis in the streptozotocin-induced model of Alzheimer's disease-like pathology. *Front. Aging Neurosci.* **2023**, *15*, 1161678. [CrossRef]

22. Yang, P.; Wang, Z.; Zhang, Z.; Liu, D.; Manolios, E.N.; Chen, C.; Yan, X.; Zuo, W.; Chen, N. The extended application of The Rat Brain in Stereotaxic Coordinates in rats of various body weight. *J. Neurosci. Methods* **2018**, *307*, 60–69. [CrossRef]
23. Zeljkovic Jovanovic, M.; Stanojevic, J.; Stevanovic, I.; Stekic, A.; Bolland, S.J.; Jasnic, N.; Ninkovic, M.; Zaric Kontic, M.; Ilic, T.V.; Rodger, J.; et al. Intermittent Theta Burst Stimulation Improves Motor and Behavioral Dysfunction through Modulation of NMDA Receptor Subunit Composition in Experimental Model of Parkinson's Disease. *Cells* **2023**, *12*, 1525. [CrossRef]
24. Zaric, M.; Drakulic, D.; Dragic, M.; Gusevac Stojanovic, I.; Mitrovic, N.; Grkovic, I.; Martinovic, J. Molecular Alterations and Effects of Acute Dehydroepiandrosterone Treatment Following Brief Bilateral Common Carotid Artery Occlusion: Relevance to Transient Ischemic Attack. *Neuroscience* **2019**, *410*, 128–139. [CrossRef]
25. Girotti, M.J.; Khan, N.; McLellan, B.A. Early measurement of systemic lipid peroxidation products in the plasma of major blunt trauma patients. *J. Trauma* **1991**, *31*, 32–35. [CrossRef]
26. Kono, Y.; Kobayashi, K.; Tagawa, S.; Adachi, K.; Ueda, A.; Sawa, Y.; Shibata, H. Antioxidant activity of polyphenolics in diets. Rate constants of reactions of chlorogenic acid and caffeic acid with reactive species of oxygen and nitrogen. *Biochim. Et Biophys. Acta* **1997**, *1335*, 335–342. [CrossRef]
27. Navarro-Gonzálvez, J.A.; García-Benayas, C.; Arenas, J. Semiautomated measurement of nitrate in biological fluids. *Clin. Chem.* **1998**, *44*, 679–681. [CrossRef]
28. Salatić, I.; Dragović, T.; Stevanović, I.; Pavlović, B.D.; Ninković, M. Is the insulin necessary for the struggle against oxidative stress in diabetes mellitus type 2–a pilot study. *Vojnosanit. Pregl.* **2022**, *79*, 433–440. [CrossRef]
29. Misra, H.P.; Fridovich, I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *J. Biol. Chem.* **1972**, *247*, 3170–3175. [CrossRef] [PubMed]
30. Kovačević, V.; Daković, D.; Stevanović, I. Oxidative/antioxidative effects of colloidal silver ions and chlorhexidine in saliva and gingival fluid of periodontal patients. *Vojnosanit. Pregl.* **2022**, *79*, 441–447. [CrossRef]
31. Góth, L. A simple method for determination of serum catalase activity and revision of reference range. *Clin. Chim. Acta; Int. J. Clin. Chem.* **1991**, *196*, 143–151. [CrossRef]
32. Stohs, S.J.; Lawson, T.A.; Anderson, L.; Bueding, E. Effects of oltipraz, BHA, ADT and cabbage on glutathione metabolism, DNA damage and lipid peroxidation in old mice. *Mech. Ageing Dev.* **1986**, *37*, 137–145. [CrossRef]
33. Ellman, G.L. Tissue sulfhydryl groups. *Arch. Biochem. Biophys.* **1959**, *82*, 70–77. [CrossRef]
34. Latchoumycandane, C.; Anantharam, V.; Jin, H.; Kanthasamy, A.; Kanthasamy, A. Dopaminergic neurotoxicant 6-OHDA induces oxidative damage through proteolytic activation of PKCδ in cell culture and animal models of Parkinson's disease. *Toxicol. Appl. Pharmacol.* **2011**, *256*, 314–323. [CrossRef]
35. Tieu, K. A guide to neurotoxic animal models of Parkinson's disease. *Cold Spring Harb. Perspect. Med.* **2011**, *1*, a009316. [CrossRef]
36. Blum, D.; Torch, S.; Lambeng, N.; Nissou, M.; Benabid, A.L.; Sadoul, R.; Verna, J.M. Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: Contribution to the apoptotic theory in Parkinson's disease. *Prog. Neurobiol.* **2001**, *65*, 135–172. [CrossRef]
37. Dexter, D.T.; Carter, C.J.; Wells, F.R.; Javoy-Agid, F.; Agid, Y.; Lees, A.; Jenner, P.; Marsden, C.D. Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *J. Neurochem.* **1989**, *52*, 381–389. [CrossRef]
38. Sriraksa, N.; Wattanathorn, J.; Muchimapura, S.; Tiamkao, S.; Brown, K.; Chaisiwamongkol, K. Cognitive-enhancing effect of quercetin in a rat model of Parkinson's disease induced by 6-hydroxydopamine. *Evid.-Based Complement. Altern. Med. Ecam* **2012**, *2012*, 823206. [CrossRef]
39. Niedzielska, E.; Smaga, I.; Gawlik, M.; Moniczewski, A.; Stankowicz, P.; Pera, J.; Filip, M. Oxidative Stress in Neurodegenerative Diseases. *Mol. Neurobiol.* **2016**, *53*, 4094–4125. [CrossRef] [PubMed]
40. Smith, M.P.; Cass, W.A. Oxidative stress and dopamine depletion in an intrastriatal 6-hydroxydopamine model of Parkinson's disease. *Neuroscience* **2007**, *144*, 1057–1066. [CrossRef] [PubMed]
41. Haleagrahara, N.; Siew, C.J.; Ponnusamy, K. Effect of quercetin and desferrioxamine on 6-hydroxydopamine (6-OHDA) induced neurotoxicity in striatum of rats. *J. Toxicol. Sci.* **2013**, *38*, 25–33. [CrossRef] [PubMed]
42. Sian, J.; Dexter, D.T.; Lees, A.J.; Daniel, S.; Agid, Y.; Javoy-Agid, F.; Jenner, P.; Marsden, C.D. Alterations in glutathione levels in Parkinson's disease and other neurodegenerative disorders affecting basal ganglia. *Ann. Neurol.* **1994**, *36*, 348–355. [CrossRef]
43. Ahmad, M.; Saleem, S.; Ahmad, A.S.; Yousuf, S.; Ansari, M.A.; Khan, M.B.; Ishrat, T.; Chaturvedi, R.K.; Agrawal, A.K.; Islam, F. Ginkgo biloba affords dose-dependent protection against 6-hydroxydopamine-induced parkinsonism in rats: Neurobehavioural, neurochemical and immunohistochemical evidences. *J. Neurochem.* **2005**, *93*, 94–104. [CrossRef] [PubMed]
44. Ximenes, J.C.; Neves, K.R.; Leal, L.K.; do Carmo, M.R.; Brito, G.A.; Naffah-Mazzacoratti, M.d.G.; Cavalheiro, É.A.; Viana, G.S. Valproic Acid Neuroprotection in the 6-OHDA Model of Parkinson's Disease Is Possibly Related to Its Anti-Inflammatory and HDAC Inhibitory Properties. *J. Neurodegener. Dis.* **2015**, *2015*, 313702. [CrossRef] [PubMed]
45. Khan, Z.; Ali, S.A. Oxidative stress-related biomarkers in Parkinson's disease: A systematic review and meta-analysis. *Iran. J. Neurol.* **2018**, *17*, 137–144. [CrossRef] [PubMed]
46. You, H.; Mariani, L.L.; Mangone, G.; Le Febvre de Nailly, D.; Charbonnier-Beaupel, F.; Corvol, J.C. Molecular basis of dopamine replacement therapy and its side effects in Parkinson's disease. *Cell Tissue Res.* **2018**, *373*, 111–135. [CrossRef]
47. Medina-Fernández, F.J.; Escribano, B.M.; Padilla-Del-Campo, C.; Drucker-Colín, R.; Pascual-Leone, Á.; Túnez, I. Transcranial magnetic stimulation as an antioxidant. *Free Radic. Res.* **2018**, *52*, 381–389. [CrossRef]

48. Cullen, C.L.; Young, K.M. How Does Transcranial Magnetic Stimulation Influence Glial Cells in the Central Nervous System? *Front. Neural Circuits* **2016**, *10*, 26. [CrossRef]
49. He, F.; Ru, X.; Wen, T. NRF2, a Transcription Factor for Stress Response and Beyond. *Int. J. Mol. Sci.* **2020**, *21*, 4777. [CrossRef]
50. Wang, Q.; Li, W.X.; Dai, S.X.; Guo, Y.C.; Han, F.F.; Zheng, J.J.; Li, G.H.; Huang, J.F. Meta-Analysis of Parkinson's Disease and Alzheimer's Disease Revealed Commonly Impaired Pathways and Dysregulation of NRF2-Dependent Genes. *J. Alzheimer's Dis. JAD* **2017**, *56*, 1525–1539. [CrossRef]

Research Article

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Prolonged intermittent theta burst stimulation restores the balance between $A_{2A}R$- and A_1R-mediated adenosine signaling in the 6-hydroxidopamine model of Parkinson's disease

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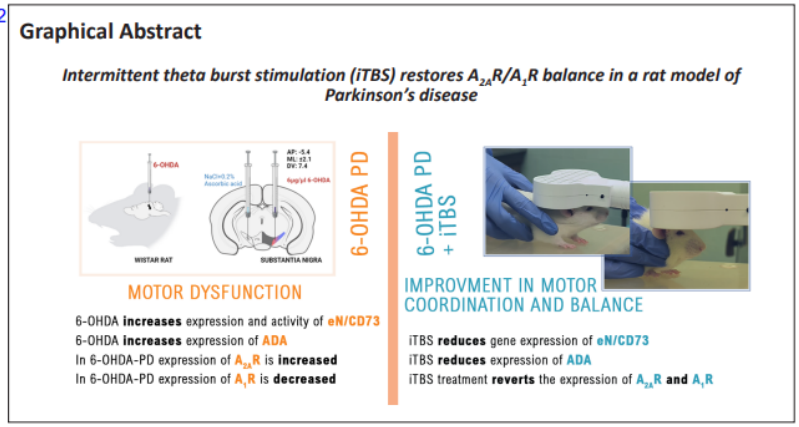
From the Contents

Graphical Abstract



Abstract

An imbalance in adenosine-mediated signaling, particularly the increased $A_{2A}R$-mediated signaling, plays a role in the pathogenesis of Parkinson's disease. Existing therapeutic approaches fail to alter disease progression, demonstrating the need for novel approaches in PD. Repetitive transcranial magnetic stimulation is a non-invasive approach that has been shown to improve motor and non-motor symptoms of Parkinson's disease. However, the underlying mechanisms of the beneficial effects of repetitive transcranial magnetic stimulation remain unknown. The purpose of this study is to investigate the extent to which the beneficial effects of prolonged intermittent theta burst stimulation in the 6-hydroxydopamine model of experimental parkinsonism are based on modulation of adenosine-mediated signaling. Animals with unilateral 6-hydroxydopamine lesions underwent intermittent theta burst stimulation for 3 weeks and were tested for motor skills using the Rotarod test. Immunoblot, quantitative reverse transcription polymerase chain reaction, immunohistochemistry, and biochemical analysis of components of adenosine-mediated signaling were performed on the synaptosomal fraction of the lesioned caudate putamen. Prolonged intermittent theta burst stimulation improved motor symptoms in 6-hydroxydopamine-lesioned animals. A 6-hydroxydopamine lesion resulted in progressive loss of dopaminergic neurons in the caudate putamen. Treatment with intermittent theta burst stimulation began seven days after the lesion, coinciding with the onset of motor symptoms. After treatment with prolonged intermittent theta burst stimulation, complete motor recovery was observed. This improvement was accompanied by downregulation of the eN/CD73-$A_{2A}R$ pathway and a return to physiological levels of A_1R-adenosine deaminase 1 after 3 weeks of intermittent theta burst stimulation. Our results demonstrated that 6-hydroxydopamine-induced degeneration reduced the expression of A_1R and elevated the expression of $A_{2A}R$. Intermittent theta burst stimulation reversed these effects by restoring the abundances of A_1R and $A_{2A}R$ to control levels. The shift in ARs expression likely restored the balance between dopamine-adenosine signaling, ultimately leading to the recovery of motor control.

Key Words: A_1R; $A_{2A}R$; adenosine receptors; adenosine; ecto-5'-nucleotidase; intermittent theta burst stimulation; non-invasive brain stimulation; Parkinson's disease; purinergic signalling

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease characterized by a range of motor symptoms as well as various nonmotor symptoms (Kumar et al., 2017). The symptoms result from the progressive loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc), leading to dopamine depletion in the basal ganglia, specifically the caudate putamen (CPu), as well as excitotoxicity and neurodegeneration (Gardoni et al., 2010; Schirinzi et al., 2016).

Purinergic signaling constitutes an intricate network of membrane receptors, ectonucleotidases, and transporters that interact with ATP and its downstream metabolites—ADP, AMP, and adenosine. Under physiological conditions, the tonic release of ATP and its subsequent action on high-affinity P2-receptors play a pivotal role in regulating numerous physiological processes (Di Virgilio et al., 2023). Dephosphorylation of ATP/ADP to AMP is catalyzed by NTPDase1/CD39, whereas hydrolysis of AMP to adenosine is catalyzed by ecto-5′-nucleotidase (eN/CD73) (Dragić et al., 2021). Adenosine regulates many physiological processes through four subtypes of G-coupled adenosine receptors (AR), associated with inhibition (A_1R, A_3R) or stimulation ($A_{2A}R$ and $A_{2B}R$) of adenylate cyclase (Fredholm et al., 2011). While A1R is the predominant adenosine receptor subtype in the brain, $A_{2A}R$ is significantly enriched in striatal nuclei (Schiffmann et al., 2007). Notably, $A_{2A}R$ is mostly expressed with D_2R at the indirect pathway synapses, whereas A_1R is co-localized with D_1R at the direct pathway neurons (Navarro et al., 2016). Because A_1R/D_1R and $A_{2A}R/D_2R$ are coupled in a reciprocal manner to inhibition/stimulation of adenylate cyclase, adenosine acts as a negative modulator of D_1R- and D_2R-mediated actions in both pathways.

There is evidence that enhanced $A_{2A}R$-mediated adenosine signalling plays a role in pathogenesis in PD (Abbracchio et al., 2009; Tóth et al., 2019). Specifically, enhanced $A_{2A}R$ activation precedes neuronal damage and the onset of motor symptoms in PD (Gonçalves et al., 2023) and promotes the inflammatory phenotype of glia, thus perpetuating chronic neuroinflammation in PD (Meng et al., 2019; Agostinho et al., 2020). Levodopa/carbidopa, a key therapeutic option for PD, is solely symptomatic, and its long-term use can lead to levodopa-induced dyskinesia. The observed beneficial effects of non-selective $A_{2A}R$ antagonists, such as methylxanthines and caffeine, prompted the development and clinical testing of novel selective $A_{2A}R$ antagonists. Among these, only istradefylline has been approved as an add-on therapy for PD (Nourianz®) (Cummins and Cates, 2022). However, existing treatments fail to alter the course of the disease or its progression, highlighting the unmet need for novel therapeutic approaches in PD.

Repetitive transcranial magnetic stimulation (rTMS) is a non-invasive brain stimulation technique that lacks a specific molecular target but rather induces global physiological changes (Edwards et al., 2008). rTMS has shown potential in the treatment of many neurodegenerative disorders, including PD. Previous studies have shown that the high-frequency rTMS can alleviate symptoms of PD (Chou et al., 2015; Xie et al., 2020). Intermittent theta burst stimulation (iTBS) is a high-frequency, excitatory protocol that has been shown to facilitate the induction of plasticity mechanisms (Suppa et al., 2016). It has short stimulation duration and low stimulation pulse intensity, while demonstrating comparable efficacy to rTMS (Suppa et al., 2016). Studies have demonstrated that iTBS modulates the subunit composition of N-methyl-D-aspartate (NMDA) glutamate receptors and attenuates inflammation and oxidative stress in experimental models of PD and Alzheimer's disease (Dragic et al., 2020; Stanojevic et al., 2022, 2023; Stekic et al., 2022; Zeljkovic Jovanovic et al., 2023). The rationale for using an iTBS protocol lies in the cerebral atrophy and hypofrontality observed in patients with PD (Jahanshahi et al., 1995; González-Redondo et al., 2014). These excitatory protocols may help reverse these conditions, leading to improvements in both motor and non-motor symptoms (Kanno et al., 2004; Edwards et al., 2008).

In the present study, we aimed to investigate the efficacy of iTBS in unilateral 6-hydroxydopamine (6-OHDA)-induced neurodegeneration in restoring motor skills by modulating adenosinergic signaling. The 6-OHDA model is the most commonly used model to study PD compared with other neurotoxic and genetic models (Kin et al., 2019). Furthermore, this model is most comparable to human disease in terms of behavior and offers the possibility of tracking the progression of dopaminergic neurodegeneration and the effects of other neurotransmitter systems in the pathogenesis of motor symptoms in PD, such as the adenosinergic system (Truong et al., 2006; Petrovic et al., 2021). It also allows for the investigation of the prodromal phase with nonmotor symptoms of PD. Finally, the 6-OHDA model recapitulates the main changes in $A_{2A}R$-mediated signaling, which are also observed in human pathology, making this model ideal for research on therapeutic interventions aimed at this signaling system (Cunha, 2005; Carmo et al., 2019; Vieira et al., 2019; Chen and Cunha, 2020; Gonçalves et al., 2023). The results of the present study show that iTBS alters eN/CD73-driven adenosine production and restores the balance between $A_{2A}R$ and A_1R signaling, which contributes to a significant improvement in motor abilities in the lesioned animals.

Methods

Animals

A total of 78 male Wistar rats (2 months old, 270 ± 30 g, specific pathogen-free (SPF)–grade) were used for this study. The rats were housed in the Center of Veterinary Services, with three rats per cage, under constant environmental conditions including a temperature of 23 ± 2°C, humidity ranging from 56% to 65%, a 12-hour light/dark cycle, and *ad libitum* access to a standard diet and tap water. All experimental procedures were approved by the Ethics Committee for Animal Experiments of the University of Belgrade - Faculty of Biology (No. 323-07-08250/2021-05) and performed in strict accordance with the principles and procedures outlined in the European Union Directives (2010/63/EU) regarding the "3Rs". Furthermore, all animal experiments were reported in compliance with the ARRIVE guidelines (Animal Research: Reporting of *In Vivo* Experiments; Percie du Sert et al., 2020). A total of 12 animals were excluded from the study based on the criteria outlined in the corresponding Methods section.

6-Hydroxydopamine-induced lesion

Following the anesthesia by intraperitoneal administration of ketamine (100 mg/kg; VetViva Richter, Wels, Austria) and xylazine (10 mg/kg; PROVET d.o.o., Belgrade, Serbia), all animals were positioned in the stereotaxic frame (Stoetling Co., Wood Dale, IL, USA). Then 2 µL of 6-hydroxydopamine (6-OHDA; Merck Millipore, Burlington, MA, USA, 6 µg/µL in sterile saline containing 0.2% ascorbic acid, 0.4 µL/min) was injected into the right substantia nigra pars compacta (rSNpc) (–5.40 mm anterior-posterior, ±2.10 mm medial-lateral, and +7.40 mm dorsal-ventral ; Paxinos and Watson, 1998), whereas vehicle was injected into the left substantia nigra pars compacta (lSNpc).

For rats weighing either more or less than 290 ± 15 g, stereotaxic references were adjusted as outlined in Yang et al. (2018). Immediately after the surgical procedure, each animal received 1 mL of sterile saline through subcutaneous hydration to maintain fluid levels. To minimize discomfort associated with the surgery, animals were injected with ibuprenorphine (0.05 mg/kg, s.c.) and were closely monitored by a responsible veterinarian for 3 consecutive days. Two animals died during anesthesia, and one animal exhibiting severe signs of distress post-surgery was euthanized. Seven days after surgery, all animals were tested on a rotarod to assess the effect of the 6-OHDA lesion, with their performance being compared with baseline values recorded for each animal prior to surgery. Any animals that exhibited a similar or even better latency to fall after surgery were excluded from the study.

The experimental design is summarized in **Figure 1**. One group of 6-OHDA-treated animals was sacrificed at 3, 5, 7, or 21 days post-intoxication (dpi; *n* = 3 per group) to assess the histopathological outcomes of the 6-OHDA lesion (**Figure 1A**). Another group of 54 animals recovered for 7 days and were then divided into two subgroups: the iTBS group (iTBS; *n* = 28), which underwent prolonged iTBS, and the sham group (Sham; *n* = 28), which was exposed to noise artifact only (**Figure 1B**). After 7 and 21 days of stimulation, the animals in both the iTBS and Sham groups were sacrificed by decapitation using a device from Harvard Apparatus (Holliston, MA, USA).

Theta burst stimulation protocol

Seven days after the injection of 6-OHDA, iTBS treatment was initiated, as this time point coincides with the near-peak of neuronal cell death and the onset of motor symptoms. iTBS was administered using the MagStim Rapid2 device (MagStim Company, Whitland, UK), following previously established protocols (Stekic et al., 2022; Zeljkovic Jovanovic et al., 2023). The stimulation protocol involved the use of a 25-mm figure-of-eight coil and comprised 20 trains, with each train consisting of 10 bursts. Each burst contained three pulses delivered at a frequency of 50 Hz, and the trains were repeated at a rate of 5 Hz, with 10-second intervals between trains. The stimulation intensity was set at 35% of the device's maximum power output, which corresponded to a magnetic field strength of 690 mT (Zeljkovic Jovanovic et al., 2023). Animals in the sham group were exposed only to the noise artifact generated by the device. This treatment protocol was repeated daily for either 7 or 21 consecutive days.

Rotarod performance test

Prior to surgery, all animals underwent three training sessions. Animals that were unable to complete these sessions were excluded from the experiment (*n* = 4). Baseline motor coordination was assessed 1 day before the operation, and animals were tested again 1 day prior to stimulation to evaluate any motor dysfunction that may have occurred due to surgery. Animals that did not exhibit motor dysfunction at this point were also excluded from the study (*n* = 5). Following 3 weeks of stimulation, the experimental animals were tested on a rotarod apparatus to assess their motor coordination. Each animal underwent three test sessions, with an acceleration from 4 to 20 revolutions per minute (r/min) and a maximum duration of 200 seconds per trial. A 30-minute interval was maintained between trials (Zeljkovic Jovanovic et al., 2023). The latency to fall and the distance traveled were recorded for each animal, and the best performance from the trial series was selected for further analysis.

Brain tissue collection and isolation of crude membrane (P2) and crude cytosolic fraction

The brains were carefully removed from the skulls of the animals (*n* = 5–6 per group). The right and left caudoputamen (rCPu and lCPu, respectively) were dissected and stored at –80°C until further processing. Preparation of the crude synaptosomal (P2) and cytosolic fractions was conducted following established protocols (Gray and Whittaker, 1962). Protein concentration was determined after isolation using the PierceTM BCA Protein Assay Kit (Thermo Fisher Scientific, Waltham, MA, USA), according to the manufacturer's instructions.

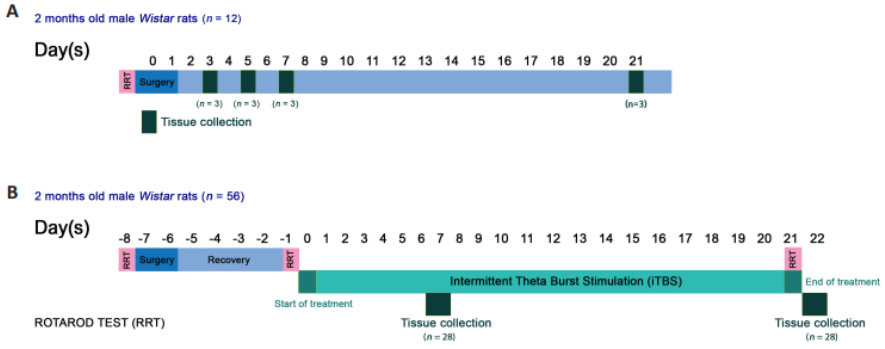

Figure 1 | Experimental design.
(A) Experimental design of 6-hydroxydopamine-induced neurodegeneration. (B) Experimental design of prolonged intermittent theta burst stimulation.

Immunoblot and dot blot analyses

Immunoblot analysis was performed with striatal P2 fractions. Prepared protein samples (20 µg, *n* = 5/group) were loaded onto a 10% sodium dodecyl-sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel, resolved, and transferred to polyvinylidene difluoride (PVDF) membranes (0.45 mm, Millipore, Darmstadt, Germany). These membranes were blocked with 5% non-fat dry milk for 1 hour (SERVA, Germany) and incubated with primary antibodies overnight at 4°C and horseradish peroxidase (HRP)-conjugated secondary antibodies for 2 hours at room temperature (**Table 1**). For each animal, the chemiluminescent intensity ratio between the target protein and GAPDH in the rCPu was expressed relative to the lCPu (%) utilizing the freely available ImageJ software (version 1.54i) at https://imagej.net/ij/download.html.

The presence of soluble inflammatory mediators was determined using a dot blot method. Briefly, a cytosolic fraction (20 µg) was applied onto a PVDF support membrane (Immobilon-P membrane, Millipore) within a vacuum-based minifold dot blot apparatus (Schleicher & Schuell Inc., Keene, NH, USA) following the protocol described by Dragic et al. (2020). After successful transfer and blocking, the PVDF membranes were incubated with primary antibodies overnight at 4°C, followed by incubation with secondary HRP-conjugated antibodies for 2 hours at room temperature. The results are then normalized using Ponceau S staining.

Brain tissue preparation and immunohistochemical staining

Brains were removed from the skull (*n* = 3/group) and fixed in 4% paraformaldehyde (PFA) for 24 hours and processed for immunohistochemistry as previously described (Dragić et al., 2021). The 25 µm thick coronal sections were probed with appropriate primary overnight at 4°C and secondary (2 hours at room temperature) antibodies and examined under the light microscope LEITZ DM RB (Leica Mikroskopie and Systems GmbH, Wetzlar, Germany; **Table 2**) equipped with the LEICA DFC320 CCD camera (Leica Microsystems Ltd., Heerbrugg, Switzerland).

The brains were carefully removed from the skulls of animals in each group (*n* = 3/group) and fixed in 4% paraformaldehyde (PFA) for 24 hours. They were then processed for immunohistochemistry following a protocol described previously by Dragić et al. (2021). Coronal sections, each 25 µm thick, were incubated with appropriate primary antibodies overnight at 4°C, followed by 2-hour incubation with secondary antibodies at room temperature. These sections were then carefully examined under the LEITZ DM RB light microscope (Leica Mikroskopie and Systems GmbH in Wetzlar, Germany; **Table 2**) equipped with a LEICA DFC320 CCD camera (Leica Microsystems Ltd. in Heerbrugg, Switzerland).

Ectonucleotidase assays

AMPase activity was assayed by quantifying the amount of inorganic phosphates (Pi) released by ectonucleotidases upon the addition of AMP to the isolated P2 fraction, following a previously established protocol (Grković et al., 2019). Briefly, aliquots containing 15 µg of protein from the P2 fraction (*n* = 5–6 per group) were incubated in a reaction buffer supplemented with 1 mM AMP (Merck Millipore). After

Table 1 | **Primary and secondary antibodies used in this study**

Antibody	Host organism and clonality	Dilution	Supplier	Cat#	RRID
TH	Rabbit, polyclonal	1:2000^{WB}, 1:500^{IHC}	Millipore, Darmstadt, Germany	AB152	AB_390204
GFAP	Rabbit, polyclonal	1:500^{IHC}, 1:7000^{WB}	Santa Clara, CA, USA	Z0334	AB_10013382
Iba-1	Goat, polyclonal	1:400^{IHC}, 1:500^{WB}	Abcam, Cambridge, MA, USA	AB5076	AB_2224402
CD73, rNu-9L(I4,I5)	Rabbit, polyclonal	1:200^{IHC}	Ectonucleotidases-ab.com,		
IL-1β	Rabbit, polyclonal	1:500^{DB}	Fine Test, Wuhan China	FNab04209	
TNF-α	Rabbit, polyclonal	1:500^{DB}	Thermo Fisher Scientific, Waltham, MA, USA	PA1-40281	AB_2204371
P2X7R	Rabbit, polyclonal	1:1000^{WB}	Alomone Labs, Jerusalem, Israel	AAR-004	AB_2040068
P2Y1R	Rabbit, polyclonal	1:1000^{WB}	Alomone Labs, Jerusalem, Israel	AAR-021	AB_10919250
P2Y12R	Rabbit, polyclonal	1:1000^{WB}	Alomone Labs, Jerusalem, Israel	AAR-012	AB_2040074
P2Y13R	Rabbit, polyclonal	1:1000^{WB}	Alomone Labs, Jerusalem, Israel	AAR-017	AB_2040076
CD73	Rabbit, polyclonal	1:1000^{WB}	Cell Signaling Technology, Danvers, MA, USA	13160	AB_2716625
ADA	Rabbit, polyclonal	1:1000^{WB}	Thermo Fisher Scientific	PA5-51572	AB_2637694
A1R	Rabbit, polyclonal	1:1000^{WB}	Alomone Labs	AAR-009	
A2AR	Rabbit, polyclonal	1:1000^{WB}	Thermo Fisher Scientific	PA1-042	AB_2257858
p-AMPK	Rabbit, polyclonal	1:2000^{WB}	Cell Signaling Technology	2535	AB_331250
t-AMPK	Rabbit, polyclonal	1:2000^{WB}	Cell Signaling Technology	2532	AB_330331
D1DR	Rabbit, polyclonal	1:500^{WB}	Abcam	AB81296	AB_2814742
D2DR	Rabbit, polyclonal	1:500^{WB}	Fine Test	FNab02533	
GAPDH	Rabbit, polyclonal	1:2000^{WB}	Thermo Fisher Scientific	PA1-987	AB_2107311
Goat anti-rabbit IgG, HRP-conjugated	Goat, polyclonal	1:30000^{WB}	Abcam	AB6721	AB_955447
Rabbit anti-goat IgG, HRP-conjugated	Rabbit, polyclonal	1:10000^{WB}	R and D Systems , Minneapolis, MN, USA	HAF017	AB_562588

DB: ; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GFAP: glial fibrillary acidic protein; HRP: horseradish peroxidase; Iba-1: ionized calcium binding adaptor molecule 1; IL-1β: interleukin-1β; TH: tyrosine hydroxylase; TNF-α: tumor necrosis factor-α; p-AMPK: phospho-adenosine monophosphate kinase, t-AMPK: total-adenosine monophosphate kinase; D1DR: dopamine 1 receptor, D2DR: dopamine 2 receptor; WB: western blotting; IHC: immunohistochemistry.

30 minutes, the reactions were terminated by adding 3 M perchloric acid (Centrohem, Novi Sad, Serbia). Subsequently, 80-µL aliquots of the reaction mixture were transferred to a 96-well plate and mixed with 20-µL of malachite green solution. Following a 30-minute incubation period, the absorbance was measured at 620 nm, and the concentration of Pi was determined using KH2PO4 (Centrohem, Novi Sad, Serbia) as a reference standard. Each biological replicate was assayed in at least two technical replicates to ensure reproducibility. Additionally, enzyme assay conditions were optimized in separate experiments to guarantee the linearity of the reactions (**Additional Figure 1**).

Quantitative reverse transcription-polymerase chain reaction analysis

Total RNA was extracted from CPu (n = 5/group) using NZYol reagent (NYZtech, Lisbon, Portugal), according to the manufacturer's instructions and quantitative reverse transcription-polymerase chain reaction (qRT-PCR) was performed as previously described (Grković et al., 2019). Briefly, the reaction mixture contained 2 µL of cDNA (10 ng/µL), 5 µL of QTM SYBR Green PCR Master Mix (Applied Biosystems, Foster City, CA, USA), 0.5 µL of primer (100 pmol/µL; **Table 2**), and 2 µL of RNase-free water (UltraPure, Invitrogen, Waltham, MA, USA). Amplification was carried out under the following optimized conditions: initial enzyme activation for 10 minutes at 95°C, followed by 40 cycles consisting of denaturation for 15 seconds at 95°C, annealing for 30 seconds at 64°C, and amplification for 30 seconds at 72°C. Fluorescence measurements were taken every 5 seconds at 72°C to monitor the progress of the reaction. The abundance of the target gene's mRNA was expressed relative to the mRNA levels of cyclophilin A (CycA), which served as a reliable reference gene for normalization purposes.

Table 2 | **Primer pairs for quantitative reverse transcription-polymerase chain reaction**

Target gene	Forward (5'–3')	Reverse (5'–3')
Nt5e	CAA ATC TGC CTC TGG AAA GC	ACC TTC CAG AAG GAC CCT GT
Adora1	GTG ATT TGG GCT GTG AAG GT	GAG CTC TGG GTG AGG ATG AG
Adora2a	TGC AGA ACG TCA CCA ACT TC	CAA AAC AGG CGA AGA AGA GG
Adora3	TTC TTG TTT GCC TTG TGC TG	AGG GTT CAT CAT GGA GTT CG
Ada	GAG CCT CAT CCT GTG AAT GG	ATG CCC ATG ATT GTC AAG GT
Slc29a1	CAC TTC CTT CGC TGT TAG GG	TGT CCC CCT ACC ACT CTG AC
Slc29a2	CCC TCA TGA CCT TCT TCC TG	CCA AGA GAC CCG GTA TAG CA
Entpd1	CCC AGC TGA ACA GCC ATT AT	GAT GAA CAG CCC TGT GAT GA
Entpd2	GGC CAA AGG GCT ACT CTA CC	GTT CCT GAC AGG CTG ACG AT
P2rx7	ATT GTT AGG CCA ATG GCA AG	AAC ACC TTC ACC GTC TCC AC
P2ry1	CTG GAT CTT CGG GGA TGT TA	CTG CCC AGA GAC TTG AGA GG
P2ry6	CAG TTA TGG AGC GGG ACA AT	GTA AAC TGG GGG TAG CAG CA
P2ry12	CGA AAC CAA GTC ACT GAG AGG A	CCA GGA ATG GAG GTG GTG TTG
P2ry13	GGC ATC AAC CGT GAA GAA AT	TTG GCA ATC ACC GTG TAA AA
CycA	CAA AGT TCC AAA GAC AGC AGA AAA	CCA CCC TGG CAC ATG AAT

Statistical analyses

No statistical methods were performed to predetermine sample sizes; however, our sample sizes are comparable to those reported in a previous study (Zeljkovic Jovanovic et al., 2023). To assess data normality, we utilized the Shapiro-Wilk test, and accordingly applied either parametric or nonparametric tests. Rotarod test results were analyzed using both paired and unpaired two-tailed Student's t-tests, whereas immunoblot, qRT-PCR, and enzyme assays were evaluated using either the unpaired two-tailed Student's t-test or the Mann-Whitney U test. For the analysis of TH expression across different time points, we used one-way ANOVA followed by the Dunnett *post hoc* test. All data are presented as mean ± SD for behavioral analysis, immunoblot, and qRT-PCR, and as mean ± SEM for enzyme assays. Statistical significance was set at $P < 0.05$. Data analysis and graphical representation were conducted using the GraphPad Prism 9.0 software package (GraphPad, San Diego, CA, USA, www.graphpad.com).

Results

Behavioral outcomes and histopathological evaluation of the unilateral 6-OHDA injection and iTBS stimulation

The animals showed marked impairment of motor skills. Because of the unilateral rSNpc lesion, the animals had difficulty using the contralateral left limbs. The animals also showed a significant reduction in latency to fall (t = 7.96, d_f = 40, $P < 0.0001$; **Figure 2A**) and the distance traveled (t = 7.30, d_f = 54, $P < 0.0001$; **Figure 2B**) compared with their baseline performance in the rotarod test assessed 1 day before lesion was induced (**Figure 2A** and **B**).

The neurodegeneration specific to certain regions, resulting from the injection of 6-OHDA into the right SNpc, was further confirmed at the protein level. The loss of dopaminergic neurons and terminals in the ipsilateral CPu was indirectly demonstrated through immunoblotting of tyrosine hydroxylase (TH), the enzyme that serves as a rate-limiting factor in the catecholamine biosynthesis pathway (**Figure 2C**). The trend of decreasing TH protein abundance in the rCPu was evident at all tested time points: 3 dpi (t = 7.56, d_f = 4, $P < 0.0001$), 5 dpi (t = 8.16, d_f = 4, $P < 0.0001$), 7 dpi (t = 7.24, d_f = 4, $P < 0.0001$), and 21 dpi (t = 8.69, d_f = 4, $P < 0.0001$; **Figure 2C**, right axis). This indicates a progressive loss of dopaminergic neurons.

The loss of dopaminergic neurons was further confirmedd through TH immunohistochemistry conducted at the caudoputamen/midbrain level. A decrease in TH immunoreactivity was evident in the rSNpc and the right substantia nigra pars reticulata, when compared with their corresponding left counterparts. Notably, the ventral tegmental area (VTA) appeared to be spared bilaterally (**Figure 2D** and **E**). Additionally, unilateral enhancements in glial fibrillary acidic protein immunostaining (**Figure 2F** and **G**) and Iba1 immunostaining (**Figure 2H** and **I**) were observed at the border between the rSNpc and the right substantia nigra pars reticulata. These findings are suggestive of reactive gliosis. However, the overall immunoreactive patterns did not indicate the presence of highly reactive glial states (**Additional Figure 2**).

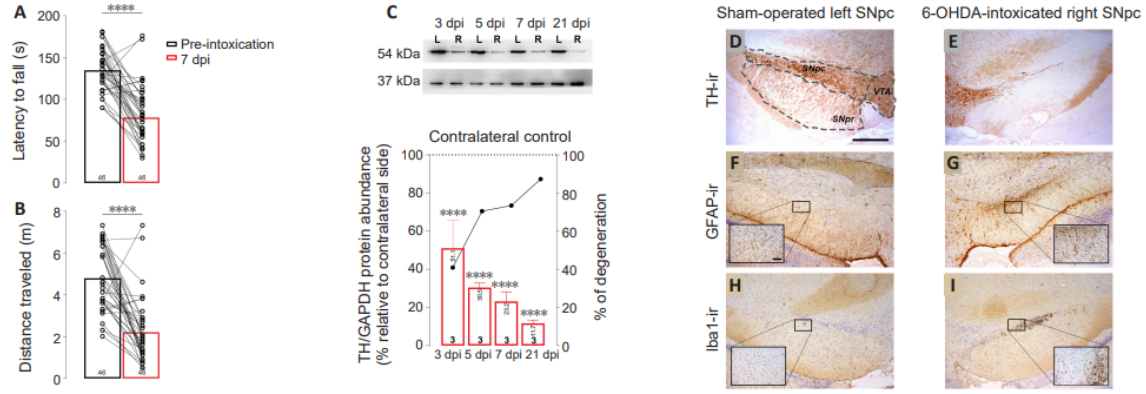

Figure 2 | **Unilateral 6-OHDA lesion induces motor impairment and progressive loss of dopaminergic neurons in caudoputamen.**
(A) Histograms showing latency to fall and (B) distance travelled on a rotarod test of animals before (pre-intoxication) and 7 days after 6-OHDA unilateral lesion (7 dpi). (C) Histograms showing the average ratio of abundance of TH (left *y*-axis) in the right caudoputamen expressed as a percentage (%) of the contralateral caudoputamen (contralateral control) and % of degeneration expressed as 100–% of TH/GAPDH protein abundance (right *y*-axis). All data are expressed as mean ± SD. Dots in the graphs represent individual values. The number at the bottom of the graphs represents the number of individual animals included in the analysis. (D) Micrographs depicting TH-ir neurons in sham-operated left SNpc, VTA, and (E) in 6-OHDA-operated right SNpc. (F) GFAP-ir cells in sham-operated left SNpc and (G) 6-OHDA-treated right SNpc. (H) Iba1-ir cells in sham-operated left SNpc and (I) 6-OHDA-treated right SNpc. Scale bars: 500 µm in D–I, and 100 µm in insets. Significance shown inside graphs: ****$P < 0.0001$. Data in A and B are analyzed by paired Student's *t*-test, while data in C are analyzed by one-way analysis of variance followed by Tukey's *post hoc* test. All experiments are repeated at least twice. 6-OHDA: 6-Hydroxydopamine; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; GFAP: glial fibrillary acidic protein; Iba1: ionized calcium binding adaptor molecule 1; ir-immunoreactive; SNPc: substantia nigra pars compacta; TH: tyrosine hydroxylase; VTA: ventral tegmental area.

