UNIVERSIDADE FEDERAL DE MINAS GERAIS
Instituto de Ciências Biológicas

Programa de Pós-graduação em Biologia Celular

Bruna da Silva Oliveira

CARACTERIZAÇÃO DE UM MODELO PRODRÔMICO DA DOENÇA DE PARKINSON E ESTUDO DO EFEITO DE MODULADORES DO SISTEMA RENINA-ANGIOTENSINA.

Belo Horizonte
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Orientador: Aline Silva de Miranda

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ATA DA DEFESA DE TESE DE DOUTORADO DE BRUNA DA SILVA OLIVEIRA

Às **nove horas** do dia **31 de outubro de 2023**, reuniu-se, no Instituto de Ciências Biológicas da UFMG, a Comissão Examinadora da Tese, indicada pelo Colegiado do Programa, para julgar, em exame final, o trabalho final intitulado: **"CARACTERIZAÇÃO DE UM MODELO PRODRÔMICO DA DOENÇA DE PARKINSON E ESTUDO DO EFEITO DE MODULADORES DO SISTEMA RENINA-ANGIOTENSINA"**, requisito final para obtenção do grau de Doutora em Biologia Celular. Abrindo a sessão, a Presidente da Comissão, **Dra. Aline Silva de Miranda**, após dar conhecimento aos presentes do teor das Normas Regulamentares do Trabalho Final, passou a palavra à candidata, para apresentação de seu trabalho. Seguiu-se a arguição pelos examinadores, com a respectiva defesa da candidata. Logo após, a Comissão se reuniu, sem a presença da candidata e do público, para julgamento e expedição de resultado final. Foram atribuídas as seguintes indicações:

Prof./Pesq.	**Instituição**	**Indicação**
Dra. Aline Silva de Miranda	UFMG	Aprovada
Dr. Victor Rodrigues Santos	UFMG	Aprovada
Dr. Thiago Verano Braga	UFMG	Aprovada
Dra. Erica Vieira	CAMH	Aprovada
Dra. Milene Alvarenga Rachid	UFMG	Aprovada

Pelas indicações, a candidata foi considerada: APROVADA

O resultado final foi comunicado publicamente à candidata pela Presidente da Comissão. Nada mais havendo a tratar, a Presidente encerrou a reunião e lavrou a presente ATA, que será assinada por

todos os membros participantes da Comissão Examinadora. **Belo Horizonte, 31 de outubro de 2023.**

Dra. Aline Silva de Miranda (Orientadora)_____

Dr. Victor Rodrigues Santos_____

Dr. Thiago Verano Braga_____

Dra. Erica Leandro Marciano Vieira_____

Dra. Milene Alvarenga Rachid_____

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“O insucesso é apenas uma oportunidade para
recomeçar com mais inteligência.

RESUMO

A doença de Parkinson (DP) é classicamente caracterizada por alterações motoras, como tremor de repouso, bradicinesia, rigidez e instabilidade postural, relacionados à perda de neurônios dopaminérgicos na substância *nigra pars compacta* (SNpc). A diminuição de dopamina também afeta núcleos não dopaminérgicos, levando a sintomas não motores relacionados à DP (SNDP), que podem aparecer até 25 anos antes dos sintomas motores. Embora SNDP reduzam a qualidade de vida dos pacientes com DP, os mecanismos que desencadeiam esses sintomas permanecem pouco esclarecidos. Pesquisas anteriores têm identificado a presença do Sistema Renina-Angiotensina (SRA) no encéfalo, levantando a hipótese de que componentes do SRA podem desempenhar um papel nas doenças neurodegenerativas, incluindo a DP. O objetivo principal do presente estudo foi investigar o potencial envolvimento do SRA em SNMDP. Primeiro, com objetivo de padronizar um modelo prodrômico da DP administramos 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) ou uma solução salina por via intranasal em camundongos C57BL/6 machos. Em seguida, realizamos uma ampla avaliação comportamental e analisamos marcadores neuroinflamatórios e dopaminérgicos em áreas chaves da DP, como SNpc e o Estriado. Uma vez estabelecido o modelo prodrômico da DP, conduzimos novos experimentos para investigar o papel do SRA nos SNMDP. Dividimos os camundongos C57BL/6 machos em cinco grupos, incluindo um grupo MPTP que recebeu solução salina a 0,9%. Os outros grupos receberam tratamentos específicos: Perindopril (5mg/Kg, um antagonista da enzima conversora de angiotensina (ECA), Telmisartan (10mg/Kg, um antagonista dos receptores AT1) e AVE099 (10mg/Kg, um agonista dos receptores Mas), administrados por gavagem. Esses tratamentos começaram cinco dias antes da infusão de MPTP e continuaram até o 11º dia após a infusão. Nossos resultados revelaram uma melhora significativa em parâmetros comportamentais, inflamatórios e neuroquímicos nos animais tratados com o AVE0991, indicando uma potencial neuroproteção conferida pelo eixo alternativo do SRA na DP experimental.

Palavras chaves: Doença de Parkinson, Sistema Renina Angiotensina, Neuroinflamação, MPTP.

ABSTRACT

Parkinson's disease (PD) is classically conceived as a motor condition characterized resting tremor, bradykinesia, rigidity and postural instability, related to the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). The decrease in dopamine also affects non-dopaminergic nuclei, leading to non-motor symptoms related to PD (NMS-PD), which can appear up to 25 years before the motor symptoms. Although NMS-PD reduce the quality of life of PD patients, the mechanisms underlying the development of these symptoms remain poorly understood. Previous research has identified the presence of the Renin-Angiotensin System (RAS) in the brain, raising the hypothesis that RAS molecules may play a role in neurodegenerative diseases, including PD. The main goal of this study was to investigate the potential involvement of the RAS in NMS-PD. First, with the aim of standardizing a prodromal model of PD, we administered 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or a saline solution intranasally to male C57BL/6 mice. Subsequently, we conducted a comprehensive behavioral assessment and analyzed neuroinflammatory and dopaminergic markers in key areas of PD, such as the SNpc and the Striatum. After this characterization, we conducted new experiments to investigate the role of RAS components in NMS-PD. We divided male C57BL/6 mice into five groups, including a MPTP group that received 0.9% saline. The other groups received specific treatments: Perindopril (5mg/Kg, an angiotensin-converting enzyme (ACE) inhibitor), Telmisartan (10mg/Kg, an AT1 receptor antagonist), and AVE099 (10mg/Kg, a Mas receptor agonist), administered by gavage. These treatments began five days before MPTP infusion and continued until the 11th day after infusion. Our results revealed a significant improvement in behavioral, inflammatory, and neurochemical parameters in animals treated with AVE0991, indicating a potential neuroprotective role of RAS alternative axis in experimental PD.

Keywords: Parkinson's Disease, Renina Angiotensin System, Neuroinflammation Non motor symptoms

LISTA DE ABREVIATURAS:

6- OHDA - 6-Hidroxidopamina

Ang – Angiotensina

AT1 – Receptor de Angiotensina tipo 1

BRA1 - Bloqueadores dos receptores de angiotensina

CL – Corpos de Lewy

DAT – Transportador de dopamina

Ddr – Receptor dopaminérgico

DP - Doença de Parkinson

Dpi – dias pós infusão

ECA – Enzima conversora de angiotensinogênio

IL – Interleucina

MPP + - 1-metil-4-phenylpyridinium

MPTP - 1-metil-4-fenil-1,2,3,6-tetra-hidropiridina

SNpc - Substância Negra parte compacta

SRA – Sistema Renina Angiotensina

APRESENTAÇÃO

O presente trabalho tem como objetivo principal apresentar os resultados obtidos durante o doutorado, no qual foram avaliadas alterações cognitivas e comportamentais durante a fase pré-clínica da Doença de Parkinson (DP), utilizando o modelo de infusão intranasal de MPTP em camundongos, bem como o efeito de estratégias farmacológicas que modulam o Sistema Renina Angiotensina (SRA) na prevenção dessas alterações.

A tese foi estruturada em 7 partes i) introdução, ii) justificativa, iii) hipótese, iv) objetivo, v) método, resultado, discussão sob forma de dois artigos e resultados adicionais que não entraram nos artigos; vi) considerações finais; vii) produções bibliográficas contendo os artigos publicados durante o doutorado, mas não relacionados diretamente com a tese.

No artigo 1 intitulado "*Nigrostriatal inflammation is associated with nonmotor symptoms in an experimental model of prodromal Parkinson's Disease"* submetido na revista Neurotoxicity Research (IF=3.7), demonstramos que uma infusão de (1mg/kg por narina) de 1-metil-4-fenil-1,2,3,6-tetra-hidropiridina (MPTP) é capaz de reproduzir sinais comportamentais e neuroquímicos da fase prodrômica da DP.

No artigo 2 intitulado "*Neuroprotective effects of Renin Angiotensin System alternative axis in non-motor symptoms of Parkinson's Disease"* em fase de correção pelos autores, demonstramos que o eixo alternativo do SRA tem um papel crucial no potencial neuroprotetor da DP.

SUMÁRIO

1. INTRODUCAO

A Doença de Parkinson (DP) é uma condição neurológica de natureza progressiva que afeta o sistema dopaminérgico, resultando em manifestações motoras como tremor de repouso, rigidez muscular e bradicinesia, caracterizada pela redução da velocidade dos movimentos, além de instabilidade postural. Esses sintomas estão correlacionados com a degeneração dos neurônios dopaminérgicos, células responsáveis pela síntese de dopamina, localizadas na região conhecida como *Pars compacta* da substância negra (SNpc) (Calne, 2005).

Além das manifestações motoras, a doença também pode apresentar sintomas não motores, como perda do olfato, ansiedade, depressão e insônia, que podem surgir até 25 anos antes dos sintomas motores propriamente ditos (Hou & Lai, 2007). Os mecanismos subjacentes ao desenvolvimento da patologia de Parkinson ainda não estão completamente elucidados. No entanto, processos inflamatórios (Araújo et al., 2022; Rocha et al., 2015) estresse oxidativo (Dias et al., 2013) e possivelmente a interação com o Sistema Renina Angiotensina (SRA)(Rocha et al., 2021) estão sendo investigados como potenciais desencadeadores da morte dos neurônios dopaminérgicos na SNpc, característica patológica definidora da doença.

1.1 Epidemiologia da Doença de Parkinson

A DP ocupa a segunda posição entre as doenças neurodegenerativas mais prevalentes em indivíduos com idade superior a 60 anos, apresentando uma prevalência inferior apenas à Doença de Alzheimer (Tysnes & Storstein, 2017). A incidência dessa condição tem exibido uma ampla variação, variando de 5 casos por cada 100.000 indivíduos para 355 casos por cada 100.000 indivíduos, o que representa um aumento de 5 a 10 vezes durante as últimas seis

décadas (Simon et al., 2020). É pertinente ressaltar que, devido ao processo de envelhecimento da população, é antecipado que o número de indivíduos afetados pela DP aumente significativamente até o ano de 2030 (Elbaz et al., 2016).

No Brasil, a obrigatoriedade de notificação da DP ainda não foi estabelecida em âmbito nacional. Essa ausência regulamentar representa um obstáculo significativo para a obtenção de uma estimativa precisa da prevalência da doença. Entretanto, de acordo com dados do Instituto Brasileiro de Geografia e Estatística (IBGE), há o surgimento de aproximadamente 36 mil novos casos por ano. Isso nos leva a uma estimativa de prevalência atual de cerca de 200 mil indivíduos convivendo com a DP. É válido ressaltar que essa estimativa considera uma taxa de incidência de 700 casos por 100.000 pessoas entre 60 e 69 anos, e de 1500 casos por 100.000 pessoas entre 70 e 79 anos (G. F. Santos et al., 2022)

É relevante, no entanto, observar que possíveis subnotificações e carências de informações podem estar afetando esses números. É imperativo reconhecer que a presente pesquisa destaca a necessidade urgente de conduzir investigações complementares. Tais estudos são cruciais para a promoção de uma compreensão mais abrangente da epidemiologia da DP dentro do contexto brasileiro.

1.2 Fisiopatologia da Doença de Parkinson

A fisiopatologia da DP envolve a degeneração progressiva de 50-70% dos neurônios dopaminérgicos na SNpc e pela presença de Corpos de Lewy (CL), inclusões citoplasmáticas formadas principalmente por α-sinucleína e ubiquitina, nos neurônios dopaminérgicos (Jellinger, 2012) (Fig.1). Essa degeneração dos neurônios dopaminérgicos leva a uma diminuição na quantidade de dopamina disponível no circuito motor resultando em sintomas motores característicos, como tremores, rigidez muscular, bradicinesia e instabilidade postural

(Simon et al., 2020). Além disso, a DP também pode envolver a disfunção de outras vias neuronais e sistemas de neurotransmissores, o qual contribuem para o aparecimento de sintomas não motores da DP, tais como perda da olfação, distúrbios do sono, depressão e ansiedade, alterações digestivas (Hussein et al., 2023).

A SNpc constitui uma região anatômica incumbida pela regulação e aprimoramento do movimento por meio da emissão de axônios dopaminérgicos excitatórios ao córtex motor primário e às áreas do estriado, nomeadamente os núcleos putâmen e globus pallidus. Essa projeção desempenha um papel crucial na modulação dos núcleos essenciais para a coordenação do movimento voluntário A supressão dos neurônios dopaminérgicos na SNpc resulta na desarticulação da via nigroestriatal, acarretando na diminuição concomitante dos níveis de dopamina no estriado (Keath et al., 2007).

Ademais, ocorre um decréscimo nos níveis de acetilcolina, glutamato e ácido gama-aminobutírico (GABA) no núcleo subtalâmico, no tálamo e no córtex cerebral. Estas regiões estão intimamente associadas à manifestação sintomatológica da Doença de Parkinson (DP), como discutido por Wichmann e De Long (2003).

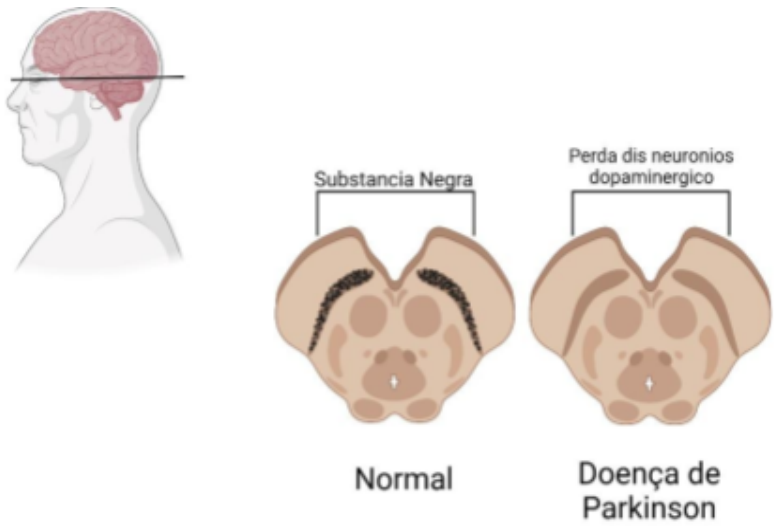

Figura 1: Neuropatologia da Doença de Parkinson. Imagem produzida pelo BioRender

As causas subjacentes a degeneração dos neurônios dopaminérgicos ainda se encontra envoltas em desconhecimento, no entanto, diversas teorias têm emergido a respeito dos possíveis desencadeadores responsáveis pelo declínio dessas células na SNpc. Entre tais teorias, destaca-se a perspectiva de que a inflamaçãoo (Vivekanantham et al., 2015) possa exercer um papel primordial enquanto catalisadora desse processo de mortalidade neuronal.

Existe uma relação intrínseca entre o avanço da idade e a manifestação da enfermidade em questão (Caslake et al., 2013). O processo de envelhecimento acarreta consigo alterações fisiológicas que frequentemente estão sujeitas a determinantes como o estilo de vida, a alimentação, o ambiente e a predisposição genética, entre outros fatores. Além disso, à medida que a idade avança, o sistema imunológico sofre modificações que o levam a adentrar em um estado conhecido como "inflamaging" (Calabrese et al., 2018). Em outras palavras, durante a trajetória de vida, agentes estressores endógenos e exógenos podem instigar respostas adaptativas locais e sistêmicas, incluindo a ativação do sistema imunológico, o que resulta em uma instância de "inflamação fisiológica" (Franceschi et al., 2000). Um equilíbrio fisiológico entre citocinas pró-inflamatórias e anti-inflamatórias se faz necessário. Contudo, à medida que a idade progride, pode ocorrer um desequilíbrio nesse balanço citocínico, tornando os indivíduos mais suscetíveis a respostas inflamatórias(Rocha et al., 2021). Adicionalmente, convém destacar que uma correlação se estabelece entre processos inflamatórios e enfermidades neurodegenerativas, conforme indicado por Franceschi et al. (2014).