Seven days after 6-OHDA injection, TH protein abundance in the rCPu had decreased by approximately 70% compared with the contralateral side. Based on this observation and the timeline outlined in **Figure 1B**, this time point was selected as the starting point for the initiation of iTBS stimulation. Briefly, animals with 6-OHDA lesions were allowed to recover for 7 days before being subjected to either iTBS stimulation (iTBS group) or sham stimulation (sham group) for 7 or 21 consecutive days. At the end of the 21-day stimulation period, motor abilities were assessed using the rotarod test. Animals in the iTBS group exhibited significant improvements in rotarod performance, demonstrating both enhanced endurance on the rotating rod ($t = 5.16$, $d_f = 19$, $P = 0.0012$; **Figure 3A**) and increased distance traveled ($t = 3.41$, $d_f = 19$, $P = 0.014$; **Figure 3A**), compared with the sham group. There were no significant differences in the expression levels of IL-1β (Mann-Whitney $U = 2$, $P = 0.4$) and TNF-α (Mann-Whitney $U = 2$, $P = 0.8$) between the lCPu and rCPu in the sham group (**Figure 3B**). However, in the iTBS group, a significant decrease in IL-1β levels was observed in the rCPu (Mann-Whitney $U = 0$, $P = 0.028$), whereas no significant change in TNF-α expression was observed (Mann-Whitney $U = 7$, $P = 0.88$). With regards to microglial (**Figure 3C–F**) and astroglial (**Figure 3G–J**) responses in iTBS animals, the ionized calcium binding adaptor molecule 1 (Iba-1) and glial fibrillary acidic protein-immunoreactive (GFAP-ir) patterns in the right SNpc were notably less pronounced compared with those in rSNpc in the sham group. Furthermore, these patterns were only slightly different from the non-lesioned lSNpc (**Figure 3C** and **G**), indicating a substantially lower level of neuroinflammation and glial response following iTBS stimulation.

Purinome expression in the 6-OHDA model of neurodegeneration and after iTBS stimulation

Purinome was analyzed at the mRNA and protein levels. Gene transcripts determined by qRT-PCR in rCPu were compared between iTBS and iTBSsh after 7- and 21-day stimulation (**Table 3**). Since Sham animals were solely exposed to noise artifact, it was presumed that any differences observed between Sham groups after 7-day and 21-day stimulation could be solely attributed to the internal processes triggered by the 6-OHDA lesion. Conversely, differences detected between the iTBS and Sham groups at the same time points were likely due to the iTBS stimulation. Notably, we observed significant differences in genes involved in adenosine signaling between the 7-day and 21-day time points within the sham group. In particular, the expression of *Nt5e* ([153 ± 16]%, $t = 2.735$, $d_f = 8$, $P = 0.014$) and *Adora2A* ([121.9 ± 6]%, $t = 2.56$, $d_f = 8$, $P = 0.026$) continued to increase in the sham group at two time points, while the expression of *Adora1* mRNA ([16.7 ± 11]%, $t = 5.72$, $d_f = 8$, $P < 0.0001$) was reduced to a minimum level. We also observed induction of Adora3 in the Sham group ([247.6 ± 37]%, $t = 9.63$, $d_f = 8$, $P < 0.0001$), although the basal expression of this gene was 10 times lower than those of *Adora1* and *Adora2a*. Taken together, the results indicate increases in extracellular adenosine levels and an imbalance in $A_1R/A_{2A}R$-mediated signaling induced by 6-OHDA lesion. With regards to P2-receptor-mediated signaling, among the genes tested, we observed a significant upregulation of *P2xr7* ([230.3 ± 25]%, $t = 6.92$, $d_f = 8$, $P < 0.0001$), *P2yr12* ([142.0 ± 27]%, $t = 2.13$, $d_f = 8$, $P = 0.046$), and P2yr13 ([156.6 ± 19]%, $t = 4.05$, $d_f = 8$, $P = 0.0008$) in response to the 6-OHDA lesion.

Table 3 | **Expression levels of purinergic genes**

Values	$2^{-\Delta Ct\ (Ct\ (Gapdh)-Ct\ (target\ gene))}$			
	Sham 7-day	iTBS 7-day	Sham 21-day	iTBS 21-day
Target gene		(% of iTBSsh 7-day)		(% of iTBSsh 21-day)
Nt5e	0.0047	0.0046 (97.9)	0.0072 (153.2)	0.0048 (66.7)**
Adora1	0.0287	0.0297 (103.5)	0.0048 (16.7)	0.0152 (316.7)***
Adora2a	0.0398	0.0506 (127.1)	0.0485 (121.9)	0.0339 (69.9)**
Adora3	0.0042	0.0044 (104.7)	0.0104 (247.6)	0.0040 (38.5)***
Ada	0.0021	0.0017 (80.9)	0.0017 (80.9)	0.0011(64.7)*
Slc29a1	0.0034	0.0035 (102.9)	0.0030 (88.2)	0.0018 (60.0)*
Slc29a2	0.002	0.0024 (120.0)	0.0024 (120.0)	0.0016 (80.0)*
Entpd1	0.0085	0.0087 (102.3)	0.0069 (81.2)	0.0107 (155.1)*
Entpd2	0.0443	0.0458 (103.4)	0.0433 (97.7)	0.0359 (82.9)
P2xr7	0.0076	0.0098 (128.9)**	0.0175 (230.3)	0.0076 (43.4)***
P2yr1	0.0046	0.0053 (115.2)*	0.0035 (76.1)	0.0036 (102.9)
P2yr6	0.0017	0.0018 (105.9)	0.0020 (117.6)	0.0018 (105.9)*
P2yr12	0.005	0.0036 (72.0)...	0.0071 (142.0)	0.0046 (64.8)**
P2yr13	0.009	0.0064 (71.1)*	0.0141 (156.6)	0.0086 (61.0)***

*P < 0.05, **P < 0.01, ***P < 0.001. iTBS: Intermittent theta burst stimulation.

iTBS stimulation completely reversed the lesion-induced changes in adenosine gene expression after 21-day stimulation, whereas the effects were not significant after 7-day stimulation. Specifically, the expression of *Nt5e* ([66.7 ± 23]%, t = 3.20, d_f = 8, P = 0.0052), *Adora2a* ([69.9 ± 13]%, t = 4.06, d_f = 8, P = 0.0012), *Adora3* ([38.5 ± 15]%, t = 9.69, d_f = 8, P < 0.0001), *Slc29a1* ([60 ± 15]%, t = 2.57, d_f = 8, P = 0.0221) and *Slc29a2* ([80.0 ± 13]%, t = 2.19, d_f = 8, P = 0.043) significantly decreased, while the abundance of *Adora1* ([316.7 ± 23]%, t = 5.69, d_f = 8, P = 0.0004) markedly increased after 21-day iTBS stimulation compared with the sham group.

With regard to ATP/ADP and P2-mediated signaling, iTBS stimulation effectively downregulated *P2xr7* ([43.4 ± 17]%, t = 6.70, d_f = 8, P < 0.0001), *P2yr12* ([64.8 ± 23]%, t = 2.94, d_f = 8, P = 0.008), and *P2yr13* ([61.0 ± 7]%, t = 4.36, d_f = 8, P = 0.004) transcripts. Additionally, it upregulated the abundance of *Entpd1* mRNA ([155.1 ± 32] %, t = 2.18, d_f = 8, P = 0.0432) after 21 days of stimulation. A summary of the expression levels of purinergic genes is presented in **Table 3**.

Changes in ATP/ADP-mediated signaling and P2 receptor expression in the 6-OHDA model of neurodegeneration and after iTBS stimulation

We determined ADPase activity in the crude membrane fraction (P2) of the caudate putamen (CPu) after 7- and 21-day iTBS. A significant increase in ADPase activity was detected in the rCPu (rCPu) of sham-treated animals (51.49 ± 3.86 nmol Pi/mg/min) compared with the lCPu (74.17 ± 3.20 nmol Pi/mg/min, t = 4.54, d_f = 23, P = 0.0001). However, after 7 days of iTBS, no significant differences were observed between the lCPu (79.77 ± 9.72 nmol Pi/mg/min) and rCPu (70.47 ± 7.08 nmol Pi/mg/min, t = 0.79, d_f = 24, P = 0.4383), although the total ADPase activity increased in both hemispheres following stimulation. Similarly, after 21 days, neither sham-treated animals nor iTBS-stimulated animals exhibited significant changes in ADPase activity between the lCPu (56.95 ± 3.39 nmol Pi/mg/min) and rCPu (61.49 ± 4.04 nmol Pi/mg/min, t = 0.86, d_f = 28, P = 0.3942) or between the lCPu (82.12 ± 3.69 nmol Pi/mg/min) and rCPu (69.45 ± 5.53 nmol Pi/mg/min; t = 1.92, d_f = 28, P = 0.06) of iTBS animals, respectively (**Figure 4E**).

Based on the quantitative reverse transcriptase polymerase chain reaction (qRT-PCR) analysis, we chose $P2X_7R$, $P2Y_1R$, $P2Y_{12}R$, and $P2Y_{13}R$ for further protein expression analysis. After 7 days of iTBS, there was no significant change in the expression of P2X7R (**Figure 4A**) between the sham and iTBS groups (t = 0.32, d_f = 8, P = 0.75). However, after 21 days of iTBS, we observed a significant increase in P2X7R expression (t = 4.05, d_f = 8, P = 0.0037). While we did not directly compare the lCPu with the rCPu within the same group, it appears that the observed increase in $P2X_7R$ expression was due to downregulation of protein expression in the sham group, which was restored to near control levels by iTBS. Similarly, there were no changes in the protein expression of striatal P2Y1R (**Figure 4B**) after 7 days of stimulation (t = 1.47, d_f = 8, P = 0.1908). However, after 21 days of stimulation, we observed an increase in receptor expression (t = 2.98, d_f = 8, P = 0.0227). The expression of $P2Y_{12}R$ (**Figure 4C**) remained unchanged after both 7 days (t = 1.43, d_f = 8, P = 0.2022) and 21 days of stimulation (t = 1.21, d_f = 8, P = 0.2605). In contrast, the expression of P2Y13R (**Figure 4D**) showed a significant increase after both 7 days (t = 5.02, d_f = 8, P = 0.0024) and 21 days of stimulation (t = 4.83, d_f = 8, P = 0.0013).

Changes in adenosine-mediated signaling and adenosine receptors expression in the 6-OHDA model of neurodegeneration and after iTBS stimulation

We further performed immunoblot analysis of adenosine receptors and adenosine-metabolizing enzymes. The analyses were performed in crude CPu membrane (P2) and cytosolic fractions (**Figure 5**). The abundance of target proteins in rCPu was expressed as a percentage of the protein abundance in lCPu in each animal. The abundance of eN/CD73 increased by approximately 50% and 25% after 7- and 21-day sham stimulation, respectively, although the increase at the mRNA level was observed only after 21-day stimulation. iTBS stimulation was able to decrease eN/CD73 protein abundance to a level comparable to the contralateral lCPu after 7-day stimulation (t = 3.25, d_f = 8, P = 0.0063). Accordingly, after 21-day stimulation, eN/CD73 protein abundances were also comparable in two hemispheres (t = 1.09, d_f = 8, P = 0.3050; **Figure 5A**). With regard to adenosine-degrading enzyme adenosine deaminase (ADA), there was a trend toward a modest increase in adenosine deaminase 1 (ADA1) protein abundance in iTBSsh after 7-day and 21-day sham stimulation (**Figure 5B**). Consistent with qRT-PCR data, iTBS reduced expression of ADA1 protein after 21-day stimulation to a level comparable to the contralateral CPu (t = 3.32, d_f = 8, P = 0.0105). Therefore, with regard to adenosine metabolizing enzymes, it appears that iTBS counteracted the impact of 6-OHDA lesion by restoring protein expression of eN/CD73 and ADA1 to the levels comparable to the contralateral CPu.

Next, we determined the functional consequences of altered gene and protein expression of ectonucleotidases after 6-OHDA lesion and iTBS stimulation. Specifically,

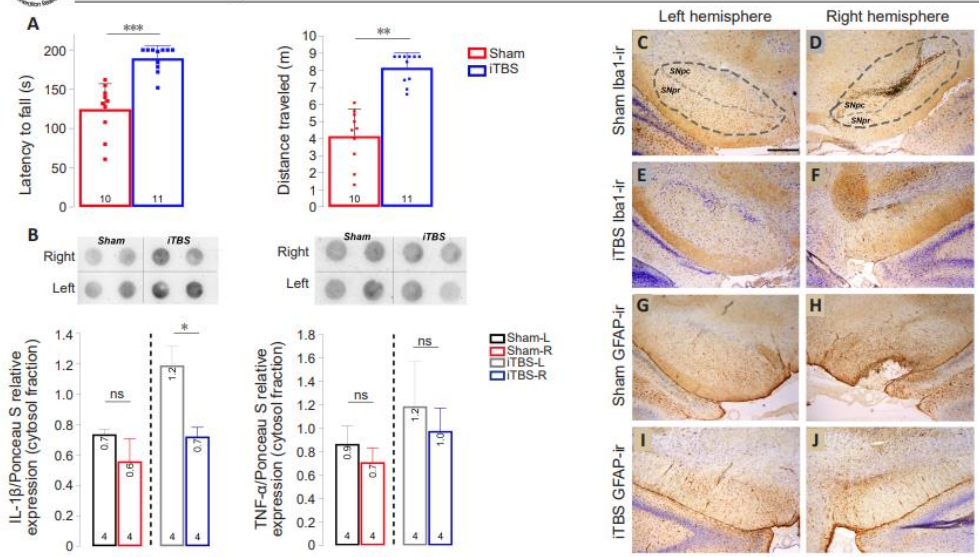

Figure 3 | **Effect of prolonged iTBS on motor behavior and neuroinflammation of 6-OHDA lesioned rats.**
(A) Histograms show latency to fall (left histogram) and distance travelled (right histogram) on a rotarod test after 3 weeks of sham stimulation (Sham) or real iTBS (iTBS). Dots in the graphs represent individual values. (B) Dot blot analysis of pro-inflammatory IL-1β (left histogram) and TNF-α (right histogram) cytokines in cytosolic fraction of left (Sham-L, iTBS-L) and right (Sham-R, iTBS-R) CPu of Sham- and iTBS-stimulated animals, expressed as relative optical density of total protein load stained by PonceauS. All data are expressed as mean ± SD. The number at the bottom of the graphs represents the number of individual animals included in the analysis. (C–F) Micrographs depicting Iba1-ir cells in the left and right SNpc of the Sham animals (C, D) and after 3 weeks of iTBS (E, F). (G-J) Micrographs depicting GFAP-ir cells in the left SNpc and right SNpc of the Sham animals (G, H) and after 3 weeks of iTBS (I, J). Dotted lines circle the SNpc. Scale bar: 500 µm. Significance shown inside graphs: $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. Data in A and B are analyzed by unpaired Student's t-test and all experiments are repeated at least twice. 6-OHDA: 6-Hydroxydopamine; CPu: caudoputamen; GFAP: glial fibrillary acidic protein; Iba1: ionized calcium binding adaptor molecule 1; ir: immunoreactive; iTBS: intermittent theta burst stimulation; SNpc: substantia nigra pars compacta.

we determined the rate of AMP hydrolysis which can be attributed to eN/CD73. Conditions for optimal enzyme activity were determined in separate experiments and are shown in **Additional Figure 1**. With regard to eN/CD73, more than 2-fold higher activity was detected in rCPu (100.20 ± 3.11 nmol Pi/mg/min) compared with lCPu (50.68 ± 2.98 nmol Pi/mg/min, $t = 11.58$, $d_f = 27$, $P < 0.0001$) after 7-day sham stimulation, while the hemispheric difference was less pronounce after 21-day sham, but still increased in rCPu [lCPu, 72.59 ± 3.51 nmol Pi/mg/min; rCPu, 95.66 ± 2.28 nmol Pi/mg/min; $t = 5.71$, $d_f = 28$, $P < 0.0001$).

The 7-day iTBS stimulation failed to produce any effect, resulting in the activity of eN/CD73 remaining unchanged in both the rCPu (61.45 ± 3.88 nmol Pi/mg/min) and the lCPu (100.30 ± 3.69 nmol Pi/mg/min, $t = 6.98$, $d_f = 28$, $P < 0.0001$) compared with the corresponding sham group. Following a 21-day stimulation, the activity of eN/CD73 in the rCPu (100.8 ± 2.01 nmol Pi/mg/min) became comparable to that in the contralateral lCPu (94.16 ± 2.52 nmol Pi/mg/min, $t = 1.99$, $d_f = 27$, $P = 0.0591$), effectively erasing the differences between the two hemispheres (**Figure 5E**).

Consistent with the qRT-PCR data, 6-OHDA induced a decrease in A_1R protein abundance by approximately 20% in the rCPu (**Figure 5C**) and an increase in $A_{2A}R$ protein abundance by approximately 30% (**Figure 5D**) after both 7-day and 21-day sham stimulation. Following iTBS stimulation, there was a consistent increase in A_1R expression at the protein level in the rCPu compared with the rCPu of sham-stimulated animals after both 7-day ($t = 2.91$, $d_f = 8$, $P = 0.0271$) and 21-day ($t = 3.47$, $d_f = 8$, $P = 0.0084$) stimulation. Furthermore, consistent with the expression analysis, $A_{2A}R$ protein decreased in the rCPu after both 7-day ($t = 3.60$, $d_f = 8$, $P = 0.007$) and 21-day ($t = 3.73$, $d_f = 8$, $P = 0.0057$) stimulation, becoming comparable to the contralateral hemisphere.

Finally, the cellular allocation of eN/CD73, A_1R, and $A_{2A}R$ was determined using immunochemistry (**Figure 5**). The immunoreactivity corresponding to eN/CD73 was evenly distributed throughout the striatal matrix in both the iTBSsh group (**Figure 5F**) and the iTBS group (**Figure 5G**), with striosomes completely devoid of immunostaining (**Figure 5F** and **G**). As for adenosine receptors, both A_1R (**Figure 5H** and **I**) and $A_{2A}R$ (**Figure 5J** and **K**) were predominantly expressed in striosomes, while the matrix remained poorly stained. Notably, there were striking differences in the immunostaining patterns between the sham and iTBS groups.

Effect of iTBS on AMP-kinase and intracellular adenosine metabolism in the 6-OHDA model of neurodegeneration

We determined the protein levels of intracellular ADA2 and the cytosolic pools of eN/CD73 (**Figure 6**). While the 6-OHDA lesion doubled the cytosolic pool of eN/CD73, iTBS stimulation decreased its abundance to less than 50% after 7-day stimulation ($t = 6.51$, $d_f = 8$, $P = 0.0002$; **Figure 6A**). Additionally, we observed a significant decrease in the

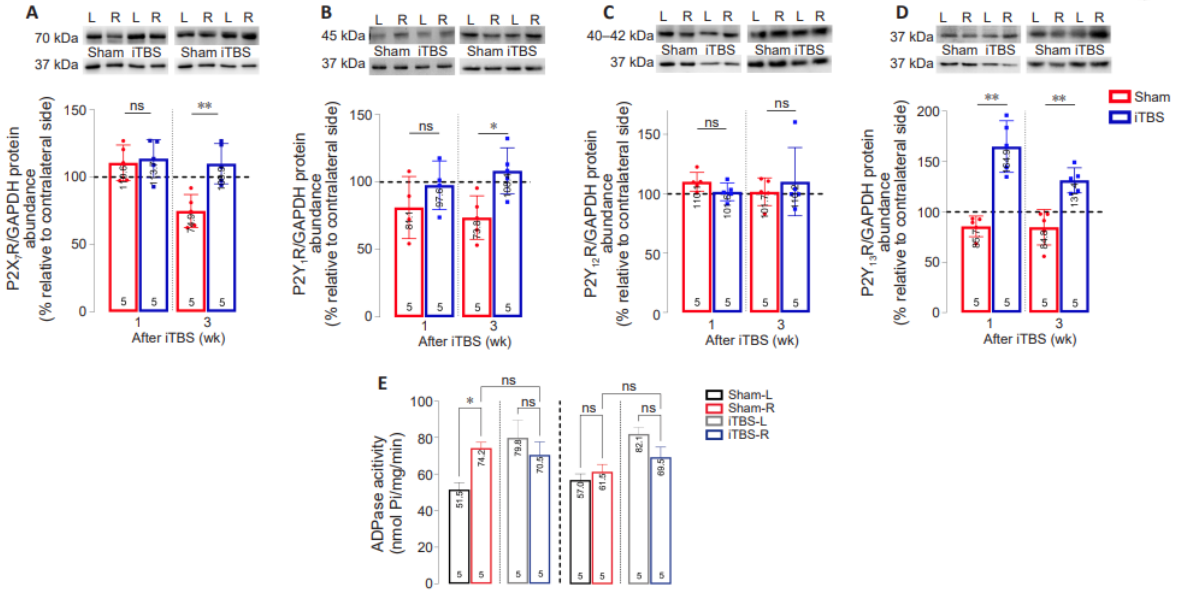

Figure 4 | **Effects of prolonged iTBS on expression of crude membrane (P2) receptors in the caudoputamen.**
(A) Representative support membranes showing density of 70-kDa ($P2X_7R$) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing $P2X_7R$ protein abundance. (B) Representative support membranes showing density of 70-kDa ($P2Y_1R$) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing $P2Y_1R$ protein abundance. (C) Representative support membranes showing density of 70-kDa ($P2Y_{12}R$) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing $P2Y_{12}R$ protein abundance stimulation. (D) Representative support membranes showing the density of 70-kDa ($P2Y_{13}R$) and 37-kDa (GAPDH) bands, as well as histograms of immunoblot analysis displaying $P2Y_{13}R$ protein abundance in crude synaptosomal fractions of the CPu of sham-stimulated and iTBS-stimulated animals, 1 and 3 weeks after stimulation. (E) ADP in crude synaptosomal fraction from CPu from Sham- and iTBS-stimulated animals 1 and 3 weeks after stimulation. Bars show mean activity (nmol Pi/mg/min) ± SEM from $n = 5$ determinations performed in duplicate. The number at the bottom of the graphs represents the number of individual animals included in analysis. $^*P < 0.05$, $^{**}P < 0.01$ (unpaired Student's t-test). All experiments were repeated at least twice. CPu: Caudoputamen; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; iTBS: intermittent theta burst stimulation; ns: not significant.

abundance of ADA2 in the iTBS group after 7-day stimulation ($t = 2.49$, $d_f = 8$, $P = 0.047$; **Figure 6B**). Regarding the cytosolic pools, our findings indicate that iTBS increased the ratio between phosphorylated and total adenosine-monophosphate kinase (AMPK) after 21-day stimulation (t = 2.68, $d_f = 8$, $P = 0.0276$; **Figure 6C**), suggestive of AMPK activation.

We also measured the activity of cytosolic 5'-nucleotidase II (cN-II) in crude cytosolic fractions from the CPu. cN-II is a soluble enzyme that catalyzes the intracellular conversion of nucleotide monophosphates, such as INP, GMP, and AMP, into their corresponding nucleosides. The cN-II activity was unaffected by the 6-OHDA lesion, as the activity was comparable between the rCPu and lCPu in the iTBS sham group after both 7-day (rCPu: 11.79 ± 0.68 nmol Pi/mg/min; lCPu: 11.82 ± 0.73 nmol Pi/mg/min; $t = 0.0297$, $d_f = 27$, $P = 0.978$) and 21-day (rCPu: 12.70 ± 1.29 nmol Pi/mg/min; lCPu: 13.20 ± 0.91 nmol Pi/mg/min; $t = 0.31$, $d_f = 28$, $P = 0.7536$) stimulation. However, in the iTBS group, cN-II activity was significantly lower in the rCPu (4.69 ± 0.51 nmol Pi/mg/min) compared with the lCPu (16.02 ± 1.19 nmol Pi/mg/min; $t = 9.31$, $d_f = 28$, $P < 0.0001$; **Figure 6D**) after 7-day stimulation. After 21-day stimulation, the activity was similar in both hemispheres [rCPu: 16.59 ± 1.47 nmol Pi/mg/min; lCPu: 15.56 ± 0.73 nmol Pi/mg/min; $t = 0.67$, $d_f = 28$, $P = 0.5083$; **Figure 6D**). The specificity of the reaction was confirmed by the addition of the specific inhibitor of eN/CD73, MRS4592 (**Figure 6E**; Mihajlovic et al., 2023).

Effect of prolonged iTBS treatment on D_1R and D_2R in caudoputamen in the 6-OHDA model of neurodegeneration

We examined the alterations in the expression of D_1R and D_2R in the CPu after 21-day iTBS (**Figure 7**). Notably, our findings indicated that there were no significant changes in D_1R expression between the Sham and iTBS groups ($t = 0.88$, $d_f = 7$, $P = 0.4043$), although there was a trend suggesting a potential increase. Conversely, D_2R expression was downregulated after prolonged iTBS stimulation ($t = 2.45$, $d_f = 8$, $P = 0.0428$).

Discussion

We demonstrated that a 21-day iTBS stimulation protocol: 1) Significantly improved motor performance in the rotarod test and decreased histopathological signs of 6-OHDA-induced neurodegeneration and neuroinflammation; 2) Reversed 6-OHDA-induced changes in adenosinergic gene expression by decreasing the expression of *Nt5e*, *Adora2a*, *Slc29a1*, and *Slc29a2* and upregulating the expression of *Adora1* in the rCPu; 3) In concordance with gene expression patterns, protein expression of eN/CD73, A_1R, and $A_{2A}R$ in the rCPu was restored to levels comparable to those observed in the contralateral control hemisphere; 4) Counteracted the lesion-induced increase in activity of NTPDase1 and eN/CD73 in the rCPu; and 5) Induced activation of AMPK, which regulates many of the central mechanisms underlying the pathology of PD (Curry et al., 2018); 6) Dopamine receptor expression was altered by prolonged iTBS stimulation.

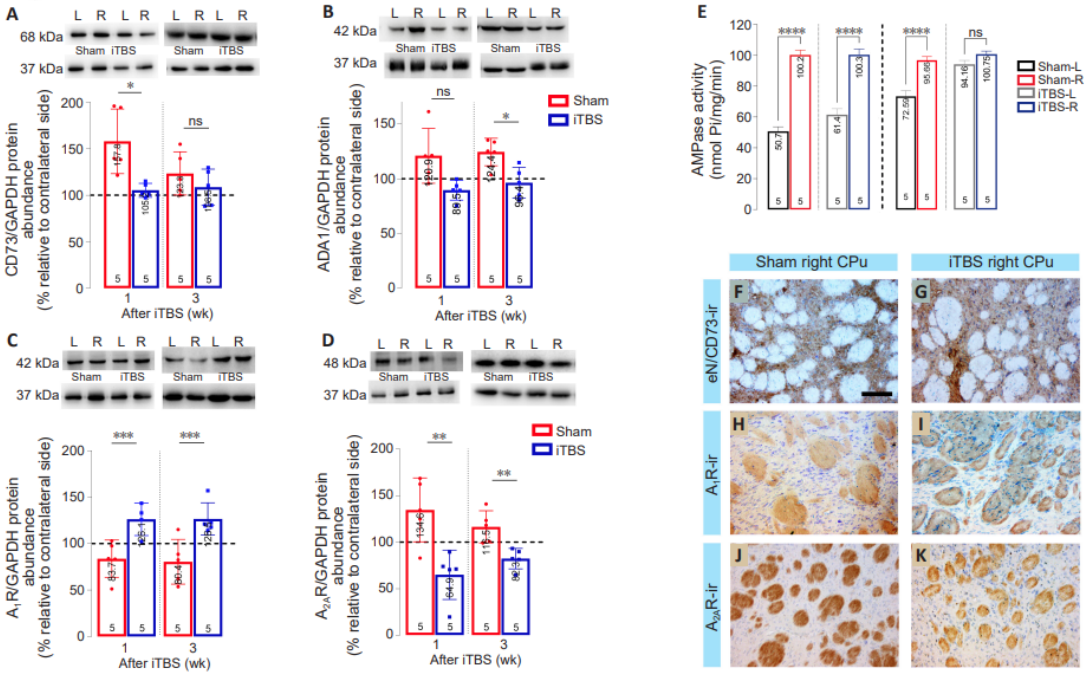

Figure 5 | Effect of prolonged iTBS on adenosine-mediated signaling in crude synaptosomal fraction of caudoputamen of 6-OHDA lesioned rats.

(A) Representative support membranes showing density of 68-kDa (eN/CD73) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing eN/CD73 protein abundance in crude synaptosomal fractions of the CPu of sham-stimulated and iTBS-stimulated animals at 1 and 3 weeks after stimulation. (B) Representative support membranes showing density of 42-kDa (ADA1) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing ADA1 protein abundance in crude synaptosomal fractions of the CPu of sham-stimulated and iTBS-stimulated animals at 1 and 3 weeks after stimulation. (C) Representative support membranes showing density of 42-kDa (A_1R) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing A1R protein abundance in crude synaptosomal fractions of the CPu of sham-stimulated and iTBS-stimulated animals at 1 and 3 weeks after stimulation. (D) Representative support membranes showing density of 48-kDa ($A_{2A}R$) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing $A_{2A}R$ protein abundance in crude synaptosomal fractions of the CPu of sham-stimulated and iTBS-stimulated animals at 1 and 3 weeks after stimulation. All data are expressed as mean ± SD. Dots in the graphs represent individual values. The number at the bottom of the graphs represents the number of individual animals included in analysis. All data are expressed as percentage (%) of contralateral (left) CPu (dotted line at 100%). (E) The AMP phosphohydrolase/eN activity in the crude synaptosomal fraction of the CPu was measured in sham-stimulated and iTBS-stimulated animals at 1 and 3 weeks after stimulation. The bars represent the mean activity (expressed as nmol Pi/mg/min) ± standard error of the mean (SEM) from $n = 5$ determinations performed in duplicate. The number at the bottom of the graphs represents the number of individual animals included in analysis. Significance shown inside graphs: $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$ (unpaired Student's *t*-test). (F, G) Micrographs illustrate the immunoreactivity of eN/CD73 in the CPu of sham animals in both the left and right hemispheres, as well as after 3 weeks of iTBS. (H, I) Micrographs depict immunoreactive cells for A1R in the left and right CPu of sham animals, as well as after 3 weeks of iTBS. Scale bar: 500 µm. All experiments were repeated at least twice. 6-OHDA: 6-Hydroxydopamine; A1R: adenosine A1 receptor; ADA1: adenosine deaminase 1; CPu: caudoputamen; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; iTBS: intermittent theta burst stimulation; ns: not significant.

The 6-OHDA model of neurodegeneration is one of the most widely used paradigms for studying PD (Hernandez-Baltazar et al., 2017). Our study demonstrated that unilateral administration of 6-OHDA results in the destruction of dopaminergic neurons and triggers a progressive loss of projections to the ipsilateral caudate putamen, while sparing the VTA. This recapitulates the primary histopathological features observed in human PD (Carman et al., 1991; Prasad and Hung, 2020). Gliosis, although present, exhibited a pattern that was more indicative of compact glial scarring filling the space formerly occupied by neurons (Sofroniew, 2009; Sofroniew and Vinters, 2010), rather than glia contributing to neuroinflammation. Interestingly, although gliosis was evident, it was much less pronounced after iTBS, suggesting the well-documented effects of iTBS/rTMS on glial cells and neuroinflammation (Cullen and Young, 2016; Cirillo et al., 2017; Dragic et al., 2020; Stekic et al., 2022). Levels of major proinflammatory cytokines remained unaltered in both the sham and iTBS groups, providing further evidence that major neurotoxic events leading to neurodegeneration and neuroinflammation subsided much earlier than the 21-day sham/stimulation time period (Hernandez-Baltazar et al., 2013; Parra et al., 2020). At the end of the 3-week iTBS protocol, motor performance was reassessed, revealing a remarkable improvement in motor symptoms (Zeljkovic Jovanovic et al., 2023).

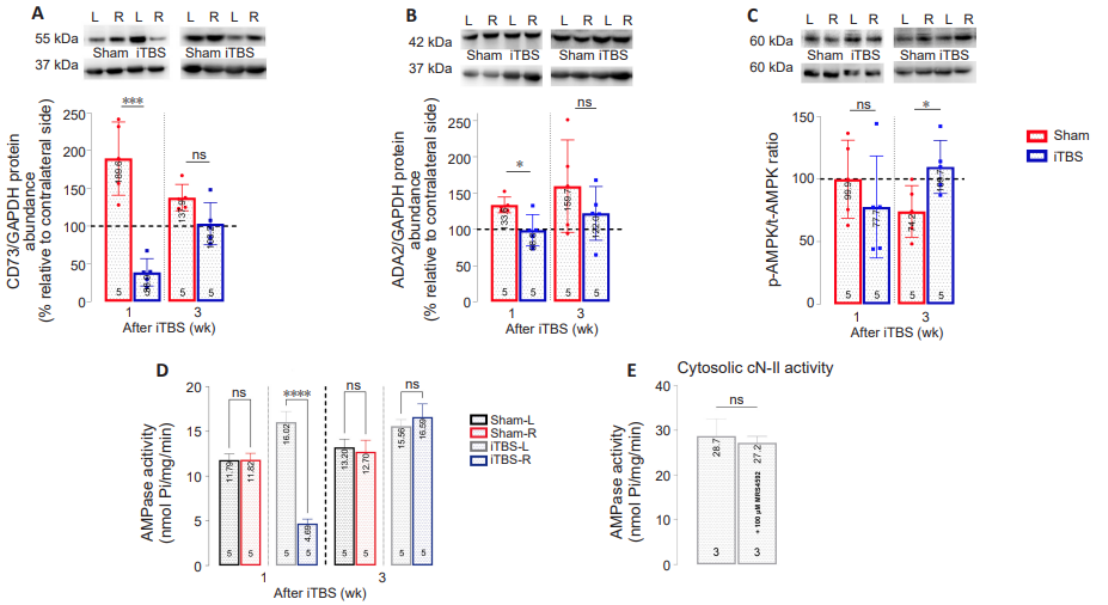

Figure 6 | Effects of iTBS on cytosolic pools of adenosine-generating and adenosine-eliminating enzymes and AMP kinase in crude synaptosomal fraction of the caudoputamen of 6-OHDA-lesioned rats.

(A) Representative support membranes showing density of 55-kDa (eN/CD73) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing eN/CD73 protein abundance in cytosolic fraction of the CPu of sham-stimulated and iTBS-stimulated animals at 1 and 3 weeks after stimulation. (B) Representative support membranes showing density of 42-kDa (ADA2) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing ADA1 protein abundance in cytosolic fraction of the CPu of sham-stimulated and iTBS-stimulated animals 1 and 3 weeks after stimulation. (C) Representative support membranes showing density of 60-kDa (phospho-AMPK) and 60-kDa (total-AMPK) bands and histograms of immunoblot analysis showing ratio of p-AMPK and t-AMPK protein abundance in cytosolic fraction fractions of the CPu of sham-stimulated and iTBS-stimulated animals at 1 and 3 weeks after stimulation. All data are represented as mean ± SD. Dots in the graphs represent individual values. The number at the bottom of the graphs represents the number of individual animals included in analysis. All data are expressed as the percentage (%) of contralateral (left) CPu (dotted line at 100%). (D) The AMP phosphohydrolase/cN-II activity was assayed in the cytosolic fraction of the CPu from both sham-stimulated and iTBS-stimulated animals, at both 1 and 3 weeks after the stimulation. The bars depicted represent the mean activity (expressed as nmol Pi/mg/min) ± SEM, based on five determinations performed in duplicate. (E) The AMP phosphohydrolase activity was measured in both the absence and presence of the specific eN/CD73 inhibitor MRS 4592. The bars represent the mean activity (expressed as nmol Pi/mg/min) ± SEM, calculated from five determinations performed in duplicate. The numbers at the bottom of the graphs indicate the total number of individual animals included in the analysis. Significance shown inside graphs: $*P < 0.05$, $***P < 0.001$, $****P < 0.0001$ (unpaired Student's *t*-test). All experiments are repeated at least twice. 6-OHDA: 6-Hydroxydopamine; CPu: caudoputamen; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; iTBS: intermittent theta burst stimulation; ns: not significant.

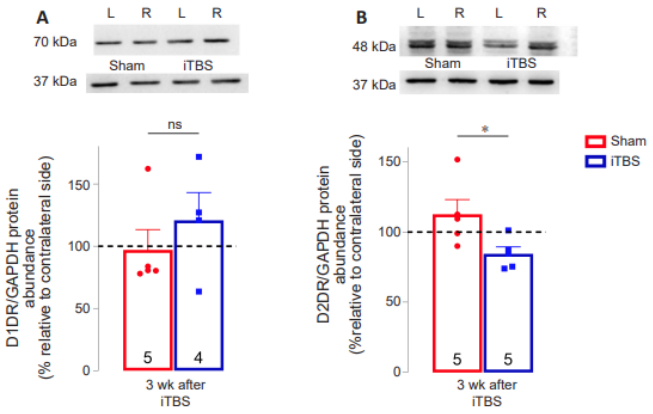

Figure 7 | Effect of prolonged iTBS on expression of D_1R and D_2R in crude synaptosomal fraction of the CPu of 6-OHDA- lesioned rats.

(A) Representative support membranes showing density of 55-kDa (D1DR) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing D1DR protein abundance in crude synaptosomal fractions of the CPu of sham-stimulated and iTBS-stimulated animals at 3 weeks after stimulation. (B) Representative support membranes showing density of 48-kDa (D2DR) and 37-kDa (GAPDH) bands and histograms of immunoblot analysis showing D2DR protein abundance in crude synaptosomal fractions of the CPu of sham-stimulated and iTBS-stimulated animals at 3 weeks after stimulation. All experiments were repeated at least twice. CPu: Caudoputamen; 6-OHDA: 6-hydroxydopamine; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; iTBS: intermittent theta burst stimulation; ns: not significant.

Purinergic signaling plays a pivotal role in synaptic plasticity and activity (Cunha, 2016; Agostinho et al., 2020), as well as in neuroinflammation (Dragić et al., 2021; Di Virgilio et al., 2023). Under pathological conditions, extracellular ATP can be released passively due to cellular injury/death, but also actively through pannexin-1, connexins, secretory vesicles, and the P2X7R (Adinolfi et al., 2018; Di Virgilio et al., 2023). Once released, it acts on P2 receptors to mediate various effects, with P2X7R-mediated neuroinflammatory actions being particularly prominent (Monif et al., 2009; Giuliani et al., 2020). Notably, cells exposed to 6-OHDA exhibit an increase in the release of eATP, accompanied by a depletion of intracellular ATP, which potentiates P2X7R-mediated signaling and contributes to the development of parkinsonian pathology (Carmo et al., 2019). Given that iTBS reversed the expression of P2X7R to levels comparable to the contralateral side and that there was no neuroinflammation and reactive gliosis was less pronounced at that time, it is conceivable that this change, in the absence/presence of low concentrations of eATP favored by rTMS (Feng et al., 2008), may potentiate P2X7R-mediated phagocytosis (Leeson et al., 2018; Campagno and Mitchell, 2021).

Once released, eATP is rapidly degraded to ADP by ectoenzymes NTPDase2/NTPDase1 (Dragic et al., 2022a). However, in a pathologically induced inflammatory environment, ADP-mediated signaling is significantly altered and may promote glial activation, proliferation, and the release of proinflammatory cytokines (Quintas et al., 2011, 2018). Consistently, an increase in ADPase activity after 7-day stimulation in the sham group suggests microglial activity, given that NTPDase 1 is predominantly expressed by microglia (Braun et al., 2000), which aligns with previously published data (Oses et al., 2011). Conversely, the absence of changes between the rCPu and lCPu after real stimulation indicates anti-inflammatory effects of iTBS (Stekic et al., 2022). Comparable ADPase activity levels between the rCPu and lCPu observed in both groups after 21-day stimulation may suggest a microglial resting state. After 7- and 21-day stimulation in the sham group, the downregulation of $P2Y_1R$ was reversed to nearly control levels of the contralateral hemisphere following 3-week iTBS. Given that $P2Y_1R$ is primarily expressed by astrocytes (Simões et al., 2018; Rodrigues et al., 2022), modulating Ca^{2+} signaling, proliferation, glutamate secretion, and inflammatory responses, as well as controlling neuronal excitability (Rodrigues et al., 2005), this reversal following iTBS may be attributed to the cumulative beneficial effects of the applied treatment. Additionally, the lack of changes in microglial $P2Y_{12}R$ protein expression in both groups further corroborates that microglia is not in a reactive state (Illes et al., 2020). Remarkably, the most significant upregulation after iTBS was observed for $P2Y_{13}R$. Although $P2Y_{13}R$ is expressed by microglia (Illes et al., 2020; Kyrargyri et al., 2020), other parameters suggestive of a microglial state, such as $P2Y_{12}R$ and Iba1 staining, led us to believe that the observed increase is more likely related to neuronal expression, which is also reported (Pérez-Sen et al., 2015). This upregulation may be linked to the resolution of neuroinflammation and recovery processes mediated by pro-survival Erk1/2 and Akt kinase pathways, as well as the Nrf2/HO-1 tandem (Pérez-Sen et al., 2015).

In contrast to limited data regarding P2-mediated effects in 6-OHDA-induced neurodegeneration, adenosine-mediated signaling, particularly $A_{2A}R$-mediated effects, has been extensively studied in PD pathology (Morelli et al., 2009). Amplification of $A_{2A}R$-mediated signaling attenuates inhibitory D_2R-mediated signaling, generating ''NO-GO'' signals that contribute to tremor and uncoordinated movements (Nazario et al., 2017). This may explain the beneficial effects of the $A_{2A}R$ antagonist istradefylline, which enhances motor function without exacerbating levodopa-induced dyskinesias (Cummins and Cates, 2022). According to this model, the attenuation of A1R-mediated signaling in PD counteracts D_1R-mediated dopamine actions in direct pathway neurons, resulting in increased spontaneous motor activity. Our findings revealed that 6-OHDA-induced neurodegeneration caused inverse changes in the expression of adenosine receptors, specifically by reducing A_1R expression and elevating $A_{2A}R$ expression. However, after 7- and 21-day stimulation, iTBS reversed these effects, restoring the abundance of A_1R and $A_{2A}R$ to control levels. This shift in AR expression likely restored the balance between D_1R-A_1R and D_2R-$A_{2A}R$ signaling, leading to the recovery of motor control.

Furthermore, eN/CD73-mediated formation of extracellular adenosine is critical for striatal $A_{2A}R$ functions and it has been shown that eN/CD73-$A_{2A}R$ are tightly spatially coupled (Augusto et al., 2013). Their spatial proximity enables functional coupling, in which eN/CD73 provides adenosine for selective $A_{2A}R$ activation (Moreira-de-Sá et al., 2021). The functional coupling of eN/CD73 and $A_{2A}R$ is evidenced by the parallel regulation of the *Nt5e* and *Adora2a* genes in various neuropathological conditions (Nedeljkovic, 2019). These data support the view that eN/CD73 is the master regulator of $A_{2A}R$ activity and suggest that eN/CD73 is a novel therapeutic target for fine-tuning $A_{2A}R$ activity in the control of neurodegeneration and neuroinflammation in PD (Carmo et al., 2019).

Adenosine deaminase 1 (ADA1) is another purinome component that is a potential target at PD. ADA1 is a rate-limiting enzyme that catalyzes the deamination of extracellular adenosine to inosine (Dragic et al., 2022b). A recent study showed that pharmacological inhibition of ADA1 protects against MPTP-induced cell death of dopaminergic neurons in PD, whereas ADA1 inhibition in combination with $A_{2A}R$ antagonism has an additive antiparkinsonian effect (Huang et al., 2019). These findings indicate that it is not the mere reduction of adenosine that accounts for the beneficial effects in PD, but rather a deficiency of adenosine specifically produced by eN/CD73 in proximity to $A_{2A}R$. Prior studies have demonstrated that, like eN/CD73-A2AR, ADA1 is functionally linked to A1R (Ciruela et al., 1996; Franco et al., 1998; Ruiz et al., 2000), thus regulating extracellular adenosine availability, ligand-induced signaling, and the internalization of A_1R (Ciruela et al., 1996). This suggests that ADA1 may also play a role in modulating the interaction between A_1R- and D_1R-mediated signaling in the direct striatal pathway. Furthermore, Ada1 and Nt5e share the same TCF/LEF site in their promoter regions, which are regulated by the same signals but in opposing manners (Spychala et al., 1999). Collectively, these results underscore the specific connections between eN/CD73-$A_{2A}R$

and ADA1-A_1R and support the rationale for targeting both eN/CD73 and ADA1 (Carmo et al., 2019; Huang et al., 2019) as a potential multi-faceted therapeutic approach in PD.

Our study demonstrated that 21-day iTBS stimulation reduced ADA1 expression, thereby contributing to control of adenosine availability for enhanced A_1R activation. As for the expression of dopamine receptors, D_1R was not altered by 6-OHDA pathology (Fornaretto et al., 1993), while iTBS did not alter its expression, although a tendency to increase it can be detected, while iTBS reduced the expression of D_2R. The mRNA and protein expression of D_2R has been shown to be increased in 6-OHDA pathology, probably as a compensatory mechanism, while the expression of D_1R, although not increasing, has a higher binding affinity in pathology, again suggesting a compensatory mechanism (Gerfen et al., 1990; Fornaretto et al., 1993; Narang and Wamsley, 1995). It appears that the expression of dopamine receptors follows the pattern of expression of adenosine receptors to which they are coupled after prolonged iTBS, suggesting a reversal of the pathological process, but this remains to be explored in depth.

Multiple electromagnetic pulses administered at a specific frequency and timing pattern are thought to affect cortical excitability and induce neuronal plasticity beyond the period of stimulation (Huang et al., 2005). Other study provided a 3D FEM model showing the geometry and gradient of magnetic and electric field density in the iTBS protocol used in the present study (Zeljkovic Jovanovic et al., 2023). The model predicted an electromagnetic density throughout the brain, including CPu and SNpc, above the level required to modulate excitability. Our study contributes to the understanding of the mechanism of action of iTBS by demonstrating that this treatment enhances the ratio of phosphorylated AMPK (p-AMPK) to total AMPK (t-AMPK). This ratio is integral to adaptive responses triggered by diminished energy availability. There is extensive evidence to support the neuroprotective effects of AMPK activation in bioenergetic disorders, including mitochondrial dysfunction, elevated reactive oxygen species, proteostatic impairment, accumulation of neurotoxic protein aggregates, and neuroinflammation (Curry et al., 2018).

This study has several limitations that should be noted. First, the technical limitations associated with the size of the coil prevent focal stimulation, instead stimulating the entire brain. Although we have previously demonstrated that our protocol reaches the CPu and the SNpc, we cannot discount the possibility that the observed effects are also attributable to the stimulation of other cortical and subcortical structures and their connectivity. Second, the 6-OHDA model, although widely used, recapitulates only one major feature of human pathology and its effects—namely, dopamine deficiency. This model lacks the neuroinflammatory component and progressivity, meaning that it often produces synaptic-level changes, or synaptopathy. Therefore, our results can only be applied to these specific aspects. Nonetheless, our data complements the existing literature on the role of purinergic signaling in 6-OHDA pathology, adding the temporal component and also revealing changes in previously unexplored P2 and P1 receptors. Finally, we have also demonstrated how purinergic signaling is modulated after iTBS application and the potential beneficial effects of such application on 6-OHDA-induced synaptopathy.

In conclusion, our study shows that prolonged iTBS stimulation leads to marked motor recovery in animals subjected to a unilateral 6-OHDA lesion. Specifically, iTBS stimulation effectively decreased expression and eN/CD73 and ADA1 activity and induced reciprocal changes in A1R and A_{2A}R expression in the rCPu, which may indicate a possible restoration of adenosinergic interference with dopaminergic signaling in striatal circuits involved in motor behavior. The study also showed that iTBS promoted AMPK activation, which may have a number of effects relevant to PD, including changes in cellular metabolism, promotion of autophagy, enhanced mitochondrial quality control, increased antioxidant capacity, and reduced inflammation (Curry et al., 2018). Given the importance of purinergic signaling in the flow of information in striatal circuits, modulation of the specific components may provide a framework for defining novel therapeutic approaches that can slow or alter disease progression (Oliveira-Giacomelli et al., 2018).

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Conflicts of interest: *The authors have no conflicts of interest to declare.*

Data availability statement: *All relevant data are within the paper and its Additional files.*

Additional files:

Additional Figure 1: *AMPase enzyme assay optimization and conditions.*

Additional Figure 2: *Effect of prolonged iTBS on expression of glial markers in crude cytosolic fraction of the caudoputamen of 6-OHDA- lesioned rats.*

References

Abbracchio MP, Burnstock G, Verkhratsky A, Zimmermann H (2009) Purinergic signalling in the nervous system: an overview. Trends Neurosci 32:19-29.

Adinolfi E, Giuliani AL, De Marchi E, Pegoraro A, Orioli E, Di Virgilio F (2018) The P2X7 receptor: A main player in inflammation. Biochem Pharmacol 151:234-244.

Agostinho P, Madeira D, Dias L, Simões AP, Cunha RA, Canas PM (2020) Purinergic signaling orchestrating neuron-glia communication. Pharmacol Res 162:105253.

Augusto E, Matos M, Sévigny J, El-Tayeb A, Bynoe MS, Müller CE, Cunha RA, Chen JF (2013) Ecto-5'-nucleotidase (CD73)-mediated formation of adenosine is critical for the striatal adenosine A2A receptor functions. J Neurosci 33:11390-11399.

Braun N, Sévigny J, Robson SC, Enjyoji K, Guckelberger O, Hammer K, Di Virgilio F, Zimmermann H (2000) Assignment of ecto-nucleoside triphosphate diphosphohydrolase-1/cd39 expression to microglia and vasculature of the brain. Eur J Neurosci 12:4357-4366.

Campagno KE, Mitchell CH (2021) The P2X(7) receptor in microglial cells modulates the endolysosomal axis, autophagy, and phagocytosis. Front Cell Neurosci 15:645244.
Carman LS, Gage FH, Shults CW (1991) Partial lesion of the substantia nigra: relation between extent of lesion and rotational behavior. Brain Res 553:275-283.
Carmo M, Gonçalves FQ, Canas PM, Oses JP, Fernandes FD, Duarte FV, Palmeira CM, Tomé AR, Agostinho P, Andrade GM, Cunha RA (2019) Enhanced ATP release and CD73-mediated adenosine formation sustain adenosine A(2A) receptor over-activation in a rat model of Parkinson's disease. Br J Pharmacol 176:3666-3680.
Chen JF, Cunha RA (2020) The belated US FDA approval of the adenosine A2A receptor antagonist istradefylline for treatment of Parkinson's disease. Purinergic Signal 16:167-174.
Chou Y, Hickey PT, Sundman M, Song AW, Chen N (2015) Effects of repetitive transcranial magnetic stimulation on motor symptoms in Parkinson disease: a systematic review and meta-analysis. JAMA Neurol 72:432-440.
Cirillo G, Di Pino G, Capone F, Ranieri F, Florio L, Todisco V, Tedeschi G, Funke K, Di Lazzaro V (2017) Neurobiological after-effects of non-invasive brain stimulation. Brain Stimul 10:1-18.
Ciruela F, Saura C, Canela EI, Mallol J, Lluis C, Franco R (1996) Adenosine deaminase affects ligand-induced signalling by interacting with cell surface adenosine receptors. FEBS Lett 380:219-223.
Cullen CL, Young KM (2016) How does transcranial magnetic stimulation influence glial cells in the central nervous system? Front Neural Circuits 10:26.
Cummins L, Cates ME (2022) Istradefylline: A novel agent in the treatment of "off" episodes associated with levodopa/carbidopa use in Parkinson disease. Ment Health Clinician 12:32-36.
Cunha RA (2005) Neuroprotection by adenosine in the brain: From A(1) receptor activation to A (2A) receptor blockade. Purinergic Signal 1:111-34.
Cunha RA (2016) How does adenosine control neuronal dysfunction and neurodegeneration? J Neurochem 139:1019-1055.
Curry DW, Stutz B, Andrews ZB, Elsworth JD (2018) Targeting AMPK signaling as a neuroprotective strategy in Parkinson's disease. J Parkinsons Dis 8:161-181.
Di Virgilio F, Vultaggio-Poma V, Falzoni S, Giuliani AL (2023) Extracellular ATP: A powerful inflammatory mediator in the central nervous system. Neuropharmacol 224:109333.
Dragic M, Zeljkovic M, Stevanovic I, Ilic T, Ilic N, Nedeljkovic N, Ninkovic M (2020) Theta burst stimulation ameliorates symptoms of experimental autoimmune encephalomyelitis and attenuates reactive gliosis. Brain Res Bull 162:208-217.
Dragić M, Mitrović N, Adžić M, Nedeljković N, Grković I (2021) Microglial- and astrocyte-specific expression of purinergic signaling components and inflammatory mediators in the rat hippocampus during trimethyltin-induced neurodegeneration. ASN Neuro 13:17590914211044882.
Dragic M, Mihajlovic K, Adzic M, Jakovljevic M, Kontic MZ, Mitrovic N, Laketa D, Lavrnja I, Kipp M, Grkovic I, Nedeljkovic N (2022a) Expression of ectonucleoside triphosphate diphosphohydrolase 2 (NTPDase2) is negatively regulated under neuroinflammatory conditions in vivo and in vitro. ASN Neuro 14:17590914221102068.
Dragic M, Stekic A, Zeljkovic M, Zaric Kontic M, Mihajlovic K, Adzic M, Grkovic I, Nedeljkovic N (2022b) Altered topographic distribution and enhanced neuronal expression of adenosine-metabolizing enzymes in rat hippocampus and cortex from early to late adulthood. Neurochem Res 47:1637-1650.
Edwards MJ, Talelli P, Rothwell JC (2008) Clinical applications of transcranial magnetic stimulation in patients with movement disorders. Lancet Neurol 7:827-840.
Feng H, Yan L, Cui L (2008) Effects of repetitive transcranial magnetic stimulation on adenosine triphosphate content and microtubule associated protein-2 expression after cerebral ischemia-reperfusion injury in rat brain. Chin Med J 121:1307-1312.
Fornaretto MG, Caccia C, Caron MG, Fariello RG (1993) Dopamine receptors status after unilateral nigral 6-OHDA lesion. Autoradiographic and in situ hybridization study in the rat brain. Mol Chem Neuropathol 19:147-162.
Franco R, Valenzuela A, Lluis C, Blanco J (1998) Enzymatic and extraenzymatic role of ecto-adenosine deaminase in lymphocytes. Immunol Rev 161:27-42.
Fredholm BB, IJzerman AP, Jacobson KA, Linden J, Müller CE (2011) International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors—an update. Pharmacol Rev 63:1-34.
Gardoni F, Ghiglieri V, Di Luca M, Calabresi P (2010) Assemblies of glutamate receptor subunits with post-synaptic density proteins and their alterations in Parkinson's disease. Prog Brain Res 183:169-182.
Gerfen CR, Engber TM, Mahan LC, Susel Z, Chase TN, Monsma FJ Jr, Sibley DR (1990) D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. Science 250:1429-1432.
Giuliani AL, Sarti AC, Di Virgilio F (2020) Ectonucleotidases in acute and chronic inflammation. Front Pharmacol 11:619458.
Gonçalves FQ, Matheus FC, Silva HB, Real JI, Rial D, Rodrigues RJ, Oses JP, Silva AC, Gonçalves N, Prediger RD, Tomé ÂR, Cunha RA (2023) Increased ATP release and higher impact of adenosine A2A receptors on corticostriatal plasticity in a rat model of presymptomatic Parkinson's disease. Mol Neurobiol 60:1659-1674.
González-Redondo R, García-García D, Clavero P, Gasca-Salas C, García-Eulate R, Zubieta JL, Arbizu J, Obeso JA, Rodríguez-Oroz MC (2014) Grey matter hypometabolism and atrophy in Parkinson's disease with cognitive impairment: a two-step process. Brain 137:2356-2367.
Gray EG, Whittaker VP (1962) The isolation of nerve endings from brain: an electron-microscopic study of cell fragments derived by homogenization and centrifugation. J Anat 96:79-88.
Grković I, Mitrović N, Dragic M, Adzic M, Drakulić D, Nedeljkovic N (2019) Spatial distribution and expression of ectonucleotidases in rat hippocampus after removal of ovaries and estradiol replacement. Mol Neurobiol 56:1933-1945.
Hernandez-Baltazar D, Mendoza-Garrido ME, Martinez-Fong D (2013) Activation of GSK-3β and caspase-3 occurs in Nigral dopamine neurons during the development of apoptosis activated by a striatal injection of 6-hydroxydopamine. PLoS One 8:e70951.
Hernandez-Baltazar D, Zavala-Flores LM, Villanueva-Olivo A (2017) The 6-hydroxydopamine model and parkinsonian pathophysiology: Novel findings in an older model. Neurologia 32:533-539.
Huang W, Xu Y, Zhang Y, Zhang P, Zhang Q, Zhang Z, Xu F (2019) Metabolomics-driven identification of adenosine deaminase as therapeutic target in a mouse model of Parkinson's disease. J Neurochem 150:282-295.
Huang YZ, Edwards MJ, Rounis E, Bhatia KP, Rothwell JC (2005) Theta burst stimulation of the human motor cortex. Neuron 45:201-206.
Illes P, Rubini P, Ulrich H, Zhao Y, Tang Y (2020) Regulation of microglial functions by purinergic mechanisms in the healthy and diseased CNS. Cells 9:1108.
Jahanshahi M, Jenkins IH, Brown RG, Marsden CD, Passingham RE, Brooks DJ (1995) Self-initiated versus externally triggered movements. I. An investigation using measurement of regional cerebral blood flow with PET and movement-related potentials in normal and Parkinson's disease subjects. Brain 118:913-933.
Kanno M, Matsumoto M, Togashi H, Yoshioka M, Mano Y (2004) Effects of acute repetitive transcranial magnetic stimulation on dopamine release in the rat dorsolateral striatum. J Neurol Sci 217:73-81.
Kin K, Yasuhara T, Kameda M, Date I (2019) Animal models for Parkinson's disease research: trends in the 2000s. Int J Mol Sci 20:5402.
Kumar S, Mishra A, Krishnamurthy S (2017) Purinergic antagonism prevents mitochondrial dysfunction and behavioral deficits associated with dopaminergic toxicity induced by 6-OHDA in rats. Neurochem Res 42:3414-3430.
Kyrargyri V, Madry C, Rifat A, Arancibia-Carcamo IL, Jones SP, Chan VTT, Xu Y, Robaye B, Attwell D (2020) P2Y13 receptors regulate microglial morphology, surveillance, and resting levels of interleukin 1β release. Glia 68:328-344.
Leeson HC, Kasherman MA, Chan-Ling T, Lovelace MD, Brownlie JC, Toppinen KM, Gu BJ, Weible MW 2nd (2018) P2X7 receptors regulate phagocytosis and proliferation in adult hippocampal and SVZ neural progenitor cells: implications for inflammation in neurogenesis. Stem Cells 36:1764-1777.

Meng F, Guo Z, Hu Y, Mai W, Zhang Z, Zhang B, Ge Q, Lou H, Guo F, Chen J, Duan S, Gao Z (2019) CD73-derived adenosine controls inflammation and neurodegeneration by modulating dopamine signalling. Brain 142:700-718.

Mihajlovic K, Bukvic MA, Dragic M, Scortichini M, Jacobson KA, Nedeljkovic N (2023) Anti-inflammatory potency of novel ecto-5'-nucleotidase/CD73 inhibitors in astrocyte culture model of neuroinflammation. Eur J Pharmacol 956:175943.

Monif M, Reid CA, Powell KL, Smart ML, Williams DA (2009) The P2X7 receptor drives microglial activation and proliferation: a trophic role for P2X7R pore. J Neurosci 29:3781-3791.

Moreira-de-Sá A, Lourenço VS, Canas PM, Cunha RA (2021) Adenosine A2A receptors as biomarkers of brain diseases. Front Neurosci 15:702581.

Morelli M, Carta AR, Jenner P (2009) Adenosine A2A receptors and Parkinson's disease. Handb Exp Pharmacol:589-615.

Narang N, Wamsley JK (1995) Time dependent changes in DA uptake sites, D1 and D2 receptor binding and mRNA after 6-OHDA lesions of the medial forebrain bundle in the rat brain. J Chem Neuroanat 9:41-53.

Navarro G, Borroto-Escuela DO, Fuxe K, Franco R (2016) Purinergic signaling in Parkinson's disease. Relevance for treatment. Neuropharmacology 104:161-168.

Nazario LR, da Silva RS, Bonan CD (2017) Targeting adenosine signaling in Parkinson's disease: from pharmacological to non-pharmacological approaches. Front Neurosci 11:658.

Nedeljkovic N (2019) Complex regulation of ecto-5'-nucleotidase/CD73 and A(2A)R-mediated adenosine signaling at neurovascular unit: a link between acute and chronic neuroinflammation. Pharmacol Res 144:99-115.

Oliveira-Giacomelli Á, Naaldijk Y, Sardá-Arroyo L, Gonçalves MCB, Corrêa-Velloso J, Pillat MM, de Souza HDN, Ulrich H (2018) Purinergic receptors in neurological diseases with motor symptoms: targets for therapy. Front Pharmacol 9:325.

Oses JP, Batassini C, Pochmann D, Böhmer AE, Vuaden FC, Silvestrin RB, Oliveira A, Bonan CD, Bogo MR, Souza DO, Portela LV, Sarkis JJ, Mello e Souza T (2011) The hydrolysis of striatal adenine- and guanine-based purines in a 6-hydroxydopamine rat model of Parkinson's disease. Neurochem Res 36:215-222.

Parra I, Martínez I, Ramírez-García G, Tizabi Y, Mendieta L (2020) Differential effects of LPS and 6-OHDA on microglia's morphology in rats: implications for inflammatory model of Parkinson's disease. Neurotox Res 37:1-11.

Paxinos G, Watson C (1998) The rat brain in stereotaxic coordinates, 4th ed. New York: Academic Press.

Percie du Sert N, et al. (2020) The ARRIVE guidelines 2.0: Updated guidelines for reporting animal research. PLoS Biol 18:e3000410.

Pérez-Sen R, Queipo MJ, Morente V, Ortega F, Delicado EG, Miras-Portugal MT (2015) Neuroprotection mediated by P2Y13 nucleotide receptors in neurons. Comput Struct Biotechnol J 13:160-168.

Petrovic J, Radovanovic L, Saponjic J (2021) Prodromal local sleep disorders in a rat model of Parkinson's disease cholinopathy, hemiparkinsonism and hemiparkinsonism with cholinopathy. Behav Brain Res 397:112957.

Prasad EM, Hung SY (2020) Behavioral tests in neurotoxin-induced animal models of Parkinson's disease. Antioxidants (Basel) 9:1007.

Quintas C, Fraga S, Gonçalves J, Queiroz G (2011) Opposite modulation of astroglial proliferation by adenosine 5'-O-(2-thio)-diphosphate and 2-methylthioadenosine-5'-diphosphate: mechanisms involved. Neuroscience 182:32-42.

Quintas C, Vale N, Gonçalves J, Queiroz G (2018) Microglia P2Y13 receptors prevent astrocyte proliferation mediated by P2Y1 receptors. Front Pharmacol 9:418.

Rodrigues RJ, Almeida T, Richardson PJ, Oliveira CR, Cunha RA (2005) Dual presynaptic control by ATP of glutamate release via facilitatory P2X1, P2X2/3, and P2X3 and inhibitory P2Y1, P2Y2, and/or P2Y4 receptors in the rat hippocampus. J Neurosci 25:6286-6295.

Rodrigues RJ, Figueira AS, Marques JM (2022) P2Y1 receptor as a catalyst of brain neurodegeneration. NeuroSci 3:604-615.

Ruiz MA, Escriche M, Lluis C, Franco R, Martín M, Andrés A, Ros M (2000) Adenosine A(1) receptor in cultured neurons from rat cerebral cortex: colocalization with adenosine deaminase. J Neurochem 75:656-664.

Schiffmann SN, Fisone G, Moresco R, Cunha RA, Ferré S (2007) Adenosine A2A receptors and basal ganglia physiology. Prog Neurobiol 83:277-292.

Schirinzi T, Madeo G, Martella G, Maltese M, Picconi B, Calabresi P, Pisani A (2016) Early synaptic dysfunction in Parkinson's disease: Insights from animal models. Mov Disord 31:802-813.

Simões AP, Silva CG, Marques JM, Pochmann D, Porciúncula LO, Ferreira S, Oses JP, Beleza RO, Real JI, Köfalvi A, Bahr BA, Lerma J, Cunha RA, Rodrigues RJ (2018) Glutamate-induced and NMDA receptor-mediated neurodegeneration entails P2Y1 receptor activation. Cell Death Dis 9:297.

Sofroniew MV (2009) Molecular dissection of reactive astrogliosis and glial scar formation. Trends Neurosci 32:638-647.

Sofroniew MV, Vinters HV (2010) Astrocytes: biology and pathology. Acta Neuropathol 119:7-35.

Spychala J, Zimmermann AG, Mitchell BS (1999) Tissue-specific regulation of the ecto-5'-nucleotidase promoter. Role of the camp response element site in mediating repression by the upstream regulatory region. J Biol Chem 274:22705-22712.

Stanojevic JB, Zeljkovic M, Dragic M, Stojanovic IR, Ilic TV, Stevanovic ID, Ninkovic MB (2023) Intermittent theta burst stimulation attenuates oxidative stress and reactive astrogliosis in the streptozotocin-induced model of Alzheimer's disease-like pathology. Front Aging Neurosci 15:1161678.

Stanojevic J, Dragic M, Stevanovic I, Ilic T, Stojanovic I, Zeljkovic M, Ninkovic M (2022) Intermittent theta burst stimulation ameliorates cognitive impairment and hippocampal gliosis in the Streptozotocin-induced model of Alzheimer's disease. Behav Brain Res 433:113984.

Stekic A, Zeljkovic M, Zaric Kontic M, Mihajlovic K, Adzic M, Stevanovic I, Ninkovic M, Grkovic I, Ilic TV, Nedeljkovic N, Dragic M (2022) Intermittent theta burst stimulation ameliorates cognitive deficit and attenuates neuroinflammation via PI3K/Akt/mTOR signaling pathway in Alzheimer's-like disease model. Front Aging Neurosci 14:889983.

Suppa A, Huang YZ, Funke K, Ridding MC, Cheeran B, Di Lazzaro V, Ziemann U, Rothwell JC (2016) Ten years of theta burst stimulation in humans: established knowledge, unknowns and prospects. Brain Stimul 9:323-335.

Tóth A, Antal Z, Bereczki D, Sperlágh B (2019) Purinergic signalling in Parkinson's disease: a multi-target system to combat neurodegeneration. Neurochem Res 44:2413-2422.

Truong L, Allbutt H, Kassiou M, Henderson JM (2006) Developing a preclinical model of Parkinson's disease: a study of behaviour in rats with graded 6-OHDA lesions. Behav Brain Res 169:1-9.

Vieira JCF, Bassani TB, Santiago RM, de O Guaita G, Zanoveli JM, da Cunha C, Vital MABF (2019) Anxiety-like behavior induced by 6-OHDA animal model of Parkinson's disease may be related to a dysregulation of neurotransmitter systems in brain areas related to anxiety. Behav Brain Res 371:111981.

Xie YJ, Gao Q, He CQ, Bian R (2020) Effect of repetitive transcranial magnetic stimulation on gait and freezing of gait in Parkinson disease: a systematic review and meta-analysis. Arch Phys Med Rehabil 101:130-140.

Yang P, Wang Z, Zhang Z, Liu D, Manolios EN, Chen C, Yan X, Zuo W, Chen N (2018) The extended application of The Rat Brain in Stereotaxic Coordinates in rats of various body weight. J Neurosci Methods 307:60-69.

Zeljkovic Jovanovic M, Stanojevic J, Stevanovic I, Stekic A, Bolland SJ, Jasnic N, Ninkovic M, Zaric Kontic M, Ilic TV, Rodger J, Nedeljkovic N, Dragic M (2023) Intermittent theta burst stimulation improves motor and behavioral dysfunction through modulation of NMDA receptor subunit composition in experimental model of Parkinson's disease. Cells 12:1525.

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4. DISKUSIJA

U kontekstu sve većeg opterećenja koji PB kao multisistemska neurodegenerativna bolest predstavlja na globalnom nivou, potreba za dubljim razumevanjem mehanizama koji leže u osnovi ovog oboljenja, kao i potreba za razvojem novih terapijskih strategija, nikada nije bila izraženija. Sa prevalencom koja se povećava paralelno sa starenjem populacije, PB ostaje jedan od vodećih uzroka invaliditeta, smanjenog kvaliteta života i smrtnosti, ne samo zbog svoje složene patofiziologije i raznovrsnosti simptoma, već i zbog ograničenih mogućnosti trenutno dostupnih terapija koje primarno utiču samo na olakšanje simptoma i znakova bolesti. Doprinos ove doktorske disertacije navedenim problemima ogleda se u njenom sveobuhvatnom pristupu, koji ne istražuje samo potencijal iTBS-a kao inovativne terapijske opcije već teži dubljem razumevanju mehanizama koji doprinose njenom terapijskom efektu. Iz ispitivanja kako direktnih, tako i indirektnih efekata iTBS-a na neuroprotekciju DA neurona, glutamatne receptore, komponente purinskog signalnog sistema, sistem antioksidativne zaštite, ali i sagledavanja kompleksnih interakcija unutar ovih sistema, proistekli rezultati, koji će biti komentarisani u nastavku, doprinose širenju znanja o mogućim putevima koji mogu biti potencijalne mete za delovanje različitih terapeutika na tok bolesti.

4.1. Promene nastale dejstvom 6-OHDA

Kako je osnovni cilj ove doktorske disertacije bio da rasvetli efekte produžene stimulacije iTBS protokola u 6-OHDA modelu PB, bilo je nephodno potvrditi već dobro opisane promene koje nastaju nakon unilateralne aplikacije toksina, lokalno, u desni SNpc pri dozi od 12 μg. Već je pomenuto, da se primenom ovog toksina u zavisnosti od doze i izabranog regiona može postići različit stepen degeneracije nigrostrijatalnog sistema. U dosadašnjoj literaturi je pokazano da navedeni metodološki pristup proizvodi gotovo potpunu leziju DA neurona unutar SNpc. Smrt neurona se dešava u okviru prvih 12 sati od lokalne mikroinjekcije, a progresivna lezija anterogradnih projekcija, paralelno sa deplecijom dopamina, uspostavlja se unutar 2-3 dana (Fabio Blandini et al., 2008; Deumens et al., 2002). Iako se lezija razvija kroz svega par dana, stepen degeneracije se kontroliše pomoću količine toksina koja se injecira. Doza koja je izabrana za ovu doktorsku disertaciju, dovodi do brzog umiranja DA neurona u SNpc, te se već trećeg dana od injeciranja uočava pad ekspresije TH na oko 50% u odnosu na kontrolnu hemisferu, koji se nastavlja kroz tri nedelje kada je stepen degeneracije oko 90%. Ovaj progresivni gubitak DA neurona u SNpc i strijatumu u ispitivanim vremenskim tačkama nije bio praćen gubitkom neurona u ventralnom tegmentalnom području, što dodatno potvrđuje preciznost injeciranja u strukturu od interesa, ali i olakšava pripisivanje promena degeneracije DA sistema uočenim simptomima, s obzirom da projekcije neurona ventralnog tegmentalnog pripadaju mezokoritkolimbičkom sistemu koji je uključen u sistem nagrade i potkrepljenja (Breton et al., 2019). Takođe, ukupno posmatrano, unilateralna lezija sa odabranom dozom kod životinja dovodi do relativno brzog razvoja motornih simptoma, unutar sedam dana od injeciranja, što bi moglo da odgovara ranoj, početnoj fazi PB u humanoj populaciji (Bernheimer et al., 1973; Riederer & Wuketich, 1976; Schapira, 2009). Iako 6-OHDA izazvana degeneracija ne oponaša sve karakteristike PB uključujući formiranje Luijevih tela, ona svakako, kao što smo i u našem slučaju pokazali, proizvodi robustnu i relativno stabilnu leziju bez spontanog oporavka i verno oslikava najvažniji neuropatološki aspekt kliničke PB. Zbog toga model 6-OHDA ima visok stepen validnosti, što ga čini idealnim alatom za proučavanje različitih neuroprotektivnih strategija u ranim fazama PB (Slézia et al., 2023; Tieu, 2011). Bilateralna aplikacija ovog toksina generalno se izbegava zbog visoke stope smrtnosti povezane sa izraženom afagijom, adipsijom i smanjenom telesnom masom kod operisanih životinja koje usled toga zahtevaju intenzivnu negu (Ungerstedt, 1971). Iako je u literaturi gotovo jednako zastupljen i metod injeciranja 6-OHDA u strijatum, on ima i nekoliko

negativnih aspekata. Zbog veličine same strukture, injeciranje toksina u strijatum zahteva odabir dve, a najčešće tri stereotaksičke lokacije, što rezultuje sa dve do tri ubodne lezije po hemisferi. Sama ubodna lezija podrazumeva i ulazak ćelija bele krvne loze i lokalnu inflamaciju koja će biti prisutna i u kontrolnoj hemisferi. Takođe, zbog načina ulaska 6-OHDA u ćelije, njegovo apliciranje u strijatum neće rezultovati specifičnom degeneracijom samo DA neurona, nego praktično svih neurona i ćelija glije koje eksprimiraju DAT, ali u manjoj meri i NET i SERT transportere koji imaju afinitet za 6-OHDA (Chotibut et al., 2012; Conti Mazza et al., 2023). S obzirom na složenu ulogu strijatuma u brojnim motornim i nemotornim funkcijama, ovakva lezija može dovesti do razvoja različitog stepena motornih i nemotornih simptoma koji bi značajno mogli da variraju usled same operacije i preciznosti, a ne kao posledica patologije, te bi zahtevalo mnogo veći broj životinja i teže pripisivanje uočenih promena određenim fenomenima. Sa druge strane injekcija 6-OHDA u SNpc dovodi do degeneracije DA neurona i njihovih projekcija, te je mnogo pouzdanije tvrditi da su uočeni motorni i nemotorni simptomi posledica lezije DA neurona.

U pogledu motornih i nemotornih simptoma, u našoj studiji dolazi do značajnog smanjenja motorne koordinacije i ravnoteže već u prvoj nedelji od izazivanja lezije lokalnom mikroinjekcijom toksina u SNpc, smanjenog eksplorativnog ponašanja i smanjene upotrebe prednjih ekstremiteta pri vertikalnom uspinjanju i istraživanju, kao i do ponašanja nalik anksioznom i depresivnom, ali i do smanjenja performansi u testovima učenja i pamćenja. Interesantno je primetiti da, iako su promene jasno vidljive na odgovarajućim testovima ponašanja, životinje se ponašaju naizgled potpuno kao i fiziološke kontorle i nije moguće posmatranjem uočiti motorne deficite ili druge promene u ponašanju, što ide u prilog hipotezi da je ovakav pristup idealan za modelovanje rane faze bolesti. Primećene promene u ponašanju, koje su opisane primenom odgovarajućih testova ponašanja, su u skladu sa brojnim istraživanjima na ovom modelu (Miyanishi et al., 2019; E. M. Prasad & Hung, 2020). Još jedna važna karateristika pored progresivne degeneracije DA neurona je i prateća neuroinflamacija. Izražen inflamacijski odgovor uz oslobađanje proinflamacijskih citokina, primećen je u toksičnim modelima PB koji su zasnovani na sistemskoj primeni MPTP-ja ili rotenona, ili pak primenom LPS-a, bilo lokalno bilo sistemski. Sa druge strane, lokalna aplikacija 6-OHDA nije praćena visokim stepenom aktivacije glijskih ćelija, koje ima tendenciju povlačenja nakon prve nedelje od lokalne mikroinjekcije toksina, kao ni drugim parametrima inflamacije, što predstavlja jedan od nedostataka ovog modela (Hald & Lotharius, 2005; Oliynyk et al., 2023; Parra et al., 2020). Nivoi glavnih proinflamacijskih citokina, IL-1β i TNF-α, nisu bili značajno povećani ni u drugoj ni u četvrtoj nedelja od lezije SNpc što ukazuje na značajno raniji završetak inflamacijskih procesa. Takođe, imunohistohemijska analiza glavnih markera astro- i mikroglioze, GFAP i IBA-1 pozitivnih ćelija, u našoj studiji ukazala je na postojanje glioze u formi glijskog ožiljka koji ispunjava prostor koji su nekada zauzimali neuroni, ali ne u formi koja bi dodatno doprinosila daljoj neuroinflamaciji (Sofroniew, 2009). Jedno od potencijalnih obrazloženja za odsustvo inflamacijskog odgovora je i način delovanja 6-OHDA, koji ćelije ubija dominantno apoptotskim putem, odustvo progresije, ali i činjenica da samim načinom aplikacije dolazi do narušavanja krvno-moždane barijere i ulaska ćelija bele krvne loze, koje zajedno sa mikroglijom mogu da relativno brzo i efikasno uklone umiruće ćelije i ćelijski debris i doprinesu ponovnom uspostavljanju lokalne homeostaze (Carvey et al., 2005; da Fonseca et al., 2014), što nije slučaj sa LPS ili MPTP koji su i sami u stanju da inflamacijski aktiviraju ćelije glije i periferne imunske ćelije (Skrzypczak-Wiercioch & Sałat, 2022; Stojkovska et al., 2015). Opravdanost za korišćenje ovog modela u ispitivanju terapijskog efekta rTMS pored svega navedenog, uključujući i nedostatke, leži i u visokom stepenu reproducibilnosti kontrolisane lezije nigrostrijatalnog sistema, kao i u jednostavnosti same procedure i ne tako velikim finansijskim troškovima. S obzirom na to je 7 dana nakon mikroinjekcije 6-OHDA, nivo TH

proteina smanjen za ~70% u strijatumu u poređenju sa kontralateralnom stranom što je u skladu sa pojavom prvih motornih simptoma (Jeon et al., 1995), ova vremenska tačka je odabrana za početak iTBS stimulacije. Takođe, i kod pacijenata prvi motorni simptomi se javljaju nakon degeneracije 50–60% DA neurona i 70–80% smanjenja dopamina u strijatumu (Bernheimer et al., 1973; Riederer & Wuketich, 1976; Schapira, 2009). U literaturi je moguće pronaći i radove koji započinju tretman rTMS istovremeno ili čak pre (kao vid profilakse) izazivanja patologije, te je izbor da se životinje stimulišu sa pojavom prvih simptoma adekvatniji pristup sa translacionog aspekta. Dakle, sveukupno, naš izabrani eksperimentalni model PB, verno je reprodukovao glavnu neuropatološku osnovu, kao i motorne i nemotorne simptome humane bolesti uz stabilnu leziju DA neurona u SNpc bez izražene inflamacije.