Uma teoria tem sido formulada, sustentando que a liberação em pequena escala de α-sinucleína, em conjunto com o mencionado estado de "inflamaging", pode ativar as células da micróglia, desencadeando uma resposta inflamatória exacerbada, a qual poderia servir como o agente desencadeante subjacente à DP. A presença da α-sinucleína, uma proteína central nos CL, tem demonstrado ter importância significativa na etiologia da DP. A

toxicidade decorrente do aumento da α-sinucleína precede a formação de agregados volumosos ou fibrilas (Kalia et al., 2013). Logo, o aumento desta proteína exerce interferência em uma variedade de funções intracelulares, notadamente na manutenção da homeostase vesicular e no transporte neuronal(Davidson et al., 1998; Postuma, 2013; Rhoades et al., 2006). Além do mais, vale ressaltar que astrócitos portadores de inclusões de α-sinucleína, com reatividade imunológica, são identificados na DP. (D'Agostinho et al., 2012).

1.3 Doença de Parkinson e a neuroinflamação

Neuroinflamação é um fator importante nas doenças neurodegenerativas, assim como na DP. Vários autores demonstram uma relação entre a neuroinflamação e a perda dos neurônios dopaminérgicos na SNpc(Damier et al., 1993; Tansey & Goldberg, 2010; W. Zhang et al., 2023). A neuroinflamação cronica está associada com a perda neuronal através de mecanismos biológicos, como a produção excessiva de espécies reativas de oxigênio (ROS), acumulação de agregados de α-sinucleína, ativação microglial e astroglial, assim como a liberação de mediadores inflamatórios(Hy et al., 2021; W. Zhang et al., 2023).

A fisiopatologia da DP envolve a perda progressiva dos neurônios dopaminérgicos e a presença dos Corpos de Lewy, os quais são agregados proteicos de proteínas, principalmente a α-sinucleína(Jellinger, 2012). Essa super expressão da α-sinucleína ou a duplicações no gene SNCA na DP desencadeia a acumulação tóxica de fibrilas de α-sinucleína.(Braak et al., 2007) Os corpos de Lewy e neuritos são tóxicos e causam perda neuronal dopaminérgica na DP. Além disso, a patologia de α-sinucleína pode ativar a micróglia estimulando o receptor toll na superfície microglial e iniciando a resposta inflamatória(Li et al., 2021). A resposta inflamatória promove a liberação de citocinas pró-inflamatórias, tais como TNF-α, IL-1β e IL-6,e ativa a via de sinalização NF-KB, levando a uma exacerbação adicional da resposta

inflamatória (Guan & Han, 2020). Portanto, a neuroinflamação generalizada e sustentada é um componente importante da patogênese da DP.

1.4 Doença de Parkinson e os fatores neurotróficos

Os fatores neurotróficos são proteínas que desempenham um papel importante no desenvolvimento, sobrevivência e função dos neurônios(Almeida et al., 2005; Kromer, 1987; Palasz et al., 2020). Eles são produzidos por células da neuróglia e atuam em neurônios promovendo sua sobrevivência, crescimento e diferenciação. Alguns exemplos de fatores neurotróficos incluem o fator de crescimento nervoso (NGF), o fator neurotrófico derivado do cérebro (BDNF) e o fator neurotrófico derivado da glia (GDNF). Esses fatores são importantes para a manutenção da saúde do sistema nervoso e têm sido estudados como potenciais terapêuticos para doenças neurodegenerativas e lesões nervosas.(Palasz et al., 2020; Weissmiller & Wu, 2012)

Na DP, o papel dos fatores neurotróficos tem sido estudado como potencial neuroprotetor dos neurônios dopaminérgicos(Palasz et al., 2020). Estudos demonstraram que o GDNF tem efeitos neuroprotetores robustos em neurônios dopaminérgicos em modelos de primatas não humanos de DP(Allen et al., 2013; Autry & Monteggia, 2012; Kaur et al., 2018).

Além disso, estudos tem demonstrado que fatores neurotróficos, como o GDNF, BDNF e NGF, desempenham um papel neuroprotetor ativando vias de sinalização intracelular que promovem a sobrevivência e o crescimento neuronal. O GDNF, BDNF e o NGF se liga a receptores específicos nas células nervosas, incluindo o receptor tirosina quínase Ret, TrkB e TrKA respectivamente, os quais ativam uma cascata de sinalização intracelular que inclui a ativação de proteínas quínases, como a MAPK/ERK1/2 e a Akt. Essas proteínas quínases ativadas promovem a sobrevivência neuronal, inibem a apoptose, reduzem a neurotoxicidade

do glutamato e do óxido nítrico e reduzem o dano celular causado pelo estresse oxidativo(Markham et al., 2012; Nguyen et al., 2009). Além disso, os fatores neurotróficos podem promover a plasticidade sináptica e a regeneração neuronal, melhorando a função dos neurônios afetados na DP(Lykissas et al., [s.d.]).

1.5 Modelos animais da Doença de Parkinson

Os modelos animais para simular a DP podem ser categorizados em duas classes distintas: modelos genéticos e neurotóxicos. Ambos os grupos apresentam suas próprias vantagens e limitações (Fig.2).

Os modelos genéticos têm sua origem embasada em alvos que teoricamente podem induzir a DP em humanos (Blesa & Przedborski, 2014). Contudo, os atuais modelos genéticos disponíveis não conseguem simular de maneira completa os fenótipos de neurodegeneração que são característicos da patologia (Dawson et al., 2010). As limitações inerentes a esses modelos podem ser superadas mediante a adoção de abordagens que utilizam modelos

	Animal model	Motor behavior	SNc neuron loss	Striatal DA loss	Lewy body/Syn pathology
Toxin-based	MPTP Mice	Reduced locomotion, bradykinesia	↑↑↑	↑↑↑	NO
	MPTP Monkeys	Reduced locomotion, altered behavior, tremor, and rigidity	↑↑↑	↑↑↑	NO
	6-OHDA rat	Reduced locomotion, altered behavior	↑↑↑	↑↑↑	NO
	Rotenone	Reduced locomotion	↑↑	↑↑↑	YES
	Paraquat/maneb	Reduced locomotion	↑↑	↑↑↑	YES
	MET/MDMA	Reduced locomotion	↑↑	↑↑↑	NO
Genetic mutations*	α-Synuclein	Altered behavior, reduced or increased motor activity	↑ Not consistent	↑	↑ (in old animals)
	LRKK2	Mild behavioral alteration	NO	NO	NO
	PINK1	No obvious alterations or reduced locomotion	NO	NO	NO
	PARKIN	No obvious locomotion or reduced locomotion	NO	↑	NO
	DJ-1	Decreased locomotor activity	NO	NO	NO
	ATP13A2	Late onset sensorimotor deficits	NO	NO	NO
Others	SHH	Reduced locomotion	↑↑	↑↑	NO
	Nurr1	Reduced locomotion	↑↑	↑↑	NO
	Engrailed 1	Reduced locomotion	↑↑	↑	NO
	Pitx3	Reduced locomotion	↑↑↑	↑↑↑	NO
	C-Rel-NFKB	Gait, bradykinesia, rigidity	↑↑	↑↑	YES
	MitoPark	Reduced locomotion, tremor, and rigidity	↑↑	↑↑	YES
	Atg7	Late onset locomotor deficits	↑↑	↑↑	YES
	VMAT2	Reduced locomotion and altered behavior	↑↑	↑↑	YES

↑↑↑, *Severe loss*; ↑↑, *Moderate loss*; ↑, *Mild loss*.
**This table summarizes general observations for each model. See the main text for full and specific description of different animal models for each genetic mutation.*

Figura 2:: Modelos neurotóxicos utilizados para o estudo de DP. Retirado de Blessa J. et al.

neurotóxicos. Estes modelos empregam distintos agentes moleculares para provocar lesões na via nigroestriatal, o que, por sua vez, facilita a condução de novas investigações.

Através dos modelos neurotóxicos da DP, diversos compostos, tais como a 6-Hidroxidopamina (6-OHDA), a Rotenona, a 1-metil-4-fenil-1,2,3,6-tetraidropiridina (MPTP) e o Paraquat, são utilizados em diferentes vias de administração, como a intranasal, a intracraniana e a intraperitoneal, com o propósito de servirem como modelos experimentais destinados a investigar os fenótipos associados à DP. Esses modelos animais conseguem reproduzir, em certa medida, as principais características da PD, incluindo defeitos motores, perda progressiva de neurônios dopaminérgicos na substância negra pars compacta e a formação de corpos de Lewy. Entretanto, nenhum desses modelos neurotóxicos reproduz perfeitamente todas as características da PD(Chi et al., 2018) .

Cada modelo experimental terá impacto diferenciado nas vias metabólicas, desencadeando eventos relacionados ao estresse oxidativo, inflamação e comprometimento da função mitocondrial. Esses desdobramentos cumulativos culminam na degeneração dos neurônios dopaminérgicos. Como resultado, a seleção criteriosa da neurotoxina específica e do modelo animal a ser empregado revela-se um aspecto de extrema importância para a reprodução das características da afecção em análise, bem como para a minuciosa análise dos mecanismos patogênicos subjacentes(Blesa & Przedborski, 2014).

1.5.1 Modelo experimental de doença de Parkinson por administração de 1-Metil-4-fenil-1,2,3,6-tetrahidropiridina – MPTP

O composto químico conhecido como 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) induz uma neurodegeneração seletiva dos neurônios dopaminérgicos da substância negra quando administrado sistemicamente. Foi descoberto que o MPTP tem efeitos neurotóxicos e está relacionado à doença de Parkinson. Foi em 1982 que se descobriu uma

conexão entre MPTP e a doença de Parkinson, quando um grupo de indivíduos jovens que consumiram heroína contaminada com esse composto apresentarem os sinais clássicos da DP (Langston, 2017).

O MPTP é uma substancia lipossolúvel que ultrapassa a barreira hematoencefálica. Uma vez no cérebro, ele é metabolizado em um composto tóxico chamado 1-metil-4-fenilpiridínio (MPP+) por meio da ação da enzima monoamina oxidase, em particular a isoforma MAO B (Dauer & Przedborski, 2003; Przedborski et al., 2001). Esta transformação ocorre predominantemente dentro dos astrócitos. O MPP+ exibe toxicidade específica aos neurônios dopaminérgicos na substância negra, ocasionando a perda dessas células e, consequentemente, dando origem aos sintomas característicos da doença de Parkinson (Przedborski & Jackson-Lewis, 1998).

A seletividade do MPP+ aos neurônios dopaminérgicos é facilitada pela sua afinidade ao transportador de dopamina (DAT)(Michel et al., 1990). Após ser internalizado por esses neurônios, o MPP+ se acumula nas mitocôndrias, alterando a funcionalidade do Complexo I da cadeia de transporte de elétrons, o que culmina na redução da produção de ATP e na amplificação da geração de radicais livres. Essas alterações bioenergéticas e oxidativas resultam na subsequente degeneração neuronal (Dauer & Przedborski, 2003; Lotharius et al., 1999; Mustapha & Taib, 2021).

Dentre os modelos de neurotoxinas empregados para simular a DP, o uso de MPTP tem se destacado pela sua ampla utilização. Este destaque é atribuído não apenas a sua viabilidade econômica, mas também a sua correlação clínica mais significativa em comparação com os outros modelos de neurotoxinas (Mustapha & Taib, 2021).

No que diz respeito aos modelos de camundongos induzidos por MPTP para estudar a DP, diversas vias de administração têm sido empregadas. A administração intraperitoneal é comumente adotada, pois ela resulta em danos expressivos na função motora e na integridade

neuronal dopaminérgica.(Y. Zhang et al., 2018; Zhu et al., 2020) .No entanto, o uso de outras vias de administração, como a subcutânea, tem gerado resultados conflitantes quanto à formação de inclusões citoplasmáticas semelhantes a corpos de Lewy (Mustapha & Taib, 2021). E por fim, a administração intranasal tem se revelado vantajosa nos estudos da DP. Esse método é não invasivo e oferece uma representação mais próxima do ambiente fisiológico. Além disso, sua abordagem menos agressiva em relação à via intraperitoneal permite uma investigação mais abrangente, não apenas dos sintomas motores, mas também dos sintomas não motores DP(D. S. Prediger et al., 2011; B. S. Oliveira et al., 2023; R. D. S. Prediger et al., 2010).

1.6 Sistema Renina Angiotensina

O sistema renina-angiotensina (SRA) é um sistema hormonal amplamente reconhecido por sua função reguladora na pressão arterial, bem como no equilíbrio de fluidos e eletrólitos no organismo(Dhanachandra Singh & Karnik, 2017). Esse sistema é constituído por uma complexa rede de enzimas, peptídeos e receptores que colaboram de maneira sinérgica para assegurar a homeostase corporal.

O componente bioativo clássico do SRA consiste na Angiotensina (Ang) II, a qual é gerada através da ativação da renina. Essa enzima desencadeia a conversão do Angiotensinogênio em Ang I, que subsequente é cindida pela ação da enzima conversora de angiotensina (ECA). A Ang II, por sua vez, estabelece ligação com os receptores de Angiotensina do tipo I e II (AT1, AT2) (Paul et al., 2006).

Os receptores de angiotensina AT1 e AT2 desempenham papéis distintos no organismo, sendo distribuídos em diversos tecidos, como os vasos sanguíneos, o coração, os rins e o cérebro. Os receptores AT1 são responsáveis pelos efeitos sistêmicos da angiotensina II, incluindo vasoconstrição e hipertensão. Adicionalmente, esses receptores também

influenciam processos inflamatórios, apoptose e crescimento celular (referência necessária). Em contraste, os receptores AT2 são menos numerosos e encontrados em tecidos diversos. Tradicionalmente, os receptores AT2 apresentam efeitos opostos aos do AT1, como promoção da vasodilatação, efeitos anti-inflamatórios, inibição da proliferação celular e regulação da apoptose (Fyhrquist et al., 2008). Entretanto, a função precisa dos receptores AT1 e AT2 permanece parcialmente elucidada, requerendo investigações mais aprofundadas.

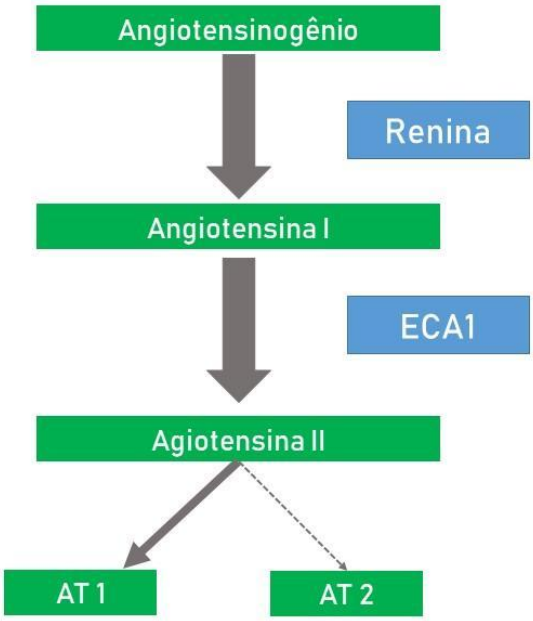

Figura 3: Componentes do Sistema Renina Angiotensina. (Adaptado Gismond et al., 2011)

Atualmente, um maior número de componentes do SRA tem sido identificado, cada um desempenhando papéis na modulação intrínseca desse sistema. Um exemplo notável é a enzima conversora de angiotensinogênio 2 (ECA2), homólogo da ECA, que tem sido observada em variados tecidos (Bacani e Frishman, 2006; Hamming et al., 2007). A ECA2, efetua a conversão da Ang II em Ang (1-7) ou Ang VI, desempenhando várias funções. A

Ang IV atua diretamente nos receptores do tipo AT4, cujas atribuições estão diretamente vinculadas a processos cognitivos como memória e aprendizagem (Wright et al. 2011; Kloet et al. 2010). Por outro lado, a Ang (1-7) age de maneira direta no receptor conhecido como Mas. Membro da família de receptores associados a proteína G, o receptor Mas antagoniza os efeitos instigados pela ativação do receptor AT1 (Clark et al., 2001; Kostenis et al., 2005). Além disso, a ativação do receptor Mas pela Ang (1-7) resulta em vasodilatação, inibição da proliferação celular e redução da inflamação (Fyhrquist et al., 2008).

1.6.1 Sistema Renina Angiotensina no Sistema Nervoso Central

Durante um longo período, o Sistema Renina-Angiotensina (SRA) tem sido tradicionalmente concebido como um sistema hormonal circulante, destinado a regular a pressão arterial e o equilíbrio osmótico. Embora a Angiotensina (Ang) II não seja capaz de atravessar a barreira hematoencefálica, os componentes do SRA estão amplamente distribuídos por todo o Sistema Nervoso Central (SNC) (Saavedra, 2012). Atualmente, é reconhecido que as ações do RAS no SNC vão além da simples regulação da pressão arterial e do equilíbrio hidroeletrolítico. De fato, o RAS desempenha um papel em diversas funções cerebrais, incluindo aspectos motores, cognitivos e de modulação emocional/comportamental (Rocha et al., 2021) Além disso, o RAS tem sido associado à fisiopatologia clínica observada em várias doenças neurodegenerativas, como a doença de Alzheimer (Kehoe, 2009)), doença de Huntington (Miranda et al., 2023), doença de Parkinson (Sekar et al., 2018), entre outras.

O RAS cerebral é composto por dois eixos principais: o eixo clássico, que inclui a enzima conversora de angiotensina (ECA), Ang II e o receptor tipo 1 de angiotensina (AT1), e o eixo de regulação contrária, composto pela enzima conversora de angiotensina 2 (ECA2), Ang(1-7) e o receptor Mas (Xue et al., 2020). Nos receptores AT1 do eixo clássico, observa-se uma expressão proeminente nas células endoteliais dos vasos cerebrais, o que explica como a

Ang II circulante promove a estimulação e inibição do receptor AT1, desempenhando um papel vital na modulação do fluxo sanguíneo cerebral (Nair et al., 2018). Adicionalmente, os receptores AT1 também estão presentes em certos circuitos neuronais, como nucleo paraventricular, trato mesencefálico (Höhle et al., 1995), micróglias (Rodriguez-Perez et al., 2015) e astrócitos (O'Connor & Clark, 2019). Dessa forma, a ativação dos receptores AT1 no cérebro pode influenciar processos como neurotransmissão (Oz et al., 2005), plasticidade sináptica(Oz et al., 2005), inflamação(Labandeira-Garcia et al., 2017a) entre outras funções cerebrais.

Por outro lado, o eixo contra-regulatório apresenta os receptores Mas, os quais são identificados em regiões como o tronco encefálico, hipotálamo e amígdala, além de outras estruturas cerebrais (Dhanachandra Singh & Karnik, 2017). Apesar de ser um componente recentemente descrito, o receptor Mas exibe um papel protetor no sistema nervoso central, sendo que sua ativação pode estar associada à modulação de vias anti-oxidantes, anti-inflamatórias, anti-apoptóticas, anti-fibróticas e anti-trombóticas, todas essas implicadas na fisiopatologia de doenças neuropsiquiátricas (Duan et al., 2021; Rukavina Mikusic et al., 2021).

1.6.2 Moduladores do Sistema Renina Angiotensina

Os bloqueadores do Sistema Renina-Angiotensina (BRAS) foram inicialmente desenvolvidos para controlar o aumento da pressão arterial, que está diretamente relacionado à ativação dos receptores do tipo 1 da angiotensina (AT1). No entanto, descobriu-se que os BRAS do SRA não só regulam a pressão arterial, mas também reduzem a resposta inflamatória, proporcionando benefícios adicionais ao metabolismo(Hollenberg, 2010; Leung, 2007).

Os BRAS englobam um conjunto de moléculas que interferem no Sistema Renina-Angiotensina, abarcando inibidores da enzima conversora de angiotensina (ECA) e bloqueadores dos receptores AT1. Os estudos relacionados aos BRAS têm sido conduzidos devido aos seus potenciais efeitos neuroprotetores (Rocha et al., 2018a).

No contexto cerebral, a ativação dos receptores AT1 desencadeia efeitos pró-inflamatórios, notadamente caracterizados pelo aumento dos níveis de citocinas pró-inflamatórias (Benicky et al., 2011) e pela ativação das células microgliais (Labandeira-Garcia et al., 2017a). Além disso, os receptores AT1 promovem efeitos pró-oxidativos por meio da ativação da enzima oxidase NADPH (Pendergrass et al., 2009), enquanto também suprimem a atividade da enzima antioxidante superóxido dismutase e de outras enzimas com propriedades antioxidantes (Saleem et al., 2016).

Dentre os BRAS, destaca-se o Telmisartan (TEL). O TEL configura-se como um antagonista seletivo não peptídico do receptor AT1, empregado como agente farmacêutico para a regulação da hipertensão, sem exercer influência sobre outros sistemas de receptores envolvidos na regulação cardiovascular (McClellan et al., 1998). Ademais, o TEL opera como agonista parcial do receptor ativado por proliferadores de peroxissoma (PPAR)-gama, conferindo-lhe efeitos anti-hipertensivos, bem como propriedades anti-inflamatórias e antioxidantes mediante a ativação do PPAR-gama (Kishi et al., 2012).

Por sua vez, o Perindopril assume a forma de um éster pró-fármaco que, quando metabolizado no fígado, transforma-se em perindoprilato. Este último composto atua inibindo a atividade da enzima conversora de angiotensina (ECA), interrompendo assim a formação da Angiotensina (Ang) II e bloqueando a ativação do eixo clássico do RAS (Dong et al., 2023)

Por fim, o AVE0991 configura-se como um composto análogo não peptídico da Angiotensina (1-7). Este agente atua como um agonista do receptor Mas da Angiotensina (1-7), mimetizando, quando ativado, os efeitos dessa angiotensina em diversas estruturas. Isso

ocorre ao potencializar a via alternativa do SRA (R. A. S. Santos & Ferreira, 2006). A via alternativa apresenta um papel central na sinalização celular de diversos processos fisiológicos, englobando a regulação da pressão arterial, a resposta inflamatória e a função cognitiva. O Mas, como receptor acoplado à proteína G, é ativado pela angiotensina (1-7), desencadeando uma cascata de sinalização intracelular que culmina em efeitos neuroprotetores. O AVE0991, ao funcionar como agonista do Mas, é capaz de ativar essa via de sinalização celular de maneira similar à angiotensina (1-7)(Mi et al., 2021, p. 202).

1.7 Tratamentos utilizados para Doença de Parkinson

A redução dos níveis de dopamina no circuito nigroestriatal da SNpc, tem sido associada aos sintomas motores na DP. Assim, apesar de os mecanismos subjacentes à degeneração dos neurônios dopaminérgicos ainda não estarem plenamente esclarecidos, as principais abordagens terapêuticas têm se centrado na modulação do sistema dopaminérgico (Armstrong & Okun, 2020).

Nesse contexto, o tratamento da DP engloba uma variedade de medicamentos com distintos mecanismos de ação, com o propósito de aumentar os níveis de dopamina. Dentre esses fármacos, incluem-se levodopa, agonistas dopaminérgicos, inibidores da monoaminoxidase-B, inibidores da catecol-O-metil transferase, amantadina e agentes anticolinérgicos. Os tratamentos visam desacelerar os sintomas, uma vez que não há cura definitiva para a DP (Dong et al., 2023).

Ademais, sintomas não motores também se associam à DP, tais como depressão, perda do olfato, ansiedade e alterações cognitivas, os quais podem afetar a qualidade de vida dos pacientes com DP (Schapira et al., 2017). Entretanto, não existe um tratamento específico para esses sintomas. Portanto, psiquiatras desempenham um papel complexo ao monitorar esses sintomas e determinar se derivam da progressão natural da DP ou se são influenciados

por outros medicamentos, estados emocionais, entre outros fatores. A maior parte dos medicamentos aprovados pela FDA (Food and Drug Administration) que se encontram disponíveis para amenizar os sintomas não motores da DP estão relacionados aos tratamento sintomático tais como depressão, ansiedade, sialorreia e distúrbios gastrointestinais (Seppi et al., 2019).

Consequentemente, pacientes com DP que experimentam depressão geralmente são tratados com antidepressivos convencionais da classe dos Inibidores Seletivos de Recaptação de Serotonina (ISRSs) ou Inibidores de Recaptação de Serotonina e Noradrenalina (IRSNs). Os ISRSs constituem uma categoria de antidepressivos que atuam aumentando os níveis de serotonina no cérebro, o que pode auxiliar na melhoria do humor e na redução dos sintomas de depressão e ansiedade. Contudo, é imperativo observar que a administração de ISRSs e outros medicamentos para gerenciar os sintomas não motores da DP deve ser rigorosamente monitorada por um profissional de saúde, uma vez que o impacto desses fármacos pode resultar em dependência, hipomania e agitação noturna (Keath et al., 2007; Nagy & Schrag, 2019; Sharma et al., 2017).

1.8 Doença de Parkinson e o Sistema Renina Angiotensina

O sistema renina-angiotensina (SRA) tem sido implicado na patofisiologia de várias doenças neurodegenerativas, tais como a doença de Alzheimer, a doença de Huntington e a doença de Parkinson (Rocha et al., 2021). Como previamente descrito, o SRA compreende dois eixos fundamentais: o eixo clássico e o contra-regulatório ou alternativo, cujas funções se antagonizam. O eixo clássico, associado à vasoconstrição, inflamação e estresse oxidativo, contrasta com o eixo contra regulatório, que ostenta propriedades anti-inflamatórias, antioxidantes e neuroprotetoras . A interação entre esses eixos exerce influência significativa na patofisiologia das enfermidades neurodegenerativas (Goldstein et al., 2016).

No tocante à doença de Parkinson (DP), a hiperatividade do eixo clássico do SRA pode exacerbar a progressão da degeneração dopaminérgica, agravando o estresse oxidativo e a neuroinflamação. Este aumento na atividade do SRA torna as células dopaminérgicas mais suscetíveis aos danos, agravando assim a condição patológica (Gelders et al., 2018; Grammatopoulos et al., 2007).

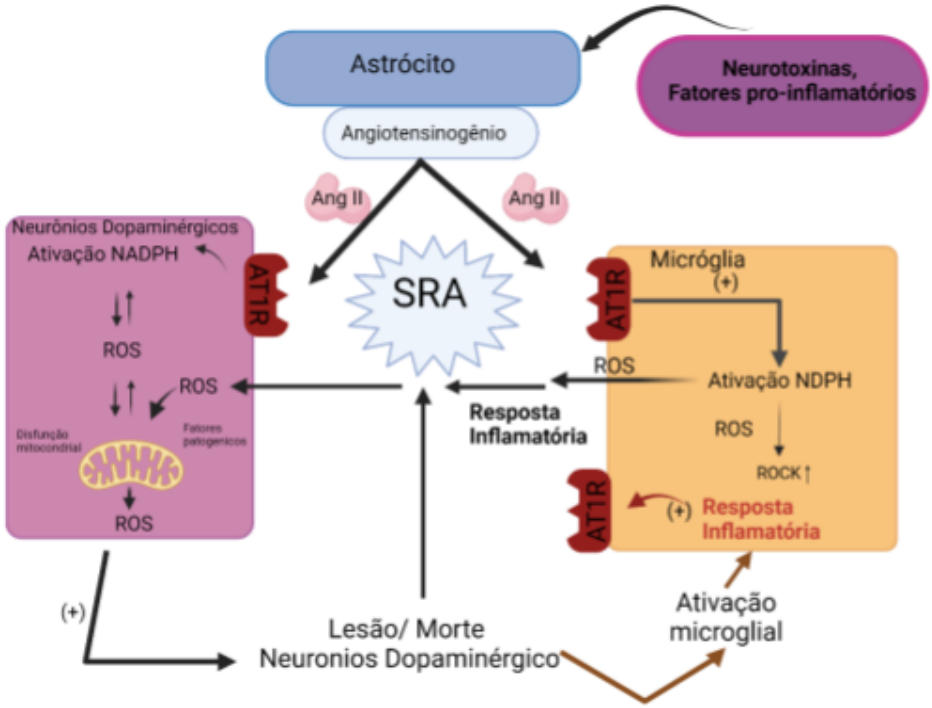

Figura 4 Efeitos da hiper ativação do SRA na progressão da neurodegeneração dopaminergica.(Adaptado Labareda-Garcia et al. 2016). Esquema feito o BioRender

No interior das células dopaminérgicas, múltiplos fatores patogênicos, a exemplo da disfunção mitocondrial, alterações relacionadas ao envelhecimento ou a exposição a neurotoxinas, podem desencadear o processo de lesão celular e, consequentemente, prejudicar a função dopaminérgica. Isso culmina em um aumento na ativação do receptor AT1 tanto em neurônios dopaminérgicos quanto em micróglias. Ademais, observa-se um aumento na produção de angiotensinogênio/angiotensina por parte dos astrócitos. A elevação da atividade do eixo clássico do SRA em células dopaminérgicas resulta na intensificação da

NADPH-oxidase, propiciando um aumento na produção de ROS (espécies reativas de oxigênio) e a liberação de sinais pró-inflamatórios. Nas micróglias, a ativação do complexo NADPH-oxidase microglial desencadeia uma resposta inflamatória robusta, promovendo a liberação extracelular de quantidades elevadas de ROS, citocinas e fatores neurotóxicos. Isso, por sua vez, propaga a progressão da neuroinflamação, o estresse oxidativo e a degeneração dos neurônios dopaminérgicos (Fig.4) (Labandeira-Garcia et al., 2016).

Embora a pesquisa sobre o uso de moduladores do SRA na doença de Parkinson ainda esteja em seus estágios iniciais, há algumas evidências sugerindo que esses medicamentos podem ter um efeito neuroprotetor e serem benéficos no tratamento da doença de Parkinson (Sathiya et al., 2013; Sekar et al., 2018).

Pesquisas como as conduzidas por Sathiya e colaboradores em 2013 demonstraram que o pré-tratamento com Telmisartan, um inibidor do receptor AT1, exibiu a capacidade de prevenir a neurodegeneração dopaminérgica induzida por MPTP e o subsequente comprometimento motor. Adicionalmente, observou-se que o Telmisartan reduziu a expressão da proteína α-sinucleína e promoveu um aumento nos níveis de fator neurotrófico derivado do cérebro (BDNF) e fator neurotrófico derivado de células gliais (GDNF). Além disso, a administração de captopril, um inibidor da enzima conversora de angiotensina (ECA), em camundongos, resultou na diminuição da perda de neurônios dopaminérgicos e na melhoria do desempenho motor (Sonsall et al., 2013).

Os moduladores do SRA podem proteger contra DP por meio de vários mecanismos neuroprotetores. Esses medicamentos podem reduzir a inflamação neurotóxica, diminuir a ativação de caspase-3 pró-apoptótica, proteger a via de sobrevivência da fosfoinositídeo-3-quinase/proteína quinase B/Akt/glicogênio sintase quinase 3 beta, reduzir a expressão do receptor N-metil-D-aspartato (NMDA) e diminuir a expressão do receptor tipo 1 da angiotensina II (AT1R), que está associado a atividade pró-apoptótica e pró-inflamatória.

Além disso, alguns bloqueadores do receptor AT1, como o telmisartan, têm propriedades antioxidantes e podem regular a expressão de microRNAs seletivos, como o microRNA-155 (miR-155), que reduz a expressão do AT1R (Villapol & Saavedra, 2015). No entanto, mais pesquisas são necessárias para entender completamente os mecanismos pelos quais os moduladores do RAS protegem contra a DP.

Entretanto estudos vêm apontando que a ativação do eixo contra regulatório ACE2/angiotensina-(1e7)/receptor mas, pode ter implicações terapêuticas no tratamento de doenças neurológicas. Estudos em animais mostraram que a ativação do receptor mas pode ter maiores efeitos neuroprotetores em modelos de doenças neurodegenerativas, como a doença de Alzheimer(Deng et al., 2023) e a doença de Parkinson(Mi et al., 2021). Além disso, a ativação do receptor Mas também pode melhorar a plasticidade sináptica e a memória (Albrecht, 2007; Hellner et al., 2005). No entanto, mais pesquisas são necessárias para determinar a eficácia e segurança do uso de moduladores do RAS nos sintomas não motores da DP, assim como o papel do eixo alternativo nos tratamentos de doenças neurológicas em humanos.