4.2. Efekti rTMS-a na dopaminske neurone i ponašajne simptome izazvane 6-OHDA

U cilju ispitivanja selektivnosti uticaja iTBS protokola u našim istraživanjima kao i razumevanja uticaja izabranog iTBS protokola na specifične regione mozga, razvili smo 3D model zasnovan na metodi konačnih elemenata (eng. *finite element method*; FEM). S obzirom da prilikom stimulacije životinja nije moguće tačno utvrditi motorni prag nadražaja niti je moguće fokalno primeniti stimulaciju, intenzitet magnetnog polja i električnog polja koje ono generiše kao i njihova distribucija po različitim regiona mozga od velike su važnosti za bolje razumevanje efekata iTBS u patologiji. Naš FEM model otkriva da nakon stimulacije kalemom oblika broja osam, unutradšnjeg dijametra 25 mm dolazi do indukcije električnog polja u skoro svim moždanim regionima, uključujući strukture od interesa u okviru bazalnih ganglija. Procenjena vrednost električnog polja na kortikalnoj površini mozga iznosila je 124,05 V/m, smanjujući se do minimuma od približno 21 V/m na donjoj, ventralnoj površini mozga. Jačina električnog polja u nivou bazalnih ganglija kretala se između 33-37 V/m što sugeriše da su navedeni intenziteti bili dovoljni za iniciranje akcionih potencijala u neuronima unutar tih struktura (Moretti & Rodger, 2022). Takođe, važno je pomenuti da pored direktnog efekta na neurone, električno polje generisano ovim protokolom utiče i na glijske ćelije, što može imati značajnu ulogu u sveukupnom odgovoru koji se opaža nakon primene iTBS-a, ali i drugih rTMS protokola (Cullen & Young, 2016). Stoga, FEM model pruža čvrstu teorijsku osnovu za pretpostavku da bi iTBS protokol, primenjen u našoj studiji, mogao direktno uticati na strijatum i SNpc, kao i da ostvaruje potencijalne indirektne efekte usled stimulacije drugih moždanih regiona. Prethodne studije su pokazale da maksimalne vrednosti električnog polja indukovane rTMS-om variraju u zavisnosti od anatomije mozga određene vrste i njegove veličine. Komparativne FEM studije otkrile su da su vrednosti indukovanog električnog polja kod glodara, majmuna i ljudi prilično slične kada se koristi kalem od 25 mm (Alekseichuk et al., 2019). Ovi nalazi podržavaju mogućnost primene ovog protokola u slučaju različitih vrsta.

U skladu sa opštim i specifičnim ciljevima ove doktorske disertacije praćeno je ponašanje životinja na testu rotirajućeg cilindra nakon svake nedelje u trajanju od tri nedelje stimulacije iTBS protokolom. Tronedeljni iTBS protokol uticao je pozitivno na motorne deficite kod životinja sa 6-OHDA lezijom, što je pokazano značajnim poboljšanjem performansi na testu rotirajućeg cilindra i u cilindar testu, standardnim testovima koji se koriste za procenu ravnože, koordinacije, motorne asimetrije i motorike prednjih udova. Ova poboljšanja motornih sposobnosti primećena su već nakon jedne nedelje iTBS stimulacije, za razliku od drugih rTMS protokola kod kojih je tek nakon dve ili više nedelja primene došlo do merljivog poboljšanja motornih simptoma (J. Y. Lee et al., 2013, 2020). Takođe, primenjeni iTBS protokol rezultirao je održivim poboljšanjem motornih sposobnosti koje je trajalo tokom celog perioda stimulacije. Druge studije pokazale su smanjenje akinezije prednjih ekstremiteta i poboljšanje hoda kod pacova i nakon akutne primene iTBS protokola (Cacace et al., 2017), što ukazuje da primena iTBS protokola ostvaruje povoljne efekte već nakon

primene jedne stimulacije. Brojne studije koje su primenjivale različite rTMS protokole u eksperimentalnim modelima različitih neurodegeneracija sugerišu da rTMS ispoljava svoje neuroprotektivne efekte i smanjuje smrt neurona blokirajući apoptozu, pretežno regulacijom genske i proteinske ekspresije BDNF, Bcl-2 i Bax proteina (Q. Dong et al., 2015; Fujiki et al., 2003; Luo et al., 2017; Uzair et al., 2022). Stoga, poboljšanje motornih sposobnosti može biti povezano sa značajnim smanjenjem gubitka DA neurona i boljim očuvanjem nigrostrijatalnih projekcija koje je primećeno i u našoj studiji nakon završetka stimulacije, imunoblot i imunohistohemijskom analizom TH u SNpc i strijatumu. Ono što zahteva dalja istraživanja i ne može se zaključiti iz naše studije je da li uočeni porast proteinske ekspresije TH potiče od smanjene smrti DA neruona ili od povećane ekspresije ovog proteina preživelih neurona. Takođe, pored direktnih neuroprotektivnih efekata, iTBS ublažava sekundarna oštećenja posredovana glijskim ćelijama, što takođe može biti razlog uočenog povećanja TH ekspresije u ispitivanim strukturama nakon tretmana (Dragic et al., 2020; J. Stanojevic et al., 2022).

Na nivou ponašanja pokazano je i da iTBS protokol utiče na poboljšanje nemotornih simptoma, uključujući ponašanje nalik anksioznom i ponašanje nalik depresivnom, kao i na deficite u procesima kratkoročne memorije izazvane neurotoksinom. Dakle, tretman iTBS-om doveo je do povećane lokomotorne aktivnosti u otvorenom polju, a poznato je da ona ne zavisi samo od motorne sposobnosti već i od motivacije za istraživanjem novih prostora i spremnosti za ulazak u centralne osvetljene delove arene, što može ukazivati na smanjenje ponašanja nalik anksioznom (Levy & Dubois, 2006; Seibenhener & Wooten, 2015). Osim toga, životinja koje su bile izložene stimulaciji češće su birale sladak napitak, što može ukazivati na promene u ponašanju kada je u pitanju sposobnost za osećaj zadovoljstva, odnosno došlo je do smanjenja anhedoničnog ponašanja, a anhedonija predstavlja jedan od osnovnih simptoma depresije, uzrokovan nedostatkom dopamina. Nekoliko studija pokazalo je da 6-OHDA izazvani gubitak DA aksonskih projekcija, kao i drugih kateholaminskih projekcija, dovodi do opšteg disbalansa neurotransmiterskog sistema i smanjenog nivoa neurotransmitera u bazalnim ganglijama, koji delimično leži u osnovi posmatranih poremećaja ponašanja (Vieira et al., 2019). U prilog tome, pokazali smo da iTBS protokol povećava nivoe dopamina u strijatumu u odnosu na nestimulisane životinje, što potvrđuju i prethodni podaci dobijeni sa drugim protokolima rTMS u životinjskim modelima neurodegenerativnih (J. Y. Lee et al., 2013; X. Yang et al., 2010) i neuropsihijatrijskih poremećaja (Lee et al., 2021; J. Yang et al., 2019). Povećanje nivoa dopamina nakon iTBS može se povezati sa očuvanjem DA strijatalnih projekcija, ali i sa smanjenom ekspresijom strijatalnog DAT transportera, čije je smanjenje ekspresije uočeno i kod lažno stimulisanih jedinki, te je ovo verovatno vid kompenzatornog mehanzima. Naime, dopaminski transporter je direktno uključen u regulaciju nivoa dopamina u strijatumu utičući na njegov ponovni unos iz sinaptičke pukotine. Inhibitori DAT-a, koji sprečavaju ponovni unos dopamina i time povećavaju njegovu dostupnost u sinaptičkoj pukotini, istraživani su kao terapijske opcije u PB (Nutt et al., 2004). Pored navedenih mehanizama, moguće je i da iTBS stimuliše oslobađanje postojećeg pula dopamina te tako doprinosi akutnim i hroničnim efektima koji se uočavaju. Naime, pokazano je da akutna stimulacija rTMS-om dovodi do povećanih koncentracija dopamina u međućelijskom prostoru mezolimbičkog i mezostrijatalnog sistema, što dodatno ukazuje na složenost mehanizma delovanja iTBS kao i višeznačnost dobijenih rezultata (Keck et al., 2002).

Dodatni dokaz složenosti i patologije i terapije dolazi od činjenice da se korisni efekti iTBS-a na pomenute poremećaje ponašanja koji se javljaju u ovom modelu delom mogu pripisati i drugim neurotransmiterskim sistema, konkretno serotoninu, čiji se nivo takođe povećavaju nakon tri nedelje stimulacije u strijatumu. Smanjenje strijatalnog serotonina i njegovih metabolita primećeno je u *post mortem* analizi moždanog tkiva pacijenata sa PB (Kish et al., 2008). Najveći broj neurona koji sintetišu serotonin smešten je u okviru rubnih

jedara (lat. *nuclei raphe*) koja se projektuju na različite regione nervnog sistema, uključujući bazalne ganglije, posebno strijatum, ali i ka prečeonoj kori i limbičkom sistemu i uključena su u regulaciju procesa raspoloženja, apetita, sna, kao i u kognitivne i motorne funkcije (Prinz et al., 2013). Serotonin može kroz direktnu interakciju sa dopaminskim neuronima da utiče na oslobađanje dopamina ili pak može kroz indirektne interakcije da utiče na smanjenje oslobađanja glutamata preko talamostrijatalnih i kortikostrijatalnih projekcija, što ima važnu ulogu u modulaciji učenja koje je posredovano nagradom, pored uloge u kontroli voljne motorike (Blomeley & Bracci, 2009; Cavaccini et al., 2018; Mathur et al., 2011). Stoga se može pretpostaviti da uočeno povećanje nivoa serotonina može uticati na poboljšane učenja i pamćenja u testu prepoznavanja novog objekta kao i drugih nemotornih simptoma.

Poremećaji ponašanja koji se manifestuju nakon lokalne mikroinjekcije 6-OHDA predstavljaju složene pojave čiji mehanizmi regulacije nisu u potpunosti razjašnjeni, što dalje ukazuje na to da samo povećanje nivoa dopamina i serotonina, iako korisno, samo po sebi ne može biti jedino odgovorno za poboljšanja uočena nakon primene iTBS-a, naročito imajući u vidu neselektivnost primenjenog tretmana. U kontekstu primećenih poboljšanja, posebno je značajno istaći da zbog nesrazmerne veličine kalema, kao što je ranije pomenuto, stimulacija obuhvata gotovo čitav mozak, što nas navodi na razmišljanje da stimulacija drugih moždanih regiona, kao i hemisferna kompenzacija, može predstavljati dodatne faktore koji doprinose opaženim poboljšanjima ponašanja (Blesa et al., 2011), a ujedno otežavaju izvođenje jasnog zaključka o direktnim i indirektnim efektima iTBS na eksperimentalnim modelima.

4.3. Efekti rTMS-a na komponente glutamatnog signalnog sistema

Jedan od glavnih uzročnika „sinaptopatije“ pored nedostatka dopamina je disregulacija homeostaze glutamata. Brzo i povećano oslobađanje glutamata iz presinaptičkih neurona koje se dešava u patološkim uslovima, prevazilazi kapacitet transportera koji ga preuzimaju usled čega dolazi do prekomerne i produžene ekscitatorne neurotransmisije, što dovodi do ekscitotoksičnosti i smrti neurona (Lau & Tymianski, 2010). Ove varijacije u koncentraciji glutamata unutar sinaptičke pukotine i u nishodnoj signalizaciji posredovanoj membranskim receptorima za koje se glutamat vezuje, kao i izmene ekspresije samih subjedinica receptora za glutamat, predstavljaju jedan od osnovnih uzročnika narušene funkcije bazalnih ganglija u PB. Naime, pored povećane koncentracije glutamata u serumu pacijenata sa PB, do danas je poznato i da su promene u sastavu NMDA receptorskih subjedinica usko povezane sa patofiziologijom i progresijom PB (Campanelli et al., 2022; Dunah et al., 2000). Kod životinja nakon 6-OHDA-izazvane lezije dolazi do značajnog povećanja vanćelijskog glutamata, što dovodi do pojačane signalizacije posredovane GluN1/GluN2B receptorima, što zajedno sa smanjenim nivoom dopamina može da dovede do motornih, kao i nemotornih deficita (Nash & Brotchie, 2002). Smanjena ekspresija GluN1 i GluN2A i povećana ekspresija GluN2B subjedinice sugerišu da i u našem slučaju 6-OHDA menja sastav subjedinica NMDA receptora i to u korist GluN2B subjedinice čija aktivacija podstiče ćelijsku smrt preko PSD-95-nNOS signalnog puta (Cao et al., 2005). Sa druge strane, pokazali smo da tretman iTBS-om dovodi do povećane ekspresije GluN1 i GluN2A i smanjene ekspresije GluN2B subjedinice. Ranije smo pomenuli da je upravo najveći broj selektivnih NMDA antagonista usmeren baš na GluN2B subjedinicu i da je pokazano da njena blokada može da ublaži simptome u modelima PB kod glodara i primata (Löschmann et al., 2004; Nash et al., 1999; Steece-Collier et al., 2000). Prilikom komentarisanja promena sastavana NMDA receptora, treba uzeti u obzir da je GluN2B subjedinica dominantno ekstrasinaptički lokalizovana, te povećana ekspresija ove subjedinice zajedno sa povećanim oslobađanjem glutamata i smanjenim kapacitetima njegovog preuzimanja mogu dovesti do „prelivanja glutamata“ izvan sinaptičke pukotine (*engl.* spill-over effect) i potenciranja GluN2B subjedinice (Hassel & Dingledine, 2012). Dakle, iTBS-om indukovana signalizacija posredovana GluN1/GluN2A subjedinicama, za koje je u

više studija pokazano da pozitivno utiču na preživljavanje ćelija i oporavak, može biti jedan od razloga za uočena poboljšanja kako motornih tako i nemotornih simptoma. Osim iTBS-a, i ostali protokoli rTMS-a demonstrirali su svoj uticaj na ekspresiju NMDAR subjedinica, pri čemu treba istaći jedan od mehanizama koji leži u osnovi ove stimulacije svakako jeste NMDAR posredovana signalizacija, što je u saglasnosti sa našim rezultatima (Brown et al., 2020; Labedi et al., 2014). Pored toga, iTBS indukuje povećanje ekspresije GLAST (EAAT1) i GLT-1 (EAAT2), transportera koji omogućavaju brzo preuzimanje glutamata iz sinaptičke pukotine, što može biti povoljan događaj u slučaju povećanog vanćelijskog nivoa glutamata, i takođe smanuje prelivanje glutamata i potencijaciju GluN2B subjedinice. GLT-1 koji se većinski eksprimira na astrocitima je dominantan transporter i odgovoran je za više od 90% ukupnog preuzimanja glutamata iz sinaptičke pukotine, dok GLAST ima daleko manji doprinos (Leek et al., 2024). Naime, iTBS izaziva istovremenu, neselektivnu aktivaciju velikog broja aksona, presinaptičkih i postsinaptičkih završetaka, zajedno s aktivacijom samih tela neurona i njihovih dendrita, što dovodi do brojnih, mahom nedovoljno poznatih promena koje su uglavnom usmerene na promene u sinaptičkoj plastičnosti i u količini oslobođenih neurotransmitera, uključujući glutamat (Pell et al., 2011). Povećanje ekspresije GLAST i GLT1 u ovom slučaju predstavlja kompenzacijski mehanizam koji može da smanji nivo glutamata u sinaptičkom prostoru. S obzirom da se oslobođeni glutamat prvobitno vezuje za AMPA receptor, što izaziva influks jona Na^+ i hipopolarizaciju postsinaptičkog neurona usled koje se uklanja blokada NMDA receptora u vidu Mg^{2+} jona i omogućava influks jona Ca^{2+} od čije količine i brzine priliva zavisi kaskada složenih biohemijskih procesa koji rezultuju povećanjem ili smanjenjem snage sinaptičke transmisije, ispitali smo i promene u ekspresiji GluA1 subjedinice AMPA receptora. Određene studije pokazale su pozitivne efekte alosteričkih modulatora AMPA receptora, poznatim i kao ampakini, koji usporavaju desenzitizaciju ili deaktivaciju AMPAR i predstavljaju obećavajući terapijski pristup neurodegenerativnim bolestima (Arai & Kessler, 2007; Johnson et al., 2009). Najzanimljiviji aspekt ovih pozitivnih alosteričkih modulatora povezan je sa njihovom sposobnošću da povećaju ekspresiju neurotrofnih faktora, posebno BDNF (Lauterborn et al., 2009). Primena tronedeljnog iTBS tretmana dovela je do povećane ekspresija GluA1 subjedinice AMPAR u desnom strijatumu iTBS životinja u odnosu na sham grupu. I druge studije pokazuju da nakon rTMS dolazi do povećane ekspresije GluA1 subjedinice AMPAR što ukazuje da rTMS može posredstvom AMPA/NMDA signalizacije da izazove koordinisanu funkcionalnu i strukturnu plastičnost ekscitatornih sinapsi (Vlachos et al., 2012). Pojednostavljeni šematski prikaz pretpostavljenog mehanizma nakon stimulacije iTBS-om na nivou glutamatnog signalnog sistema prikazan je na Slici 6.

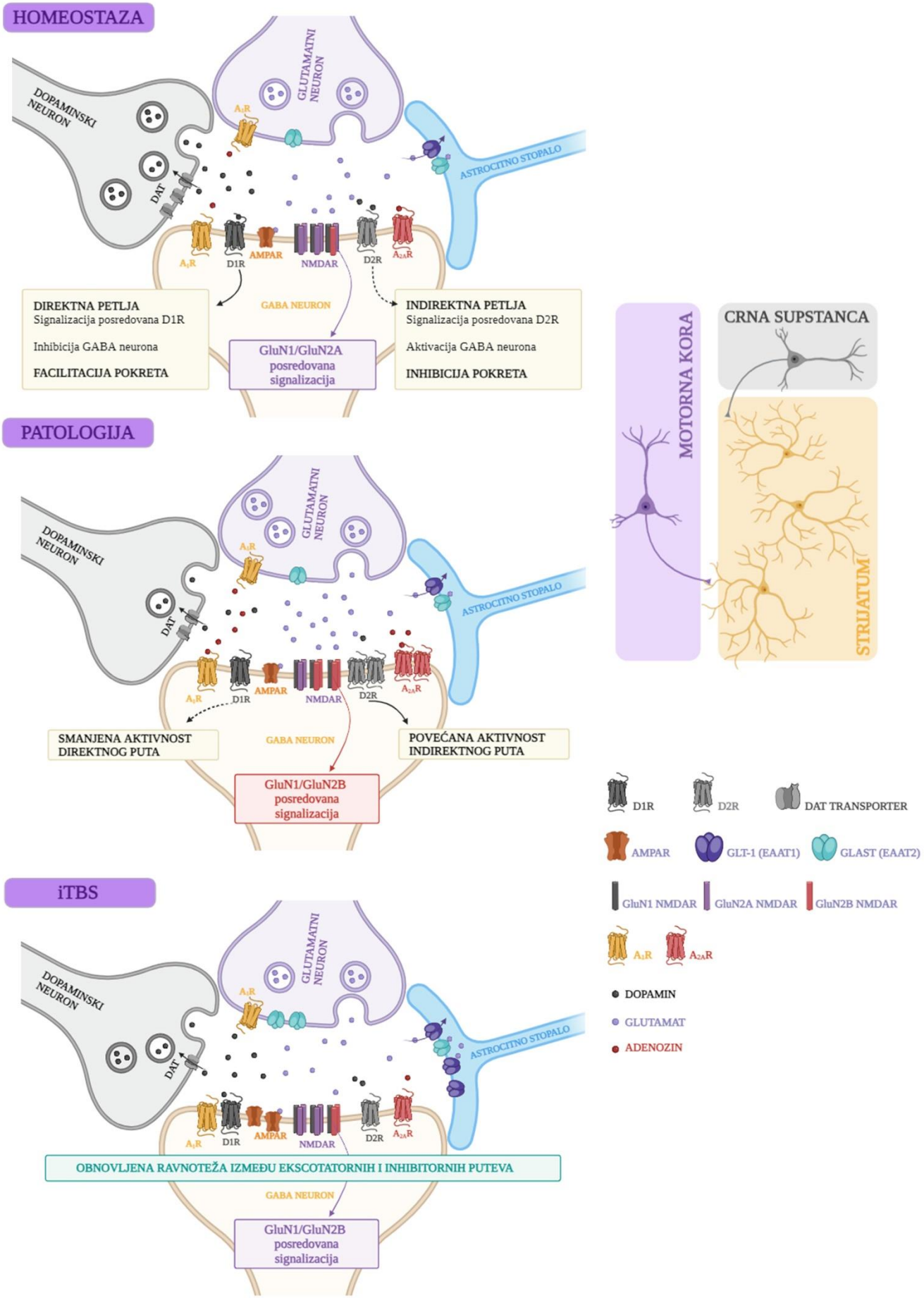
Sinaptičke promene, kao što je ranije pomenuto, se ispoljavaju u dva suprotna oblika, kao LTP ili LTD odnosno kao povećanje, odnosno smanjenje snage i funkcije sinapse u čijoj osnovi su brojni mehanizmi, ali ono što je zajedničko jeste aktivacija glutamatnih receptora (Bear & Malenka, 1994; Lüscher & Malenka, 2012). Većina dosadašnjih istraživanja je pokazala da visokofrekventni protokoli (≥ 5 Hz) rTMS-a dovode do LTP-sličnih efekata, dok niskofrekventni protokoli (≤ 5 Hz) izazivaju LTD-slične promene (Gersner et al., 2011). U skladu sa pomenutim, pokazano je da je izazvanje LTP-a putem HF-rTMS-a u strijatumu zavisno od NMDA receptora, preciznije, zahteva NMDA receptore koji sadrže GluN2A, a ne one koji sadrže GluN2B subjedinicu (Ping Li et al., 2009). Rezultati ove doktorske disertacije ukazuju da nakon tronedeljnog tretmana iTBS-om dolazi do povećane ekspresije kako presinaptičkih tako i postsinaptičkih markera, što može posredno ukazati na veći broj sinaptičkih kontakata a na taj način može biti i povezan sa uočenim promenama motornih i nemotornih simptoma (Cirillo et al., 2017).

Naime, sinaptofizin (SYN) kao prvi identifikovani integralni membranski protein nervnih završetaka koji je isključivo lokalizovan u sinaptičkim vezikulama, koristi se kao marker presinaptičke oblasti, i pokazano je da utiče na oslobađanje neurotransmitera (Thiel, 1993). Sa druge strane, PSD-95, marker postsinaptičke gustine, je neophodan za ukotvljavanje i pozicioniranje komponenti NMDA receptora kao i nishodnu signalizaciju u postsinaptičkim neuronima, zbog čega takođe ima značajnu ulogu u održavanju signalne transdukcije i u sinaptičkoj plastičnosti. Studije su pokazale da je ekspresija PSD-95 proteina regulisana aktivnošću BDNF signalnog puta (Hu et al., 2011). Ranije pomenuti BDNF pored svoje klasične uloge u podsticanju rasta i preživljavanja različitih populacija neurona, uključen je pored drugih neurotrofnih faktora u kontrolu neuroplastičnosti, kako stukturne tako i funkcionalne (Colucci-D'Amato et al., 2020). Brojne studije demonstrirale su da BDNF, kroz aktivaciju TrkB receptora, moduliše aktivnost NMDA receptora indukujući fosforilaciju NR1 subjedinice (Suen et al., 1997), kao i njegovu ulogu u procesu LTP-a (Leal et al., 2017).

Takođe, humane *post mortem* studije su pokazale smanjen nivo BDNF i njegovog TrkB receptora kod pacijenata sa PB (Mogi et al., 1999; Murer et al., 2001), kao i u modelima PB (Berghauzen-Maciejewska et al., 2015), a nekoliko studija ukazuje i na smanjenje BDNF-a u serumu pacijenata sa PB što se dovodi u vezu sa depresivnim ponašanjem i kognitivnim oštećenjima koja se javljaju u ovom oboljenju (Wang et al., 2017). Povećano oslobađanje BDNF-a, čiju smo povećanu ekspresiju nakon iTBS tretmana pokazali u rezultatima ove doktorske disertacije, može da dovode do pojačane signalizacija preko njegovog TrkB receptora što dalje aktivira nishodni mTOR signalni put koji predstavlja jedan od mehanizama regulacije sinaptogeneze, a promoviše i procese inicijacije translacije sinaptičkih proteina poput SYN i PSD-95 (Dwyer & Duman, 2013). Međutim, definitivna potvrda sinaptičke plastičnosti koja bi bila jedan od razloga poboljšanja poremećaja ponašanja nakon iTBS tretmana na našem modelu zahteva dalja istraživanja i definitivnu elektrofiziološku potvrdu.

Svakako ovaj fenomen demonstrirale su druge grupe u modelu PB nakon akutne primene iTBS (Cacace et al., 2017; Puyu Li et al., 2022). Pored toga postoje brojne studije na različitim modelima neurodegenerativnih bolesti koje ukazuju na pozitivan ishod u kontekstu neuroplastičnosti bilo funkcionalne ili strukturne nakon primene različitih modaliteta rTMS-a (Ferro et al., 2022; Hong et al., 2021; Jun Ma et al., 2013).

Iako navedeni rezultati ukazuju na pozitivne efekte iTBS stimulacije i pružaju uvid u moguće mehanizme delovanja, neophodno je osvrnuti se i na metodološko ograničenje koji se tiče analiziranih tkivnih komponenti koje upućuje na to da su razmatrane samo opšte promene u strijatalnoj regiji bez određivanja promena u specifičnim ćelijskim odeljcima (tj. ekstrasinaptičkog naspram sinaptičkog nivoa kao i membranski naspram citoplazmatskog), ukoliko se uzme u obzir da distribucija različitih receptorskih komponenti može imati pre-, post- ili ekstrasinpatičku lokalizaciju, što naglašava potrebu za daljim istraživanjima koja bi preciznije okarakterisala mehanizme koji leže u osnovi terapijskog potencijala iTBS-a u slučaju eksperimentalnog modela PB.



Slika 6. Efekti rTMS-a na komponente glutamatnog i purinskog signalnog sistema u modelu PB – pretpostavljeni mehanizam (opis u tekstu)

4.4. Efekti rTMS-a na komponente purinskog signalnog sistema

Purinska signalizacija ima važnu ulogu u mnogim fiziološkim i patološkim procesima, ali je od posebnog interesa za ovu disertaciju njena uloga u procesima sinaptičke plastičnosti (Agostinho et al., 2020; Cunha, 2016), i u procesima neuroinflamacije (Di Virgilio et al., 2023; Dragić et al., 2021). U patološkim uslovima, vanćelijski ATP (eATP) može biti pasivno oslobođen kao rezultat ćelijskog oštećenja ili smrti, ali i aktivno preko paneksina-1, koneksina, sekretornih vezikula i P2X7R (Adinolfi et al., 2018). Jednom oslobođen, eATP ostvaruje efekte posredstvom P2 receptora, od kojih su najistaknutiji efekti na procese neuroinflamacije posredovani P2X7R (Giuliani et al., 2020; Monif et al., 2009). Shodno tome, studije su pokazale da ćelije koje se izlože 6-OHDA pojačano otpuštaju eATP, uz istovremeno iscrpljivanje unutarćelijskog ATP (iATP). Ovako oslobođeni eATP pojačava P2X7R-posredovanu signalizaciju koja doprinosi razvoju patologije nalik PB (Carmo et al., 2019). Pored ove uloge, pokazano je da P2X7R, u prisustvu niskih koncentracija eATP ima ulogu i u procesima fagocitoze (Leeson et al., 2018). Dosadašnja istraživanja pokazala su da rTMS utiče na metabolizam ugljenih hidrata i lipida kako kod zdravih ispitanika tako i kod pacijenata sa moždanim udarom. Ekscitatorni protokoli kakav je i iTBS ubrzavaju potrošnju glukoze i lipida kao i ATP, tako da dugotrajna stimulacija dovodi do smanjenja unutarćelijskih nivoa ATP (Horimoto et al., 2022; Michael et al., 2003; Ren et al., 2017). Nakon tronedeljne stimulacije iTBS je vratio ekspresiju P2X7R na nivoe uporedive sa kontrolom hemisferom. Pored svoje dobro okarakterisane uloge u pokretanju inflamazoma i posledične inflamacije, P2X7R u odsustvu *e*ATP kao signala može da služi kao receptor za fagocitozu. S obzirom da stimulacije nije uspela da spreči smrt dopaminskih neurona u velikoj meri, a da je P2X7R, osim na ćelijama glije eksprimiran i na sinapsama, sasvim je moguće da je uočeno povećanje ekspresije u odnosu na sham zapravo na ćelijama mikroglije. Takodje, s obzirom da u tom trenutku nije bilo neuroinflamacije, kao i da je prisutna glioza značajno manje izražena nakon iTBS tretmana, moguće je da je ova promena, u odsustvu, odnosno niskim koncentracijama eATP-a koje favorizuje rTMS (Feng et al., 2008), možda pojačala fagocitozu posredovanu P2X7R receptorom (Campagno & Mitchell, 2021), što svakako zahteva dalja ispitivanja.

Oslobođeni ATP, bilo u homeostatskim bilo u patološkim uslovima u vanćelijskom prostoru podleže brzoj enzimskoj razgradnji do ADP-a posredstvom ektonukleotidaza NTPDaza2 i NTPDaza1/CD39 (Dragic, Mihajlovic, et al., 2022). U tom smislu, NTPDaza1 hidrolizuje ATP i ADP podjednako dobro, dok se NTPDaza2 odlikuje nešto većim afinitetom prema ATP, ali pokazuje i vrlo nizak afinitet prema ADP, zbog čega ovaj enzim predstavlja glavni izvor ADP u vanćelijskoj sredini (Zimmermann et al., 2012). U fiziološkim uslovima, dominantnu ulogu u održavanju homestaze ima NTPDaza2 koja omogućava toničnu aktivnost ADP-zavisnih P2 receptora (Dragic, Mihajlovic, et al., 2022). Sa druge strane, u patološkim uslovima dolazi do smanjenja aktivnosti NTPDaza2 zajedno sa povećanjem aktivnosti NTPDaza1/CD39 što bi moglo da dovede do smanjenog nivoa ADP i posledičnog smanjenja aktivacije ADP-zavisnih purinskih receptora. Posledično, ADP-posredovana signalizacija može promovisati aktivaciju glijskih ćelija, kao i proliferaciju i otpuštanje proinflamacijskih citokina (Quintas et al., 2011). Shodno tome, povećanje aktivnosti ADPaze sedmog dana od početka stimulacije u sham grupi ukazuje na aktivnost mikroglije jer je upravo NTPDaza1 visoko eksprimirana na mikrogliji (Braun et al., 2000), što je u skladu sa prethodno objavljenim podacima (Oses et al., 2011). Sa druge strane odsustvo promena između desnog i levog strijatuma u iTBS grupi nakon 7 dana stimulacije ukazuju na potencijalne anti-inflamacijske efekte iTBS-a (Stekic et al., 2022), dok slični nivoi aktivnosti ADPaze između levog i desnog strijatuma, primećeni u obe grupe nakon tri nedelje stimulacije, mogu da ukažu na mirujuću mikrogliju. Nadalje su analizirane i promene u genskoj i proteinskoj ekspresiji ADP-zavisnih receptora $P2Y_1$, $P2Y_{12}$ i $P2Y_{13}$. U fiziološkim uslovima, sva tri receptora se eksprimiraju i na mikrogliji i na astrocitima, s tim što se na mikrogliji eksprimiraju u istoj meri, dok je na

astrocitima najviše eksprimiran $P2Y_1$ (Quintas et al., 2018). $P2Y_1$ reguliše inflamacijski status kao i astrocit-astrocit signalizaciju putem jona Ca^{2+}, kao i sekreciju glutamata i ekscitabilnost neurona (Kuboyama et al., 2011; Simões et al., 2018). Smanjena genska ekspresija $P2Y_1R$ u 7. i 21. danu od početka stimulacije u sham grupi vraćena je na gotovo kontrolne nivoe u kontralateralnoj hemisferi. Ni u prvoj ni u trećoj nedelji nakon iTBS stimulacije nema promena u proteinskoj ekspresiji $P2Y_{12}R$, receptora koji predstavlja marker mirujuće mikroglije, što dodatno potvrđuje da mikroglija najverovatnije nije u svom reaktivnom stanju (Goldmann et al., 2016; Illes et al., 2020). Rezultati ove disertacije takođe pokazuju da dolazi do značajnog porasta ekspresije $P2Y_{13}$ receptora. Ovaj receptor prisutan je na mikrogliji gde zajedno sa $P2Y_{12}$ inhibira proliferaciju astrocita i indukuje neuroprotektivni fenotip (Quintas et al., 2018). Međutim, iako mikroglija pretežno eksprimira $P2Y_{13}R$ (Kyrargyri et al., 2020), drugi parametri koji ukazuju na mirujuću mikrogliju, kao što su $P2Y_{12}R$ i Iba-1 imunohistohemijsko bojenje, navode nas da verujemo da je posmatrano povećanje verovatnije povezano sa neuroprotekcijom koja je posredovana neuronskim $P2Y_{13}R$, što je takođe pokazano u literaturi (Pérez-Sen et al., 2015). Naime, pokazano je da aktivacija $P2Y_{13}R$ može da indukuje glavne signalne puteve vezane za preživljavanje neurona, odnosno kinaze aktivirane mitogenima poput ERK1/2 i PI3K/Akt/GSK3, koje između ostalog mogu dovesti i do aktivacije Nrf-2/HO-1 tandema koji ove ćelije štiti od ekscitotoksičnog oštećenja, koje je prisutno u 6-OHDA modelu usled pojačanog oslobađanja glutamata (Espada et al., 2010). Stoga, uočeno povećanje ekspresije ovog receptora na proteinskom nivou u strijatumu nakon 21-dnevne stimulacije iTBS protokolom može da ukaže na njegovu ulogu u neuroprotekciji, odnosno preživljavanju i otpornosti neurona na različite štetne uticaje, ali svakako zahteva dalja istraživanja.

Za razliku od oskudnih podataka o efektima koji se ostvaruju posredstvom P2 receptora u neurodegeneraciji izazvanoj 6-OHDA-om, signalizacija posredovana P1 receptorima za adenozin, posebno efekti posredovani signalizacijom preko $A_{2A}R$ privukli su značajno više pažnje i u literaturi su bolje okarakterisani (Morelli et al., 2009). Povećana strijatalna ekspresija $A_{2A}R$ primećena je u *post mortem* studijama na uzorcima moždanog tkiva pacijenata obolelih od PB, kao i u eksperimentalnim modelima PB (Villar-Menéndez et al., 2014). Jedan od glavnih razloga „sinaptopatije“ jeste upravo povećana proteinska ekspresija $A_{2A}R$ na postinaptičkim neuronima indirektne petlje. Ovo povećanje kuplovano je i sa povećanjem ekspresije eN/CD73 koja proizvodi adenozin, a ujedno i sa prostornim kuplovanjem ova dva proteina. Ukupni efekat u patologiji je pojačana signalizacija preko $A_{2A}R$, što, u odsustvu dopamina koji bi trebalo da inhibira ovaj put, pojačava verovatnoću aktivacije indirektne petlje kada bi ona trebalo da bude inhibirana i generiše "NO-GO" signale koji dovode do tremora i nekoordinisanih pokreta, jer se praktično prepliću sa zadatim motornim planom i prekidaju ga (Nazario et al., 2017). Ovo bi moglo da objasniti korisne efekte antagoniste $A_{2A}R$, istradefilina, koji dovodi do poboljšanja motornih funkcija uz smanjen rizik za razvoj odnosno pogoršanje diskinezija koje se javljaju prilikom upotrebe levodope (Cummins & Cates, 2022), što je veoma interesantno jer je efekat $A_{2A}R$ u PB dominantno sinaptički, a ne neuroinflamacijski-glijski kao u mnogim drugim patologijama (Gomes et al., 2011). Takođe, još jedan faktor koji doprinosi kompleksnosti cele patologije jeste heteromerna interakcija dopaminskih i adenozinskih receptora i to u vidu heterodimera koji se najčešće sastoje od D1R-A_1R i D2R-$A_{2A}R$. Upravo zbog postojanja ovih interakcija, adenozinski sistem je veoma interesantna kao ciljna meta za lečenje simptoma PB. Dosadašnja istraživanja su pokazala da agonisti A_1R smanjuju mogućnost vezivanja dopamina za D1R i redukuju proizvodnju cAMP-a, pa bi ovo smanjenje dopaminske signalizacije potencijalno moglo da umanji prekomernu stimulaciju D1 receptora i time smanjiti rizik od diskinezija koje se javljaju pri dugotrajnoj upotrebi levodope, dok antagonisti A_1R aktiviraju D1R povećavajući nivoe cAMP-a što dovodi do povećanja spontane motorne aktivnosti (Ferré et al., 1998).

Interakcija između strijatalnih A_{2A} i D2 receptora je jedna od najviše ispitivanih receptorskih interakcija. A_{2A}R-D2R dimer je funkcionalni heteroreceptor koji indukuje alosternu inhibiciju, dakle kada se A_{2A}R aktivira adenozinom, afinitet D2R za dopamin se smanjuje (Ferre et al., 1991). U fiziološkim uslovima A_{2A}R je blago aktiviran adenozinom pa afinitet D2R nije oslabljen, i signalizacija posredovana preko cAMP-zavisne protein kinaze (PKA) ne doprinosi aktivnosti GABA neurona i ne dolazi do ometanja kompleksne kontrole voljne motorike (Ferré et al., 2007). Međutim, u patološkim uslovima kada dolazi do prekomerne aktivacije A_{2A}R i uz nedostatak dopamina, dolazi do povećanja nivoa cAMP i posledične aktivacije PKA, čija dalja signalna kaskada dovodi do aktivacije GABA neurona i posledične inhibicije D2R-posredovane kontrole pokreta (Prasad et al., 2021). Precizan mehanizam na koji dopamin i adenozin zajednički regulišu aktivnost PKA i dalje je u većoj meri nepoznat. Takođe, promene koje nastaju kao kompenzacijski mehanizam u drugim signalnim sistemima poput serotoninskog, glutamatnog i GABA dodatno otežavaju razumevanje promena nastalih usled patologije. Rezultati ove disertacije su pokazali da neurodegeneracija izazvana 6-OHDA indukuje smanjenje ekspresije A_1R i povećava ekspresiju A_{2A}R, što je potvrđeno i u humanoj patologiji i u drugim eksperimentalnim modelima (Calon et al., 2004; Massari et al., 2021; Reyhani-Rad & Mahmoudi, 2016). Tretman iTBS i nakon 7. i 21. dana rezultirao je vraćanjem ekspresije A_1R i A_{2A}R na nivoe primećene u kontrolnoj levoj hemisferi.