2. JUSTIFICATIVA

Para entendermos a relação entre o SRA e a DP, pretendemos buscar por alterações nos componentes do SRA na DP, com enfoque na regulação do sistema imune e associação com o desenvolvimento dos sintomas (não motores e motores) da doença. Para isso usaremos o modelo experimental de parkinsonismo, induzido pela pró-neurotoxina 1-metil-4-fenil-1,2,3,6-tetraidropiridina (MPTP), administrado pela via intranasal. Este modelo é considerado sub-clínico, pois apresenta danos olfatórios, danos na memória, mas pouco prejuízo motor, como os observados nas fases iniciais da DP(R. D. S. Prediger et al., 2010). Poucos estudos relacionando o SRA, regulação do sistema imune e o desenvolvimento dos sintomas da DP em modelos experimentais têm sido relatados. O conhecimento acerca do processo neuroinflamatório e o SRA, na DP, também é bastante restrito. Além disso, é fundamental ressaltar que até o presente momento, não existe tratamento para os sintomas não motores da DP, tornando o estudo de potenciais alvos terapêuticos um objetivo científico urgente.

Durante o meu mestrado, demonstramos que a infusão intranasal de MPTP (1mg/narina) em camundongos C57BL/6 induziu sintomas da fase pré-clínica da DP, tais como ansiedade e perda de memória olfatória. Os sintomas foram prevenidos ou reduzimos nos animais tratados com o agonista dos receptores do tipo Mas, AVE0991. Por isso, acreditamos que a abordagem proposta nos auxiliará na compreensão da fisiopatologia da DP associada aos sinais não motores e, possivelmente, na detecção de potenciais alternativas terapêuticas.

3. HIPÓTESE

"A ativação do eixo contra-regulatório do Sistema Renina-Angiotensina é mais eficaz em amenizar os sintomas não motores da Doença de Parkinson do que a inibição do eixo clássico".

4. OBJETIVOS

4.1 Objetivo Geral

Investigar os processos inflamatórios e neuroquímicos relacionados ao desenvolvimento de alterações cognitivas e comportamentais durante a fase prodômica da Doença de Parkinson, utilizando o modelo de infusão intranasal de MPTP em camundongos, bem como o efeito de estratégias farmacológicas que modulam o Sistema Renina Angiotensina na prevenção dessas alterações.

4.2 Objetivos específicos

4.2.1 Investigar transtornos cognitivos e comportamentais durante a fase pré-clínica da DP, assim como avaliar os possíveis efeitos protetores dos moduladores do SRA.

4.2.2 Avaliar a concentração dos componentes do SRA e a expressão dos receptores AT1, AT2 e Mas, no estriado, SNpc, hipocampo e córtex pré-frontal de camundongos C57BL/6 submetidos a infusão intranasal com MPTP e os efeitos dos tratamentos com os moduladores do SRA.

4.2.3 Avaliar os processos inflamatórios localizados no SNC durante a fase pré-clínica da DP, por meio da análise dos níveis de citocinas e de fatores neurotróficos no estriado, SNpc, hipocampo e córtex pré-frontal de camundongos C57BL/6 submetidos a infusão intranasal com MPTP e tratados com os moduladores do SRA.

4.2.4 Avaliar alterações do sistema dopaminérgicos, por meio da expressão dos receptores dopaminérgicos e da tirosina hidroxilase nas regiões da SNpc e estriado, na fase não motora da DP e a ação dos moduladores do SRA.

4.2.5 Avaliar morte de neurônios dopaminérgicos na Substancia Negra e Estriado, além de investigar o efeito dos moduladores do SRA, na proteção neuronal.

4.2.6 Avaliar a ativação das células da gliais, tais como micróglia e astrócito, através de imunohistoquímica e imunofluorescência durante a fase não motora da DP e investigar a ação do moduladores do SRA.

5. MÉTODOS/ RESULTADOS / DISCUSSÃO

Os resultados, métodos e discussão pertinentes à tese serão apresentados em dois artigos distintos, além de um capítulo que engloba os dados analisados durante o doutorado, mas que não foram incorporados nos referidos artigos. O primeiro artigo intitulado "*Nigrostriatal inflammation is associated with nonmotor symptoms in an experimental model of prodromal Parkinson's disease*", já se encontra submetido na revista Neurotoxicity Research (IF=3.7), enquanto o segundo artigo intitulado "*Neuroprotective effects of Renin Angiotensin System alternative axis in non-motor symptoms of Parkinson's disease"*, encontra-se atualmente em processo de revisão pelos autores.

5.1 Artigo Científico 1**: Nigrostriatal inflammation is associated with nonmotor symptoms in an experimental model of prodromal Parkinson's disease**

Nigrostriatal inflammation is associated with nonmotor symptoms in an experimental model of prodromal Parkinson's disease

Bruna Silva Oliveira
Universidade Federal de Minas Gerais
Eliana Cristina Brito Toscano
Universidade Federal de Juiz de Fora
Larissa Katharina Sabino Abreu
Universidade Federal de Minas Gerais
Heliana Barros Fernandes
Universidade Federal de Minas Gerais
Renan Florindo Amorim
Universidade Federal de Minas Gerais
Rodrigo Novaes Ferreira
Universidade Federal de Minas Gerais
Caroline Amaral Machado
Universidade Federal de Minas Gerais
Brener Cunha Carvalho
Universidade Federal de Minas Gerais
Maria Carolina Machado Silva
Universidade Federal de Minas Gerais
Antônio Carlos Pinheiro Oliveira
Universidade Federal de Minas Gerais
Milene Alvarenga Rachid
Universidade Federal de Minas Gerais
Natália Pessoa Rocha
The University of Texas Health Science Center at Houston
Antônio Lúcio Teixeira
University of Texas Health Science Center at Houston
Elizabeth Ribeiro Silva
Universidade Federal de Minas Gerais
Aline Silva Miranda (mirandas.aline@gmail.com)
Universidade Federal de Minas Gerais

Research Article

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Abstract

Recent evidence has supported a pathogenic role for neuroinflammation in Parkinson's disease (PD). However, it is unclear whether the immune changes are involved in the initial physiopathology of PD, leading to the non-motor symptoms (NMS) observed in the prodromal PD stage. The current study aimed to characterize the behavioral and cognitive changes in a toxic-induced model of prodromal PD-like syndrome. We also sought to investigate the role of neuroinflammation in prodromal PD-related NMS. Male mice were subjected to bilateral intranasal (i.n.) infusion with 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) or saline (control group), followed by comprehensive behavioral and neurochemical analysis. Intranasal MPTP infusion was able to cause the loss of dopaminergic neurons. In parallel, it induced impairment in olfactory discrimination and social memory consolidation, compulsive and anxious-like behaviors, but did not influence motor function. In addition, iba-1 and GFAP expressions were increased in the SNpc, suggesting an activated state of microglia and astrocytes. Consistent with this finding, MPTP mice had increased levels of IL-10 and IL-17A, and decreased levels of BDNF and tropomyosin receptor kinase (Trk) A mRNA in the SNpc. The striatum showed increased IL-17A and decreased BDNF and NFG levels compared to control mice. In conclusion, our results suggest that neuroinflammation may play an important role in the early stage of experimental PD-like syndrome. Our data also indicate that i.n. administration of MPTP may represents a valuable mouse model for prodromal PD.

Introduction

Parkinson's disease (PD) is an aging-related neurodegenerative disease of insidious onset (Schapira et al. 2017), characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc) and the presence of intracytoplasmic insoluble alpha-synuclein aggregates, which are the primary components of Lewy bodies (Simon et al. 2020). The loss of dopaminergic neurons in the SNpc leads to reduced dopamine input into the *striatum*, inducing the classic motor symptoms of the disease, i.e. bradykinesia, resting tremor, and rigidity. A long prodromal period might precede the onset of this classic PD manifestation. The more prodromal symptoms, the higher the risk of developing parkinsonism. The prodromal period can start decades before the onset of motor parkinsonism (Bloem et al. 2021). The decrease in dopamine levels and the subsequent dysfunction of cholinergic and noradrenergic pathways are implicated in the development of early PD-related nonmotor symptoms (NMS) (Emre 2003; Dexter and Jenner 2013). Although motor symptoms are the main characteristic of PD, NMS may affect the quality of life of patients. Neuropsychiatric features, such as anxiety and depression, often occur in PD from the prodromal pre-motor phase to the late stages of the disease (Tolosa et al. 2009; Schapira et al. 2017). The main PD-related NMS are cognitive impairment, hyposmia, depression, anxiety, constipation, and sleep disorders (Hayes 2019).

Several experimental models have been employed to investigate the molecular and cellular mechanisms involved in the pathophysiology of PD, especially underlying motor symptoms (Jackson-Lewis et al. 2012; McDowell and Chesselet 2012). Nevertheless, the understanding of the pathophysiology of early NMS is

still limited partly because of the lack of models mimicking the pathological changes in the prodromal stage of PD, i.e. before the development of typical motor symptoms (Taguchi et al. 2020). The proneurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) is widely used to induce a PD-like syndrome in rodents. MPTP crosses the blood–brain barrier (BBB) and is converted into the neurotoxic metabolite 1-methyl-4-phenylpyridinium (MPP^+) by glial monoamine oxidases (MAOs). The MPP^+ is taken up by dopaminergic neurons *via* dopamine transporters (DAT) and accumulates in the mitochondria (Hare et al. 2013). MPP^+ impairs ATP production and, thus, energy homeostasis inducing the loss of dopaminergic neurons (Drechsel and Patel 2008). Prediger et al. (2010) proposed a new MPTP mouse model based on the olfactory vector hypothesis (DS Prediger et al. 2011; Doty 2008). In this model, olfactory, cognitive, and behavioral impairments were observed up to 20 days after a single intranasal (*i.n.*) MPTP administration without significant motor symptoms. These results indicate that the i.n. administration of MPTP represents a valuable mouse model for the study of the early stages of PD (DS Prediger et al. 2011; Tristao et al. 2014).

Reactive microglia were first found in the substantia nigra of human post-mortem PD brains in 1988 (McGeer et al. 1988). Subsequently, imaging studies also found activated microglia in brainstem, basal ganglia, and frontal areas (Gerhard et al. 2006). Microglial activation alongside to increased brain levels of pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interferon-gamma (IFN-γ), suggest a role for neuroinflammation in the pathophysiology of PD (Zhu et al. 2022; Rocha et al. 2015). A growing body of evidence have demonstrated changes in peripheral blood immune cells and postmortem studies showing infiltration of T-cells and natural killer cells in the patients' brains (Rocha et al. 2015; MacMahon Copas et al. 2021). In addition, blood levels of pro-inflammatory cytokines correlated with physical and cognitive performance in patients with PD (Rocha et al. 2014; Scalzo et al. 2010b). Despite the previous efforts, the majority of studies investigating inflammatory response in PD were conducted after the onset of motor symptoms. Thus, a potential temporal relationship between inflammation and the development and progression of PD-related motor deficits were not addressed. In this scenario, neuroinflammatory response at prodromal stages of PD deserve further investigation (Terkelsen et al. 2022).

Herein, we aimed at characterizing behavioral and cognitive functions a few days after MPTP-induced PD-like syndrome in mice. In addition, we investigated the involvement of inflammatory and neurotrophic pathways. This study is the first to characterize the neuroinflammatory response and its association with NMS in the prodromal PD-like syndrome in the experimental model induced by intranasal administration of MPTP.

Materials and Methods

Intranasal Administration of MPTP

Male C57BL/6 mice, aged 8–9 weeks, were obtained from the Animal Care Facilities of Instituto de Ciências Biológicas (ICB) of the Universidade Federal de Minas Gerais (UFMG), Belo Horizonte, Brazil. The

animals were housed in groups of five mice per cage at room temperature (22°C) and were provided with food and water *ad libitum*. All experiments were approved by the Animal Ethics Committee of UFMG, protocol number 255/2017.

One hundred milligrams of MPTP hydrochloride (MPTP-HCl) (Sigma Chemical Co., USA) was dissolved in 2.5 mL of sterile 0.9% NaCl (saline). Intranasal administration of MPTP (1 mg) was performed as described by Prediger and colleagues (DS Prediger et al. 2011). Briefly, tubing was connected to a pump, and the flow rate was set to 12.5 µl/min. The mice were restrained, and a 7-mm piece of PE-10 tubing was inserted through the left nostril. A dose of 1 mg of MPTP was infused for 2 min. Animals were given a 3-hour interval to recover, and then 1 mg of MPTP was infused through the right nostril. The same procedure was carried out on control animals using saline as the infused solution. Mice were submitted to behavioral tests, and on the 11th day after i.n. administration of MPTP or saline, the mice were killed using an overdose of ketamine (150 mg/kg) and xylazine (10 mg/kg) solution by the intraperitoneal route. A cohort of mice performed the olfactory recognition test, the social recognition test and the marble-burying test. A different cohort of animals undergoes the forced swimming test and the open field test at 11 dpi. A third cohort of mice performed the open field test at 30 dpi and the rotarod test from 36 to 40 dpi (Fig. 1A).

Olfactory Recognition Test

To assess olfactory memory, we used the discriminative olfactory recognition test five days after bilateral intranasal infusion. The test was performed as previously described by Prediger and colleagues (DS Prediger et al. 2011). Each animal was placed in a box divided equally into two compartments separated by an open door for five minutes. In one of the compartments, clean wood shavings were placed (new environment), while the dirty wood shavings (occupied by the animal for at least three days before the test) were placed in the other one, creating a familiar environment. The animals were free to choose compartments.

Social Recognition Test

The social recognition test was performed on the 6th day post-i.n. infusion to assess sociability and social memory skills as described elsewhere (Stanojlovic et al. 2019). In this test, the animals were placed in a box containing three connected compartments with a free transition between them. First, one mouse was placed in the box for a training session. After the training session, another mouse (juvenile) was placed in one of the compartments, and the behavior of the animals was evaluated (sociability skill). To assess social memory, a second unknown juvenile mouse was introduced to the environment, and the time that the first mouse spent in front of the unknown animal was measured. Images were recorded and analyzed using EthoVision software (EthoVision HTP 2.1.2.0, based on EthoVision XT 4.1, Noldus Information Technology, Wageningen, Netherlands).

Marble Burying Test

On the 8th day post-i.n. infusion, the animals were placed in a standard plastic cage box containing 25 clean marbles organized in a row. The compulsive-like behavior was evaluated based on the number of marbles buried in the shaved wood. A higher number of marbles buried indicates compulsive-like behavior (Bahi and Dreyer 2012).

Forced Swimming test

The Forced Swimming Test (FST) was performed to evaluate depressive-like behavior at 11 dpi, as described elsewhere (de Miranda et al. 2017). The paradigm was performed in a round glass beaker (18 cm in diameter and 30 cm in height) filled with tap water at 25 ± 0.5°C. The water level was approximately 20 cm to prevent the animal from touching the bottom of the glass. The mouse was also unable to climb out of the beaker. The animal was carefully lowered into the water for 6 min. The first 2 min were not evaluated; however, floating behavior was scored for the following 4 min by an experimenter blind to experimental and control groups. Floating was defined by immobility of the animal and minimal movements to keep the body's balance. After the swim session, mice were dried and placed in a cage surrounded by a heating pad. The water was changed between each animal. Images were recorded and the time of immobility (s) analyzed using EthoVision software (EthoVision HTP 2.1.2.0, based on EthoVision XT 4.1, Noldus Information Technology, Wageningen, Netherlands).

Open field test

To assess the possible effects of MPTP on locomotor activity, the animals were submitted to the open field test immediately before and 11 days after i.n. administration of MPTP (Takahashi et al. 2006). Each animal was gently placed in the cage and allowed to explore the arena for 15 minutes. The total distance traveled in the cage was recorded. In addition, to evaluate anxiety like-behavior, time spent in the center was quantified. Video tracking was performed by an infrared-sensitive video camera installed in the top unit of each cage. The X-Y coordinates of each mouse were acquired using EthoVision software (EthoVision HTP 2.1.2.0, based on EthoVision XT 4.1, Noldus Information Technology, Wageningen, The Netherlands).

Rotarod test

The rotarod (Insight, Ribeirao Preto, Brazil) was performed to evaluate motor coordination and balance from 36 to 40 dpi as previously described by Kao and colleagues (Kao et al. 2015). Briefly, mice were placed on the rotarod and rotation was accelerated linearly from 4 to 40 rpm over 5 min with no reverse. Each mouse was tested for 3 trials during 5 days in a row. Mice were returned to home cage and given at least 30-min breaks between trials. Latency to fall was recorded automatically and the mean of the three trials was used for statistical analysis for each day.

Immunostaining and morphometric analysis

After euthanasia, the animals were intracardially perfused with 4% paraformaldehyde in phosphate buffer, pH 7.4. The brain was removed and fixed in the same perfusion solution for 24 hours. Next, fixed

brains were transferred to a 0.1 M phosphate saline buffer (PBS), pH 7.4, containing sucrose at 30% for 48 hours at 4°C. Then, they were frozen in isopentane and stored at -70°C until further proceedings. Briefly, serial coronal 30µm thick sections of SNpc and striatum regions were cut on a cryostat (Leica SM2000 R) and identified according to Paxinos and Franklin's Mouse Brain in Stereotaxic Coordinates (Paxinos and Franklin 2019).

Free-floating sections were incubated with a citrate buffer for 60 min at 70°C for antigenic recovery, washed with TBS, blocked for 2h with blocking solution (4% BSA in 0.5% TBST), and incubated with anti-TH monoclonal antibody (1:500, Abcam, Cambridge, UK). After overnight incubation with the primary antibody at 4°C, sections were incubated in 0.1 M PBS Sections were washed with TBS and incubated with a secondary antibody (anti-rabbit IgG, 1:500) for 2h and then processed using an avidin/biotin-peroxidase complex (1:100, Vectastain - Elite ABC kit, Vector Laboratories, Burlingame, USA). The resultant antigen-antibody complexes were visualized by reaction with a solution of 25 mM Tris-buffered saline (TBS) containing 0.5 mg/mL 3,30-diaminobenzidine (DAB) plus 0.025% H_2O_2.

The TH immunostaining area and the integrated density of gray values in the SNpc and striatum were quantified using digital morphometry. A total of eight frames measuring 1443520 μm^2 were digitalized using a ×20 objective (Olympus, Japan, JP) for each mouse (n = 4 per group). The obtained images were analyzed using ImageJ software (National Institute of Health, USA). Brown pixels, indicating positive staining, were selected for making a binary image, digital processing, measuring area (μm^2), and optical density of the integrated density of gray value. The threshold applied was 0–58 pixels.

In order to quantify the number of microglial and astroglial cells in the SNpc, slices (30 µm) were incubated with a citrate buffer for 60 min at 70°C for antigenic recovery and washed with TBS and blocked for 2 h with blocking solution (4% BSA in 0.5% TBST). Slices were then incubated overnight with primary antibodies rabbit anti-Iba-1 (1:500; Wako Chemicals, Osaka, Japan) and mouse anti-GFAP (1:150; MAB3402, abcam, Waltham, Boston, USA). Thereafter, slices were washed with TBS and incubated for 2 h with secondary antibodies goat anti-rabbit (1:1000; Alexa Fluor 594, Life Technologies, Carlsbad, USA) and goat anti-mouse (1:750; Alexa Fluor 488, Life Technologies, Carlsbad, USA). Immediately after, they were washed, incubated with DAPI 1.75 µg/mL for 30 min, washed again and mounted in gelatinized slides with Fluoromount (Sigma-Aldrich, St. Louis, USA). Slices were observed under fluorescence with LSM880 Zeiss microscope in 20X magnification. The Paxinos and Watson mouse brain atlas was used to localize the SNpc. The labeled area (µm2) was quantified using using the software ImageJ 1.52a (National Institute of Health, USA) and the results are represented as the area of positive cell-bodies per field for Iba-1^+ and $GFAP^+$ (Bellozi et al. 2019).

Assessment of cytokine and neurotrophic factor concentrations.

The SNpc and striatum regions of controls and MPTP mice (n = 6 per group) were dissected and homogenized in a PBS buffer containing a protease inhibitor cocktail. The concentrations of brain-derived neurotrophic factor (BDNF) and nerve growth factor (NGF) were determined using commercially

available enzyme-linked immunosorbent assay (ELISA) DuoSet kits (R&D Systems; Minneapolis). In addition, the levels of the cytokines TNF-α, IFN-γ, IL-4, IL-6, IL-10, IL-12, and IL-17A were determined using a BD™ CBA Mouse Inflammation Kit (CBA; BD Biosciences, San Diego, CA) by the manufacturer's instructions and analyzed on a FACSCalibur flow cytometer (Becton Dickinson, San Jose, USA). Results are expressed as picograms per 100 mg of tissue. The detection limit of the CBA and ELISA assays was 0.2 and 5 pg/mL, respectively.

mRNA expression of NGF and BDNF receptors

The mRNA expression of tyrosine kinase receptor (Trk) 1 and Trk2 in the SNpc and striatum of MPTP and control mice (n = 4 per group) was estimated using real-time quantitative PCR (qPCR). First, total RNA was extracted using TRIzol® reagent according to the manufacturer's protocol (Sigma Aldrich, St. Louis, USA). Then, reverse transcription was performed from 1 µg total RNA and using an MMLV-reverse transcriptase kit according to the manufacturer's instructions (Invitrogen, Waltham, USA). The resulting cDNA was used for the qPCR reaction using iTaq Universal SYBR Green Supermix and a CFX96 TouchTM Real-Time detection system (both from Bio-Rad, Hercules, USA). The respective forward and reverse sequences are described as follow: *Ntrk1 (GCCTAACCATCGTGAAGAGTG, CCAACGCATTGGAGGACAGAT); Ntrk2 (CCGCTAGGATTTGGTGTACTG; CCGGGTCAACGCTGTTAGG) and Rpl32 (GCTGCCATCTGTTTTACGG; TGACTGGTGCCTGATGAACT).* Primer synthesis was carried by Integrated DNA Technologies (IDT Inc., USA). The data were analyzed according to the method described by Schmittgen and Livak using the Eq. 2 − ΔCt (Livak and Schmittgen 2001).

Statistical analysis

The Kolmogorov–Smirnov test was used to test if data were normally distributed. Comparisons between groups were made by Student's t-test when the data were determined to follow a normal distribution or the Mann–Whitney test when variables did not follow a normal distribution. The rotarod data were analyzed by repeated measures two-way ANOVA. Data were analyzed using GraphPad Prism v6 software (GraphPad, San Diego, USA) and expressed as the mean ± standard error of the mean (SEM). Significant differences were set at $p < 0.05$ in a bicaudal analysis.

Results

Behavioral changes post i.n. infusion of MPTP

After MPTP or saline i.n. infusion, mice were subjected to a comprehensive behavioral analysis (Fig. 1A).

In the olfactory recognition test, control mice remained longer in the familiar compartment than in the nonfamiliar chamber (Unpaired t-test: $t = 12.91$ $df = 8$ $p < 0.0001$). Conversely, MPTP-treated mice had no preference for a specific compartment, i.e., there was no significant difference in the time remaining in each environment (Unpaired t-test: $t = 1.43$ $df = 6$ $p = 0.20$) (Fig. 1B). These results suggest that the MPTP group had impairment in olfactory recognition as early as the 5th day post-i.n. infusion.

In the social recognition test, both control and MPTP-treated mice spent a similar amount of time in the left and right empty chambers (Saline-Unpaired t-test: $t = 0.60$ $df = 8$ $p = 0.56$; MPTP- Unpaired t-test: $t = 0.88$ $df = 8$ $p = 0.40$, Fig. 1C). When a stranger mouse was introduced into one of these chambers, the animals spent more time in the chamber with the new mouse (S1) than in the empty chamber, exhibiting a seeking behavior towards a new individual (Saline-Mann Whitney test: $p < 0.01$; MPTP- Unpaired t-test: $t = 12.78$ $df = 8$ $p < 0.0001$, Fig. 1D). Social novelty preference analysis was performed by introducing a second strange animal (S2) into the test, and control mice spent significantly more time in the chamber with the unknown mouse (S2) (Unpaired t-test: $t = 2.67$ $df = 6$ $p = 0.04$). In contrast, the MPTP-treated group spent similar time in each chamber (Unpaired t-test: $t = 0.97$ $df = 8$ $p = 0.36$, Fig. 1E). These observations reflect a decrease in social memory consolidation in treated animals on the 6th day after MPTP infusion.

We next examined whether MPTP treatment induces compulsive behavior by the marble burying test. MPTP-treated mice buried more marbles in the wood shavings than control animals (Unpaired t-test: $t = 3.40$ $df = 7$ $p = 0.01$, Fig. 1F), suggesting compulsive behavior. Conversely, both control and MPTP mice submitted to the forced swimming test remained immobile during a similar time in the water tank, i.e., the MPTP i.n. The infusion did not induce depressive-type behavior (Unpaired t-test: $t = 0.84$ $df = 14$ $p = 0.41$, Fig. 1G).

The total distance traveled in the open field arena was the same for the control and MPTP groups (Unpaired t-test: $t = 0.42$ $df = 15$ $p = 0.68$, Fig. 1H). This result indicates confirms that motor symptoms did not appear within 11 days after MPTP infusion, characterizing the experimental model used in the current study as a pre-motor PD model. Conversely, the percentage of time spent in the center of the arena was decreased in the MPTP group compared with controls, indicating anxiety-like behavior (Unpaired t-test: $t = 3.80$ $df = 14$ $p < 0.01$, Fig. 1I). Importantly, no significant motor changes were found in the total distance traveled in the open field at 30 dpi (Unpaired t-test: $t = 1.57$ $df = 15$ $p = 0.14$, Fig. 1J) or in the latency to fall in the rotarod task from 36 dpi to 40 dpi (Days: $F(4, 69) = 1.66$ $p = 0.17$; Groups: $F(1, 69) = 2.55$ $p = 0.11$; Interaction: $F(4, 69) = 0.11$ $p = 0.98$, Fig. 1K), reinforcing the prodromal feature of the PD model proposed in the current study.

MPTP-induced loss of dopaminergic neurons and astroglial activation in the SNpc

Eleven days post-i.n. infusion with saline or MPTP, mice were euthanized, and the nigrostriatal areas were collected. Tyrosine hydroxylase (TH) immunostaining was observed in the SNpc of both control and MPTP-treated mice (Fig. 2A and B, respectively). There was a decrease in the stained area (Unpaired t-test: $t = 2.60$ $df = 6$ $p = 0.04$, Fig. 2C) and integrated density in the gray value (Unpaired t-test: $t = 2.60$ $df = 6$ $p = 0.04$, Fig. 2D) for TH in the SNpc of MPTP-treated mice in comparison to the control mice. The SNpc also showed positive cells for Iba-1 and GFAP in both groups (Fig. 3A, B, D, and E). In addition, MPTP mice had an increased immunofluorescent area compared with the control mice for Iba-1 (Unpaired t-test:

t = 12.07 df = 6 p < 0.0001, Fig. 3C) and GFAP (Unpaired t-test: t = 9.60 df = 6 p < 0.0001, Fig. 3F), suggesting astromicroglial activation.

Effects of i.n. infusion of MPTP on the levels of cytokines and neurotrophic factors in the SNpc andstriatum

We found increased levels of IL-10 and IL-17A in the SNpc of MPTP mice compared with control mice (unpaired t-test- IL-10: t = 2.30, df = 10, p = 0.01; IL-17A: t = 2.43, df = 10, p = 0.03; Fig. 4D and E). Conversely, BDNF levels and mRNA TrkA expression were decreased in MPTP mice (unpaired t-test- BDNF-: t = 3.92, df = 5, p = 0.01; TrkA: t = 2.63, df = 6, p = 0.04; Fig. 4H and J). The concentrations of other measured cytokines were similar between groups (unpaired t-test- IL-2: t = 0.80, df = 10, p = 0.50; IL-4: t = 1.23, df = 10, p = 0.25; IL-6: t = 0.67, df = 10, p = 0.52; IFN-γ: t = 0.97, df = 10, p = 0.36; TNF-α: t = 0.16, df = 10, p = 0.87; NGF: t = 0.01, df = 6, p = 0.10, Fig. 4A, B, C, F-I, respectively) as well as the mRNA expression of TrkB (unpaired t-test: t = 0.50, df = 7, p = 0.64, Fig. 4K).

In the striatum, i.n. infusion of MPTP induced increase in IL-17A, BDNF, and NGF levels (unpaired t test: IL-17A: t = 2.34 df = 10 p = 0.04; BDNF: t = 8.52 df = 5 p < 0.001; NGF: t = 3.80 df = 6 p = 0.01, Fig. 5E, H, and I). The concentration of other cytokines and mRNA expression of TrkA and TrkB were similar between groups in this brain area (unpaired t-test- IL2: t = 0.76 df = 10 p = 0.47; IL4: t = 0.55 df = 10 p = 0.60; IL-6: t = 0.17 df = 10 p = 0.87; IL-10: t = 0.25 df = 9 p = 0.81; IFN-γ: t = 0.92 df = 10 p = 0.38; TNF-α: t = 0.18 df = 9 p = 0.86; Mann Whitney test-TrkA: p = 0.49; Unpaired t test-TrkB: t = 0.40 df = 7 p = 0.72, Fig. 5A-D, F-H, J, and K).

Discussion

In the current study, we demonstrated that bilateral i.n. infusion with MPTP did not influence motor function of mice but impaired olfactory recognition and social memory consolidation. In addition, MPTP infusion induced compulsive- and anxiety-like behaviors. These NMS were associated with astromicroglial activation and changed levels of inflammatory mediators in the SNpc and striatum prior to the development of motor symptoms.

Loss of dopaminergic neurons in the SNpc can promote NSM. These neurons produce TH, an important enzyme in the dopamine production cascade, converting tyrosine to DOPA (Dunkley and Dickson 2019). We showed decreased neuronal immunostaining for TH in the SNpc of animals treated with a single low dose of MPTP with preserved motor function. Considering that the first motor symptoms of PD can appear decades after the onset of dopaminergic cell loss and NMS, our results suggest that the acute MPTP parkinsonism model can mimic the prodromal stage of PD (Schapira et al. 2017).

The diagnosis of PD relies on motor deficits, including bradykinesia, rigidity, and resting tremor. These motor features result from the loss of dopaminergic neurons in the SNpc (Berardelli et al. 2013). However, PD is associated with a series of remarkable NMS that can precede the motor symptoms. Herein, we demonstrated a decrease in social memory consolidation on the 6th day after MPTP i.n. infusion, using

olfactory recognition test. Similar results were observed by Prediger and colleagues using the same murine model (DS Prediger et al. 2011). Hyposmia is present in more than 90% of patients with PD. The presence of hyposmia can be regarded as a biomarker for early premotor PD, particularly if it is combined with other early symptoms, such as cognitive dysfunction (Gaenslen et al. 2014; Doty 2012; Baba et al. 2011). Impaired olfaction following i.n. administration of MPTP could simply reflect damage to the olfactory epithelium (Kurtenbach et al. 2013). However, previous studies with MPTP found hyposmia without damage to the olfactory epithelium, i.e. the cause of MPTP-related olfactory impairment is still controversial (Dluzen 1992). In the open field test, MPTP treated mice spent less time in the center of the arena compared with controls, indicating anxiety-like behavior. Bilateral 6-OHDA-induced injury of the nigrostriatal pathway in rats promoted anxiety-like behavior associated with reductions in dopamine (Tadaiesky et al. 2008). In addition, individuals with anxiety have a higher risk of developing PD (Lin et al. 2015). Anxiety affects up to 60% of patients with PD and, often in association with depression, can occur before the onset of motor signs of PD.

The neuroanatomical and pathophysiological bases of nonmotor abnormalities in PD are complex, seem to involve non-dopaminergic pathways and remain largely undefined (Schapira et al. 2017). Studies using animal models have supported that neuroinflammation can increase dopaminergic neurodegeneration in PD. Microglial activation and increased levels of inflammatory mediators in the SNpc and striatum have been related to MPTP administration in mice (Tristao et al. 2014; Machado et al. 2016; Mustapha and Taib 2021). Interestingly, Yasuda and colleagues reported that MPTP-sensitive C57BL/6 mice showed increased inflammatory cytokine levels in the cerebrospinal fluid, while it was not observed in MPTP-resilient BALB mice (Yasuda et al. 2008). These results indicate that the neuroinflammatory response might trigger the susceptibility of C57BL/6 mice to MPTP-induced loss of dopaminergic neurons. Studies of inflammatory response in the early stage of parkinsonism syndrome (i.e., before the motor symptoms occurrence) are still missing (Yasuda et al. 2008). In the current study, we demonstrated increased immunostaining for Iba-1 and GFAP in the SNpc of MPTP-treated mice compared to control mice previously to the motor symptoms, corroborating the hypothesis of an early role of astromicrogliosis in PD pathophysiology (Tristao et al. 2014).