Glavni izvor adenozina, koji je odgovoran za prekomernu aktivaciju A_{2A}R, predstavlja eN/CD73, koji hidrolizuje vanćelijske adeninske nukleotide do adenozina zbog čega se ovaj enzim našao u fokusu mnogih farmakoloških studija kao meta potencijalne blokade (Gessi et al., 2011). Kao što je već pomenuto, brojne studije upućuju na blisku fizičku i funkcionalnu povezanost između A_{2A}R i eN/CD73, koja je još izraženija u patološkim uslovima što je potvrđeno paralelnom regulacijom gena *Nt5e* i *Adora2a* (Augusto et al., 2013; Nedeljkovic, 2019). I druge studije su u 6-OHDA indukovanom modelu PB uputile na povećanu aktivnost eN/CD73 koja se dešava paralelno sa povećanom ekspresijom A_{2A}R (Carmo et al., 2019), što je rezultat i ove disertacije, gde su se nakon primene iTBS-a i u prvoj i trećoj nedelji od početka stimulacije smanjili ekspresija ali ne i aktivnost ovog enzima. Načelno, aktivnost eN/CD73 identifikovana je kao faktor koji može direktno uticati na neurodegeneraciju kroz regulaciju preživljavanja neurona, ali i indirektno kroz modulaciju inflamacijskih procesa (Meng et al., 2019). Interesantno je da je aktivnot eN/CD73 u obe hemisfere bila povećana nakon tronedeljnog iTBS, što ukazuje na njegovo neselektivno delovanje kao i na povećanje metaboličke aktivnosti. Međutim iako se povećana aktivnost eN/CD73 načelno smatra kao nepovoljan događaj, efekat tog povećanja zavisi od distribucije i odnosna ekspresije adenozinskih receptora. S obzirom da je tronedeljni tretman vratio nivoe adenozinskih receptora na one slične kontrolnim vrednostima, ovo povećanje se može staviti u kontekst povoljnih efekata na sinaptičku aktivnost.

Adenozin nastao hidrolizom AMP uklanja se iz vanćelijske sredine delovanjem ektoenzima adenozin deaminaze 1 (ADA1) te je ona još jedna komponenta purinoma koja predstavlja potencijalni cilj u PB (Dragic, Stekic, et al., 2022). Nedavna studija pokazala je da farmakološka blokada ADA1 u eksperimentalnom modelu PB indukovanom pomoću MPTP-ja dovodi do značajnog poboljšanja motorinih deficita, kao i do povećanja nivoa dopamina i smanjene smrti DA neurona u strijatumu, dok je neuroprotektivni efekat bio još izraženiji uz sinergističko korišćenje antagoniste A_{2A}R (Huang et al., 2019). Ovi rezultati sugerišu da primećena poboljšanja u PB nisu direktno uslovljena samo smanjenjem nivoa adenozina, već verovatnije nedostatkom adenozina koji proizvodi eN/CD73 u neposrednoj blizini A_{2A}R. Istraživanja su pokazala da je ADA1, slično kao eN/CD73-A_{2A}R, funkcionalno spregnut sa A_1R čime kontroliše dostupnost vanćelijskog adenozina i samim tim ligand-zavisnu signalizaciju posredovanu ovim receptorom (Ciruela et al., 1996; Ruiz et al., 2000). Ova dinamika

interakcije je od posebnog značaja u direktnom putu važnom za kontrolu voljne motorike, jer funkcionalna povezanost ADA1 sa A_1R ukazuje na to da ADA1 ne samo da utiče na nivo adenozina i njegovo dalje vezivanje za A_1R i sledstvenu signalizaciju već ima uticaj i na heterodimer A_1R-D1R. S tim u vezi, multimodalni pristup koji bi uključivao i inhibiciju eN/CD73 i ADA1 i samim tim specifično uticao na aktivnost $A_{2A}R$ i A_1R pokazao bi se kao moguća inovativna strategija za kontrolu neuroinflamacije, neuroprotekcije, i motornih funkcija koje su narušene u PB.

Naša studija pokazala je da 21-dnevna stimulacija iTBS-om smanjuje ekspresiju ADA1, što bi moglo da dovede do veće dostupnosti adenozina za aktivaciju A_1R. Aktivacija A_1R je povezana sa neuroprotektivnim efektima, što bi moglo biti posebno korisno u kontekstu PB. Takođe, A_1R se dominantno eksprimira presinaptički, te njegova povećana ekspresija može uticati i na stabilizaciju preterano aktivnih presinaptičkih kortikostrijatalnih ulaza i time doprineti smanjeju i stabilizaciji neuronskih kola direktne i indirektne petlje (Cunha, 2016).

Kada je u pitanju ekspresija DA receptora poznato je da nakon unilateralne lezije SNpc indukovane 6-OHDA dolazi do povećane genske i proteinske ekspresije D2R, dok ekspresija D1R ostaje nepromenjena, što je verovatno vid kompenzatornog mehanizam, jer iako se ekspresija D1R ne povećava, on ima veći afinitet za vezivanja dopamina u patologiji (Fornaretto et al., 1993; Gerfen et al., 1990; Narang & Wamsley, 1995). Ista promena pokazana je i u ovoj disertaciji, a iTBS nije doveo do statistički značajne promene u ekspresiji D1R, ali postoji indikacija o mogućem trendu povećanja ekspresije. Sa druge strane iTBS tretman rezultovao je smanjenom ekspresijom D2R. Navedene promene u ekspresiji adenozinskih i DA receptora najverovatnije utiču na obnavljanje ravnoteže između D1R-A_1R i D2R-$A_{2A}R$, što je mogući razlog oporavka testiranih motornih funkcija.

Imajući u vidu sve navedeno, rezultati ove disertaciju ukazuju da je stimulacija iTBS protokolom efikasno smanjila ekspresiju i povećala aktivnost eN/CD73, ali i smanjila ekspresiju ADA1 i indukovala promene u ekspresiji A_1R i $A_{2A}R$ u strijatumu, što dalje usmerava na moguće obnavljanje interakcije i ravnoteže između adenozinskih i DA receptora čija je signalizacija ključna u strijatalnim neuronskim krugovima uključenim u kontrolu motornih funkcija.

Nadalje, naša istraživanja doprinela su rasvetljavanju mogućih mehanizama delovanja rTMS-a ističući još i specifično povećanje odnosa p-AMPK/t-AMPK kao rezultat 21-dnevnog tretmana iTBS-om. Protein kinaza aktivirana AMP-om (eng. *adenosine monophosphate-activated protein kinase*, AMPK) je važan unutarćelijski regulator kada je u pitanju održavanje energetske homeostaze u ćeliji. Povećani nivoi fosfoforme ovog enzima dodatno potvrđuju da iTBS izaziva depleciju unutarćelijskih nivoa ATP-a. S obzirom na ranije pomenute mehanizme uključene u patogenezu PB, mitohondrijsku i lizozomsku disfunkciju, oksidativni stres i neuroinflamaciju, kao i akumulaciju α-sinukleina, modulacija signalnog puta AMPK može se pokazati kao odličan pristup za postizanje neuroprotektivnih efekata. Brojni dokazi sugerišu da aktivacija AMPK može imati široki spektar povoljnih efekata, uključujući regulaciju biogeneze mitohondrija, indukciju autofagije i razgradnje nagomilanog α-sinukleina, povećanje antioksidativnog kapaciteta i smanjenje inflamacijskog odgovora (Curry et al., 2018).

4.5. Efekti rTMS-a na nivou oksidativnog stresa i antioksidativne zaštite

Oksidativni stres kao posledica i/ili uzrok svih patoloških procesa ima značajnu ulogu u etiologiji PB, o čemu je bilo reči. Dodatno, kontinuirana upotreba levodope koja uspešno ublažava motorne simptome vremenom gubi efikasnost i dovodi do neželjenih efekata u vidu diskinezija, ali i neurotoksičnih efekata (Dorszewska et al., 2021; Gesi et al., 2001) kojim

delom potiču i od procesa auto-oksidacije levodope, čiji je indirektni ishod disregulacija nivoa biotiola, generisanje ROS-a i indukcija inflamacijskih i apoptotskih procesa (Dorszewska et al., 2014). U svetlu ovih izazova, u okviru ove disertacije ispitivana je i mogućnost da iTBS, kao neselektivni tretman koji verovatno utiče na sve funkcionalne aspekte jedne ćelije, ostvaruje antioksidativne efekte zaustavljajući štetne efekte oksidativnog stresa bilo kroz smanjenje ROS ili stimuliaciju endogenog antioksidativnog sistema ciljajući i nervne i glijske ćelije (Medina-Fernández et al., 2018). Pokazali smo da su se nakon tronedeljne iTBS stimulacije desile značajne promene u antioksidativnim i pro-oksidativnim parametrima u desnoj hemisferi SNpc-a i u strijatumu. U homogenatima pomenutih struktura analizirani enzimski antioksidansi, uključujući ukupnu aktivnost SOD i CAT, kao i neenzimski antioksidansi kao što su GSH i sulfhidrilne grupe (SH^-) pokazali su značajno povećanje. Sa druge strane, sadržaj pro-oksidativnih pokazatelja, MDA, NO i $O_2^{\bullet -}$ su se smanjili. Ove promene su bile konzistentne i u SNpc i u strijatumu kod životinja tretiranih iTBS-om. Međutim, iznenađujuće odsustvo razlika između leve i desne hemisfere unutar Sham i iTBS grupe sugeriše da neurotoksični događaji povezani sa oksidativnim stresom verovatno prestaju pre završetka perioda simulacije, što je u skladu sa postojećom literaturom koja ukazuje da se oksidativni stres vraća na bazalne nivoe nakon jedne nedelje od intoksikacije 6-OHDA (Smith & Cass, 2007). Ovo dodatno potkrepljuje i naše histopatološke nalaze koji ukazuju na odsustvo aktivne inflamacije u ispitivanim regionima.

U konktekstu tumačenja rezultata treba uzeti u obzir i šire efekte iTBS-a. Shodno tome, analiza seruma otkrila je smanjenje koncentracije MDA i NO zajedno sa povećanjem u antioksidativnom odbrambenom parametru SH^-, potvrđujući pozitivne sistemske efekte iTBS-a. Takođe, tretman iTBS-om u drugim modelima neurodegeneracija, kao što su Alchajmerova bolest indukovana streptozotocinom (STZ) i neurodegeneracija indukovana trimetil-kalejem (TMT), pokazao je značajno smanjenje markera oksidativnog stresa i povećan antioksidativni kapacitet (Stanojevic et al., 2023; Stekic et al., 2022). Jedno od mogućih objašnjenja za opaženo poboljšanje u opštem oksidativnom statusu nakon iTBS leži u aktivaciji nuklearnog faktora 2 (Nrf2), koji indukuje ekspresiju mnogih citoprotektivnih proteina uključujući i antioksidativne enzime, zbog čega može imati važnu ulogu u upravljanju odgovorom na oksidativni stres (Ma, 2013). U uslovima oksidativnog stresa fosforilisan Nrf2 se premešta iz citoplazme u jedro gde zajedno sa Maf (eng. *musculo aponeurotic fibrosarcoma*) proteinima formira heterodimerni kompleks. Ovaj heterodimer se zatim vezuje za elemente antioksidativnog odgovora, ARE (eng. *antioxidant response element*, ARE) u promotoru mnogih citoprotektivnih gena čime se pokreće mašinerija za njihovu transkripciju (Zhang et al., 2010). Ovako koordinisana regulacija ARE-kontrolisanih gena omogućava održavanje bazalnog nivoa citoprotektivnih enzima, ali i efikasnu adaptaciju ćelije na povećanu koncentraciju ROS, RNS i brojnih elektrofilnih jedinjenja (He et al., 2020). Ključna uloga signalnih puteva posterovanih Nrf2 u PB je demonstrirana mikročip analizom različitih tkiva pacijenata, kojom je otkriveno smanjenje ekspresije 31 gena sa ARE sekvencama za koje se Nrf2 vezuje (Wang et al., 2017). Aktivacija Nrf2 signalnog puta od strane rTMS ima potencijal da menja ekspresiju antioksidativnih proteina kao što su HO-1 i SOD1 (Liang et al., 2021; Villavicencio Tejo & Quintanilla, 2021), što posledično dovodi do poboljšanja funkcije mitohondrija, sinteze ATP-a i smanjenja oštećenja uzrokovanog oksidativnim stresom (Dinkova-Kostova & Abramov, 2015). U skladu sa ovom tvrdnjom, povećana ekspresija $P2Y_{13}R$ nakon tronedeljne iTBS stimulacije može takođe da dovode do aktivacije Nrf-2/HO-1 ose. Studije koje su koristile iTBS protokol u STZ modelu neurodegeneracije pokazale su povećanje Nrf2 faktora, kao i druge studije koje su u neuroinflamatornim uslovima nakon primene rTMS-a pokazale povećanu ekspresiju ovog faktora (Stanojevic et al., 2023).

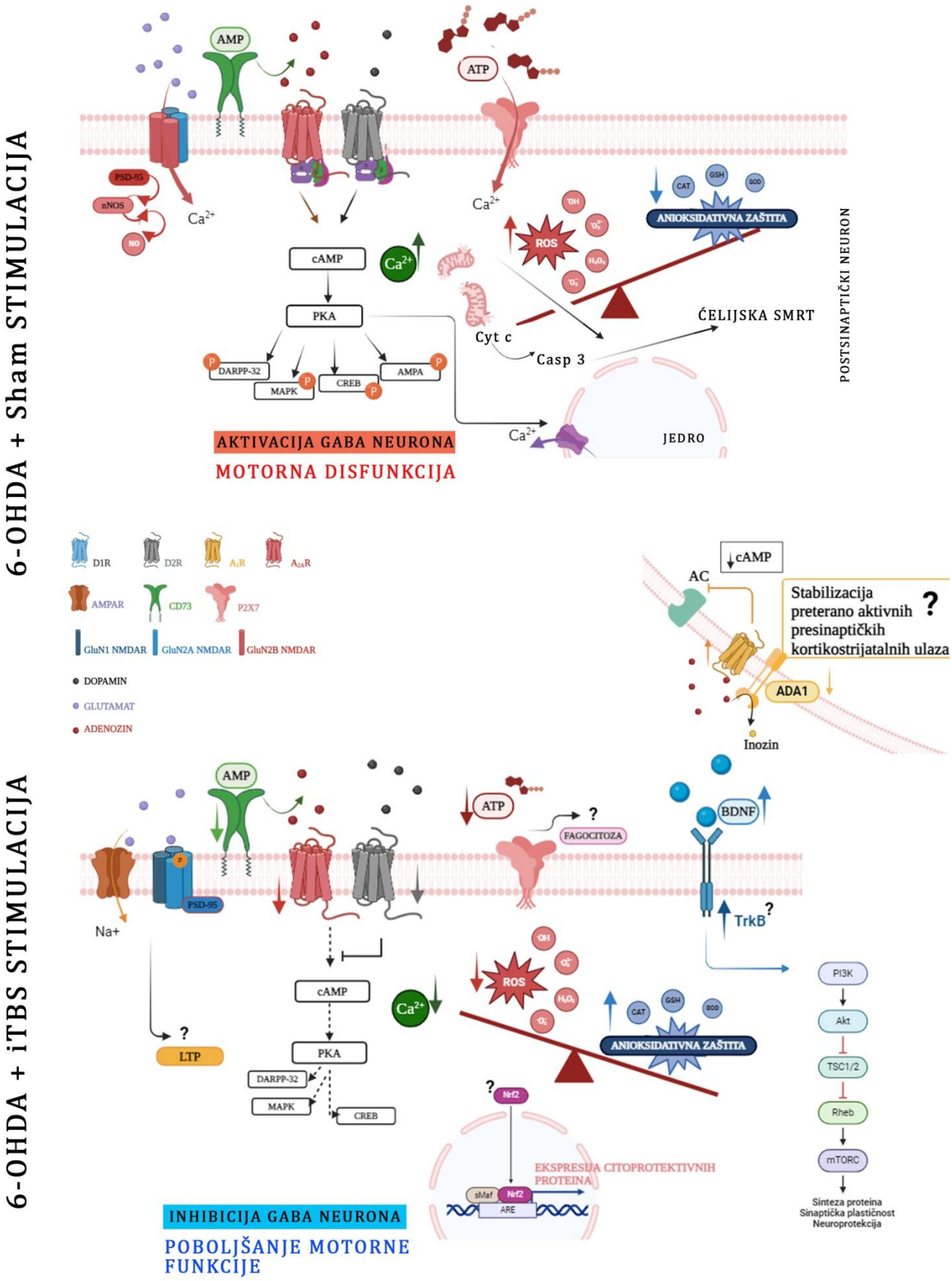
Nadalje, BDNF koji je poznat po svojoj ulozi kao modulator sinaptičke plastičnosti i koji utiče na preživljavanje neurona, može i da indukuje translokaciju Nrf2 u jezgro, a mi smo upravo pokazali njegovu povećanu ekspresiju nakon 21-og dana stimulacije iTBS-om. BDNF ispoljava svoje brojne efekte aktivirajući TrkB receptore, što dalje pokreće signalni put PI3K-Akt kojim se ushodno reguliše sinteza ključnih proteina koji učestvuju u procesima sinaptičke plastičnosti i neurogeneze, što zajedno rezultira neuroprotekcijom (Rothman & Mattson, 2013). Brojne studije su pokazale da rTMS indukuje povećanje BDNF-a i TrkB (Lee et al., 2021; Stevanovic et al., 2019; Uzair et al., 2022). Povećano oslobađanje BDNF-a i pojačana signalizacija preko TrkB-a mogu biti odgovorni za dalju indukciju signalnog puta PI3K/Akt/mTOR što može imati povoljne efekte u kontekstu neuroprotektivnih efekata, sinteze antioksidativnih proteina, poboljšanja ponašanja nalik anksioznom i poboljšanju učenja i pamćenja (Martin et al., 2004; Stekic et al., 2022; Wang et al., 2008). Takođe, u PB kao i u drugim neurodegenerativnim bolestima osim povećane produkcije ROS, povećana je i sinteza NO kao posledica oksidativnog stresa, ali njegova proizvodnja može biti i rezultat povećanog oslobađanja i smanjenog uklanjanja glutamata, što dovodi do hiperstimulacije NMDA receptora i povećanog influksa Ca^{2+}, što narušava prenos signala posredovan TrkB, aktivira metalotioneine koji dalje oštećuju ekstracelularni matriks što za posledicu ima apoptozu ćelije (Dong et al., 2009). Sve navedene promene bi mogle da uzrokuju oporavak oksidativnog sistema i uspostavljanja oksidativne ravnoteže nakon produžene stimulacije iTBS-om. Svakako, dobijeni rezultati ove doktorske disertacije ukazuju na važnost daljeg istraživanja specifičnih signalnih puteva i molekulskih kaskada uključenih u ovu iTBS posredovanu modulaciju pro-oksidativnih i antioksidativnih parametara.

4.6. Generalna diskusija

Prekomerno oslobađanje glutamata sa ekscitatornih vlakana koja polaze iz motorne zone do putamena dovode do pojačane inhibicije talamokortikalnih projekcija što uz smanjenje dopamina i posledično smanjenje aktivnosti direktnog puta dovodi do pojave brojnih hipokinetičkih simptoma, prepoznatljivih za PB. Pomenuti motorni poremećaji koji uključuju akineziju i bradikineziju, asimetrično kretanje i narušenu ravnotežu mogu se uočiti i u 6-OHDA modelu ove bolesti. U 6-OHDA modelu PB, degeneracija DA neurona u SNpc, koja je uglavnom uzrokovana masivnim oksidativnim stresom, i gubitak strijatalnih aksona, rezultuju smanjenjem nivoa dopamina u regionu bazalnih ganglija. Naime, nagomilavanje ROS-a uzrokovano toksinom uz smanjen antioksidativni kapacitet, može da izazove fosforilaciju c-Jun N-terminalne kinaze (JNK) koja pripada familiji mitogenom aktiviranih proteinskih kinaza (MAPK) koje se aktiviraju u odgovoru na stres, što dalje dodatno narušava mitohondrijski membranski potencijal i propustljivost mitohondrijskih membrana, i za posledicu ima oslobađanje citohroma c koji pokreće kaskadnu aktivnost kaspaza što na kraju vodi do apoptoze. Takođe, i narušena kalcijumska homeostaza za posledicu ima ćelijsku smrt. Povećanjo oslobađanje glutamata koje je zabeleženo u PB, kao i u 6-OHDA modelu, dovodi do prekomerne aktivacije NMDAR/AMPAR što za posledicu ima povećan influks kalcijuma i dovodi do ćelijske smrti kroz mehanizam ekscitotoksičnosti. U 6-OHDA modelu je zabeleženo i povećanje nivoa adenozina kao i povećana ekspresija $A_{2A}R$ u strijatumu. Ovo povećanje kuplovano je i sa povećanjem ekspresije eN/CD73 koja je glavni izvor adenozina, koji je odgovoran za prekomernu aktivaciju $A_{2A}R$. Aktivacija $A_{2A}R$ osim povećanja nivoa cAMP, aktivacije PKA i dalje inhibicije pokreta, aktivira i fosfolipazu C (PLC) i posledično dovodi do povećanja Ca^{2+} i aktivacije apoptotskih signalnih puteva. Pored toga, $A_{2A}R$ fizički i funkcijski interaguje sa glutamatnim receptorima, pretežno sa mGlu5 subjedinicom AMPA receptora. Ova interakcija pospešuje oslobađanje glutamata, što dalje opet dovodi do aktivacije NMDAR i povećanog influksa Ca^{2+}. Ovo povećanje nivoa Ca^{2+} aktivira dalju kaskadu i omogućava proizvodnju NO putem aktivnosti azot-oksid sintaze (nNOS), što značajno doprinosi smrti neurona. Prekomernoj koncentraciji Ca^{2+} doprinosi i P2X7R, nespecifičan katjonski kanal koji

se otvara pod uticajem ATP-a, a u 6-OHDA modelu je upravo primećena povećana koncentracija vanćelijskog ATP-a. Sve navedene promene nakon mikroinjekcije 6-OHDA od kojih je većina i primećena u rezultatima ove doktorske disertacije odgovorne su za degeneraciju DA neurona kao i za prekomernu aktivaciju GABA neurona indirektnog puta što za posledicu ima inhibiciju pokreta odnosno narušavanje motornih funkcija.

Imajući u vidu da su glutamat, dopamin, adenozin i ATP najodgovorniji za kontrolu direktne i indirektne motorne petlje, kao i da je od posebne važnosti u tom procesu i formiranje dopaminsko-adenozinskih receptorskih dimera, možemo da zaključimo da iTBS tretman 6-OHDA životinja u trajanju od tri nedelje ima povoljne efekte i dovodi do značajnih promena na nivou svih gorepomenutih sistema, koje dalje mogu da nas navedu da upravo ovaj vid stimulacije može da preokrene neke od navedenih mehanizama koji dovode do degeneracije DA neurona i narušavanja motornih funkcija. Povećano oslobađanje BDNF-a nakon iTBS tretmana može da dovode do pojačane signalizacija preko njegovog TrkB receptora što dalje aktivira nishodni signalni put PI3K/Akt/mTOR što može imati neuroprotektivne efekte. Pored toga aktivacija ove signalne kaskade može da moduliše aktivnost NMDA receptora kroz fosforilaciju GluN1 subjedinice i samim tim utiče na procese sinaptičke plastičnosti. Takođe, jedno od mogućih objašnjenja za opaženo poboljšanje u opštem oksidativnom statusu nakon iTBS leži u aktivaciji Nrf2, koji indukuje ekspresiju mnogih citoprotektivnih proteina uključujući i antioksidativne enzime, zbog čega može imati važnu ulogu u upravljanju odgovorom na oksidativni stres. Pored toga i povećana ekspresija $P2Y_{13}R$ nakon tronedeljne iTBS stimulacije može da dovode do aktivacije Nrf-2/HO-1. Imajući u vidu da iTBS ubrzava potrošnju glukoze i lipida kao i ATP-a, možemo pretpostaviti da je u niskim koncentracijama eATP-a pojačana fagocitozu posredovanu P2X7R čija je ekspresija bila vraćena na kontrolne nivoe nakon iTBS tretmana. Nadalje, nakon iTBS tretmana smanjena je i ekspresija $A_{2A}R$, kao i D2R što može da dovede do smanjenog afiniteta za formiranje heteromernog $A_{2A}R$-D2R kompleksa koji nepovoljno utiče na kontrolu pokreta. Takođe, iTBS je smanjio i ekspresiju CD73 koji obezbeđuje dostupnost adenozina $A_{2A}R$, i samim može da smanji njegovu preteranu aktivaciju. Postoje i pretpostavke da $A_{2A}R$–D2R heteroreceptorski kompleksi postoje ne samo na GABA neuronima (Fuxe et al., 2005) već i na kortikostrijatalnim glutamatnim projekcijama, gde $A_{2A}R$ inhibira D2R inhibiciju oslobađanja glutamata, što u slučaju smanjene ekspresije oba receptora kao i adenozina dostupnog $A_{2A}R$ nakon iTBS tretmana može imati neuroprotektivne efekte. Sa druge strane tronedeljna stimulacija iTBS-om smanjuje ekspresiju ADA1, što bi moglo da dovede do veće dostupnosti adenozina za aktivaciju A_1R, čija je ekspresija povećana. Aktivacija A_1R je povezana sa neuroprotektivnim efektima kroz smanjenje oslobađanja glutamata odnosno kroz stabilizaciju pteretano aktivnih glutamatnih kortikostrijatalnih projekcija. Sve navedene promene nakon tronedeljnog iTBS tretmana mogu biti odgovorne za protekciju DA neurona kao i za inhibiciju GABA neurona indirektnog puta i uspostavljanje ravnoteže između dopaminsko-adenozinskih receptorskih dimera, što za posledicu ima promociju pokreta odnosno poboljšanje prethodno narušenih motornih funkcija. Opisani pretpostavljeni mehanizmi delovanja iTBS prikazani su na slici 7.



Slika 7. Pretpostavljeni mehanizam delovanja iTBS u 6-OHDA modelu PB

5. ZAKLJUČCI

U skladu sa postavljenim ciljevima ove doktorske disertacije, a na osnovu dobijenih rezultata izvedeni su sledeći zaključci:

1) Nakon unilateralne mikroinjekcije 2 μL toksina 6-OHDA u desni SNpc u dozi 6 μg/μL proizvedena je specifična i stabilna lezija nigrostrijatalnog sistema praćena motornim deficitom. Takođe, ove životinje su ispoljile ponašanje nalik anksioznom, depresivnom, sa oslabljenom kratkoročnom memorijom. Upotreba 6-OHDA modela oslikava najvažnije karakteristike humane PB zbog čega je opradvana upotreba ovog modela za dalje ispitivanje neuroprotektivnih strategija.

2) iTBS je imao pozitivan efekat na motorne i nemotorne simptome izazvane 6-OHDA lezijom, pokazujući značajno poboljšanje motornih sposobnosti već nakon prve nedelje stimulacije koje je trajalo je do kraja perioda stimulacije. Takođe, uočena su poboljšanja u testovima za procenu ponašanja nalik anksionznom i depresivnom, ali i poboljšanje parametara u testu za procenu učenja i pamćenja.

3) iTBS stimulacija ispoljila je neuroprotektivne efekte koji su uočeni kroz povećanu ekspresiju TH markera DA neurona, kao i pojačanu imunoreaktivnost TH-pozitivnih ćelija u desnom SNpc-u i strijatumu, praćeno povećanjem strijatalnih nivoa dopamina i serotonina.
4) Analiza glavnih markera astro- i mikroglioze, nakon lezije 6-OHDA-om ukazala je na postojanje glioze u formi glijskog ožiljka bez naznaka morfoloških karakteristika reaktivne glije povezane sa neuroinflamacijom. Međutim, prisutna glioza bila je značajno manje izražena nakon iTBS tretmana, ukazujući na potencijalna anti-inflamacijska dejstva.

5) Tretman iTBS-om dovodi do povećane ekspresije GluN1 i GluN2A i smanjene ekspresije GluN2B subjedinice NMDAR što može pozitivno da utiče na preživljavanje DA neurona. Pored toga, iTBS indukuje povećanje ekspresije GLAST (EAAT1) i GLT-1 (EAAT2) transportera što može biti kompenzatorni mehanizam koji smanjuje nivo glutamata u sinaptičkom prostoru.

6) iTBS je povratila ekspresiju P2X7R na kontrolne nivoe što pri niskim koncentracijama eATP-a može da dovede do pojačane fagocitoze posredovane ovim receptorom. Uočene su i promene u ekspresiji ADP-zavisnih receptora povezanih sa glijskim ćelijama na genskom i na proteinskom nivou i to u pravcu povećanja $P2Y_1$, kao i povećanja $P2Y_{13}$ uz odsustvo promene $P2Y_{12}$, što zajedno ukazuje na antiinflamcijsko okruženje i potencijalnu ulogu u neuroprotekciji.

7) Stimulacija iTBS protokolom utiče na komponente purinskog signalnog sistema što se ogleda u smanjenju ekspresije eN/CD73 i ADA1 i povećanju ekspresije A_1R i smanjenju $A_{2A}R$ u strijatumu. Takođe, ekspresiju D1R i D2R menja se na istovetan način, što dalje usmerava na moguće obnavljanje ravnoteže između adenozinskih i DA receptora čija je signalizacija ključna u strijatalnim neuronskim krugovima uključenim u kontrolu motornih funkcija.

8) Četiri nedelje od intoksikacije 6-OHDA nije uočeno prisustvo narušene antioksidativne zaštite i nije bilo promena u parametrima oksidativnog stresa.

9) Nakon tronedeljne iTBS stimulacije analizirani enzimski i neenzimski antioksidansi pokazali su značajno povećanje. Sa druge strane, pro-oksidativni markeri su se smanjili. Takođe, analiza seruma otkrila je smanjenju koncentraciju MDA i NO praćenu povećanjem SH^- grupa, potvrđujući postojanje i pozitivnih sistemskih efekata iTBS-a.

Zaključci ove disertacije proistekli iz dobijenih rezultata ukazuju da iTBS-a utiče na ključne patofiziološke procese uključene u PB. iTBS je pokazao potencijal u regulaciji ključnih neurotransmitera, kao i u regulaciji komponenti purinskog i glutamatnog signalnog sistema, neuroprotektivni efekat, ali i potencijal u povećanju antioksidativnog kapaciteta.

Dobijeni rezultati naglašavaju važnost multisistemskog pristupa u izučavanju mehanizama kako same patologije tako i primenjenog terapeutika, te se i ova doktorska disertacija osvrće na tri glavna identifikovana signalna sistema u bolesti.

Rezultati disertacije pružaju osnovu za dalja istraživanja i doprinose postojećim znanjima o efektima rTMS pri čemu pružaju dodatne argumente za primenu ovog terapijskog modaliteta u daljim kliničkim ispitivanjima PB, tako i na drugim neurodegenerativnim bolestima.

6. LITERATURA

Abbott, L. F., & Nelson, S. B. (2000). Synaptic plasticity: taming the beast. *Nature Neuroscience*, *3 Suppl*, 1178–1183. https://doi.org/10.1038/81453

Abdeen, M. A., & Stuchly, M. A. (1994). Modeling of magnetic field stimulation of bent neurons. *IEEE Transactions on Bio-Medical Engineering*, *41*(11), 1092–1095. https://doi.org/10.1109/10.335848

Abraham, W. C., Jones, O. D., & Glanzman, D. L. (2019). Is plasticity of synapses the mechanism of long-term memory storage? *NPJ Science of Learning*, *4*, 9. https://doi.org/10.1038/s41539-019-0048-y

Adinolfi, E., Giuliani, A. L., De Marchi, E., Pegoraro, A., Orioli, E., & Di Virgilio, F. (2018). The P2X7 receptor: A main player in inflammation. *Biochemical Pharmacology*, *151*, 234–244. https://doi.org/10.1016/j.bcp.2017.12.021

Agostinho, P., Madeira, D., Dias, L., Simões, A. P., Cunha, R. A., & Canas, P. M. (2020). Purinergic signaling orchestrating neuron-glia communication. *Pharmacological Research*, *162*, 105253. https://doi.org/10.1016/j.phrs.2020.105253

Alafuzoff, I., & Hartikainen, P. (2017). Alpha-synucleinopathies. *Handbook of Clinical Neurology*, *145*, 339–353. https://doi.org/10.1016/B978-0-12-802395-2.00024-9

Alekseichuk, I., Mantell, K., Shirinpour, S., & Opitz, A. (2019). Comparative modeling of transcranial magnetic and electric stimulation in mouse, monkey, and human. *NeuroImage*, *194*, 136–148. https://doi.org/10.1016/j.neuroimage.2019.03.044

Amadio, S., Montilli, C., Picconi, B., Calabresi, P., & Volonté, C. (2007). Mapping P2X and P2Y receptor proteins in striatum and substantia nigra: An immunohistological study. *Purinergic Signalling*, *3*(4), 389–398. https://doi.org/10.1007/s11302-007-9069-8

Antczak, J., Rusin, G., & Słowik, A. (2021). Transcranial Magnetic Stimulation as a Diagnostic and Therapeutic Tool in Various Types of Dementia. *Journal of Clinical Medicine*, *10*(13). https://doi.org/10.3390/jcm10132875

Anwar, A., Saleem, S., Akhtar, A., Ashraf, S., & Ahmed, M. F. (2019). Juvenile Parkinson Disease. In *Cureus* (Vol. 11, Issue 8, p. e5409). https://doi.org/10.7759/cureus.5409

Arai, A. C., & Kessler, M. (2007). Pharmacology of ampakine modulators: from AMPA receptors to synapses and behavior. *Current Drug Targets*, *8*(5), 583–602. https://doi.org/10.2174/138945007780618490

Armstrong, M. J., & Okun, M. S. (2020). Diagnosis and Treatment of Parkinson Disease: A Review. *JAMA*, *323*(6), 548–560. https://doi.org/10.1001/jama.2019.22360

Ascherio, A., & Schwarzschild, M. A. (2016). The epidemiology of Parkinson's disease: risk factors and prevention. *The Lancet. Neurology*, *15*(12), 1257–1272. https://doi.org/10.1016/S1474-4422(16)30230-7

Augusto, E., Matos, M., Sévigny, J., El-Tayeb, A., Bynoe, M. S., Müller, C. E., Cunha, R. A., & Chen, J.-F. (2013). Ecto-5'-nucleotidase (CD73)-mediated formation of adenosine is critical for the striatal adenosine A2A receptor functions. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *33*(28), 11390–11399. https://doi.org/10.1523/JNEUROSCI.5817-12.2013

Ba, M., Ma, G., Ren, C., Sun, X., & Kong, M. (2017). Repetitive transcranial magnetic stimulation for treatment of lactacystin-induced Parkinsonian rat model. *Oncotarget*, *8*(31), 50921–50929. https://doi.org/10.18632/oncotarget.17285

Banerjee, J., Sorrell, M. E., Celnik, P. A., & Pelled, G. (2017). Immediate Effects of Repetitive Magnetic Stimulation on Single Cortical Pyramidal Neurons. *PloS One*, *12*(1), e0170528. https://doi.org/10.1371/journal.pone.0170528

Baquet, Z. C., Gorski, J. A., & Jones, K. R. (2004). Early striatal dendrite deficits followed by

neuron loss with advanced age in the absence of anterograde cortical brain-derived neurotrophic factor. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *24*(17), 4250–4258. https://doi.org/10.1523/JNEUROSCI.3920-03.2004

Barker, A. T., Jalinous, R., & Freeston, I. L. (1985). Non-Invasive Magnetic Stimulation of Human Motor Cortex. *The Lancet*, *325*(8437), 1106–1107. https://doi.org/10.1016/S0140-6736(85)92413-4

Bear, M. F., & Malenka, R. C. (1994). Synaptic plasticity: LTP and LTD. *Current Opinion in Neurobiology*, *4*(3), 389–399. https://doi.org/10.1016/0959-4388(94)90101-5

Belluzzi, E., Bisaglia, M., Lazzarini, E., Tabares, L. C., Beltramini, M., & Bubacco, L. (2012). Human SOD2 modification by dopamine quinones affects enzymatic activity by promoting its aggregation: possible implications for Parkinson's disease. *PloS One*, *7*(6), e38026. https://doi.org/10.1371/journal.pone.0038026

Berghauzen-Maciejewska, K., Wardas, J., Kosmowska, B., Głowacka, U., Kuter, K., & Ossowska, K. (2015). Alterations of BDNF and trkB mRNA expression in the 6-hydroxydopamine-induced model of preclinical stages of Parkinson's disease: an influence of chronic pramipexole in rats. *PloS One*, *10*(3), e0117698. https://doi.org/10.1371/journal.pone.0117698

Bernheimer, H., Birkmayer, W., Hornykiewicz, O., Jellinger, K., & Seitelberger, F. (1973). Brain dopamine and the syndromes of Parkinson and Huntington. Clinical, morphological and neurochemical correlations. *Journal of the Neurological Sciences*, *20*(4), 415–455. https://doi.org/10.1016/0022-510x(73)90175-5

Blandini, F, Porter, R. H., & Greenamyre, J. T. (1996). Glutamate and Parkinson's disease. *Molecular Neurobiology*, *12*(1), 73–94. https://doi.org/10.1007/BF02740748

Blandini, Fabio, Armentero, M.-T., & Martignoni, E. (2008). The 6-hydroxydopamine model: news from the past. *Parkinsonism & Related Disorders*, *14 Suppl 2*, S124-9. https://doi.org/10.1016/j.parkreldis.2008.04.015

Blesa, J., Juri, C., García-Cabezas, M. Á., Adánez, R., Sánchez-González, M. Á., Cavada, C., & Obeso, J. A. (2011). Inter-hemispheric asymmetry of nigrostriatal dopaminergic lesion: a possible compensatory mechanism in Parkinson's disease. *Frontiers in Systems Neuroscience*, *5*, 92. https://doi.org/10.3389/fnsys.2011.00092

Blesa, J., & Przedborski, S. (2014). Parkinson's disease: animal models and dopaminergic cell vulnerability. *Frontiers in Neuroanatomy*, *8*, 155. https://doi.org/10.3389/fnana.2014.00155

Blomeley, C. P., & Bracci, E. (2009). Serotonin excites fast-spiking interneurons in the striatum. *The European Journal of Neuroscience*, *29*(8), 1604–1614. https://doi.org/10.1111/j.1460-9568.2009.06725.x

Blum, D., Torch, S., Lambeng, N., Nissou, M., Benabid, A. L., Sadoul, R., & Verna, J. M. (2001). Molecular pathways involved in the neurotoxicity of 6-OHDA, dopamine and MPTP: contribution to the apoptotic theory in Parkinson's disease. *Progress in Neurobiology*, *65*(2), 135–172. https://doi.org/10.1016/s0301-0082(01)00003-x

Boonzaier, J., van Tilborg, G. A. F., Neggers, S. F. W., & Dijkhuizen, R. M. (2018). Noninvasive Brain Stimulation to Enhance Functional Recovery After Stroke: Studies in Animal Models. *Neurorehabilitation and Neural Repair*, *32*(11), 927–940. https://doi.org/10.1177/1545968318804425

Braak, H., Del Tredici, K., Rüb, U., de Vos, R. A. I., Jansen Steur, E. N. H., & Braak, E. (2003). Staging of brain pathology related to sporadic Parkinson's disease. *Neurobiology of Aging*, *24*(2), 197–211. https://doi.org/10.1016/s0197-4580(02)00065-9

Braun, N., Sévigny, J., Robson, S. C., Enjyoji, K., Guckelberger, O., Hammer, K., Di Virgilio, F., & Zimmermann, H. (2000). Assignment of ecto-nucleoside triphosphate diphosphohydrolase-1/cd39 expression to microglia and vasculature of the brain. *The European Journal of Neuroscience*, *12*(12), 4357–4366.