MPTP intoxication induces neuronal loss, triggering microglial activation and proliferation and releasing inflammatory mediators that contribute to neurodegeneration in a cyclical process (Machado et al. 2016). Alongside microglial activation, we found MPTP-induced increase of nigrostriatal levels of IL-17A. Similarly, increased brain levels of IL-17A following blood brain-barrier disruption were observed in MPTP-treated mice. In an in vitro study, IL-17A induced microglial activation, death of TH-positive neurons, and decreased dopamine levels. Interestingly, IL-17A exacerbated dopaminergic neuronal loss only in the presence of microglia, and silencing the IL-17A receptor gene in microglia abolished the IL-17A effect (Liu et al. 2019). In line with this, Ginkgolide K had a dopaminergic neuroprotective effect in MPTP-treated mice, decreasing microglia-mediated inflammation and consequent infiltration of peripheral $CD4^{+}IL\text{-}17^{+}$ T cells into the mice's brain (Miao et al. 2022). These founds suggest that, by activating microglia, IL-17A indirectly potentiates dopaminergic neuronal death, since microglia definitely express IL-17R and play a

pivotal role in neuroinflammation and neurodegeneration in PD (Garcia-Esparcia et al. 2014; Liu et al. 2019). Astrocytes also respond to IL-17 through generating chemokines to promote the recruitment of peripheral leukocytes. In addition, IL-17 reduces the ability of astrocytes to metabolize glutamate, inducing glutamatergic excitotoxicity (Chen et al. 2020). We also observed a higher concentration of IL-10, an anti-inflammatory cytokine, in the MPTP group compared to the control. The transfection of Treg cells and the consequent increase of IL-10 levels into the brain of MPTP-treated mice decreased neuronal loss and microglial activation, as well as increased production of both BDNF and GDNF, suggesting a neuroprotective role for IL-10 in the MPTP model (Reynolds et al. 2007). Therefore, the elevation of IL-10 in the SNpc might be an attempt to counterbalance the proinflammatory effects of increased astromicrogliosis and IL-17 levels (Kwilasz et al. 2015).

In the current study, we also demonstrated the reduction of BDNF levels and mRNA expression of TrkA in the SNpc of MPTP-treated mice. BDNF is involved in the activity and survival of dopaminergic and motor neurons. Cell-mediated delivery of BDNF increased dopamine levels, and nigral infusion of BDNF reversed the reduction of dopamine in the murine MPTP model (Hung and Lee 1996; Isacson et al. 1995). In addition, BDNF gene ablation led to impaired striatal development and severe motor dysfunction in mice (Li et al. 2012). BDNF and NGF concentrations were also decreased in the nigrostriatal system and serum of patients with PD (Chmielarz and Saarma 2020; Nasrolahi et al. 2018; Scalzo et al. 2010a). Although NGF levels remained similar to those in the control group, the MPTP-induced reduction of mRNA TrkA expression may inhibit the neuroprotective effects of this neurotrophic factor. TrkA is the receptor most related to NGF and its regulation in the CNS (Nasrolahi et al. 2018). Conversely, in the striatum of MPTP-treated animals, we demonstrated increased levels of NGF and BDNF. Once again, this may be an attempt to counterbalance the neuroinflammatory and neurotoxic response induced by MPTP intoxication in the early-stage model of PD.

In conclusion, our results suggest that MPTP can induce neuronal loss and neuroinflammation causing a series of cognitive and behavioral changes in the early stage of experimental PD. Our data also indicate that acute MPTP intoxication by i.n. route represents a valid model of prodromal PD.

Declarations

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ETHICS APPROVAL AND CONSENT TO PARTICIPATE

All animal studies were approved by the Ethics Committee on Animal Experimentation of UFMG (under the protocol 255/2017).

CONSENT FOR PUBLICATION

Not Applicable.

COMPETING INTERESTS

The authors have no relevant financial or non-financial interests to disclose.

DATA AVAILABILITY

Data will be made available on reasonable request.

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AUTHORS' CONTRIBUTIONS

BSO, ECBT, LKSA, HBF, RFA, RNF, CAM, BCC, MCMS, ACPO, MAR, NPR, ALT, ERS, ASM conducted experiments, contributed to writing and editing the manuscript and/or analyzed data. All authors contributed to research design and reviewed the final manuscript.

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Figures

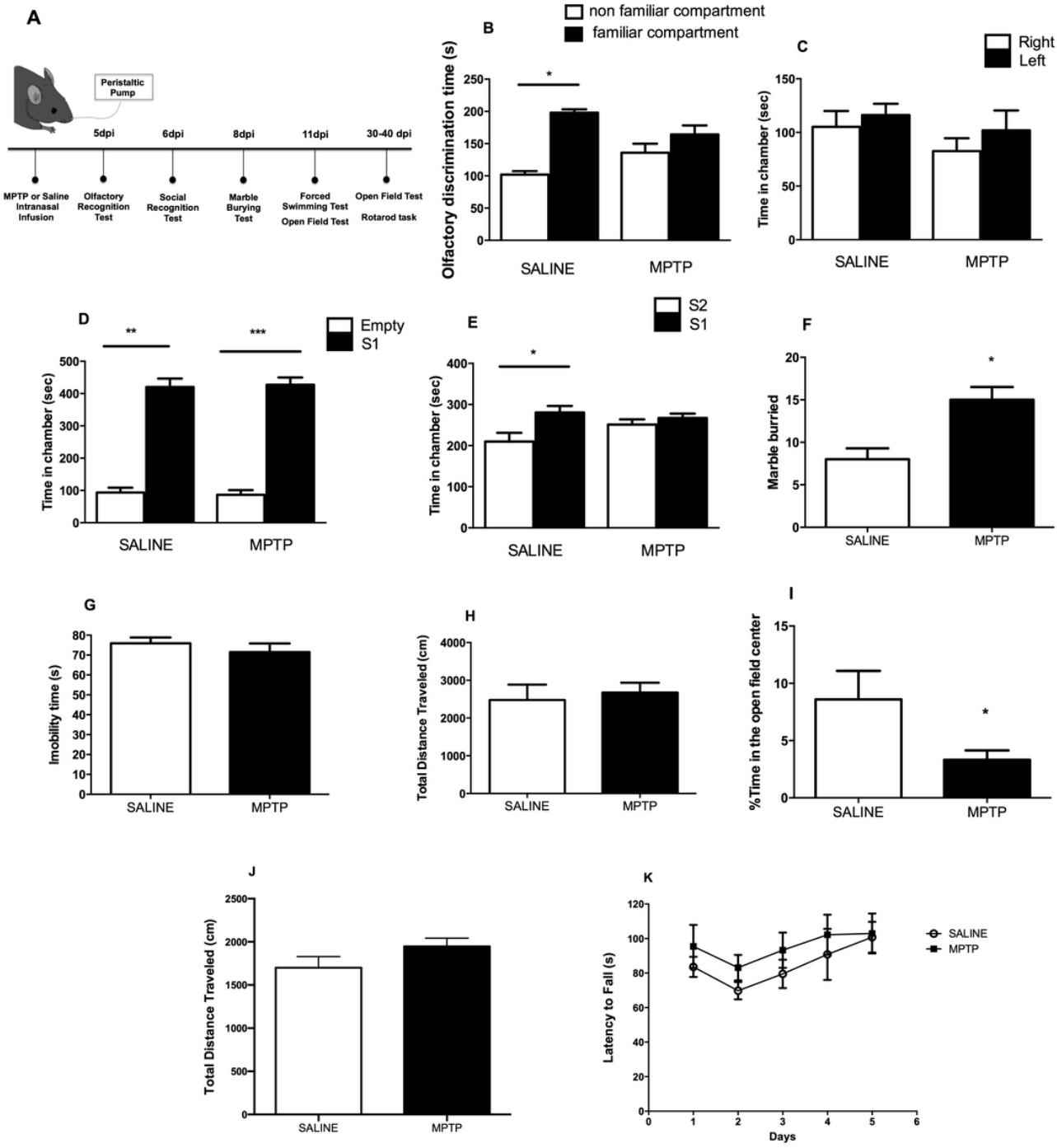

Figure 1

Effect of intranasal infusion of MPTP on mouse behavior. Experimental design of behavioral evaluation (n=5-10 per group) (A). Control mice spent more time in the familiar compartment, while MPTP-treated mice spent similar time in nonfamiliar and familiar environments (B). During the training session, both groups spent similar amounts of time in the right and left chambers in the social recognition test (C). After the introduction of the first strange mouse (S1), both groups spent more time in the chamber

occupied by this S1 mouse than in the empty one in the social recognition test (D). When a second strange mouse (S2) was introduced, only control animals showed a preference for the chamber occupied by the S2 mouse in the social recognition test (E). MPTP-treated mice showed obsessive-like behavior, burying more marbles in the wood shaves in comparison to control mice (F). Conversely, MPTP infusion was not associated with depressive-like behavior in the forced swimming test, in which MPTP and control mice remained immobile for a similar amount of time (G). In the open field test, MPTP mice traveled the same distance as the control mice (H) but spent more time in the center of the arena (I). No motor changes were found in the open field test at 30 dpi (J) or in the rotarod task from 36 dpi to 40 dpi (K). The results are expressed as mean ± SEM, and asterisks indicate significant differences, where *p<0.05, **p<0.01, and ***p<0.001

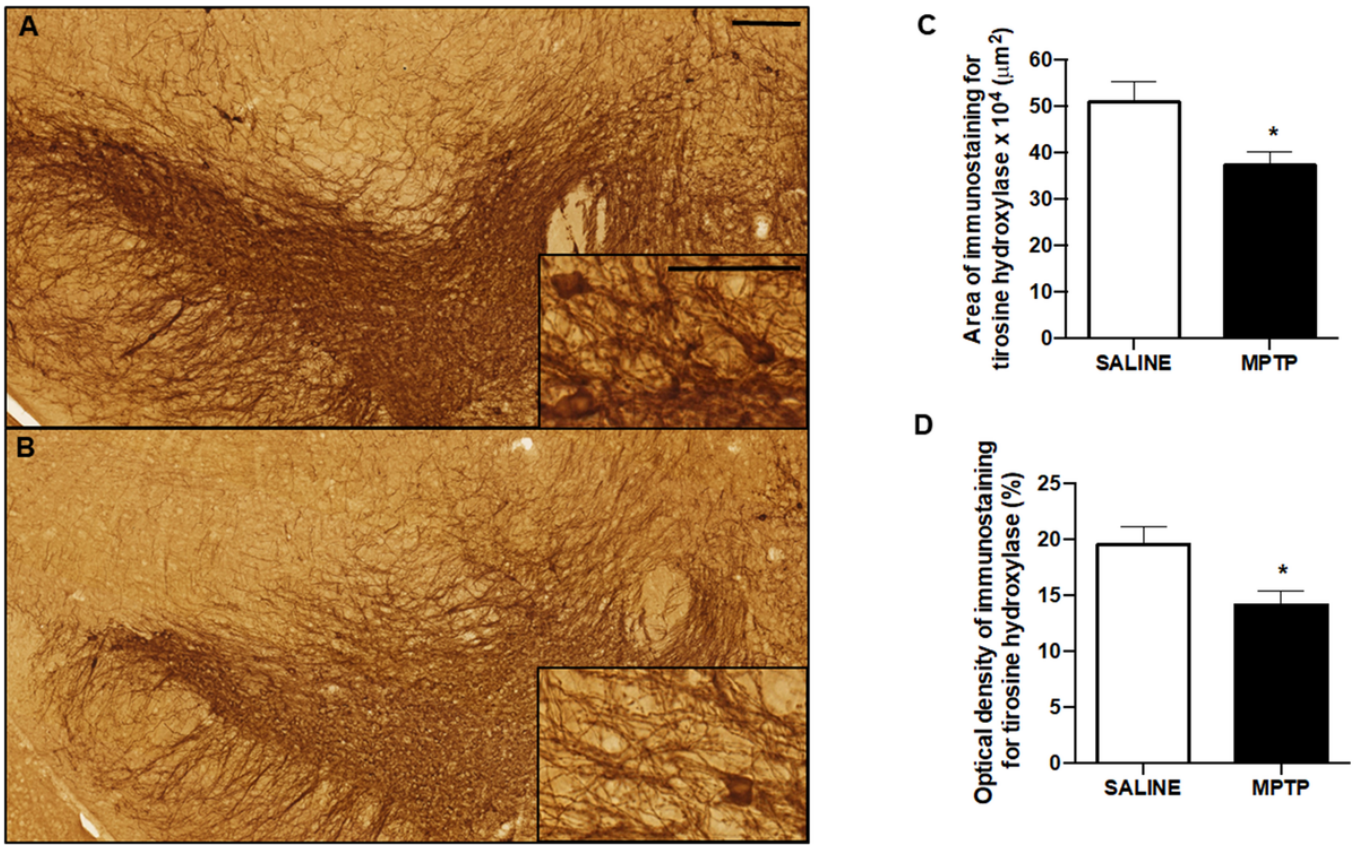

Figure 2

TH immunostaining and morphometric analysis in the substantia nigra of control and MPTP treatment groups. Immunohistochemistry for the detection of TH expression revealed neuronal and fibrillary immunostaining in the substantia nigra of control (A) and MPTP (B) mice (scale bars = 100 μm). Using digital morphometry, we demonstrated that MPTP-treated mice had a lower area (C) and integrated density of gray value (D) for TH immunostaining, compared with control mice. The results are expressed as mean ± SEM, and asterisks indicate significant differences, where *p<0.05; n=4 per group.

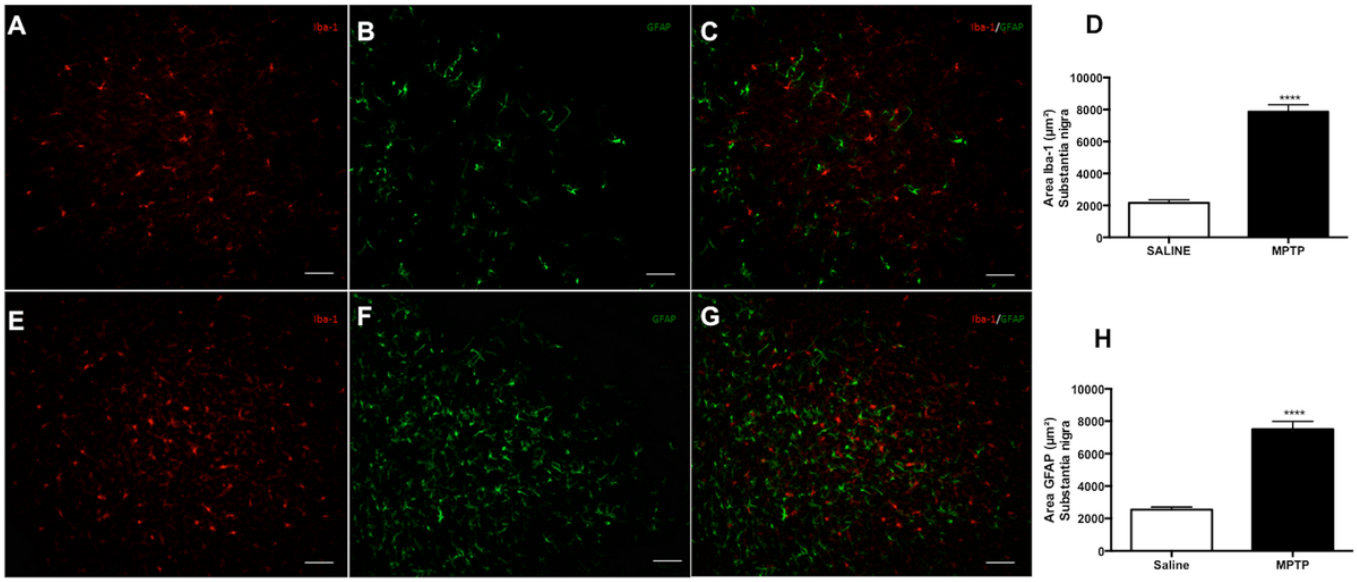

Figure 3

Iba-1 and GFAP immunofluorescent staining in the substantia nigra of the control and MPTP treatment groups. Staining for the detection of Iba-1 and GFAP expression revealed immunopositive microglial and astroglial cells in the substantia nigra of the control (Fig. 3A-C) and MPTP (Fig. 3E-G) mice (scale bars = 100 µm). Using digital morphometry by ImageJ software, we demonstrated that MPTP mice had a higher immunostaining area for both proteins Iba-1 (Fig. 3D) and GFAP (Fig. 3H) compared with control mice. The results are expressed as mean ± SEM, and asterisks indicate significant differences, where**** $p<0.0001$; n=4 per group.

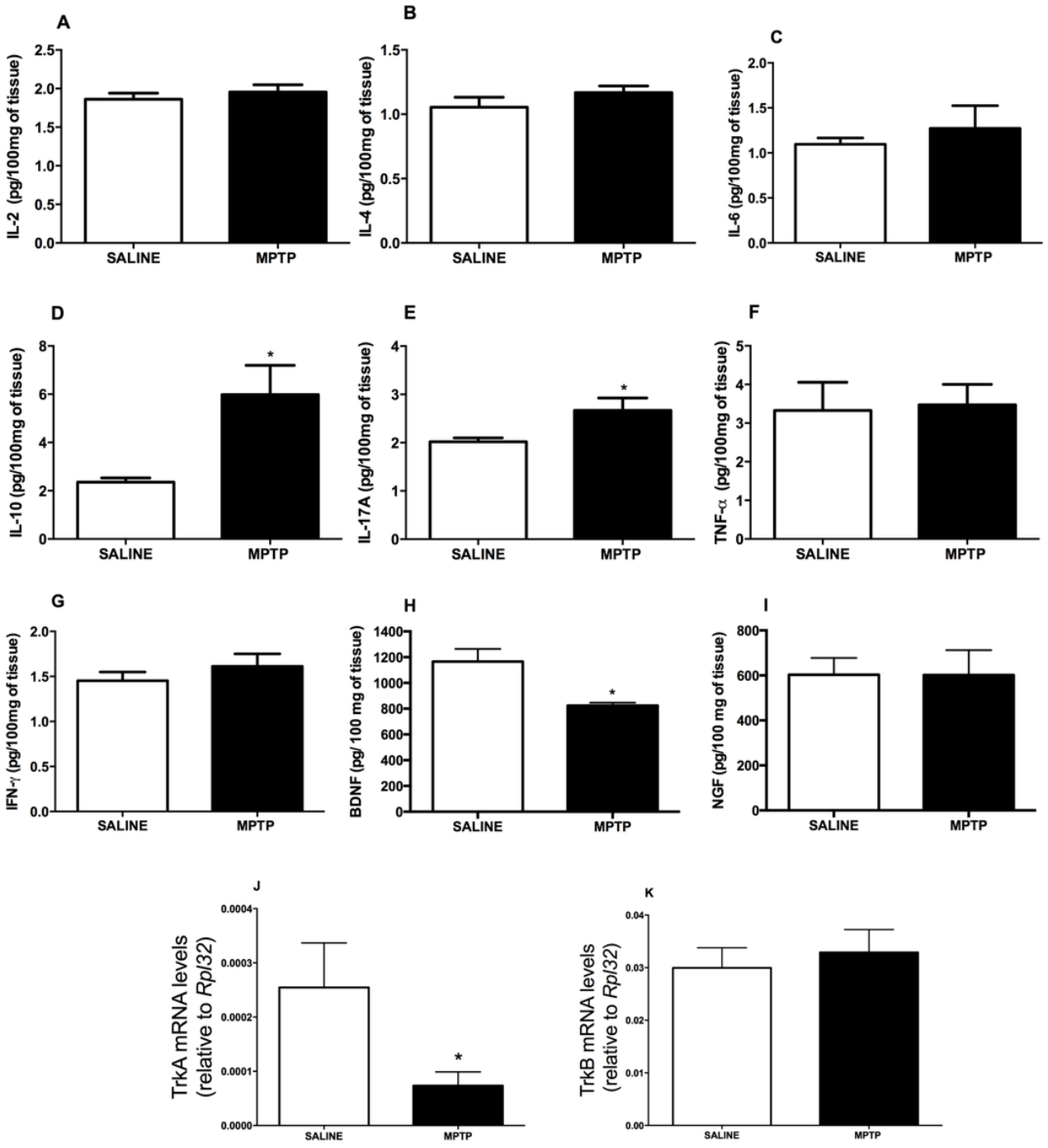

Figure 4

Effects of MPTP on the inflammatory response in the SNpc. The levels of IL-2 (A), IL-4 (B), IL-6 (C), IL-10 (D), IL-17A (E), TNF-α (F), IFN- (G), BDNF (H), and NGF (I) were measured using CBA (for the cytokines) and ELISA (for the neurotrophic factors). The mRNA expression of TrkA and TrkB (J and K) was also quantified using real-time PCR. Results are expressed as mean ± SEM, and asterisks indicate significant differences, where *p<0.05; n=5 per group.

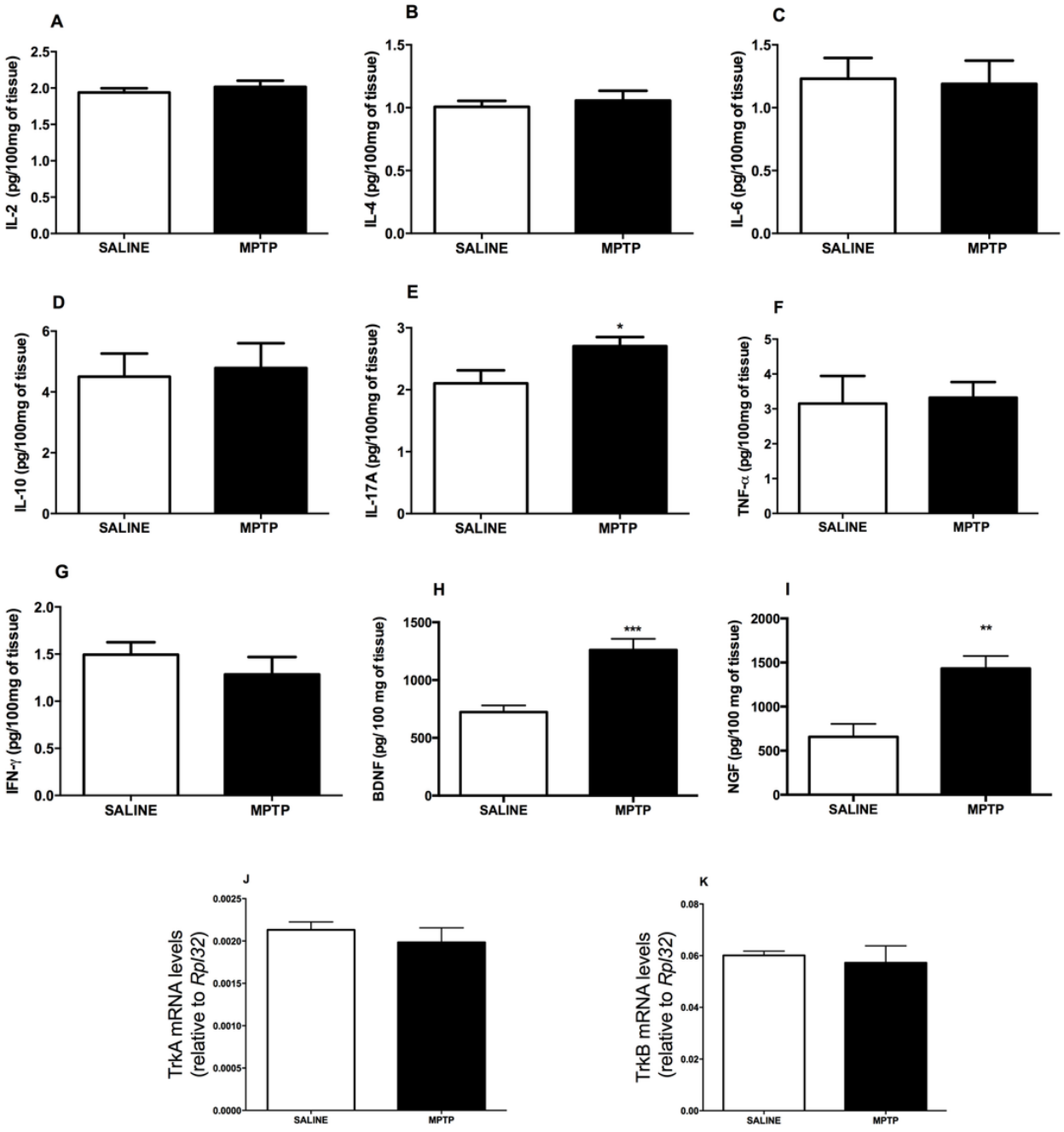

Figure 5

Effects of MPTP on the inflammatory response in the striatum. The levels of IL-2 (A), IL-4 (B), IL-6 (C), IL-10 (D), IL-17A (E), TNF-α (F), IFN- (G), BDNF (H), and NGF (I) were measured using CBA (for the cytokines) and ELISA (for the neurotrophic factors). The mRNA expression of TrkA (J) and TrkB (K) was also quantified using real-time qPCR. The results are expressed as mean ± SEM, and asterisks indicate significant differences, where *p<0.05, **p<0.01; ***p<0.001; n=5 per group.

Supplementary Files

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5.2 Artigo Científico 2: **Neuroprotective effects of Renin Angiotensin System alternative axis in non-motor symptoms of Parkinson's disease**

Neuroprotective effects of Renin-Angiotensin System alternative axis in non-motor symptoms of Parkinson's disease

Oliveira B.S, Machado C.A, Ferreira R.N, Fernandes H.B., Carvalho[5] B.C., Machado[6] M.C., Oliveira[6] A.C., Toscano[4] E., Rachid[4] M.A, S, Simões e Silva A.C, Camargos E.R ,Rocha , N.P,, Teixeira[2,3] A.L.,., Miranda[1,2] A.S.

1Laboratório de Neurobiologia, Departamento de Morfologia, Instituto de Ciências Biológicas.

2 - Laboratório Interdisciplinar de Investigação Médica, Faculdade de Medicina, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.

3 - Neuropsychiatry Program, Department of Psychiatry & Behavioral Sciences, McGovern Medical School, University of Texas Health Science Center at Houston, Houston, TX, USA.

4 - Departamento de Patologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.

5 - Laboratory of Inflammatory Genes, Department of Morphology, Universidade Federal de Minas Gerais.

6 - Laboratorio Neurofarmacologia, Departamento do Farmacologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Belo Horizonte, Brasil.

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ABSTRACT
Parkinson's disease (PD) is a prevalent neurodegenerative disorder characterized by dopaminergic neuron loss in the substantia nigra pars compacta (SNpc). It presents with motor symptoms such as resting tremor, bradykinesia, rigidity, and postural instability, but also non-motor symptoms like olfactory deficits, sleep disturbances, anxiety, depression, and cognitive impairments. The pathophysiology of PD remains incompletely understood, with genetic, environmental, neuroinflammatory, and oxidative stress factors implicated in dopaminergic neuron demise. The Renin-Angiotensin System (RAS), primarily known for its role in cardiovascular and renal homeostasis, has recently emerged as a potential player in central nervous system-related conditions, including PD. Herein, we aimed to investigate the role of both RAS axes in non-motor symptoms of PD. We used a 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) mouse model of PD induced in C57BL/6 mice by intranasal infusion of the neurotoxin MPTP (1mg/nostril). MPTP-mice received saline, Perindopril (5mg/Kg, an antagosti of ACE); Telmisartan (10mg/Kg, an antagonist of AT1 receptors) and AVE099 (3mg/Kg, an agonist of Mas receptors) by gavage. The treatments initiated 5 days before the MPTP infusion and remained until the 11th day post-infusion. Control animals received saline.. Behavioral tests, gene expression analysis, and cytokine measurements were conducted to assess the impact of these treatments on PD-associated symptoms. AVE0991 treatment effectively prevented olfactory and social memory impairments, anxiety, and compulsive-like behaviors in MPTP-treated mice. Telmisartan also mitigated social memory loss and enhanced muscle strength. However, Perindopril did not significantly affect non-motor symptoms. Furthermore, AVE0991 treatment counteracted the increased expression of dopamine receptors in the substantia nigra and prevented dopaminergic neuron loss. While AVE0991 did not suppress pro-inflammatory cytokines, it upregulated anti-inflammatory molecules in response to MPTP-induced inflammation. Our findings

suggest that activation of the alternative RAS axis, particularly through AVE0991 treatment, exerts neuroprotective effects against MPTP-induced neurodegeneration in a PD mouse model. This study highlights the potential of RAS modulation as a therapeutic approach for alleviating non-motor symptoms and preserving motor function in PD. Further research is warranted to fully elucidate the intricate mechanisms underlying RAS involvement in PD pathophysiology and to validate its clinical relevance.

Introduction

Parkinson's disease (PD) is a movement disorder characterized by loss of dopaminergic neurons in the substantia nigra pars compact (SNpc). It's the second most common neurodegenerative disease affecting 1-2 per 1000 of the population each year (Tysnes e Storstein 2017). The clinical hallmarks of PD are resting tremor, bradykinesia, rigidity and postural instability (Jankoivic et. al.,2008). However, non-motor symptoms may appear years before the motor symptoms, including olfactory deficits, sleep disturbance, anxiety, depression as well as learning and memory impairment (Ferrer et al. 2011). The mechanisms underlying PD pathophysiology remain to be fully clarified. There is evidence that genetic and environment factor, neuroinflammation and oxidative stress might be involved in the death of dopaminergic neurons in the SNpc (Labandeira-GarcÃ-a et al. 2014).

The Renin-Angiotensin System (RAS) is classically conceived as a circulating hormonal system involved in cardiovascular and renal homeostasis. (Saavedra 1992). The RAS is composed by two major axis: 1) the classical axis formed by the angiotensin-converting enzyme (ACE), by the active peptide Angiotensin (Ang) II that mediates the classical axis actions by binding to its high affinity receptor, the Angiotensin receptor type 1 (AT1R) (Arroja, Reid, e McCabe 2016). The activation of AT1R by Ang-II induce,

among others, oxidative stress, pro-inflammatory, pro-thrombotic and pro-apoptotic processes (Rocha et al. 2018). The RAS counter-regulatory axis seem to opposite the Ang-II effects and is composed by the ACE2, the active peptide Ang-(1-7) and by its receptor Mas(Labandeira-Garcia et al. 2017).

The discovery that RAS components are expressed in several organs and tissues, including the brain, opens the road for the hypothesis that RAS may play a role in the pathophysiology of the central nervous system (CNS)-related conditions like PD (Rocha N.P. et al., 2018). Previous studies have demonstrated neuroprotective effects of blocking the RAS classical axis in PD-related motor symptoms (Sathiya S. et al., 2013; Reardon K.A. et al., 2000). Although some studies imply a role of the RAS in the motor symptoms of PD, fewer studies evaluated the involvement of this system in PD non-motor symptoms. At the moment, there is no recognized treatment to prevent or minimize non-motor symptoms in this neurodegenerative disease (Nemade, Subramanian, e Shivkumar 2021). Thus, investigation of RAS role in PD might contribute for the identification of new therapeutic targets as well as for the development of more promising therapies.

The RAS alternative axis enhances the activation of Mas receptor, which has been associated with anti-inflammatory effects and protection against neurodegeneration (Costa-Besada et al. 2018). Importantly, the RAS alternative axis seem to opposite, among others, the inflammatory, pro-apoptotic and pro-fibrotic effects of the classical axis, acting as a counter-regulatory system (Costa-Besada et al. 2018). Accumulating evidence suggests that the blockade of the RAS classical axis may contribute to the activation of the alternative axis, promoting neuroprotection (Santos R.A.S. et al., 2017; Mcfall A.2020). The current study was designed to investigate the potential role of both RAS classical and alternative axis in PD-associated non-motor symptoms.

Material and Methods

Animals

Male C57BL/6 mice, aged 8-10 weeks, were obtained from Cebio - of Institute of Biological Sciences, Federal University of Minas Gerais (ICB-UFMG), weighing 20-25g. The animals were housed in groups of five in each cage in a controlled room (22 ± 2°C) temperature, with free access to food and water and a 12 hours light/dark cycle (lights on 6:00 A.M.).

These animals were distributed into five distinct groups following the treatment:

Saline + Intranasal administration Saline (SAL+SAL)

Saline + Intranasal administration MPTP (SAL+MPTP)

Telmisartan + Intranasal administration MPTP(TEL+MPTP)

Perindopril+ Intranasal administration MPTP (PE+MPTP)

AVE0991 + Intranasal administration MPTP (AVE+MPTP)

Care and anesthesia obeyed the guidelines for Laboratory Animals established by The National Institute of Health (Bethesda, MD, USA), as recommended by the Institute of Biological Sciences at the Federal University of Minas Gerais, Belo Horizonte, Brazil. The Ethical Review Board of our institution approved the study protocol and all experimental procedures (CEU/UFMG, Permit Protocol Number 255/2017).