Breit, S., Bouali-Benazzouz, R., Benabid, A. L., & Benazzouz, A. (2001). Unilateral lesion of the nigrostriatal pathway induces an increase of neuronal activity of the pedunculopontine nucleus, which is reversed by the lesion of the subthalamic nucleus in the rat. *The European Journal of Neuroscience*, *14*(11), 1833–1842. https://doi.org/10.1046/j.0953-816x.2001.01800.x

Breton, J. M., Charbit, A. R., Snyder, B. J., Fong, P. T. K., Dias, E. V, Himmels, P., Lock, H., & Margolis, E. B. (2019). Relative contributions and mapping of ventral tegmental area dopamine and GABA neurons by projection target in the rat. *The Journal of Comparative Neurology*, *527*(5), 916–941. https://doi.org/10.1002/cne.24572

Brown, J. C., DeVries, W. H., Korte, J. E., Sahlem, G. L., Bonilha, L., Short, E. B., & George, M. S. (2020). NMDA receptor partial agonist, d-cycloserine, enhances 10 Hz rTMS-induced motor plasticity, suggesting long-term potentiation (LTP) as underlying mechanism. In *Brain stimulation* (Vol. 13, Issue 3, pp. 530–532). https://doi.org/10.1016/j.brs.2020.01.005

Brusa, L., Finazzi Agrò, E., Petta, F., Sciobica, F., Torriero, S., Lo Gerfo, E., Iani, C., Stanzione, P., & Koch, G. (2009). Effects of inhibitory rTMS on bladder function in Parkinson's disease patients. *Movement Disorders : Official Journal of the Movement Disorder Society*, *24*(3), 445–448. https://doi.org/10.1002/mds.22434

Burnstock, G. (1972). Purinergic nerves. *Pharmacological Reviews*, *24*(3), 509–581.

Burnstock, G. (1997). The past, present and future of purine nucleotides as signalling molecules. *Neuropharmacology*, *36*(9), 1127–1139. https://doi.org/10.1016/s0028-3908(97)00125-1

Burnstock, G, Campbell, G., Satchell, D., & Smythe, A. (1970). Evidence that adenosine triphosphate or a related nucleotide is the transmitter substance released by non-adrenergic inhibitory nerves in the gut. *British Journal of Pharmacology*, *40*(4), 668–688. https://doi.org/10.1111/j.1476-5381.1970.tb10646.x

Burnstock, Geoffrey. (2014). Purinergic signalling: from discovery to current developments. *Experimental Physiology*, *99*(1), 16–34. https://doi.org/10.1113/expphysiol.2013.071951

Cacace, F., Mineo, D., Viscomi, M. T., Latagliata, E. C., Mancini, M., Sasso, V., Vannelli, A., Pascucci, T., Pendolino, V., Marcello, E., Pelucchi, S., Puglisi-Allegra, S., Molinari, M., Picconi, B., Calabresi, P., & Ghiglieri, V. (2017). Intermittent theta-burst stimulation rescues dopamine-dependent corticostriatal synaptic plasticity and motor behavior in experimental parkinsonism: Possible role of glial activity. *Movement Disorders : Official Journal of the Movement Disorder Society*, *32*(7), 1035–1046. https://doi.org/10.1002/mds.26982

Calon, F., Dridi, M., Hornykiewicz, O., Bédard, P. J., Rajput, A. H., & Di Paolo, T. (2004). Increased adenosine A2A receptors in the brain of Parkinson's disease patients with dyskinesias. *Brain : A Journal of Neurology*, *127*(Pt 5), 1075–1084. https://doi.org/10.1093/brain/awh128

Campagno, K. E., & Mitchell, C. H. (2021). The P2X(7) Receptor in Microglial Cells Modulates the Endolysosomal Axis, Autophagy, and Phagocytosis. *Frontiers in Cellular Neuroscience*, *15*, 645244. https://doi.org/10.3389/fncel.2021.645244

Campanelli, F., Natale, G., Marino, G., Ghiglieri, V., & Calabresi, P. (2022). Striatal glutamatergic hyperactivity in Parkinson's disease. *Neurobiology of Disease*, *168*, 105697. https://doi.org/10.1016/j.nbd.2022.105697

Canolty, R. T., Edwards, E., Dalal, S. S., Soltani, M., Nagarajan, S. S., Kirsch, H. E., Berger, M. S., Barbaro, N. M., & Knight, R. T. (2006). High gamma power is phase-locked to theta oscillations in human neocortex. *Science (New York, N.Y.)*, *313*(5793), 1626–1628. https://doi.org/10.1126/science.1128115

Cao, J., Viholainen, J. I., Dart, C., Warwick, H. K., Leyland, M. L., & Courtney, M. J. (2005). The

PSD95-nNOS interface: a target for inhibition of excitotoxic p38 stress-activated protein kinase activation and cell death. *The Journal of Cell Biology*, *168*(1), 117–126. https://doi.org/10.1083/jcb.200407024

CARLSSON, A., LINDQVIST, M., & MAGNUSSON, T. (1957). 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature*, *180*(4596), 1200. https://doi.org/10.1038/1801200a0

Carmo, M., Gonçalves, F. Q., Canas, P. M., Oses, J.-P., Fernandes, F. D., Duarte, F. V, Palmeira, C. M., Tomé, A. R., Agostinho, P., Andrade, G. M., & Cunha, R. A. (2019). Enhanced ATP release and CD73-mediated adenosine formation sustain adenosine A(2A) receptor over-activation in a rat model of Parkinson's disease. *British Journal of Pharmacology*, *176*(18), 3666–3680. https://doi.org/10.1111/bph.14771

Carmo, M. R. S., Menezes, A. P. F., Nunes, A. C. L., Pliássova, A., Rolo, A. P., Palmeira, C. M., Cunha, R. A., Canas, P. M., & Andrade, G. M. (2014). The P2X7 receptor antagonist Brilliant Blue G attenuates contralateral rotations in a rat model of Parkinsonism through a combined control of synaptotoxicity, neurotoxicity and gliosis. *Neuropharmacology*, *81*, 142–152. https://doi.org/10.1016/j.neuropharm.2014.01.045

Carvey, P. M., Zhao, C. H., Hendey, B., Lum, H., Trachtenberg, J., Desai, B. S., Snyder, J., Zhu, Y. G., & Ling, Z. D. (2005). 6-Hydroxydopamine-induced alterations in blood-brain barrier permeability. *The European Journal of Neuroscience*, *22*(5), 1158–1168. https://doi.org/10.1111/j.1460-9568.2005.04281.x

Caulfield, K. A. (2020). Is accelerated, high-dose theta burst stimulation a panacea for treatment-resistant depression? *Journal of Neurophysiology*, *123*(1), 1–3. https://doi.org/10.1152/jn.00537.2019

Cavaccini, A., Gritti, M., Giorgi, A., Locarno, A., Heck, N., Migliarini, S., Bertero, A., Mereu, M., Margiani, G., Trusel, M., Catelani, T., Marotta, R., De Luca, M. A., Caboche, J., Gozzi, A., Pasqualetti, M., & Tonini, R. (2018). Serotonergic Signaling Controls Input-Specific Synaptic Plasticity at Striatal Circuits. *Neuron*, *98*(4), 801-816.e7. https://doi.org/10.1016/j.neuron.2018.04.008

Chang, W. H., Kim, M. S., Cho, J. W., Youn, J., Kim, Y. K., Kim, S. W., Lee, A., & Kim, Y.-H. (2016). Effect of cumulative repetitive transcranial magnetic stimulation on freezing of gait in patients with atypical Parkinsonism: A pilot study. *Journal of Rehabilitation Medicine*, *48*(9), 824–828. https://doi.org/10.2340/16501977-2140

Chang, Y., Du, C., Han, L., Lv, S., Zhang, J., Bian, G., Tang, G., Liu, Y., Chen, T., & Liu, J. (2019). Enhanced AMPA receptor-mediated excitatory transmission in the rodent rostromedial tegmental nucleus following lesion of the nigrostriatal pathway. *Neurochemistry International*, *122*, 85–93. https://doi.org/10.1016/j.neuint.2018.11.007

Chen, J.-F., & Cunha, R. A. (2020). The belated US FDA approval of the adenosine A(2A) receptor antagonist istradefylline for treatment of Parkinson's disease. *Purinergic Signalling*, *16*(2), 167–174. https://doi.org/10.1007/s11302-020-09694-2

Chen, R., Classen, J., Gerloff, C., Celnik, P., Wassermann, E. M., Hallett, M., & Cohen, L. G. (1997). Depression of motor cortex excitability by low-frequency transcranial magnetic stimulation. *Neurology*, *48*(5), 1398–1403. https://doi.org/10.1212/wnl.48.5.1398

Choi, M. L., Chappard, A., Singh, B. P., Maclachlan, C., Rodrigues, M., Fedotova, E. I., Berezhnov, A. V, De, S., Peddie, C. J., Athauda, D., Virdi, G. S., Zhang, W., Evans, J. R., Wernick, A. I., Zanjani, Z. S., Angelova, P. R., Esteras, N., Vinokurov, A. Y., Morris, K., … Gandhi, S. (2022). Pathological structural conversion of α-synuclein at the mitochondria induces neuronal toxicity. *Nature Neuroscience*, *25*(9), 1134–1148. https://doi.org/10.1038/s41593-022-01140-3

Chotibut, T., Apple, D. M., Jefferis, R., & Salvatore, M. F. (2012). Dopamine transporter loss in 6-OHDA Parkinson's model is unmet by parallel reduction in dopamine uptake. *PloS One*, *7*(12), e52322. https://doi.org/10.1371/journal.pone.0052322

Chou, Y.-H., Ton That, V., & Sundman, M. (2020). A systematic review and meta-analysis of rTMS effects on cognitive enhancement in mild cognitive impairment and Alzheimer's disease. *Neurobiology of Aging*, *86*, 1–10. https://doi.org/10.1016/j.neurobiolaging.2019.08.020

Chung, E. K. Y., Chen, L. W., Chan, Y. S., & Yung, K. K. L. (2008). Downregulation of glial glutamate transporters after dopamine denervation in the striatum of 6-hydroxydopamine-lesioned rats. *The Journal of Comparative Neurology*, *511*(4), 421–437. https://doi.org/10.1002/cne.21852

Ciric, J., Kapor, S., Perovic, M., & Saponjic, J. (2019). Alterations of Sleep and Sleep Oscillations in the Hemiparkinsonian Rat. *Frontiers in Neuroscience*, *13*, 148. https://doi.org/10.3389/fnins.2019.00148

Cirillo, G., Di Pino, G., Capone, F., Ranieri, F., Florio, L., Todisco, V., Tedeschi, G., Funke, K., & Di Lazzaro, V. (2017). Neurobiological after-effects of non-invasive brain stimulation. *Brain Stimulation*, *10*(1), 1–18. https://doi.org/10.1016/j.brs.2016.11.009

Ciruela, F., Saura, C., Canela, E. I., Mallol, J., Lluis, C., & Franco, R. (1996). Adenosine deaminase affects ligand-induced signalling by interacting with cell surface adenosine receptors. *FEBS Letters*, *380*(3), 219–223. https://doi.org/10.1016/0014-5793(96)00023-3

Colucci-D'Amato, L., Speranza, L., & Volpicelli, F. (2020). Neurotrophic Factor BDNF, Physiological Functions and Therapeutic Potential in Depression, Neurodegeneration and Brain Cancer. *International Journal of Molecular Sciences*, *21*(20). https://doi.org/10.3390/ijms21207777

Conti Mazza, M. M., Centner, A., Werner, D. F., & Bishop, C. (2023). Striatal serotonin transporter gain-of-function in L-DOPA-treated, hemi-parkinsonian rats. *Brain Research*, *1811*, 148381. https://doi.org/10.1016/j.brainres.2023.148381

Cordes, J., Arends, M., Mobascher, A., Brinkmeyer, J., Kornischka, J., Eichhammer, P., Klimke, A., Winterer, G., & Agelink, M. W. (2006). Potential clinical targets of repetitive transcranial magnetic stimulation treatment in schizophrenia. *Neuropsychobiology*, *54*(2), 87–99. https://doi.org/10.1159/000096990

Cortés, A., Casadó-Anguera, V., Moreno, E., & Casadó, V. (2019). The heterotetrameric structure of the adenosine A(1)-dopamine D(1) receptor complex: Pharmacological implication for restless legs syndrome. *Advances in Pharmacology (San Diego, Calif.)*, *84*, 37–78. https://doi.org/10.1016/bs.apha.2019.01.001

Cotzias, G. C., Van Woert, M. H., & Schiffer, L. M. (1967). Aromatic amino acids and modification of parkinsonism. *The New England Journal of Medicine*, *276*(7), 374–379. https://doi.org/10.1056/NEJM196702162760703

Cullen, C. L., & Young, K. M. (2016). How Does Transcranial Magnetic Stimulation Influence Glial Cells in the Central Nervous System? *Frontiers in Neural Circuits*, *10*, 26. https://doi.org/10.3389/fncir.2016.00026

Cummins, L., & Cates, M. E. (2022). Istradefylline: A novel agent in the treatment of "off" episodes associated with levodopa/carbidopa use in Parkinson disease. *The Mental Health Clinician*, *12*(1), 32–36. https://doi.org/10.9740/mhc.2022.01.032

Cunha, R. A. (2016). How does adenosine control neuronal dysfunction and neurodegeneration? *Journal of Neurochemistry*, *139*(6), 1019–1055. https://doi.org/10.1111/jnc.13724

Curry, D. W., Stutz, B., Andrews, Z. B., & Elsworth, J. D. (2018). Targeting AMPK Signaling as a Neuroprotective Strategy in Parkinson's Disease. *Journal of Parkinson's Disease*, *8*(2), 161–181. https://doi.org/10.3233/JPD-171296

d'Arsonval, A. (1893). Influence de la fréquence sur les effets physiologiques des courants alternatifs. *Comptes Rendus Acad Sci Paris*, *116*, 830–833.

da Fonseca, A. C. C., Matias, D., Garcia, C., Amaral, R., Geraldo, L. H., Freitas, C., & Lima, F. R. S. (2014). The impact of microglial activation on blood-brain barrier in brain diseases.

Frontiers in Cellular Neuroscience, *8*, 362. https://doi.org/10.3389/fncel.2014.00362
da Silva, F. L., Coelho Cerqueira, E., de Freitas, M. S., Gonçalves, D. L., Costa, L. T., & Follmer, C. (2013). Vitamins K interact with N-terminus α-synuclein and modulate the protein fibrillization in vitro. Exploring the interaction between quinones and α-synuclein. *Neurochemistry International*, *62*(1), 103–112. https://doi.org/10.1016/j.neuint.2012.10.001
Dagan, M., Herman, T., Harrison, R., Zhou, J., Giladi, N., Ruffini, G., Manor, B., & Hausdorff, J. M. (2018). Multitarget transcranial direct current stimulation for freezing of gait in Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *33*(4), 642–646. https://doi.org/10.1002/mds.27300
Dantuma, N. P., & Bott, L. C. (2014). The ubiquitin-proteasome system in neurodegenerative diseases: precipitating factor, yet part of the solution. *Frontiers in Molecular Neuroscience*, *7*, 70. https://doi.org/10.3389/fnmol.2014.00070
Dauer, W., & Przedborski, S. (2003). Parkinson's disease: mechanisms and models. *Neuron*, *39*(6), 889–909. https://doi.org/10.1016/s0896-6273(03)00568-3
Del-Bel, E., Padovan-Neto, F. E., Szawka, R. E., da-Silva, C. A., Raisman-Vozari, R., Anselmo-Franci, J., Romano-Dutra, A. C., & Guimaraes, F. S. (2014). Counteraction by nitric oxide synthase inhibitor of neurochemical alterations of dopaminergic system in 6-OHDA-lesioned rats under L-DOPA treatment. *Neurotoxicity Research*, *25*(1), 33–44. https://doi.org/10.1007/s12640-013-9406-3
Del Felice, A., Bellamoli, E., Formaggio, E., Manganotti, P., Masiero, S., Cuoghi, G., Rimondo, C., Genetti, B., Sperotto, M., Corso, F., Brunetto, G., Bricolo, F., Gomma, M., & Serpelloni, G. (2016). Neurophysiological, psychological and behavioural correlates of rTMS treatment in alcohol dependence. *Drug and Alcohol Dependence*, *158*, 147–153. https://doi.org/10.1016/j.drugalcdep.2015.11.018
DeLong, M. R., & Wichmann, T. (2007). Circuits and circuit disorders of the basal ganglia. *Archives of Neurology*, *64*(1), 20–24. https://doi.org/10.1001/archneur.64.1.20
Deumens, R., Blokland, A., & Prickaerts, J. (2002). Modeling Parkinson's disease in rats: an evaluation of 6-OHDA lesions of the nigrostriatal pathway. *Experimental Neurology*, *175*(2), 303–317. https://doi.org/10.1006/exnr.2002.7891
Dexter, D. T., Carter, C. J., Wells, F. R., Javoy-Agid, F., Agid, Y., Lees, A., Jenner, P., & Marsden, C. D. (1989). Basal lipid peroxidation in substantia nigra is increased in Parkinson's disease. *Journal of Neurochemistry*, *52*(2), 381–389. https://doi.org/10.1111/j.1471-4159.1989.tb09133.x
Di Lazzaro, V, Dileone, M., Pilato, F., Capone, F., Musumeci, G., Ranieri, F., Ricci, V., Bria, P., Di Iorio, R., de Waure, C., Pasqualetti, P., & Profice, P. (2011). Modulation of motor cortex neuronal networks by rTMS: comparison of local and remote effects of six different protocols of stimulation. *Journal of Neurophysiology*, *105*(5), 2150–2156. https://doi.org/10.1152/jn.00781.2010
Di Lazzaro, Vincenzo, Ziemann, U., & Lemon, R. N. (2008). State of the art: Physiology of transcranial motor cortex stimulation. *Brain Stimulation*, *1*(4), 345–362. https://doi.org/10.1016/j.brs.2008.07.004
Di Virgilio, F., Dal Ben, D., Sarti, A. C., Giuliani, A. L., & Falzoni, S. (2017). The P2X7 Receptor in Infection and Inflammation. *Immunity*, *47*(1), 15–31. https://doi.org/10.1016/j.immuni.2017.06.020
Di Virgilio, F., Vultaggio-Poma, V., Falzoni, S., & Giuliani, A. L. (2023). Extracellular ATP: A powerful inflammatory mediator in the central nervous system. *Neuropharmacology*, *224*, 109333. https://doi.org/10.1016/j.neuropharm.2022.109333
Dias, A. E., Barbosa, E. R., Coracini, K., Maia, F., Marcolin, M. A., & Fregni, F. (2006). Effects of repetitive transcranial magnetic stimulation on voice and speech in Parkinson's disease. *Acta Neurologica Scandinavica*, *113*(2), 92–99. https://doi.org/10.1111/j.1600-

0404.2005.00558.x
Dieler, A. C., Dresler, T., Joachim, K., Deckert, J., Herrmann, M. J., & Fallgatter, A. J. (2014). Can intermittent theta burst stimulation as add-on to psychotherapy improve nicotine abstinence? Results from a pilot study. *European Addiction Research*, *20*(5), 248–253. https://doi.org/10.1159/000357941
Dileone, M., Profice, P., Pilato, F., Ranieri, F., Capone, F., Musumeci, G., Florio, L., Di Iorio, R., & Di Lazzaro, V. (2010). Repetitive transcranial magnetic stimulation for ALS. *CNS & Neurological Disorders Drug Targets*, *9*(3), 331–334. https://doi.org/10.2174/187152710791292620
Dinkova-Kostova, A. T., & Abramov, A. Y. (2015). The emerging role of Nrf2 in mitochondrial function. *Free Radical Biology & Medicine*, *88*(Pt B), 179–188. https://doi.org/10.1016/j.freeradbiomed.2015.04.036
Dixon, A. K., Gubitz, A. K., Sirinathsinghji, D. J., Richardson, P. J., & Freeman, T. C. (1996). Tissue distribution of adenosine receptor mRNAs in the rat. *British Journal of Pharmacology*, *118*(6), 1461–1468. https://doi.org/10.1111/j.1476-5381.1996.tb15561.x
Dong-Chen, X., Yong, C., Yang, X., Chen-Yu, S., & Li-Hua, P. (2023). Signaling pathways in Parkinson's disease: molecular mechanisms and therapeutic interventions. *Signal Transduction and Targeted Therapy*, *8*(1), 73. https://doi.org/10.1038/s41392-023-01353-3
Dong, Q., Wang, Y., Gu, P., Shao, R., Zhao, L., Liu, X., Wang, Z., & Wang, M. (2015). The Neuroprotective Mechanism of Low-Frequency rTMS on Nigral Dopaminergic Neurons of Parkinson's Disease Model Mice. *Parkinson's Disease*, *2015*, 564095. https://doi.org/10.1155/2015/564095
Dong, X., Wang, Y., & Qin, Z. (2009). Molecular mechanisms of excitotoxicity and their relevance to pathogenesis of neurodegenerative diseases. *Acta Pharmacologica Sinica*, *30*(4), 379–387. https://doi.org/10.1038/aps.2009.24
Dorsey, E. R., Sherer, T., Okun, M. S., & Bloem, B. R. (2018). The Emerging Evidence of the Parkinson Pandemic. *Journal of Parkinson's Disease*, *8*(s1), S3–S8. https://doi.org/10.3233/JPD-181474
Dorszewska, J., Kowalska, M., Prendecki, M., Piekut, T., Kozłowska, J., & Kozubski, W. (2021). Oxidative stress factors in Parkinson's disease. *Neural Regeneration Research*, *16*(7), 1383–1391. https://doi.org/10.4103/1673-5374.300980
Dorszewska, J., Prendecki, M., Lianeri, M., & Kozubski, W. (2014). Molecular Effects of L-dopa Therapy in Parkinson's Disease. *Current Genomics*, *15*(1), 11–17. https://doi.org/10.2174/1389202914666131210213042
Dragic, M., Mihajlovic, K., Adzic, M., Jakovljevic, M., Kontic, M. Z., Mitrović, N., Laketa, D., Lavrnja, I., Kipp, M., Grković, I., & Nedeljkovic, N. (2022). Expression of Ectonucleoside Triphosphate Diphosphohydrolase 2 (NTPDase2) Is Negatively Regulated Under Neuroinflammatory Conditions In Vivo and In Vitro. *ASN Neuro*, *14*, 17590914221102068. https://doi.org/10.1177/17590914221102068
Dragić, M., Mitrović, N., Adžić, M., Nedeljković, N., & Grković, I. (2021). Microglial- and Astrocyte-Specific Expression of Purinergic Signaling Components and Inflammatory Mediators in the Rat Hippocampus During Trimethyltin-Induced Neurodegeneration. *ASN Neuro*, *13*, 17590914211044882. https://doi.org/10.1177/17590914211044882
Dragic, M., Stekic, A., Zeljkovic, M., Zaric Kontic, M., Mihajlovic, K., Adzic, M., Grkovic, I., & Nedeljkovic, N. (2022). Altered Topographic Distribution and Enhanced Neuronal Expression of Adenosine-Metabolizing Enzymes in Rat Hippocampus and Cortex from Early to late Adulthood. *Neurochemical Research*, *47*(6), 1637–1650. https://doi.org/10.1007/s11064-022-03557-5
Dragic, M., Zeljkovic, M., Stevanovic, I., Ilic, T., Ilic, N., Nedeljkovic, N., & Ninkovic, M. (2020). Theta burst stimulation ameliorates symptoms of experimental autoimmune

encephalomyelitis and attenuates reactive gliosis. *Brain Research Bulletin*, *162*, 208–217. https://doi.org/10.1016/j.brainresbull.2020.06.013

Du, J., Yang, F., Hu, J., Hu, J., Xu, Q., Cong, N., Zhang, Q., Liu, L., Mantini, D., Zhang, Z., Lu, G., & Liu, X. (2019). Effects of high- and low-frequency repetitive transcranial magnetic stimulation on motor recovery in early stroke patients: Evidence from a randomized controlled trial with clinical, neurophysiological and functional imaging assessments. *NeuroImage. Clinical*, *21*, 101620. https://doi.org/10.1016/j.nicl.2018.101620

Dubin, M. (2017). Imaging TMS: antidepressant mechanisms and treatment optimization. *International Review of Psychiatry (Abingdon, England)*, *29*(2), 89–97. https://doi.org/10.1080/09540261.2017.1283297

Dunah, A. W., Wang, Y., Yasuda, R. P., Kameyama, K., Huganir, R. L., Wolfe, B. B., & Standaert, D. G. (2000). Alterations in subunit expression, composition, and phosphorylation of striatal N-methyl-D-aspartate glutamate receptors in a rat 6-hydroxydopamine model of Parkinson's disease. *Molecular Pharmacology*, *57*(2), 342–352.

Dungo, R., & Deeks, E. D. (2013). Istradefylline: first global approval. *Drugs*, *73*(8), 875–882. https://doi.org/10.1007/s40265-013-0066-7

Dwyer, J. M., & Duman, R. S. (2013). Activation of mammalian target of rapamycin and synaptogenesis: role in the actions of rapid-acting antidepressants. *Biological Psychiatry*, *73*(12), 1189–1198. https://doi.org/10.1016/j.biopsych.2012.11.011

Edwards, M. J., Talelli, P., & Rothwell, J. C. (2008). Clinical applications of transcranial magnetic stimulation in patients with movement disorders. *The Lancet. Neurology*, *7*(9), 827–840. https://doi.org/10.1016/S1474-4422(08)70190-X

Elahi, B., Elahi, B., & Chen, R. (2009). Effect of transcranial magnetic stimulation on Parkinson motor function--systematic review of controlled clinical trials. *Movement Disorders : Official Journal of the Movement Disorder Society*, *24*(3), 357–363. https://doi.org/10.1002/mds.22364

Espada, S., Ortega, F., Molina-Jijón, E., Rojo, A. I., Pérez-Sen, R., Pedraza-Chaverri, J., Miras-Portugal, M. T., & Cuadrado, A. (2010). The purinergic P2Y(13) receptor activates the Nrf2/HO-1 axis and protects against oxidative stress-induced neuronal death. *Free Radical Biology & Medicine*, *49*(3), 416–426. https://doi.org/10.1016/j.freeradbiomed.2010.04.031

Feng, H., Yan, L., & Cui, L. (2008). Effects of repetitive transcranial magnetic stimulation on adenosine triphosphate content and microtubule associated protein-2 expression after cerebral ischemia-reperfusion injury in rat brain. *Chinese Medical Journal*, *121*(14), 1307–1312.

Fernández-Dueñas, V., Gómez-Soler, M., Valle-León, M., Watanabe, M., Ferrer, I., & Ciruela, F. (2019). Revealing Adenosine A(2A)-Dopamine D(2) Receptor Heteromers in Parkinson's Disease Post-Mortem Brain through a New AlphaScreen-Based Assay. *International Journal of Molecular Sciences*, *20*(14). https://doi.org/10.3390/ijms20143600

Ferre, S., von Euler, G., Johansson, B., Fredholm, B. B., & Fuxe, K. (1991). Stimulation of high-affinity adenosine A2 receptors decreases the affinity of dopamine D2 receptors in rat striatal membranes. *Proceedings of the National Academy of Sciences of the United States of America*, *88*(16), 7238–7241. https://doi.org/10.1073/pnas.88.16.7238

Ferré, S, Torvinen, M., Antoniou, K., Irenius, E., Civelli, O., Arenas, E., Fredholm, B. B., & Fuxe, K. (1998). Adenosine A1 receptor-mediated modulation of dopamine D1 receptors in stably cotransfected fibroblast cells. *The Journal of Biological Chemistry*, *273*(8), 4718–4724. https://doi.org/10.1074/jbc.273.8.4718

Ferré, Sergi, Ciruela, F., Quiroz, C., Luján, R., Popoli, P., Cunha, R. A., Agnati, L. F., Fuxe, K., Woods, A. S., Lluis, C., & Franco, R. (2007). Adenosine receptor heteromers and their integrative role in striatal function. *TheScientificWorldJournal*, *7*, 74–85. https://doi.org/10.1100/tsw.2007.211

Ferreira, J. J., Katzenschlager, R., Bloem, B. R., Bonuccelli, U., Burn, D., Deuschl, G., Dietrichs, E., Fabbrini, G., Friedman, A., Kanovsky, P., Kostic, V., Nieuwboer, A., Odin, P., Poewe, W., Rascol, O., Sampaio, C., Schüpbach, M., Tolosa, E., Trenkwalder, C., … Oertel, W. H. (2013). Summary of the recommendations of the EFNS/MDS-ES review on therapeutic management of Parkinson's disease. *European Journal of Neurology*, *20*(1), 5–15. https://doi.org/10.1111/j.1468-1331.2012.03866.x

Ferreira, S. A., Pinto, N., Serrenho, I., Pato, M. V., & Baltazar, G. (2024). Contribution of glial cells to the neuroprotective effects triggered by repetitive magnetic stimulation: a systematic review. *Neural Regeneration Research*, *19*(1), 116–123. https://doi.org/10.4103/1673-5374.374140

Ferro, M., Lamanna, J., Spadini, S., Nespoli, A., Sulpizio, S., & Malgaroli, A. (2022). Synaptic plasticity mechanisms behind TMS efficacy: insights from its application to animal models. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *129*(1), 25–36. https://doi.org/10.1007/s00702-021-02436-7

Figura, M., Kuśmierska, K., Bucior, E., Szlufik, S., Koziorowski, D., Jamrozik, Z., & Janik, P. (2018). Serum amino acid profile in patients with Parkinson's disease. *PloS One*, *13*(1), e0191670. https://doi.org/10.1371/journal.pone.0191670

Filipović, S. R., Rothwell, J. C., van de Warrenburg, B. P., & Bhatia, K. (2009). Repetitive transcranial magnetic stimulation for levodopa-induced dyskinesias in Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *24*(2), 246–253. https://doi.org/10.1002/mds.22348

Fornaretto, M. G., Caccia, C., Caron, M. G., & Fariello, R. G. (1993). Dopamine receptors status after unilateral nigral 6-OHDA lesion. Autoradiographic and in situ hybridization study in the rat brain. *Molecular and Chemical Neuropathology*, *19*(1–2), 147–162. https://doi.org/10.1007/BF03160175

Franco, R., Reyes-Resina, I., & Navarro, G. (2021). Dopamine in Health and Disease: Much More Than a Neurotransmitter. *Biomedicines*, *9*(2). https://doi.org/10.3390/biomedicines9020109

Fredholm, B. B., IJzerman, A. P., Jacobson, K. A., Linden, J., & Müller, C. E. (2011). International Union of Basic and Clinical Pharmacology. LXXXI. Nomenclature and classification of adenosine receptors--an update. *Pharmacological Reviews*, *63*(1), 1–34. https://doi.org/10.1124/pr.110.003285

Fregni, F., Simon, D. K., Wu, A., & Pascual-Leone, A. (2005). Non-invasive brain stimulation for Parkinson's disease: a systematic review and meta-analysis of the literature. *Journal of Neurology, Neurosurgery, and Psychiatry*, *76*(12), 1614–1623. https://doi.org/10.1136/jnnp.2005.069849

Frenguelli, B. G. (2019). The Purine Salvage Pathway and the Restoration of Cerebral ATP: Implications for Brain Slice Physiology and Brain Injury. *Neurochemical Research*, *44*(3), 661–675. https://doi.org/10.1007/s11064-017-2386-6

Fujiki, M., Kobayashi, H., Abe, T., & Kamida, T. (2003). Repetitive transcranial magnetic stimulation for protection against delayed neuronal death induced by transient ischemia. *Journal of Neurosurgery*, *99*(6), 1063–1069. https://doi.org/10.3171/jns.2003.99.6.1063

Fukuda, A. M., Hindley, L. E., Kang, J. W. D., Tirrell, E., Tyrka, A. R., Ayala, A., & Carpenter, L. L. (2020). Peripheral vascular endothelial growth factor changes after transcranial magnetic stimulation in treatment-resistant depression. *Neuroreport*, *31*(16), 1121–1127. https://doi.org/10.1097/WNR.0000000000001523

Fuxe, K., Ferré, S., Canals, M., Torvinen, M., Terasmaa, A., Marcellino, D., Goldberg, S. R., Staines, W., Jacobsen, K. X., Lluis, C., Woods, A. S., Agnati, L. F., & Franco, R. (2005). Adenosine A2A and dopamine D2 heteromeric receptor complexes and their function. *Journal of Molecular Neuroscience : MN*, *26*(2–3), 209–220. https://doi.org/10.1385/JMN:26:2-3:209

Gan, J., Qi, C., Mao, L.-M., & Liu, Z. (2014). Changes in surface expression of N-methyl-D-aspartate receptors in the striatum in a rat model of Parkinson's disease. *Drug Design, Development and Therapy*, *8*, 165–173. https://doi.org/10.2147/DDDT.S51559

Gerfen, C R, Engber, T. M., Mahan, L. C., Susel, Z., Chase, T. N., Monsma, F. J. J., & Sibley, D. R. (1990). D1 and D2 dopamine receptor-regulated gene expression of striatonigral and striatopallidal neurons. *Science (New York, N.Y.)*, *250*(4986), 1429–1432. https://doi.org/10.1126/science.2147780

Gerfen, Charles R. (2022). Segregation of D1 and D2 dopamine receptors in the striatal direct and indirect pathways: An historical perspective. *Frontiers in Synaptic Neuroscience*, *14*, 1002960. https://doi.org/10.3389/fnsyn.2022.1002960

Gersner, R., Kravetz, E., Feil, J., Pell, G., & Zangen, A. (2011). Long-term effects of repetitive transcranial magnetic stimulation on markers for neuroplasticity: differential outcomes in anesthetized and awake animals. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(20), 7521–7526. https://doi.org/10.1523/JNEUROSCI.6751-10.2011

Gesi, M., Santinami, A., Ruffoli, R., Conti, G., & Fornai, F. (2001). Novel aspects of dopamine oxidative metabolism (confounding outcomes take place of certainties). *Pharmacology & Toxicology*, *89*(5), 217–224. https://doi.org/10.1034/j.1600-0773.2001.d01-151.x

Gessi, S., Merighi, S., Fazzi, D., Stefanelli, A., Varani, K., & Borea, P. A. (2011). Adenosine receptor targeting in health and disease. *Expert Opinion on Investigational Drugs*, *20*(12), 1591–1609. https://doi.org/10.1517/13543784.2011.627853

Girotto, S., Sturlese, M., Bellanda, M., Tessari, I., Cappellini, R., Bisaglia, M., Bubacco, L., & Mammi, S. (2012). Dopamine-derived quinones affect the structure of the redox sensor DJ-1 through modifications at Cys-106 and Cys-53. *The Journal of Biological Chemistry*, *287*(22), 18738–18749. https://doi.org/10.1074/jbc.M111.311589

Giuliani, A. L., Sarti, A. C., & Di Virgilio, F. (2020). Ectonucleotidases in Acute and Chronic Inflammation. *Frontiers in Pharmacology*, *11*, 619458. https://doi.org/10.3389/fphar.2020.619458

Glajch, K. E., Fleming, S. M., Surmeier, D. J., & Osten, P. (2012). Sensorimotor assessment of the unilateral 6-hydroxydopamine mouse model of Parkinson's disease. *Behavioural Brain Research*, *230*(2), 309–316. https://doi.org/10.1016/j.bbr.2011.12.007

Goldmann, T., Wieghofer, P., Jordão, M. J. C., Prutek, F., Hagemeyer, N., Frenzel, K., Amann, L., Staszewski, O., Kierdorf, K., Krueger, M., Locatelli, G., Hochgerner, H., Zeiser, R., Epelman, S., Geissmann, F., Priller, J., Rossi, F. M. V, Bechmann, I., Kerschensteiner, M., … Prinz, M. (2016). Origin, fate and dynamics of macrophages at central nervous system interfaces. *Nature Immunology*, *17*(7), 797–805. https://doi.org/10.1038/ni.3423

Gomes, C. V, Kaster, M. P., Tomé, A. R., Agostinho, P. M., & Cunha, R. A. (2011). Adenosine receptors and brain diseases: neuroprotection and neurodegeneration. *Biochimica et Biophysica Acta*, *1808*(5), 1380–1399. https://doi.org/10.1016/j.bbamem.2010.12.001

Gonçalves, F. Q., Matheus, F. C., Silva, H. B., Real, J. I., Rial, D., Rodrigues, R. J., Oses, J.-P., Silva, A. C., Gonçalves, N., Prediger, R. D., Tomé, Â. R., & Cunha, R. A. (2023). Increased ATP Release and Higher Impact of Adenosine A(2A) Receptors on Corticostriatal Plasticity in a Rat Model of Presymptomatic Parkinson's Disease. *Molecular Neurobiology*, *60*(3), 1659–1674. https://doi.org/10.1007/s12035-022-03162-1

Götz, T., Kraushaar, U., Geiger, J., Lübke, J., Berger, T., & Jonas, P. (1997). Functional properties of AMPA and NMDA receptors expressed in identified types of basal ganglia neurons. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *17*(1), 204–215. https://doi.org/10.1523/JNEUROSCI.17-01-00204.1997

Grandi, L. C., Di Giovanni, G., & Galati, S. (2018). Animal models of early-stage Parkinson's disease and acute dopamine deficiency to study compensatory neurodegenerative mechanisms. *Journal of Neuroscience Methods*, *308*, 205–218.

https://doi.org/10.1016/j.jneumeth.2018.08.012
Gröger, A., Kolb, R., Schäfer, R., & Klose, U. (2014). Dopamine reduction in the substantia nigra of Parkinson's disease patients confirmed by in vivo magnetic resonance spectroscopic imaging. *PloS One*, *9*(1), e84081. https://doi.org/10.1371/journal.pone.0084081
Groiss, S. J., Wojtecki, L., Südmeyer, M., & Schnitzler, A. (2009). Deep brain stimulation in Parkinson's disease. *Therapeutic Advances in Neurological Disorders*, *2*(6), 20–28. https://doi.org/10.1177/1756285609339382
Groppa, S., Oliviero, A., Eisen, A., Quartarone, A., Cohen, L. G., Mall, V., Kaelin-Lang, A., Mima, T., Rossi, S., Thickbroom, G. W., Rossini, P. M., Ziemann, U., Valls-Solé, J., & Siebner, H. R. (2012). A practical guide to diagnostic transcranial magnetic stimulation: Report of an IFCN committee. *Clinical Neurophysiology*, *123*(5), 858–882. https://doi.org/10.1016/j.clinph.2012.01.010
Guo, F., Lou, J., Han, X., Deng, Y., & Huang, X. (2017). Repetitive Transcranial Magnetic Stimulation Ameliorates Cognitive Impairment by Enhancing Neurogenesis and Suppressing Apoptosis in the Hippocampus in Rats with Ischemic Stroke. *Frontiers in Physiology*, *8*, 559. https://doi.org/10.3389/fphys.2017.00559
Hald, A., & Lotharius, J. (2005). Oxidative stress and inflammation in Parkinson's disease: is there a causal link? *Experimental Neurology*, *193*(2), 279–290. https://doi.org/10.1016/j.expneurol.2005.01.013
Hallett, M. (2007). Transcranial Magnetic Stimulation: A Primer. *Neuron*, *55*(2), 187–199. https://doi.org/10.1016/j.neuron.2007.06.026
Hamada, M., Ugawa, Y., & Tsuji, S. (2008). High-frequency rTMS over the supplementary motor area for treatment of Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *23*(11), 1524–1531. https://doi.org/10.1002/mds.22168
Hassel, B., & Dingledine, R. (2012). Glutamate and glutamate receptors. In *Basic neurochemistry* (pp. 342–366). Elsevier.
Hauser, R. A. (2009). Levodopa: past, present, and future. *European Neurology*, *62*(1), 1–8. https://doi.org/10.1159/000215875
He, F., Ru, X., & Wen, T. (2020). NRF2, a Transcription Factor for Stress Response and Beyond. *International Journal of Molecular Sciences*, *21*(13). https://doi.org/10.3390/ijms21134777
Hernandez-Baltazar, D., Zavala-Flores, L. M., & Villanueva-Olivo, A. (2017). The 6-hydroxydopamine model and parkinsonian pathophysiology: Novel findings in an older model. *Neurologia (Barcelona, Spain)*, *32*(8), 533–539. https://doi.org/10.1016/j.nrl.2015.06.011
Herring, W. J., Assaid, C., Budd, K., Vargo, R., Mazenko, R. S., Lines, C., Ellenbogen, A., & Verhagen Metman, L. (2017). A Phase Ib Randomized Controlled Study to Evaluate the Effectiveness of a Single-Dose of the NR2B Selective N-Methyl-D-Aspartate Antagonist MK-0657 on Levodopa-Induced Dyskinesias and Motor Symptoms in Patients With Parkinson Disease. *Clinical Neuropharmacology*, *40*(6), 255–260. https://doi.org/10.1097/WNF.0000000000000241
Herweg, N. A., Solomon, E. A., & Kahana, M. J. (2020). Theta Oscillations in Human Memory. *Trends in Cognitive Sciences*, *24*(3), 208–227. https://doi.org/10.1016/j.tics.2019.12.006
Höllerhage, M. (2019). Secondary parkinsonism due to drugs, vascular lesions, tumors, trauma, and other insults. *International Review of Neurobiology*, *149*, 377–418. https://doi.org/10.1016/bs.irn.2019.10.010
Holtzheimer, P. E. 3rd, McDonald, W. M., Mufti, M., Kelley, M. E., Quinn, S., Corso, G., & Epstein, C. M. (2010). Accelerated repetitive transcranial magnetic stimulation for treatment-resistant depression. *Depression and Anxiety*, *27*(10), 960–963. https://doi.org/10.1002/da.20731
Hong, J., Chen, J., Li, C., An, D., Tang, Z., & Wen, H. (2021). High-Frequency rTMS Improves

Cognitive Function by Regulating Synaptic Plasticity in Cerebral Ischemic Rats. *Neurochemical Research*, *46*(2), 276–286. https://doi.org/10.1007/s11064-020-03161-5

Hoogendam, J. M., Ramakers, G. M. J., & Di Lazzaro, V. (2010). Physiology of repetitive transcranial magnetic stimulation of the human brain. *Brain Stimulation*, *3*(2), 95–118. https://doi.org/10.1016/j.brs.2009.10.005

Horimoto, Y., Sato, C., Inagaki, A., Hayashi, E., Nozue, T., Morita, S., Kondo, Y., Funaki, M., Iida, A., Tajima, T., Hibino, H., Yamada, K., & Kabasawa, H. (2022). Effects of repetitive transcranial magnetic stimulation on cerebral glucose metabolism. *Neurological Sciences : Official Journal of the Italian Neurological Society and of the Italian Society of Clinical Neurophysiology*, *43*(3), 1879–1883. https://doi.org/10.1007/s10072-021-05539-x

Hu, X., Ballo, L., Pietila, L., Viesselmann, C., Ballweg, J., Lumbard, D., Stevenson, M., Merriam, E., & Dent, E. W. (2011). BDNF-induced increase of PSD-95 in dendritic spines requires dynamic microtubule invasions. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(43), 15597–15603. https://doi.org/10.1523/JNEUROSCI.2445-11.2011

Huang, W., Xu, Y., Zhang, Y., Zhang, P., Zhang, Q., Zhang, Z., & Xu, F. (2019). Metabolomics-driven identification of adenosine deaminase as therapeutic target in a mouse model of Parkinson's disease. *Journal of Neurochemistry*, *150*(3), 282–295. https://doi.org/10.1111/jnc.14774

Huang, Y.-Z., Edwards, M. J., Rounis, E., Bhatia, K. P., & Rothwell, J. C. (2005). Theta burst stimulation of the human motor cortex. *Neuron*, *45*(2), 201–206. https://doi.org/10.1016/j.neuron.2004.12.033

Huang, Y.-Z., & Rothwell, J. C. (2004). The effect of short-duration bursts of high-frequency, low-intensity transcranial magnetic stimulation on the human motor cortex. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *115*(5), 1069–1075. https://doi.org/10.1016/j.clinph.2003.12.026

Huerta, P. T., & Volpe, B. T. (2009). Transcranial magnetic stimulation, synaptic plasticity and network oscillations. *Journal of NeuroEngineering and Rehabilitation*, *6*(1), 7. https://doi.org/10.1186/1743-0003-6-7

Hunt, D. L., & Castillo, P. E. (2012). Synaptic plasticity of NMDA receptors: mechanisms and functional implications. *Current Opinion in Neurobiology*, *22*(3), 496–508. https://doi.org/10.1016/j.conb.2012.01.007

Hunt, J., Coulson, E. J., Rajnarayanan, R., Oster, H., Videnovic, A., & Rawashdeh, O. (2022). Sleep and circadian rhythms in Parkinson's disease and preclinical models. *Molecular Neurodegeneration*, *17*(1), 2. https://doi.org/10.1186/s13024-021-00504-w

Hwang, O. (2013). Role of oxidative stress in Parkinson's disease. *Experimental Neurobiology*, *22*(1), 11–17. https://doi.org/10.5607/en.2013.22.1.11

Iezzi, E., Suppa, A., Conte, A., Li Voti, P., Bologna, M., & Berardelli, A. (2011). Short-term and long-term plasticity interaction in human primary motor cortex. *The European Journal of Neuroscience*, *33*(10), 1908–1915. https://doi.org/10.1111/j.1460-9568.2011.07674.x

Illes, P., Rubini, P., Ulrich, H., Zhao, Y., & Tang, Y. (2020). Regulation of Microglial Functions by Purinergic Mechanisms in the Healthy and Diseased CNS. *Cells*, *9*(5). https://doi.org/10.3390/cells9051108

Ingham, C. A., Hood, S. H., Taggart, P., & Arbuthnott, G. W. (1998). Plasticity of synapses in the rat neostriatum after unilateral lesion of the nigrostriatal dopaminergic pathway. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *18*(12), 4732–4743. https://doi.org/10.1523/JNEUROSCI.18-12-04732.1998

Iwasaki, Y., Ikeda, K., Shiojima, T., & Kinoshita, M. (1992). Increased plasma concentrations of aspartate, glutamate and glycine in Parkinson's disease. *Neuroscience Letters*, *145*(2), 175–177. https://doi.org/10.1016/0304-3940(92)90015-y

Jana, S., Sinha, M., Chanda, D., Roy, T., Banerjee, K., Munshi, S., Patro, B. S., & Chakrabarti, S. (2011). Mitochondrial dysfunction mediated by quinone oxidation products of dopamine: Implications in dopamine cytotoxicity and pathogenesis of Parkinson's disease. *Biochimica et Biophysica Acta*, *1812*(6), 663–673. https://doi.org/10.1016/j.bbadis.2011.02.013

Janks, L., Sharma, C. V. R., & Egan, T. M. (2018). A central role for P2X7 receptors in human microglia. *Journal of Neuroinflammation*, *15*(1), 325. https://doi.org/10.1186/s12974-018-1353-8

Jeon, B. S., Jackson-Lewis, V., & Burke, R. E. (1995). 6-Hydroxydopamine lesion of the rat substantia nigra: time course and morphology of cell death. *Neurodegeneration : A Journal for Neurodegenerative Disorders, Neuroprotection, and Neuroregeneration*, *4*(2), 131–137. https://doi.org/10.1006/neur.1995.0016

Jiang, T., Hoekstra, J., Heng, X., Kang, W., Ding, J., Liu, J., Chen, S., & Zhang, J. (2015). P2X7 receptor is critical in α-synuclein--mediated microglial NADPH oxidase activation. *Neurobiology of Aging*, *36*(7), 2304–2318. https://doi.org/10.1016/j.neurobiolaging.2015.03.015

Jiang, Y., Guo, Z., McClure, M. A., He, L., & Mu, Q. (2020). Effect of rTMS on Parkinson's cognitive function: a systematic review and meta-analysis. *BMC Neurology*, *20*(1), 377. https://doi.org/10.1186/s12883-020-01953-4

Johnson, K. A., Conn, P. J., & Niswender, C. M. (2009). Glutamate receptors as therapeutic targets for Parkinson's disease. *CNS & Neurological Disorders Drug Targets*, *8*(6), 475–491. https://doi.org/10.2174/187152709789824606

Kalia, L. V, & Lang, A. E. (2015). Parkinson's disease. *Lancet (London, England)*, *386*(9996), 896–912. https://doi.org/10.1016/S0140-6736(14)61393-3

Kalinderi, K., Bostantjopoulou, S., & Fidani, L. (2016). The genetic background of Parkinson's disease: current progress and future prospects. *Acta Neurologica Scandinavica*, *134*(5), 314–326. https://doi.org/10.1111/ane.12563

Kammer, T., Beck, S., Thielscher, A., Laubis-Herrmann, U., & Topka, H. (2001). Motor thresholds in humans: A transcranial magnetic stimulation study comparing different pulse waveforms, current directions and stimulator types. *Clinical Neurophysiology*, *112*(2), 250–258. https://doi.org/10.1016/S1388-2457(00)00513-7

Kanno, M., Matsumoto, M., Togashi, H., Yoshioka, M., & Mano, Y. (2004). Effects of acute repetitive transcranial magnetic stimulation on dopamine release in the rat dorsolateral striatum. *Journal of the Neurological Sciences*, *217*(1), 73–81. https://doi.org/10.1016/j.jns.2003.08.013

Keck, M. E., Welt, T., Müller, M. B., Erhardt, A., Ohl, F., Toschi, N., Holsboer, F., & Sillaber, I. (2002). Repetitive transcranial magnetic stimulation increases the release of dopamine in the mesolimbic and mesostriatal system. *Neuropharmacology*, *43*(1), 101–109. https://doi.org/10.1016/s0028-3908(02)00069-2

Keener, A. M., & Bordelon, Y. M. (2016). Parkinsonism. *Seminars in Neurology*, *36*(4), 330–334. https://doi.org/10.1055/s-0036-1585097

Khan, Z., & Ali, S. A. (2018). Oxidative stress-related biomarkers in Parkinson's disease: A systematic review and meta-analysis. *Iranian Journal of Neurology*, *17*(3), 137–144.

Khedr, E. M., Rothwell, J. C., Shawky, O. A., Ahmed, M. A., Foly, N., & Hamdy, A. (2007). Dopamine levels after repetitive transcranial magnetic stimulation of motor cortex in patients with Parkinson's disease: preliminary results. *Movement Disorders : Official Journal of the Movement Disorder Society*, *22*(7), 1046–1050. https://doi.org/10.1002/mds.21460

Kim, J. Y., Choi, G.-S., Cho, Y.-W., Cho, H., Hwang, S.-J., & Ahn, S.-H. (2013). Attenuation of spinal cord injury-induced astroglial and microglial activation by repetitive transcranial magnetic stimulation in rats. *Journal of Korean Medical Science*, *28*(2), 295–299.

https://doi.org/10.3346/jkms.2013.28.2.295
King, E. S., & Tang, A. D. (2024). Intrinsic Plasticity Mechanisms of Repetitive Transcranial Magnetic Stimulation. *The Neuroscientist : A Review Journal Bringing Neurobiology, Neurology and Psychiatry*, *30*(2), 260–274. https://doi.org/10.1177/10738584221118262
Kirik, D., Rosenblad, C., & Björklund, A. (1998). Characterization of behavioral and neurodegenerative changes following partial lesions of the nigrostriatal dopamine system induced by intrastriatal 6-hydroxydopamine in the rat. *Experimental Neurology*, *152*(2), 259–277. https://doi.org/10.1006/exnr.1998.6848
Kish, S. J., Tong, J., Hornykiewicz, O., Rajput, A., Chang, L.-J., Guttman, M., & Furukawa, Y. (2008). Preferential loss of serotonin markers in caudate versus putamen in Parkinson's disease. *Brain : A Journal of Neurology*, *131*(Pt 1), 120–131. https://doi.org/10.1093/brain/awm239
Ko, J. H., Monchi, O., Ptito, A., Bloomfield, P., Houle, S., & Strafella, A. P. (2008). Theta burst stimulation-induced inhibition of dorsolateral prefrontal cortex reveals hemispheric asymmetry in striatal dopamine release during a set-shifting task: a TMS-[(11)C]raclopride PET study. *The European Journal of Neuroscience*, *28*(10), 2147–2155. https://doi.org/10.1111/j.1460-9568.2008.06501.x
Kobayashi, M., & Pascual-Leone, A. (2003). Transcranial magnetic stimulation in neurology. *Lancet Neurology*, *2*(3), 145–156. https://doi.org/10.1016/S1474-4422(03)00321-1
Kolin, A., Brill, N. Q., & Broberg, P. J. (1959). Stimulation of irritable tissues by means of an alternating magnetic field. *Proceedings of the Society for Experimental Biology and Medicine*, *102*(1), 251–253.
Koponen, L. M., Goetz, S. M., Tucci, D. L., & Peterchev, A. V. (2020). Sound comparison of seven TMS coils at matched stimulation strength. *Brain Stimulation*, *13*(3), 873–880. https://doi.org/10.1016/j.brs.2020.03.004
Koponen, L. M., Stenroos, M., Nieminen, J. O., Jokivarsi, K., Gröhn, O., & Ilmoniemi, R. J. (2020). Individual head models for estimating the TMS-induced electric field in rat brain. *Scientific Reports*, *10*(1), 17397. https://doi.org/10.1038/s41598-020-74431-z
Kuboyama, K., Harada, H., Tozaki-Saitoh, H., Tsuda, M., Ushijima, K., & Inoue, K. (2011). Astrocytic P2Y(1) receptor is involved in the regulation of cytokine/chemokine transcription and cerebral damage in a rat model of cerebral ischemia. *Journal of Cerebral Blood Flow and Metabolism : Official Journal of the International Society of Cerebral Blood Flow and Metabolism*, *31*(9), 1930–1941. https://doi.org/10.1038/jcbfm.2011.49
Kuhn, D. M., Arthur, R. E. J., Thomas, D. M., & Elferink, L. A. (1999). Tyrosine hydroxylase is inactivated by catechol-quinones and converted to a redox-cycling quinoprotein: possible relevance to Parkinson's disease. *Journal of Neurochemistry*, *73*(3), 1309–1317. https://doi.org/10.1046/j.1471-4159.1999.0731309.x
Kuter, K., Olech, Ł., & Głowacka, U. (2018). Prolonged Dysfunction of Astrocytes and Activation of Microglia Accelerate Degeneration of Dopaminergic Neurons in the Rat Substantia Nigra and Block Compensation of Early Motor Dysfunction Induced by 6-OHDA. *Molecular Neurobiology*, *55*(4), 3049–3066. https://doi.org/10.1007/s12035-017-0529-z
Kyrargyri, V., Madry, C., Rifat, A., Arancibia-Carcamo, I. L., Jones, S. P., Chan, V. T. T., Xu, Y., Robaye, B., & Attwell, D. (2020). P2Y(13) receptors regulate microglial morphology, surveillance, and resting levels of interleukin 1β release. *Glia*, *68*(2), 328–344. https://doi.org/10.1002/glia.23719
Labedi, A., Benali, A., Mix, A., Neubacher, U., & Funke, K. (2014). Modulation of inhibitory activity markers by intermittent theta-burst stimulation in rat cortex is NMDA-receptor dependent. *Brain Stimulation*, *7*(3), 394–400. https://doi.org/10.1016/j.brs.2014.02.010
Ladagu, A. D., Olopade, F. E., Adejare, A., & Olopade, J. O. (2023). GluN2A and GluN2B N-Methyl-D-Aspartate Receptor (NMDARs) Subunits: Their Roles and Therapeutic

Antagonists in Neurological Diseases. *Pharmaceuticals (Basel, Switzerland)*, *16*(11). https://doi.org/10.3390/ph16111535

Lan, L., Zhang, X., Li, X., Rong, X., & Peng, Y. (2017). The efficacy of transcranial magnetic stimulation on migraine: a meta-analysis of randomized controlled trails. *The Journal of Headache and Pain*, *18*(1), 86. https://doi.org/10.1186/s10194-017-0792-4

Lanciego, J. L., Luquin, N., & Obeso, J. A. (2012). Functional neuroanatomy of the basal ganglia. *Cold Spring Harbor Perspectives in Medicine*, *2*(12), a009621. https://doi.org/10.1101/cshperspect.a009621

Langston, J. W., Ballard, P., Tetrud, J. W., & Irwin, I. (1983). Chronic Parkinsonism in humans due to a product of meperidine-analog synthesis. *Science (New York, N.Y.)*, *219*(4587), 979–980. https://doi.org/10.1126/science.6823561

Lau, A., & Tymianski, M. (2010). Glutamate receptors, neurotoxicity and neurodegeneration. *Pflugers Archiv : European Journal of Physiology*, *460*(2), 525–542. https://doi.org/10.1007/s00424-010-0809-1

Lauterborn, J. C., Pineda, E., Chen, L. Y., Ramirez, E. A., Lynch, G., & Gall, C. M. (2009). Ampakines cause sustained increases in brain-derived neurotrophic factor signaling at excitatory synapses without changes in AMPA receptor subunit expression. *Neuroscience*, *159*(1), 283–295. https://doi.org/10.1016/j.neuroscience.2008.12.018

Leal, G., Bramham, C. R., & Duarte, C. B. (2017). BDNF and Hippocampal Synaptic Plasticity. *Vitamins and Hormones*, *104*, 153–195. https://doi.org/10.1016/bs.vh.2016.10.004

Lee, C.-W., Wu, H.-F., Chu, M.-C., Chung, Y.-J., Mao, W.-C., Li, C.-T., & Lin, H.-C. (2021). Mechanism of Intermittent Theta-Burst Stimulation in Synaptic Pathology in the Prefrontal Cortex in an Antidepressant-Resistant Depression Rat Model. *Cerebral Cortex (New York, N.Y. : 1991)*, *31*(1), 575–590. https://doi.org/10.1093/cercor/bhaa244

Lee, J. Y., Kim, H. S., Kim, S. H., Kim, H.-S., & Cho, B. P. (2020). Combination of Human Mesenchymal Stem Cells and Repetitive Transcranial Magnetic Stimulation Enhances Neurological Recovery of 6-Hydroxydopamine Model of Parkinsonian's Disease. *Tissue Engineering and Regenerative Medicine*, *17*(1), 67–80. https://doi.org/10.1007/s13770-019-00233-8

Lee, J. Y., Kim, S. H., Ko, A.-R., Lee, J. S., Yu, J. H., Seo, J. H., Cho, B. P., & Cho, S.-R. (2013). Therapeutic effects of repetitive transcranial magnetic stimulation in an animal model of Parkinson's disease. *Brain Research*, *1537*, 290–302. https://doi.org/10.1016/j.brainres.2013.08.051

Leek, A. N., Quinn, J. A., Krapf, D., & Tamkun, M. M. (2024). GLT-1a glutamate transporter nanocluster localization is associated with astrocytic actin and neuronal Kv2 clusters at sites of neuron-astrocyte contact. *Frontiers in Cell and Developmental Biology*, *12*, 1334861. https://doi.org/10.3389/fcell.2024.1334861

Leeson, H. C., Kasherman, M. A., Chan-Ling, T., Lovelace, M. D., Brownlie, J. C., Toppinen, K. M., Gu, B. J., & Weible, M. W. 2nd. (2018). P2X7 Receptors Regulate Phagocytosis and Proliferation in Adult Hippocampal and SVZ Neural Progenitor Cells: Implications for Inflammation in Neurogenesis. *Stem Cells (Dayton, Ohio)*, *36*(11), 1764–1777. https://doi.org/10.1002/stem.2894

Levy, R., & Dubois, B. (2006). Apathy and the functional anatomy of the prefrontal cortex-basal ganglia circuits. *Cerebral Cortex (New York, N.Y. : 1991)*, *16*(7), 916–928. https://doi.org/10.1093/cercor/bhj043

Li, Ping, Li, Y.-H., & Han, T.-Z. (2009). NR2A-containing NMDA receptors are required for LTP induction in rat dorsolateral striatum in vitro. *Brain Research*, *1274*, 40–46. https://doi.org/10.1016/j.brainres.2009.04.016

Li, Puyu, Luo, N., Sun, S., Li, Y., Shen, D., Zhu, X., Zhou, L., Zhou, H., & Liu, J. (2022). Neuroprotective Effects of Intermittent Theta Burst Stimulation in Parkinson's Disease (NET-PD): A Study Protocol for a Delayed-Start Randomized Double-Blind Sham-

Controlled Trial. *Journal of Clinical Medicine*, *11*(17). https://doi.org/10.3390/jcm11174972

Liang, H., Xu, C., Hu, S., Wen, G., Lin, J., Liu, T., & Xu, J. (2021). Repetitive Transcranial Magnetic Stimulation Improves Neuropathy and Oxidative Stress Levels in Rats with Experimental Cerebral Infarction through the Nrf2 Signaling Pathway. *Evidence-Based Complementary and Alternative Medicine : ECAM*, *2021*, 3908677. https://doi.org/10.1155/2021/3908677

Liu, W., Wang, X., O'Connor, M., Wang, G., & Han, F. (2020). Brain-Derived Neurotrophic Factor and Its Potential Therapeutic Role in Stroke Comorbidities. *Neural Plasticity*, *2020*, 1969482. https://doi.org/10.1155/2020/1969482

López-Otín, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell*, *153*(6), 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

Löschmann, P.-A., De Groote, C., Smith, L., Wüllner, U., Fischer, G., Kemp, J. A., Jenner, P., & Klockgether, T. (2004). Antiparkinsonian activity of Ro 25-6981, a NR2B subunit specific NMDA receptor antagonist, in animal models of Parkinson's disease. *Experimental Neurology*, *187*(1), 86–93. https://doi.org/10.1016/j.expneurol.2004.01.018

Luo, J., Zheng, H., Zhang, L., Zhang, Q., Li, L., Pei, Z., & Hu, X. (2017). High-Frequency Repetitive Transcranial Magnetic Stimulation (rTMS) Improves Functional Recovery by Enhancing Neurogenesis and Activating BDNF/TrkB Signaling in Ischemic Rats. *International Journal of Molecular Sciences*, *18*(2). https://doi.org/10.3390/ijms18020455

Lüscher, C., & Malenka, R. C. (2012). NMDA receptor-dependent long-term potentiation and long-term depression (LTP/LTD). *Cold Spring Harbor Perspectives in Biology*, *4*(6). https://doi.org/10.1101/cshperspect.a005710

Ma, Jiangnan, Gao, J., Niu, M., Zhang, X., Wang, J., & Xie, A. (2020). P2X4R Overexpression Upregulates Interleukin-6 and Exacerbates 6-OHDA-Induced Dopaminergic Degeneration in a Rat Model of PD. *Frontiers in Aging Neuroscience*, *12*, 580068. https://doi.org/10.3389/fnagi.2020.580068

Ma, Jun, Zhang, Z., Su, Y., Kang, L., Geng, D., Wang, Y., Luan, F., Wang, M., & Cui, H. (2013). Magnetic stimulation modulates structural synaptic plasticity and regulates BDNF-TrkB signal pathway in cultured hippocampal neurons. *Neurochemistry International*, *62*(1), 84–91. https://doi.org/10.1016/j.neuint.2012.11.010

Ma, Q. (2013). Role of nrf2 in oxidative stress and toxicity. *Annual Review of Pharmacology and Toxicology*, *53*, 401–426. https://doi.org/10.1146/annurev-pharmtox-011112-140320

Mango, D., Bonito-Oliva, A., Ledonne, A., Cappellacci, L., Petrelli, R., Nisticò, R., Berretta, N., Fisone, G., & Mercuri, N. B. (2014). Adenosine A1 receptor stimulation reduces D1 receptor-mediated GABAergic transmission from striato-nigral terminals and attenuates l-DOPA-induced dyskinesia in dopamine-denervated mice. *Experimental Neurology*, *261*, 733–743. https://doi.org/10.1016/j.expneurol.2014.08.022

Marcellino, D., Suárez-Boomgaard, D., Sánchez-Reina, M. D., Aguirre, J. A., Yoshitake, T., Yoshitake, S., Hagman, B., Kehr, J., Agnati, L. F., Fuxe, K., & Rivera, A. (2010). On the role of P2X(7) receptors in dopamine nerve cell degeneration in a rat model of Parkinson's disease: studies with the P2X(7) receptor antagonist A-438079. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *117*(6), 681–687. https://doi.org/10.1007/s00702-010-0400-0

Marín-Valencia, I., Serrano, M., Ormazabal, A., Pérez-Dueñas, B., García-Cazorla, A., Campistol, J., & Artuch, R. (2008). Biochemical diagnosis of dopaminergic disturbances in paediatric patients: analysis of cerebrospinal fluid homovanillic acid and other biogenic amines. *Clinical Biochemistry*, *41*(16–17), 1306–1315. https://doi.org/10.1016/j.clinbiochem.2008.08.077

Marras, C., Lang, A., van de Warrenburg, B. P., Sue, C. M., Tabrizi, S. J., Bertram, L., Mercimek-Mahmutoglu, S., Ebrahimi-Fakhari, D., Warner, T. T., Durr, A., Assmann, B., Lohmann, K.,

Kostic, V., & Klein, C. (2016). Nomenclature of genetic movement disorders: Recommendations of the international Parkinson and movement disorder society task force. *Movement Disorders : Official Journal of the Movement Disorder Society*, *31*(4), 436–457. https://doi.org/10.1002/mds.26527

Martin, D., Rojo, A. I., Salinas, M., Diaz, R., Gallardo, G., Alam, J., De Galarreta, C. M. R., & Cuadrado, A. (2004). Regulation of heme oxygenase-1 expression through the phosphatidylinositol 3-kinase/Akt pathway and the Nrf2 transcription factor in response to the antioxidant phytochemical carnosol. *The Journal of Biological Chemistry*, *279*(10), 8919–8929. https://doi.org/10.1074/jbc.M309660200

Masini, D., Plewnia, C., Bertho, M., Scalbert, N., Caggiano, V., & Fisone, G. (2021). A Guide to the Generation of a 6-Hydroxydopamine Mouse Model of Parkinson's Disease for the Study of Non-Motor Symptoms. *Biomedicines*, *9*(6). https://doi.org/10.3390/biomedicines9060598

Massari, C. M., Constantino, L. C., & Tasca, C. I. (2021). Adenosine A(1) and A(2A) receptors are involved on guanosine protective effects against oxidative burst and mitochondrial dysfunction induced by 6-OHDA in striatal slices. *Purinergic Signalling*, *17*(2), 247–254. https://doi.org/10.1007/s11302-021-09765-y

Mathur, B. N., Capik, N. A., Alvarez, V. A., & Lovinger, D. M. (2011). Serotonin induces long-term depression at corticostriatal synapses. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(20), 7402–7411. https://doi.org/10.1523/JNEUROSCI.6250-10.2011

Medina-Fernández, F. J., Escribano, B. M., Padilla-Del-Campo, C., Drucker-Colín, R., Pascual-Leone, Á., & Túnez, I. (2018). Transcranial magnetic stimulation as an antioxidant. *Free Radical Research*, *52*(4), 381–389. https://doi.org/10.1080/10715762.2018.1434313

Mellone, M., Stanic, J., Hernandez, L. F., Iglesias, E., Zianni, E., Longhi, A., Prigent, A., Picconi, B., Calabresi, P., Hirsch, E. C., Obeso, J. A., Di Luca, M., & Gardoni, F. (2015). NMDA receptor GluN2A/GluN2B subunit ratio as synaptic trait of levodopa-induced dyskinesias: from experimental models to patients. *Frontiers in Cellular Neuroscience*, *9*, 245. https://doi.org/10.3389/fncel.2015.00245

Mellone, M., Zianni, E., Stanic, J., Campanelli, F., Marino, G., Ghiglieri, V., Longhi, A., Thiolat, M.-L., Li, Q., Calabresi, P., Bezard, E., Picconi, B., Di Luca, M., & Gardoni, F. (2019). NMDA receptor GluN2D subunit participates to levodopa-induced dyskinesia pathophysiology. *Neurobiology of Disease*, *121*, 338–349. https://doi.org/10.1016/j.nbd.2018.09.021

Meng, F., Guo, Z., Hu, Y., Mai, W., Zhang, Z., Zhang, B., Ge, Q., Lou, H., Guo, F., Chen, J., Duan, S., & Gao, Z. (2019). CD73-derived adenosine controls inflammation and neurodegeneration by modulating dopamine signalling. *Brain : A Journal of Neurology*, *142*(3), 700–718. https://doi.org/10.1093/brain/awy351

Meredith, G E, Totterdell, S., Beales, M., & Meshul, C. K. (2009). Impaired glutamate homeostasis and programmed cell death in a chronic MPTP mouse model of Parkinson's disease. *Experimental Neurology*, *219*(1), 334–340. https://doi.org/10.1016/j.expneurol.2009.06.005

Meredith, Gloria E, & Kang, U. J. (2006). Behavioral models of Parkinson's disease in rodents: a new look at an old problem. *Movement Disorders : Official Journal of the Movement Disorder Society*, *21*(10), 1595–1606. https://doi.org/10.1002/mds.21010

Merton, P. A., & Morton, H. B. (1980). Stimulation of the cerebral cortex in the intact human subject. In *Nature* (Vol. 285, Issue 5762, p. 227). https://doi.org/10.1038/285227a0

Meshul, C. K., Emre, N., Nakamura, C. M., Allen, C., Donohue, M. K., & Buckman, J. F. (1999). Time-dependent changes in striatal glutamate synapses following a 6-hydroxydopamine lesion. *Neuroscience*, *88*(1), 1–16. https://doi.org/10.1016/s0306-4522(98)00189-4

Michael, N., Gösling, M., Reutemann, M., Kersting, A., Heindel, W., Arolt, V., & Pfleiderer, B. (2003). Metabolic changes after repetitive transcranial magnetic stimulation (rTMS) of

the left prefrontal cortex: a sham-controlled proton magnetic resonance spectroscopy (1H MRS) study of healthy brain. *The European Journal of Neuroscience*, *17*(11), 2462–2468. https://doi.org/10.1046/j.1460-9568.2003.02683.x

Miyanishi, K., Choudhury, M. E., Watanabe, M., Kubo, M., Nomoto, M., Yano, H., & Tanaka, J. (2019). Behavioral tests predicting striatal dopamine level in a rat hemi-Parkinson's disease model. *Neurochemistry International*, *122*, 38–46. https://doi.org/10.1016/j.neuint.2018.11.005

Mogi, M., Togari, A., Kondo, T., Mizuno, Y., Komure, O., Kuno, S., Ichinose, H., & Nagatsu, T. (1999). Brain-derived growth factor and nerve growth factor concentrations are decreased in the substantia nigra in Parkinson's disease. *Neuroscience Letters*, *270*(1), 45–48. https://doi.org/10.1016/s0304-3940(99)00463-2

Monif, M., Reid, C. A., Powell, K. L., Smart, M. L., & Williams, D. A. (2009). The P2X7 receptor drives microglial activation and proliferation: a trophic role for P2X7R pore. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *29*(12), 3781–3791. https://doi.org/10.1523/JNEUROSCI.5512-08.2009

Morelli, M., Carta, A. R., & Jenner, P. (2009). Adenosine A2A receptors and Parkinson's disease. *Handbook of Experimental Pharmacology*, *193*, 589–615. https://doi.org/10.1007/978-3-540-89615-9_18

Moretti, J., & Rodger, J. (2022). A little goes a long way: Neurobiological effects of low intensity rTMS and implications for mechanisms of rTMS. *Current Research in Neurobiology*, *3*, 100033. https://doi.org/10.1016/j.crneur.2022.100033

Morissette, M., Dridi, M., Calon, F., Hadj Tahar, A., Meltzer, L. T., Bédard, P. J., & Di Paolo, T. (2006). Prevention of dyskinesia by an NMDA receptor antagonist in MPTP monkeys: effect on adenosine A2A receptors. *Synapse (New York, N.Y.)*, *60*(3), 239–250. https://doi.org/10.1002/syn.20295

Muñoz, P., Huenchuguala, S., Paris, I., & Segura-Aguilar, J. (2012). Dopamine oxidation and autophagy. *Parkinson's Disease*, *2012*, 920953. https://doi.org/10.1155/2012/920953

Murer, M. G., Yan, Q., & Raisman-Vozari, R. (2001). Brain-derived neurotrophic factor in the control human brain, and in Alzheimer's disease and Parkinson's disease. *Progress in Neurobiology*, *63*(1), 71–124. https://doi.org/10.1016/s0301-0082(00)00014-9

Narang, N., & Wamsley, J. K. (1995). Time dependent changes in DA uptake sites, D1 and D2 receptor binding and mRNA after 6-OHDA lesions of the medial forebrain bundle in the rat brain. *Journal of Chemical Neuroanatomy*, *9*(1), 41–53. https://doi.org/10.1016/0891-0618(95)00064-e

Nash, J E, Hill, M. P., & Brotchie, J. M. (1999). Antiparkinsonian actions of blockade of NR2B-containing NMDA receptors in the reserpine-treated rat. *Experimental Neurology*, *155*(1), 42–48. https://doi.org/10.1006/exnr.1998.6963

Nash, Joanne E, & Brotchie, J. M. (2002). Characterisation of striatal NMDA receptors involved in the generation of parkinsonian symptoms: intrastriatal microinjection studies in the 6-OHDA-lesioned rat. *Movement Disorders : Official Journal of the Movement Disorder Society*, *17*(3), 455–466. https://doi.org/10.1002/mds.10107

Natale, G., Pignataro, A., Marino, G., Campanelli, F., Calabrese, V., Cardinale, A., Pelucchi, S., Marcello, E., Gardoni, F., Viscomi, M. T., Picconi, B., Ammassari-Teule, M., Calabresi, P., & Ghiglieri, V. (2021). Transcranial Magnetic Stimulation Exerts "Rejuvenation" Effects on Corticostriatal Synapses after Partial Dopamine Depletion. *Movement Disorders : Official Journal of the Movement Disorder Society*, *36*(10), 2254–2263. https://doi.org/10.1002/mds.28671

Nazario, L. R., da Silva, R. S., & Bonan, C. D. (2017). Targeting Adenosine Signaling in Parkinson's Disease: From Pharmacological to Non-pharmacological Approaches. *Frontiers in Neuroscience*, *11*, 658. https://doi.org/10.3389/fnins.2017.00658

Nedeljkovic, N. (2019). Complex regulation of ecto-5'-nucleotidase/CD73 and A(2A)R-

mediated adenosine signaling at neurovascular unit: A link between acute and chronic neuroinflammation. *Pharmacological Research*, *144*, 99–115. https://doi.org/10.1016/j.phrs.2019.04.007

Neyman, S., & Manahan-Vaughan, D. (2008). Metabotropic glutamate receptor 1 (mGluR1) and 5 (mGluR5) regulate late phases of LTP and LTD in the hippocampal CA1 region in vitro. *The European Journal of Neuroscience*, *27*(6), 1345–1352. https://doi.org/10.1111/j.1460-9568.2008.06109.x

Nutt, J. G., Carter, J. H., & Sexton, G. J. (2004). The dopamine transporter: importance in Parkinson's disease. *Annals of Neurology*, *55*(6), 766–773. https://doi.org/10.1002/ana.20089

O'Gorman Tuura, R. L., Baumann, C. R., & Baumann-Vogel, H. (2018). Beyond Dopamine: GABA, Glutamate, and the Axial Symptoms of Parkinson Disease. *Frontiers in Neurology*, *9*, 806. https://doi.org/10.3389/fneur.2018.00806

Ochi, M., Koga, K., Kurokawa, M., Kase, H., Nakamura, J., & Kuwana, Y. (2000). Systemic administration of adenosine A(2A) receptor antagonist reverses increased GABA release in the globus pallidus of unilateral 6-hydroxydopamine-lesioned rats: a microdialysis study. *Neuroscience*, *100*(1), 53–62. https://doi.org/10.1016/s0306-4522(00)00250-5

Oliveira-Giacomelli, Á., M Albino, C., de Souza, H. D. N., Corrêa-Velloso, J., de Jesus Santos, A. P., Baranova, J., & Ulrich, H. (2019). P2Y6 and P2X7 Receptor Antagonism Exerts Neuroprotective/ Neuroregenerative Effects in an Animal Model of Parkinson's Disease. *Frontiers in Cellular Neuroscience*, *13*, 476. https://doi.org/10.3389/fncel.2019.00476

Oliynyk, Z., Rudyk, M., Dovbynchuk, T., Dzubenko, N., Tolstanova, G., & Skivka, L. (2023). Inflammatory hallmarks in 6-OHDA- and LPS-induced Parkinson's disease in rats. *Brain, Behavior, & Immunity - Health*, *30*, 100616. https://doi.org/10.1016/j.bbih.2023.100616

Oses, J. P., Batassini, C., Pochmann, D., Böhmer, A. E., Vuaden, F. C., Silvestrin, R. B., Oliveira, A., Bonan, C. D., Bogo, M. R., Souza, D. O., Portela, L. V. C., Sarkis, J. J. de F., & Mello e Souza, T. (2011). The hydrolysis of striatal adenine- and guanine-based purines in a 6-hydroxydopamine rat model of Parkinson's disease. *Neurochemical Research*, *36*(2), 215–222. https://doi.org/10.1007/s11064-010-0305-1

Pagonabarraga, J., Tinazzi, M., Caccia, C., & Jost, W. H. (2021). The role of glutamatergic neurotransmission in the motor and non-motor symptoms in Parkinson's disease: Clinical cases and a review of the literature. *Journal of Clinical Neuroscience : Official Journal of the Neurosurgical Society of Australasia*, *90*, 178–183. https://doi.org/10.1016/j.jocn.2021.05.056

Pandey, S., & Srivanitchapoom, P. (2017). Levodopa-induced Dyskinesia: Clinical Features, Pathophysiology, and Medical Management. *Annals of Indian Academy of Neurology*, *20*(3), 190–198. https://doi.org/10.4103/aian.AIAN_239_17

Paoletti, P., Bellone, C., & Zhou, Q. (2013). NMDA receptor subunit diversity: impact on receptor properties, synaptic plasticity and disease. *Nature Reviews. Neuroscience*, *14*(6), 383–400. https://doi.org/10.1038/nrn3504

Paoletti, P., & Neyton, J. (2007). NMDA receptor subunits: function and pharmacology. *Current Opinion in Pharmacology*, *7*(1), 39–47. https://doi.org/10.1016/j.coph.2006.08.011

Parra, I., Martínez, I., Ramírez-García, G., Tizabi, Y., & Mendieta, L. (2020). Differential Effects of LPS and 6-OHDA on Microglia's Morphology in Rats: Implications for Inflammatory Model of Parkinson's Disease. *Neurotoxicity Research*, *37*(1), 1–11. https://doi.org/10.1007/s12640-019-00104-z

Pascual-Leone, A., Valls-Solé, J., Wassermann, E. M., & Hallett, M. (1994). Responses to rapid-rate transcranial magnetic stimulation of the human motor cortex. *Brain : A Journal of Neurology*, *117 (Pt 4*, 847–858. https://doi.org/10.1093/brain/117.4.847

Pell, G. S., Roth, Y., & Zangen, A. (2011). Modulation of cortical excitability induced by repetitive transcranial magnetic stimulation: influence of timing and geometrical

parameters and underlying mechanisms. *Progress in Neurobiology*, *93*(1), 59–98. https://doi.org/10.1016/j.pneurobio.2010.10.003

Pérez-Sen, R., Queipo, M. J., Morente, V., Ortega, F., Delicado, E. G., & Miras-Portugal, M. T. (2015). Neuroprotection Mediated by P2Y13 Nucleotide Receptors in Neurons. *Computational and Structural Biotechnology Journal*, *13*, 160–168. https://doi.org/10.1016/j.csbj.2015.02.002

Petrovic, J., Radovanovic, L., & Saponjic, J. (2021). Prodromal local sleep disorders in a rat model of Parkinson's disease cholinopathy, hemiparkinsonism and hemiparkinsonism with cholinopathy. *Behavioural Brain Research*, *397*, 112957. https://doi.org/10.1016/j.bbr.2020.112957

Poewe, W., Seppi, K., Tanner, C. M., Halliday, G. M., Brundin, P., Volkmann, J., Schrag, A.-E., & Lang, A. E. (2017). Parkinson disease. *Nature Reviews. Disease Primers*, *3*, 17013. https://doi.org/10.1038/nrdp.2017.13

Prasad, E. M., & Hung, S.-Y. (2020). Behavioral Tests in Neurotoxin-Induced Animal Models of Parkinson's Disease. *Antioxidants (Basel, Switzerland)*, *9*(10). https://doi.org/10.3390/antiox9101007

Prasad, K., de Vries, E. F. J., Elsinga, P. H., Dierckx, R. A. J. O., & van Waarde, A. (2021). Allosteric Interactions between Adenosine A(2A) and Dopamine D(2) Receptors in Heteromeric Complexes: Biochemical and Pharmacological Characteristics, and Opportunities for PET Imaging. *International Journal of Molecular Sciences*, *22*(4). https://doi.org/10.3390/ijms22041719

Prinz, A., Selesnew, L.-M., Liss, B., Roeper, J., & Carlsson, T. (2013). Increased excitability in serotonin neurons in the dorsal raphe nucleus in the 6-OHDA mouse model of Parkinson's disease. *Experimental Neurology*, *248*, 236–245. https://doi.org/10.1016/j.expneurol.2013.06.015

Quintas, C., Fraga, S., Gonçalves, J., & Queiroz, G. (2011). P2Y receptors on astrocytes and microglia mediate opposite effects in astroglial proliferation. *Purinergic Signalling*, *7*(2), 251–263. https://doi.org/10.1007/s11302-011-9235-x

Quintas, C., Vale, N., Gonçalves, J., & Queiroz, G. (2018). Microglia P2Y(13) Receptors Prevent Astrocyte Proliferation Mediated by P2Y(1) Receptors. *Frontiers in Pharmacology*, *9*, 418. https://doi.org/10.3389/fphar.2018.00418

Ramalingam, M., & Kim, S.-J. (2012). Reactive oxygen/nitrogen species and their functional correlations in neurodegenerative diseases. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *119*(8), 891–910. https://doi.org/10.1007/s00702-011-0758-7

Ren, W., Ma, J., Li, J., Zhang, Z., & Wang, M. (2017). Repetitive Transcranial Magnetic Stimulation (rTMS) Modulates Lipid Metabolism in Aging Adults. *Frontiers in Aging Neuroscience*, *9*, 334. https://doi.org/10.3389/fnagi.2017.00334

Reyhani-Rad, S., & Mahmoudi, J. (2016). Effect of adenosine A2A receptor antagonists on motor disorders induced by 6-hydroxydopamine in rat. *Acta Cirurgica Brasileira*, *31*(2), 133–137. https://doi.org/10.1590/S0102-865020160020000008

Riederer, P., & Wuketich, S. (1976). Time course of nigrostriatal degeneration in parkinson's disease. A detailed study of influential factors in human brain amine analysis. *Journal of Neural Transmission*, *38*(3–4), 277–301. https://doi.org/10.1007/BF01249445

Rodrigues, R. J., Tomé, A. R., & Cunha, R. A. (2015). ATP as a multi-target danger signal in the brain. *Frontiers in Neuroscience*, *9*, 148. https://doi.org/10.3389/fnins.2015.00148

Rodríguez Díaz, M., Abdala, P., Barroso-Chinea, P., Obeso, J., & González-Hernández, T. (2001). Motor behavioural changes after intracerebroventricular injection of 6-hydroxydopamine in the rat: an animal model of Parkinson's disease. *Behavioural Brain Research*, *122*(1), 79–92. https://doi.org/10.1016/s0166-4328(01)00168-1

Rossi, S., Hallett, M., Rossini, P. M., Pascual-Leone, A., Avanzini, G., Bestmann, S., Berardelli, A., Brewer, C., Canli, T., Cantello, R., Chen, R., Classen, J., Demitrack, M., Di Lazzaro, V.,

Epstein, C. M., George, M. S., Fregni, F., Ilmoniemi, R., Jalinous, R., … Ziemann, U. (2009). Safety, ethical considerations, and application guidelines for the use of transcranial magnetic stimulation in clinical practice and research. *Clinical Neurophysiology*, *120*(12), 2008–2039. https://doi.org/10.1016/j.clinph.2009.08.016

Rossini, P. M., Barker, A. T., Berardelli, A., Caramia, M. D., Caruso, G., Cracco, R. Q., Dimitrijević, M. R., Hallett, M., Katayama, Y., & Lücking, C. H. (1994). Non-invasive electrical and magnetic stimulation of the brain, spinal cord and roots: basic principles and procedures for routine clinical application. Report of an IFCN committee. *Electroencephalography and Clinical Neurophysiology*, *91*(2), 79–92. https://doi.org/10.1016/0013-4694(94)90029-9

Rossini, P. M., Burke, D., Chen, R., Cohen, L. G., Daskalakis, Z., Di Iorio, R., Di Lazzaro, V., Ferreri, F., Fitzgerald, P. B., George, M. S., Hallett, M., Lefaucheur, J. P., Langguth, B., Matsumoto, H., Miniussi, C., Nitsche, M. A., Pascual-Leone, A., Paulus, W., Rossi, S., … Ziemann, U. (2015). Non-invasive electrical and magnetic stimulation of the brain, spinal cord, roots and peripheral nerves: Basic principles and procedures for routine clinical and research application: An updated report from an I.F.C.N. Committee. *Clinical Neurophysiology*, *126*(6), 1071–1107. https://doi.org/10.1016/j.clinph.2015.02.001

Rothman, S. M., & Mattson, M. P. (2013). Activity-dependent, stress-responsive BDNF signaling and the quest for optimal brain health and resilience throughout the lifespan. *Neuroscience*, *239*, 228–240. https://doi.org/10.1016/j.neuroscience.2012.10.014

Ruiz, M. A., Escriche, M., Lluis, C., Franco, R., Martín, M., Andrés, A., & Ros, M. (2000). Adenosine A(1) receptor in cultured neurons from rat cerebral cortex: colocalization with adenosine deaminase. *Journal of Neurochemistry*, *75*(2), 656–664. https://doi.org/10.1046/j.1471-4159.2000.0750656.x

Savio, L. E. B., de Andrade Mello, P., da Silva, C. G., & Coutinho-Silva, R. (2018). The P2X7 Receptor in Inflammatory Diseases: Angel or Demon? *Frontiers in Pharmacology*, *9*, 52. https://doi.org/10.3389/fphar.2018.00052

Schapira, A. H. V. (2009). Neurobiology and treatment of Parkinson's disease. *Trends in Pharmacological Sciences*, *30*(1), 41–47. https://doi.org/10.1016/j.tips.2008.10.005

Schmid, U. D., Møller, A. R., & Schmid, J. (1995). Transcranial magnetic stimulation of the trigeminal nerve: intraoperative study on stimulation characteristics in man. *Muscle & Nerve*, *18*(5), 487–494. https://doi.org/10.1002/mus.880180503

Schulz, R., Gerloff, C., & Hummel, F. C. (2013). Non-invasive brain stimulation in neurological diseases. *Neuropharmacology*, *64*, 579–587. https://doi.org/10.1016/j.neuropharm.2012.05.016

Schwarting, R. K., & Huston, J. P. (1996). Unilateral 6-hydroxydopamine lesions of meso-striatal dopamine neurons and their physiological sequelae. *Progress in Neurobiology*, *49*(3), 215–266. https://doi.org/10.1016/s0301-0082(96)00015-9

Sebastião, A. M., & Ribeiro, J. A. (2009). Adenosine receptors and the central nervous system. *Handbook of Experimental Pharmacology*, *193*, 471–534. https://doi.org/10.1007/978-3-540-89615-9_16

Segura-Aguilar, J., Paris, I., Muñoz, P., Ferrari, E., Zecca, L., & Zucca, F. A. (2014). Protective and toxic roles of dopamine in Parkinson's disease. *Journal of Neurochemistry*, *129*(6), 898–915. https://doi.org/10.1111/jnc.12686

Seibenhener, M. L., & Wooten, M. C. (2015). Use of the Open Field Maze to measure locomotor and anxiety-like behavior in mice. *Journal of Visualized Experiments : JoVE*, *96*, e52434. https://doi.org/10.3791/52434

Shahmoradian, S. H., Lewis, A. J., Genoud, C., Hench, J., Moors, T. E., Navarro, P. P., Castaño-Díez, D., Schweighauser, G., Graff-Meyer, A., Goldie, K. N., Sütterlin, R., Huisman, E., Ingrassia, A., Gier, Y. de, Rozemuller, A. J. M., Wang, J., Paepe, A. De, Erny, J., Staempfli, A., … Lauer, M. E. (2019). Lewy pathology in Parkinson's disease consists of crowded organelles and lipid membranes. *Nature Neuroscience*, *22*(7), 1099–1109. https://doi.org/10.1038/s41593-

019-0423-2
Shipton, O. A., & Paulsen, O. (2014). GluN2A and GluN2B subunit-containing NMDA receptors in hippocampal plasticity. *Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences*, *369*(1633), 20130163. https://doi.org/10.1098/rstb.2013.0163
Shirota, Y., Ohtsu, H., Hamada, M., Enomoto, H., & Ugawa, Y. (2013). Supplementary motor area stimulation for Parkinson disease: a randomized controlled study. *Neurology*, *80*(15), 1400–1405. https://doi.org/10.1212/WNL.0b013e31828c2f66
Sibon, I., Strafella, A. P., Gravel, P., Ko, J. H., Booij, L., Soucy, J. P., Leyton, M., Diksic, M., & Benkelfat, C. (2007). Acute prefrontal cortex TMS in healthy volunteers: effects on brain 11C-alphaMtrp trapping. *NeuroImage*, *34*(4), 1658–1664. https://doi.org/10.1016/j.neuroimage.2006.08.059
Siebner, H R, Mentschel, C., Auer, C., & Conrad, B. (1999). Repetitive transcranial magnetic stimulation has a beneficial effect on bradykinesia in Parkinson's disease. *Neuroreport*, *10*(3), 589–594. https://doi.org/10.1097/00001756-199902250-00027
Siebner, Hartwig R, Funke, K., Aberra, A. S., Antal, A., Bestmann, S., Chen, R., Classen, J., Davare, M., Di Lazzaro, V., Fox, P. T., Hallett, M., Karabanov, A. N., Kesselheim, J., Beck, M. M., Koch, G., Liebetanz, D., Meunier, S., Miniussi, C., Paulus, W., … Ugawa, Y. (2022). Transcranial magnetic stimulation of the brain: What is stimulated? - A consensus and critical position paper. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *140*, 59–97. https://doi.org/10.1016/j.clinph.2022.04.022
Simões, A. P., Silva, C. G., Marques, J. M., Pochmann, D., Porciúncula, L. O., Ferreira, S., Oses, J. P., Beleza, R. O., Real, J. I., Köfalvi, A., Bahr, B. A., Lerma, J., Cunha, R. A., & Rodrigues, R. J. (2018). Glutamate-induced and NMDA receptor-mediated neurodegeneration entails P2Y1 receptor activation. *Cell Death & Disease*, *9*(3), 297. https://doi.org/10.1038/s41419-018-0351-1
Skrzypczak-Wiercioch, A., & Sałat, K. (2022). Lipopolysaccharide-Induced Model of Neuroinflammation: Mechanisms of Action, Research Application and Future Directions for Its Use. *Molecules (Basel, Switzerland)*, *27*(17). https://doi.org/10.3390/molecules27175481
Slézia, A., Hegedüs, P., Rusina, E., Lengyel, K., Solari, N., Kaszas, A., Balázsfi, D., Botzanowski, B., Acerbo, E., Missey, F., Williamson, A., & Hangya, B. (2023). Behavioral, neural and ultrastructural alterations in a graded-dose 6-OHDA mouse model of early-stage Parkinson's disease. *Scientific Reports*, *13*(1), 19478. https://doi.org/10.1038/s41598-023-46576-0
Smith, M. P., & Cass, W. A. (2007). Oxidative stress and dopamine depletion in an intrastriatal 6-hydroxydopamine model of Parkinson's disease. *Neuroscience*, *144*(3), 1057–1066. https://doi.org/10.1016/j.neuroscience.2006.10.004
Smith, Y., Wichmann, T., Factor, S. A., & DeLong, M. R. (2012). Parkinson's disease therapeutics: new developments and challenges since the introduction of levodopa. *Neuropsychopharmacology : Official Publication of the American College of Neuropsychopharmacology*, *37*(1), 213–246. https://doi.org/10.1038/npp.2011.212
Sofroniew, M. V. (2009). Molecular dissection of reactive astrogliosis and glial scar formation. *Trends in Neurosciences*, *32*(12), 638–647. https://doi.org/10.1016/j.tins.2009.08.002
Solomon, E. A., Sperling, M. R., Sharan, A. D., Wanda, P. A., Levy, D. F., Lyalenko, A., Pedisich, I., Rizzuto, D. S., & Kahana, M. J. (2021). Theta-burst stimulation entrains frequency-specific oscillatory responses. *Brain Stimulation*, *14*(5), 1271–1284. https://doi.org/10.1016/j.brs.2021.08.014
Soto-Otero, R., Méndez-Alvarez, E., Hermida-Ameijeiras, A., Muñoz-Patiño, A. M., & Labandeira-Garcia, J. L. (2000). Autoxidation and neurotoxicity of 6-hydroxydopamine in the presence of some antioxidants: potential implication in relation to the pathogenesis of Parkinson's disease. *Journal of Neurochemistry*, *74*(4), 1605–1612.

https://doi.org/10.1046/j.1471-4159.2000.0741605.x
Speer, A. M., Kimbrell, T. A., Wassermann, E. M., D Repella, J., Willis, M. W., Herscovitch, P., & Post, R. M. (2000). Opposite effects of high and low frequency rTMS on regional brain activity in depressed patients. *Biological Psychiatry*, *48*(12), 1133–1141. https://doi.org/10.1016/s0006-3223(00)01065-9
Stanojevic, J. B., Zeljkovic, M., Dragic, M., Stojanovic, I. R., Ilic, T. V, Stevanovic, I. D., & Ninkovic, M. B. (2023). Intermittent theta burst stimulation attenuates oxidative stress and reactive astrogliosis in the streptozotocin-induced model of Alzheimer's disease-like pathology. *Frontiers in Aging Neuroscience*, *15*, 1161678. https://doi.org/10.3389/fnagi.2023.1161678
Stanojevic, J., Dragic, M., Stevanovic, I., Ilic, T., Stojanovic, I., Zeljkovic, M., & Ninkovic, M. (2022). Intermittent theta burst stimulation ameliorates cognitive impairment and hippocampal gliosis in the Streptozotocin-induced model of Alzheimer's disease. *Behavioural Brain Research*, *433*, 113984. https://doi.org/10.1016/j.bbr.2022.113984
Stateman, W. A., Knöppel, A., Potolicchio, S. J., & Henkin, R. I. (2014). Repetitive transcranial magnetic stimulation (Rtms) increases plasma calcium both in-vivo and in-vitro. *J Clin Exp Pathol*, *4*(187), 681–2161.
Steece-Collier, K., Chambers, L. K., Jaw-Tsai, S. S., Menniti, F. S., & Greenamyre, J. T. (2000). Antiparkinsonian actions of CP-101,606, an antagonist of NR2B subunit-containing N-methyl-d-aspartate receptors. *Experimental Neurology*, *163*(1), 239–243. https://doi.org/10.1006/exnr.2000.7374
Stefanis, L. (2012). α-Synuclein in Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine*, *2*(2), a009399. https://doi.org/10.1101/cshperspect.a009399
Stekic, A., Zeljkovic, M., Zaric Kontic, M., Mihajlovic, K., Adzic, M., Stevanovic, I., Ninkovic, M., Grkovic, I., Ilic, T. V, Nedeljkovic, N., & Dragic, M. (2022). Intermittent Theta Burst Stimulation Ameliorates Cognitive Deficit and Attenuates Neuroinflammation via PI3K/Akt/mTOR Signaling Pathway in Alzheimer's-Like Disease Model. *Frontiers in Aging Neuroscience*, *14*, 889983. https://doi.org/10.3389/fnagi.2022.889983
Stevanovic, I., Mancic, B., Ilic, T., Milosavljevic, P., Lavrnja, I., Stojanovic, I., & Ninkovic, M. (2019). Theta burst stimulation influence the expression of BDNF in the spinal cord on the experimental autoimmune encephalomyelitis. *Folia Neuropathologica*, *57*(2), 129–145. https://doi.org/10.5114/fn.2019.86294
Stojkovska, I., Wagner, B. M., & Morrison, B. E. (2015). Parkinson's disease and enhanced inflammatory response. *Experimental Biology and Medicine (Maywood, N.J.)*, *240*(11), 1387–1395. https://doi.org/10.1177/1535370215576313
Stoker, T. B., Torsney, K. M., & Barker, R. A. (2018). Emerging Treatment Approaches for Parkinson's Disease. *Frontiers in Neuroscience*, *12*, 693. https://doi.org/10.3389/fnins.2018.00693
Su, H., Zhong, N., Gan, H., Wang, J., Han, H., Chen, T., Li, X., Ruan, X., Zhu, Y., Jiang, H., & Zhao, M. (2017). High frequency repetitive transcranial magnetic stimulation of the left dorsolateral prefrontal cortex for methamphetamine use disorders: A randomised clinical trial. *Drug and Alcohol Dependence*, *175*, 84–91. https://doi.org/10.1016/j.drugalcdep.2017.01.037
Suen, P. C., Wu, K., Levine, E. S., Mount, H. T., Xu, J. L., Lin, S. Y., & Black, I. B. (1997). Brain-derived neurotrophic factor rapidly enhances phosphorylation of the postsynaptic N-methyl-D-aspartate receptor subunit 1. *Proceedings of the National Academy of Sciences of the United States of America*, *94*(15), 8191–8195. https://doi.org/10.1073/pnas.94.15.8191
Sulzer, D., & Zecca, L. (2000). Intraneuronal dopamine-quinone synthesis: a review. *Neurotoxicity Research*, *1*(3), 181–195. https://doi.org/10.1007/BF03033289
Sveinbjornsdottir, S. (2016). The clinical symptoms of Parkinson's disease. *Journal of*

Neurochemistry, *139 Suppl*, 318–324. https://doi.org/10.1111/jnc.13691
Tan, T., Xie, J., Liu, T., Chen, X., Zheng, X., Tong, Z., & Tian, X. (2013). Low-frequency (1 Hz) repetitive transcranial magnetic stimulation (rTMS) reverses Aβ(1-42)-mediated memory deficits in rats. *Experimental Gerontology*, *48*(8), 786–794. https://doi.org/10.1016/j.exger.2013.05.001
Tanaka, K., Choi, J., Cao, Y., & Stacey, G. (2014). Extracellular ATP acts as a damage-associated molecular pattern (DAMP) signal in plants. *Frontiers in Plant Science*, *5*, 446. https://doi.org/10.3389/fpls.2014.00446
Terao, Y., & Ugawa, Y. (2002). Basic mechanisms of TMS. *Journal of Clinical Neurophysiology*, *19*(4), 322–343. https://doi.org/10.1097/00004691-200208000-00006
Thiel, G. (1993). Synapsin I, synapsin II, and synaptophysin: marker proteins of synaptic vesicles. *Brain Pathology (Zurich, Switzerland)*, *3*(1), 87–95. https://doi.org/10.1111/j.1750-3639.1993.tb00729.x
Thompson, S. P. (1910). A physiological effect of an alternating magnetic field. *Proceedings of the Royal Society of London. Series B, Containing Papers of a Biological Character*, *82*(557), 396–398.
Thomsen, T. R., & Rodnitzky, R. L. (2010). Juvenile parkinsonism: epidemiology, diagnosis and treatment. *CNS Drugs*, *24*(6), 467–477. https://doi.org/10.2165/11533130-000000000-00000
Tieu, K. (2011). A guide to neurotoxic animal models of Parkinson's disease. *Cold Spring Harbor Perspectives in Medicine*, *1*(1), a009316. https://doi.org/10.1101/cshperspect.a009316
Tolosa, E., Garrido, A., Scholz, S. W., & Poewe, W. (2021). Challenges in the diagnosis of Parkinson's disease. *The Lancet. Neurology*, *20*(5), 385–397. https://doi.org/10.1016/S1474-4422(21)00030-2
Tomiyama, M., Kimura, T., Maeda, T., Tanaka, H., Kannari, K., & Baba, M. (2004). Upregulation of striatal adenosine A2A receptor mRNA in 6-hydroxydopamine-lesioned rats intermittently treated with L-DOPA. *Synapse (New York, N.Y.)*, *52*(3), 218–222. https://doi.org/10.1002/syn.20011
Toodayan, N. (2018). James Parkinson's Essay on the shaking palsy, 1817-2017. *The Medical Journal of Australia*, *208*(9), 384–386. https://doi.org/10.5694/mja17.01085
Tronci, E., & Francardo, V. (2018). Animal models of L-DOPA-induced dyskinesia: the 6-OHDA-lesioned rat and mouse. *Journal of Neural Transmission (Vienna, Austria : 1996)*, *125*(8), 1137–1144. https://doi.org/10.1007/s00702-017-1825-5
Túnez, I., Drucker-Colín, R., Jimena, I., Medina, F. J., Muñoz, M. del C., Peña, J., & Montilla, P. (2006). Transcranial magnetic stimulation attenuates cell loss and oxidative damage in the striatum induced in the 3-nitropropionic model of Huntington's disease. *Journal of Neurochemistry*, *97*(3), 619–630. https://doi.org/10.1111/j.1471-4159.2006.03724.x
Uitti, R. J., Rajput, A. H., Ahlskog, J. E., Offord, K. P., Schroeder, D. R., Ho, M. M., Prasad, M., Rajput, A., & Basran, P. (1996). Amantadine treatment is an independent predictor of improved survival in Parkinson's disease. *Neurology*, *46*(6), 1551–1556. https://doi.org/10.1212/wnl.46.6.1551
Ungerstedt, U. (1968). 6-Hydroxy-dopamine induced degeneration of central monoamine neurons. *European Journal of Pharmacology*, *5*(1), 107–110. https://doi.org/10.1016/0014-2999(68)90164-7
Ungerstedt, U. (1971). Adipsia and aphagia after 6-hydroxydopamine induced degeneration of the nigro-striatal dopamine system. *Acta Physiologica Scandinavica. Supplementum*, *367*, 95–122. https://doi.org/10.1111/j.1365-201x.1971.tb11001.x
Uzair, M., Abualait, T., Arshad, M., Yoo, W.-K., Mir, A., Bunyan, R. F., & Bashir, S. (2022). Transcranial magnetic stimulation in animal models of neurodegeneration. *Neural Regeneration Research*, *17*(2), 251–265. https://doi.org/10.4103/1673-5374.317962

van Dijk, K. D., Møst, E. I. S., Van Someren, E. J. W., Berendse, H. W., & van der Werf, Y. D. (2009). Beneficial effect of transcranial magnetic stimulation on sleep in Parkinson's disease. *Movement Disorders : Official Journal of the Movement Disorder Society*, *24*(6), 878–884. https://doi.org/10.1002/mds.22462

Vandenberg, R. J., & Ryan, R. M. (2013). Mechanisms of glutamate transport. *Physiological Reviews*, *93*(4), 1621–1657. https://doi.org/10.1152/physrev.00007.2013

Vasquez, V., Mitra, J., Wang, H., Hegde, P. M., Rao, K. S., & Hegde, M. L. (2020). A multi-faceted genotoxic network of alpha-synuclein in the nucleus and mitochondria of dopaminergic neurons in Parkinson's disease: Emerging concepts and challenges. *Progress in Neurobiology*, *185*, 101729. https://doi.org/10.1016/j.pneurobio.2019.101729

Vazana, U., Schori, L., Monsonego, U., Swissa, E., Pell, G. S., Roth, Y., Brodt, P., Friedman, A., & Prager, O. (2020). TMS-Induced Controlled BBB Opening: Preclinical Characterization and Implications for Treatment of Brain Cancer. *Pharmaceutics*, *12*(10). https://doi.org/10.3390/pharmaceutics12100946

Vieira, J. C. F., Bassani, T. B., Santiago, R. M., de O Guaita, G., Zanoveli, J. M., da Cunha, C., & Vital, M. A. B. F. (2019). Anxiety-like behavior induced by 6-OHDA animal model of Parkinson's disease may be related to a dysregulation of neurotransmitter systems in brain areas related to anxiety. *Behavioural Brain Research*, *371*, 111981. https://doi.org/10.1016/j.bbr.2019.111981

Villar-Menéndez, I., Porta, S., Buira, S. P., Pereira-Veiga, T., Díaz-Sánchez, S., Albasanz, J. L., Ferrer, I., Martín, M., & Barrachina, M. (2014). Increased striatal adenosine A2A receptor levels is an early event in Parkinson's disease-related pathology and it is potentially regulated by miR-34b. *Neurobiology of Disease*, *69*, 206–214. https://doi.org/10.1016/j.nbd.2014.05.030

Villavicencio Tejo, F., & Quintanilla, R. A. (2021). Contribution of the Nrf2 Pathway on Oxidative Damage and Mitochondrial Failure in Parkinson and Alzheimer's Disease. *Antioxidants (Basel, Switzerland)*, *10*(7). https://doi.org/10.3390/antiox10071069

Vlachos, A., Müller-Dahlhaus, F., Rosskopp, J., Lenz, M., Ziemann, U., & Deller, T. (2012). Repetitive magnetic stimulation induces functional and structural plasticity of excitatory postsynapses in mouse organotypic hippocampal slice cultures. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *32*(48), 17514–17523. https://doi.org/10.1523/JNEUROSCI.0409-12.2012

Wakabayashi, K., Tanji, K., Odagiri, S., Miki, Y., Mori, F., & Takahashi, H. (2013). The Lewy body in Parkinson's disease and related neurodegenerative disorders. *Molecular Neurobiology*, *47*(2), 495–508. https://doi.org/10.1007/s12035-012-8280-y

Wang, C.-C., Wu, T.-L., Lin, F.-J., Tai, C.-H., Lin, C.-H., & Wu, R.-M. (2022). Amantadine treatment and delayed onset of levodopa-induced dyskinesia in patients with early Parkinson's disease. *European Journal of Neurology*, *29*(4), 1044–1055. https://doi.org/10.1111/ene.15234

Wang, H.-Y., Crupi, D., Liu, J., Stucky, A., Cruciata, G., Di Rocco, A., Friedman, E., Quartarone, A., & Ghilardi, M. F. (2011). Repetitive transcranial magnetic stimulation enhances BDNF-TrkB signaling in both brain and lymphocyte. *The Journal of Neuroscience : The Official Journal of the Society for Neuroscience*, *31*(30), 11044–11054. https://doi.org/10.1523/JNEUROSCI.2125-11.2011

Wang, L., Chen, Y., Sternberg, P., & Cai, J. (2008). Essential roles of the PI3 kinase/Akt pathway in regulating Nrf2-dependent antioxidant functions in the RPE. *Investigative Ophthalmology & Visual Science*, *49*(4), 1671–1678. https://doi.org/10.1167/iovs.07-1099

Wang, Q., Li, W.-X., Dai, S.-X., Guo, Y.-C., Han, F.-F., Zheng, J.-J., Li, G.-H., & Huang, J.-F. (2017). Meta-Analysis of Parkinson's Disease and Alzheimer's Disease Revealed Commonly Impaired Pathways and Dysregulation of NRF2-Dependent Genes. *Journal of Alzheimer's*

Disease : JAD, *56*(4), 1525–1539. https://doi.org/10.3233/JAD-161032
Wang, Y., Liu, H., Du, X.-D., Zhang, Y., Yin, G., Zhang, B.-S., Soares, J. C., & Zhang, X. Y. (2017). Association of low serum BDNF with depression in patients with Parkinson's disease. *Parkinsonism & Related Disorders*, *41*, 73–78. https://doi.org/10.1016/j.parkreldis.2017.05.012
Weber, M., & Eisen, A. A. (2002). Magnetic stimulation of the central and peripheral nervous systems. *Muscle and Nerve*, *25*(2), 160–175. https://doi.org/10.1002/mus.10038
Wessell, R. H., Ahmed, S. M., Menniti, F. S., Dunbar, G. L., Chase, T. N., & Oh, J. D. (2004). NR2B selective NMDA receptor antagonist CP-101,606 prevents levodopa-induced motor response alterations in hemi-parkinsonian rats. *Neuropharmacology*, *47*(2), 184–194. https://doi.org/10.1016/j.neuropharm.2004.03.011
Wise, R. A. (2004). Dopamine, learning and motivation. *Nature Reviews. Neuroscience*, *5*(6), 483–494. https://doi.org/10.1038/nrn1406
Wu, A. D., Fregni, F., Simon, D. K., Deblieck, C., & Pascual-Leone, A. (2008). Noninvasive brain stimulation for Parkinson's disease and dystonia. *Neurotherapeutics : The Journal of the American Society for Experimental NeuroTherapeutics*, *5*(2), 345–361. https://doi.org/10.1016/j.nurt.2008.02.002
Yang, J., Wang, L., Wang, F., Tang, X., Zhou, P., Liang, R., Zheng, C., & Ming, D. (2019). Low-Frequency Pulsed Magnetic Field Improves Depression-Like Behaviors and Cognitive Impairments in Depressive Rats Mainly via Modulating Synaptic Function. *Frontiers in Neuroscience*, *13*, 820. https://doi.org/10.3389/fnins.2019.00820
Yang, X., Song, L., & Liu, Z. (2010). The effect of repetitive transcranial magnetic stimulation on a model rat of Parkinson's disease. *Neuroreport*, *21*(4), 268–272. https://doi.org/10.1097/WNR.0b013e328335b411
Yegutkin, G. G. (2008). Nucleotide- and nucleoside-converting ectoenzymes: Important modulators of purinergic signalling cascade. *Biochimica et Biophysica Acta*, *1783*(5), 673–694. https://doi.org/10.1016/j.bbamcr.2008.01.024
Yulug, B., Hanoglu, L., Kilic, E., Polat, B., & Schabitz, W. R. (2016). The Neuroprotective Role of Repetitive Transcranial Magnetic Stimulation (rTMS) for Neurodegenerative Diseases: A Short Review on Experimental Studies. *Mini Reviews in Medicinal Chemistry*, *16*(16), 1269–1273. https://doi.org/10.2174/1389557516666160523145154
Zangen, A., Roth, Y., Voller, B., & Hallett, M. (2005). Transcranial magnetic stimulation of deep brain regions: evidence for efficacy of the H-coil. *Clinical Neurophysiology : Official Journal of the International Federation of Clinical Neurophysiology*, *116*(4), 775–779. https://doi.org/10.1016/j.clinph.2004.11.008
Zehiroglu, C., & Ozturk Sarikaya, S. B. (2019). The importance of antioxidants and place in today's scientific and technological studies. *Journal of Food Science and Technology*, *56*(11), 4757–4774. https://doi.org/10.1007/s13197-019-03952-x
Zesiewicz, T. A. (2019). Parkinson Disease. *Continuum (Minneapolis, Minn.)*, *25*(4), 896–918. https://doi.org/10.1212/CON.0000000000000764
Zhang, N, Xing, M., Wang, Y., Tao, H., & Cheng, Y. (2015). Repetitive transcranial magnetic stimulation enhances spatial learning and synaptic plasticity via the VEGF and BDNF-NMDAR pathways in a rat model of vascular dementia. *Neuroscience*, *311*, 284–291. https://doi.org/10.1016/j.neuroscience.2015.10.038
Zhang, Nianping, Yan, Z., Xin, H., Shao, S., Xue, S., Cespuglio, R., & Wang, S. (2024). Relationship among α-synuclein, aging and inflammation in Parkinson's disease (Review). *Experimental and Therapeutic Medicine*, *27*(1), 23. https://doi.org/10.3892/etm.2023.12311
Zhang, Q., Pi, J., Woods, C. G., & Andersen, M. E. (2010). A systems biology perspective on Nrf2-mediated antioxidant response. *Toxicology and Applied Pharmacology*, *244*(1), 84–97. https://doi.org/10.1016/j.taap.2009.08.018

Zhang, W., Deng, B., Xie, F., Zhou, H., Guo, J.-F., Jiang, H., Sim, A., Tang, B., & Wang, Q. (2022). Efficacy of repetitive transcranial magnetic stimulation in Parkinson's disease: A systematic review and meta-analysis of randomised controlled trials. *EClinicalMedicine*, *52*, 101589. https://doi.org/10.1016/j.eclinm.2022.101589

Zhang, X., Wang, J., Gao, J.-Z., Zhang, X.-N., Dou, K.-X., Shi, W.-D., & Xie, A.-M. (2021). P2X4 receptor participates in autophagy regulation in Parkinson's disease. *Neural Regeneration Research*, *16*(12), 2505–2511. https://doi.org/10.4103/1673-5374.313053

Zhang, Y., Tan, F., Xu, P., & Qu, S. (2016). Recent Advance in the Relationship between Excitatory Amino Acid Transporters and Parkinson's Disease. *Neural Plasticity*, *2016*, 8941327. https://doi.org/10.1155/2016/8941327

Zhang, Z., Zhang, S., Fu, P., Zhang, Z., Lin, K., Ko, J. K.-S., & Yung, K. K.-L. (2019). Roles of Glutamate Receptors in Parkinson's Disease. *International Journal of Molecular Sciences*, *20*(18). https://doi.org/10.3390/ijms20184391

Zhou, X., Li, K., Chen, S., Zhou, W., Li, J., Huang, Q., Xu, T., Gao, Z., Wang, D., Zhao, S., & Dong, H. (2022). Clinical application of transcranial magnetic stimulation in multiple sclerosis. *Frontiers in Immunology*, *13*, 902658. https://doi.org/10.3389/fimmu.2022.902658

Zhu, B., Yin, D., Zhao, H., & Zhang, L. (2022). The immunology of Parkinson's disease. *Seminars in Immunopathology*, *44*(5), 659–672. https://doi.org/10.1007/s00281-022-00947-3

Zimmermann, H. (2006). Ectonucleotidases in the nervous system. *Novartis Foundation Symposium*, *276*, 113-130,233-237,275-281.

Zimmermann, H., Zebisch, M., & Sträter, N. (2012). Cellular function and molecular structure of ecto-nucleotidases. *Purinergic Signalling*, *8*(3), 437–502. https://doi.org/10.1007/s11302-012-9309-4

Zucca, F. A., Basso, E., Cupaioli, F. A., Ferrari, E., Sulzer, D., Casella, L., & Zecca, L. (2014). Neuromelanin of the human substantia nigra: an update. *Neurotoxicity Research*, *25*(1), 13–23. https://doi.org/10.1007/s12640-013-9435-y

BIOGRAFIJA AUTORA

Milica B. Zeljković Jovanović rođena je u Nišu 07.07.1993. Osnovnu školu "Ujedinjene nacije" u Beogradu i XIII beogradsku gimnaziju završila je sa odličnim uspehom. Biološki fakultet Univerziteta u Beogradu, studijski program Biologija, upisala je 2012. godine, a diplomirala je 2016. godine sa prosečnom ocenom 9,54. Iste godine nastavlja master akademske studije, smer Molekularna biologija i fiziologija na Biološkom fakultetu, modul Biofizika koji završava sa prosečnom ocenom 9,83 i odbranom master rada "Elektrofiziološka evaluacija brzine provođenja nervnog impulsa duž kortikospinalnog trakta kod zdravih osoba". Nakon završenih master studija, 2017. godine upisuje doktorske studije na Biološkom fakultetu, studijski program Biologija, modul Eksperimentalna neurobiologija.

Od marta 2019. godine do aprila 2021. godine bila je zaposlena kao istraživač pripravnik na Institutu za biološka istraživanja „Siniša Stanković" - Institut od nacionalnog značaja za Republiku Srbiju, Univerzitet u Beogradu. Od maja 2021. godine zaposlena je kao istraživač-pripravnik na Katedri za opštu fiziologiju i biofiziku, Biološki fakultet Univerzitet u Beogradu gde je i uradila eksperimentalni deo svoje doktorske teze. Bila je angažovana na tri nacionalna projekta i dve COST akcije.

Milica Zeljković Jovanović je član Društva za Neuronauke Srbije, Evropske federacije društava za neuronauke (FENS), Evropskog društva za neurohemiju (ESN), Internacionalnog društva za neurohemiju (ISN), kao i Srpskog biološkog društva. Autor je ili koautor 16 naučnih radova u međunarodnim vodećim časopisima M20 kategorije (2 kategorije M21a, 9 kategorije M21 i 5 kategorije M22). Autor je više saopštenja na međunarodnim naučnim skupovima iz kategorije M34.

PRILOZI

Прилог 1.

Изјава о ауторству

Име и презиме аутора **Милица Б. Зељковић Јовановић**

Број индекса **Б3006/2017**

Изјављујем

да је докторска дисертација под насловом

„Ефекти репетитивне транскранијалне магнетне стимулације на неуродегенерацију, неуроинфламацију и компоненте пуринског сигналног система у моделу Паркинсонове болести изазване 6-хидроксидопамином код пацова“

- резултат сопственог истраживачког рада;
- да дисертација у целини ни у деловима није била предложена за стицање друге дипломе према студијским програмима других високошколских установа;
- да су резултати коректно наведени и
- да нисам кршио/ла ауторска права и користио/ла интелектуалну својину других лица.

У Београду, _____

Потпис аутора

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Прилог 2.

Изјава о истоветности штампане и електронске верзије докторског рада

Име и презиме аутора **Милица Б. Зељковић Јовановић**

Број индекса **Б3006/2017**

Студијски програм **Биологија**

Наслов рада **„Ефекти репетитивне транскранијалне магнетне стимулације на неуродегенерацију, неуроинфламацију и компоненте пуринског сигналног система у моделу Паркинсонове болести изазване 6-хидроксидопамином код пацова“**

Ментори **др Данијела Лакета и др Милорад Драгић**

Изјављујем да је штампана верзија мог докторског рада истоветна електронској верзији коју сам предао/ла ради похрањивања у **Дигиталном репозиторијуму Универзитета у Београду.**

Дозвољавам да се објаве моји лични подаци везани за добијање академског назива доктора наука, као што су име и презиме, година и место рођења и датум одбране рада.

Ови лични подаци могу се објавити на мрежним страницама дигиталне библиотеке, у електронском каталогу и у публикацијама Универзитета у Београду.

У Београду, _____

Потпис аутора

_____

Прилог 3.

Изјава о коришћењу

Овлашћујем Универзитетску библиотеку „Светозар Марковић“ да у Дигитални репозиторијум Универзитета у Београду унесе моју докторску дисертацију под насловом:

„Ефекти репетитивне транскранијалне магнетне стимулације на неуродегенерацију, неуроинфламацију и компоненте пуринског сигналног система у моделу Паркинсонове болести изазване 6-хидроксидопамином код пацова“

која је моје ауторско дело.

Дисертацију са свим прилозима предала сам у електронском формату погодном за трајно архивирање.

Моју докторску дисертацију похрањену у Дигиталном репозиторијуму Универзитета у Београду и доступну у отвореном приступу могу да користе сви који поштују одредбе садржане у одабраном типу лиценце Креативне заједнице (Creative Commons) за коју сам се одлучила.

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6. Ауторство – делити под истим условима. Дозвољавате умножавање, дистрибуцију и јавно саопштавање дела, и прераде, ако се наведе име аутора на начин одређен од стране аутора или даваоца лиценце и ако се прерада дистрибуира под истом или сличном лиценцом. Ова лиценца дозвољава комерцијалну употребу дела и прерада. Слична је софтверским лиценцама, односно лиценцама отвореног кода.