Treatment

The drug has a hydrophobic character, then they were diluted in Poly (ethylene glycol) and maintained frozen until the moment used. Mice received 100 µl of drug solution by gavage at the following daily dose: Telmisartan (10mg / kg), Perindopril (5mg / kg) AVE0991 (3mg / kg). Control mice received the same volume of 0.9% saline by gavage. The treatments started five days before the MPTP infusion and were finalized on the day of the euthanasia, corresponding to the 11th day after the infusion (dpi).

Intranasal administration of MPTP

1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)(Sigma Chemical Co., USA) administered by intranasal route according to the procedure described by (R. Prediger et al. 2006; D. S. Prediger et al. 2011; R. D. S. Prediger et al. 2010). Firstly, we used a 7-mm piece of PE-10 tubing inserted through the nostrils. The tubing was connected to a peristaltic pump, set in 25mg/nostril at a flow rate of 12.5 µl/min. The MPTP was administered in two doses at 1mg in 25 µl. The control solution consisted of saline. Animals had 4hours intervals to regain normal respiratory function.

Behavior tests

Olfactory discrimination test

Mice at 5th-day post MPTP infusion were tested to olfactory memory in the olfactory discrimination test such as previously described by Predger 2010. The olfactory discrimination apparatus is an acrylic box with two equal compartments separated for an open door, which allowed the animal to choose between them. One familiar compartment, with unchanged sawdust of at least 3 days before. Another one, a non-familiar compartment, with fresh sawdust. The animals were allowed to explore for 5 minutes. The time spent in each chamber were recorded and analyzed by the EthoVision XT (Noldus, Technology, Leesburg, VA, USA). This test is based on fact that rodents prefer to stay in locals with their dour (familiar compartment) than others, non-familiar.

Three-Chamber Social Recognition Test

Mice at 6th-day post MPTP infusion were tested in the Three-chamber Social Recognition test, which evaluates short-term social memory. The Three-Chamber social recognition apparatus is a rectangular box containing three chambers, 20 X 40 X 20 cm in size for each chamber, which there are some opens that allow the mouse to explore all three chambers. Three sections were recorded, habituation, sociability and memory. First

habituation, in this section, the animal had 5 min to explore all empty chambers. The second was sociability. An unfamiliar mouse (Stranger 1) aged match was placed on one side of the right chamber. The stranger mouse was restrained in a clear plastic cylinder (80 mm in diameter, 150 mm in height) with numerous holes on the wall, which allow nose contact but prevent fighting. In this section, the animals were allowed to explore all the chambers for 10 minutes. In the last section memory. The animal back to the arena after one hour and thirty minutes, and a second new stranger (stranger2) was placed in the chamber that was empty in the second section, left the chamber. The trajectory and time spent in each chamber were recorded and analyzed by the EthoVision XT (Noldus, Technology, Leesburg, VA, USA).

Open field

Mice at 6th and 11th-day post MPTP infusion were tested in the Open field test, which evaluates locomotor activity and anxiety-like behavior in the open field test such as previously described (Podhorna, McCabe, e Brown 2002). Briefly, the mice were videotaped with the Phenotyper apparatus (Noldus, Information Technology, Leesburg, VA, USA). There were 4 four test analysis cages in the room, so four mice were observed individually at the same time. Each cage (30X30X50 cm) contained a top unit with a digital video camera and infrared lights. Center and border zones were created by the software. The distance was moved (cm) was acquired by the EthoVision video tracking. In the beginning the test, each mouse was placed in the cage to free movement for ten minutes. Parameters such as locomotion activity, the percentage of time spent in the arena center (anxiety measure) were recorded and analyzed by a tracking software (EthoVision XT, Noldus Information Technology, Leesburg, VA, USA).

Marble buried

Mice at 8th-day post-MPTP infusion were tested in the Marble buried test, which evaluates compulsive-like behavior (Oliveira et al. 2021). Mice were placed in a rectangular cage (30x30x50cm) with 20cm of fresh beddings and 25 marbles placed equidistant to each other. Briefly, the animals were allowed to explore and burry the marbles for 30 minutes. At the end of the section, the animals were removed and measured the number of marble buried. Only the balls buried by more than 2 cm were considered.

Force swimming

Mice at 9th-day post MPTP infusion were tested in the forced swimming test, which evaluates the depressive-like behavior (Camargos et al. 2020). Mice were placed in a cylindrical tank (30 cm height x 20 cm diameters) and recorded for 6 minutes. After, the vigorous activity of swimming the animal, the duration of immobility time was recorded during the last 4-min of the 6-min testing period, after a 2-min habituation period by the EthoVision XT (Noldus, Technology, Leesburg, VA, USA).

Y-Maze test

Mice at 11th-day post MPTP infusion were tested in the Y-maze test, which evaluates evaluate short-term spatial memory(Ribeiro et al. 2023). Y-maze apparatus has 3 arms that cross each other with 120° between them. Mice were gently placed in the apparatus for 5 minutes to free exploration. Between each trial, the maze was wiped clean with 70% alcohol and dried with paper towels. Mice were expected to explore the new arms with a higher frequency than a recently explored arm. The frequency of exploration of 3 consecutive new arms (A, B, and C) was analyzed and the scores were calculated by the formula: $(\text{actual alternation}/\text{maximal alternation} - 2) \times 100$. Also, all numbers of entries were recorded.

Object Recognition Test

Mice at 10/11th-day post MPTP infusion were tested in the Object recognition test, which evaluates the long and short memory (De Miranda et al. 2015). The animals were placed in a box of (30cm X 30cm X 50cm). The test was executed for 3 days: habituation, training and testing days. On the habituation day, the animals were allowed to explore the empty box for 10 minutes. On the training day, the mice were allowed two explore identical objects for 10 min. Following the test, one hour and a half after training, the animals returned to the box, where one of the two familiar objects was switched to a novel one and recorded for 10 min. In the second phase of the test, the animals returned to the box after the training, a second novel object was placed in the box. The animals were allowed to explore for 10 minutes. The time spent on each object was measured and analyzed by the EthoVision XT (Noldus, Technology, Leesburg, VA, USA).

Measurement of cytokine and inflammatory factors in the Brain

The concentration of the cytokines interleukin 6 (IL-6), IL-10, IL- 12p70, interferon γ (IFN-γ), tumor necrosis factor α (TNF- α), and chemokine monocyte chemoattractant protein 1 (MCP- 1) was determined using a mouse CBA kit (BD Biosciences, San Diego, CA) and acquired on a FACS CANTO II flow cytometer (Becton Dickinson, San Jose, CA). The samples were incubated with capture microspheres (beads) covered by specific antibodies to the respective cytokines and chemokines, as well as the proteins of the standard curve. Then, the colour reagent was added, and the samples were incubated for 3 h, at room temperature, and protected from light. Then, the plate wells were washed with the Wash BufferR washing solution, provided in the kit, and subjected to centrifugation for 5 min at 200 rpm at room temperature. The supernatant was then aspirated and discarded. The precipitate containing the microspheres was then suspended with 300 μL of Wash BufferR . The CBA results were analyzed by employing

the software FCAP Array version 3.0 (Soft Flow Inc., Pécs, Hungary). Leptin, adiponectin, and insulin levels were detected by enzyme-linked immunosorbent assay (R&D Systems, Minneapolis, MN) by the manufacturer's instructions. Total cholesterol and triglyceride assays were measured by specific kits from BioclinR (MG, Brazil). The monoreagent cholesterol and triglycerides kit is based on a colourimetric enzymatic test, in which a substrate is formed in which the color produced is directly proportional to the concentration of analysis, and its intensity is determined in a spectrophotometer at 500 nm.

Dopaminergic system menssegers RNA (PCR real time)

Anesthetized animals had their brain dissected for isolation of prefrontal cortex, hippocampus, striatum and substancia nigra. The samples were immediately snap frozen and total RNA was extracted using TRIzol reagent (Life Technologies, ThermoFisher Scientific, MA, USA) according to manufacturer's instructions. One microgram of RNA was used for cDNA synthesis using M-MLV reverse transcriptase (Invitrogen, ThermoFisher Scientific, MA, USA). Quantitative PCR was performed using iTaq Universal SYBER Green Supermix kit (BioRad) in the CFX96 Touch™ Real Time detection system (BioRad). Oligonucleotides sequences were obtained from the PrimerBank public database (Wang et al. 2012) or designed through freely available Primer3 website. The respective forward and reverse sequences are described as follow: *Agtr1a* (TGCCATGCCCATAACCATCTG, CGTGCTCATTTTCGTAGACAGG), *Agtr1b* (CTGTGAAATTGCGGACGTAGT, GAAGGGCGGTAGGAAAGAGTA), *Agtr2* (AACTGGCACCAATGAGTCCG, CCAAAAGGAGTAAGTCAGCCAAG); *Mas1* (CCTATCGGTCCTCTACCCCAT; AGAAGGGCACAGACGAATGC) *Ntrk1* (GCCTAACCATCGTGAAGAGTG, CCAACGCATTGGAGGACAGAT); *Ntrk2* (CCGCTAGGATTTGGTGTACTG; CCGGGTCAACGCTGTTAGG); *Drd1*

(ATGGCTCCTAACACTTCTACCA; GGGTATTCCCTAAGAGAGTGGAC); *Drd2* (ACCTGTCCTGGTACGATGATG; GCATGGCATAGTAGTTGTAGTGG), *Drd4* (GCCTGGAGAACCGAGACTATG; CGGCTGTGAAGTTTGGTGTG), *Drd5* (CTCGGCAACGTCCTAGTGTG; AATGCCACGAAGAGGTCTGAG), *Th* (CCAGAGAGGACAAGGTTCCC; ATACGCCTGGTCAGAGAAGC) *Slc6a3* (ACTTCAGGGAAGGTGGTGTGGAT; GTAGAAGTCCACACTGAGGTATGC), and *Rpl32* (GCTGCCATCTGTTTTACGG; TGACTGGTGCCTGATGAACT). Primer synthesis was carried by Integrated DNA Technologies (IDT Inc., USA). Expression values were obtained by the difference between Cts of the target gene and *Rpl32* reference gene (−ΔCt).

Statistics Analysis

Data are presented as mean ± SEM. All data were analyzed by GraphPad Prism 8.2 (GraphPad Software, San Diego). The distribution of data was analyzed by normality test. A comparison between two groups was performed by Student t-test. Three or more groups were analyzed by one-way analysis of variance (ANOVA) followed by Turkey post-test. In all cases, significance was defined by $p < 0.05$.

Results

AVE0991 was able to prevent behavioral and cognitive changes in a prodromic model Parkinson's disease.

MPTP mice displayed a significant impairment in the discriminatory olfactory memory test on the 5th day following intranasal infusion of MPTP compared with controls that received saline (Fig. 1B). Importantly, the treatment with the Mas receptor agonist AVE0991 was the only one capable of preventing the olfactory memory impairment induce by MPTP. The intranasal infusion of MPTP also induced a significant

deficit in the social memory, which was prevented by the treatment with AVE0991 or telmisartan, an antagonist of AT1 receptors (Fig. 1C).

Anxiety-like behaviour was evaluated by the percentage of time spent in the centre area in the open field test. The MPTP animals presented anxiety-like behaviour at 6th and 11th days post-infusion of MPTP. Interestingly, only the AVE0991 treatment was able to revert the anxiety-like behaviour at both 6 and 11 days following MPTP infusion.

The marble burying test was employed to investigate compulsive -like behaviour following MPTP infusion. The MPTP- mice buried more marbles than controls, indicating a compulsive-like behaviour. The AVE0991 treatment prevented the compulsive-like behaviour induced by MPTP infusion .Unexpected, TEL treatment induced a decrease in the marble buried compared to controls, suggesting a depressive-like behaviour.

Furthermore, we evaluated depressive-like behavior and working memory in the mice. The results showed no significant differences between the groups on the 9th and 8th day post-infusion in terms of depressive-like behavior and working memory (Supplementary Figure 1a-b).

Finally, the locomotor activity was evaluated by employing the Open Field task at the 6th and 11th days post-infusion of MPTP. Locomotor activity was indicated as the total distance (cm) travelled by the animal in the open field for 30 min. No significant differences were found between the mice receiving MPTP and controls receiving saline or the RAS modulators.

Evaluation of the Dopaminergic system (mRNA) in the brain

Substantia Nigra

mRNA expression of dopaminergic receptors was measured in the substantia nigra on the 11th day-post infusion by PCR Real-time. The mRNA levels of Ddr1 were significantly higher in the MPTP, but the RAS modulators treatment prevented this alteration back to the control standard. Similar results were observed when the mRNA levels of Ddr4, Ddr5 and DAT were tested. However, mRNA levels of Ddr2 did not present a significant difference between the groups ($p<0,05$). Furthermore, the MPTP mice group presented a decrease in the mRNA levels of Tyrosine Hydroxylase compared with controls. This reduction was prevented by the RAS modulators treatments (Fig. 2a-f)

Striatum

mRNA expression of dopaminergic receptors was measured in the striatum on the 11th day-post infusion by PCR Real-time. The mRNA levels of Ddr1 were lower in the AVE0991 group compared to control. Similar results were observed when the mRNA levels of Ddr4, Ddr5 and DAT were found. No significant diferences in the Ddr2 expression was found between groups. Furthermore, the MPTP group presented a decrease in the mRNA levels of Tyrosine Hydroxylase compared with controls, which the RAS modulators treatments prevented. (Fig.3 a-f)

Evaluation of Renin-Angiotensin System Components

Substantia Nigra

The local concentrations of RAS components were measured in the substantia nigra on the 11th-day post-infusion by ELISA. In the MPTP group, ACE1 levels were significantly lower compared to the control group. Additionally, the ACE1/ACE2 ratio decreased in the MPTP group. On the other hand, Ang II levels were significantly higher

in the AVE0991 group compared to the control group. Furthermore, Ang(1-7) and ACE2 levels were higher in the Telmisartan (Tel), Perindopril (PE), and AVE0991 (AVE) groups compared to the control group. The ratio of Ang II/Ang(1-7) was significantly higher in the Telmisartan group compared to the control group(Fig.4 A-F).

In terms of the expression levels of RAS receptors, there was no difference between all groups for the Angiotensin receptor type 1a, Angiotensin receptor type 1b, and Angiotensin receptor type 2. However, the Mas receptors were increased in the MPTP group compared to the control group. Nonetheless, the AVE0991, Perindopril, and Telmisartan groups prevented this alteration and brought the Mas receptor levels back to the control standard (Fig.4 G-J).

Striatum

The local concentrations of RAS components were measured at the Striatum on the 11th day post-infusion by ELISA test. In the MPTP group, there was no significant difference in the levels of ACE1, ACE2, Ang (1-7), and Ang II compared to the control group. However, in the AVE0991 group, ACE1 levels were significantly higher compared to both the control and MPTP groups. On the other hand, in the Telmisartan group, ACE2 and Ang (1-7) levels were significantly lower compared to both the control and MPTP groups. There was no significant difference in Ang II concentration between the groups. The ratio of Ang II to Ang-(1–7) was only increased in the TEL group. Additionally, the ratio of ACE1 to ACE2 was increased in both the TEL and AVE0991 groups (Fig. 5 A-F).

In terms of the expression levels of RAS receptors, there was no difference in the expression levels of the Angiotensin receptor type 1a, Angiotensin receptor type 1b, Angiotensin receptor type 2, and Mas receptor in the MPTP mice compared to the control group. However, treatment with AVE0991 decreased the expression levels of the

Angiotensin receptor type 1a, Angiotensin receptor type 1b, and Angiotensin receptor type 2, as well as the Mas receptor, compared to both the control and MPTP groups. These findings suggest that in the MPTP group, there are no significant changes in the local concentrations of RAS components and the expression levels of RAS receptors in the Striatum. However, treatment with AVE0991 leads to an increase in ACE1 levels and a decrease in the expression levels of RAS receptors. Additionally, treatment with Telmisartan results in lower levels of ACE2 and Ang (1-7) and an imbalance in the ratio of Ang II to Ang-(1–7) and ACE1 to ACE2. These results indicate potential alterations in the RAS system in response to the different treatments in the MPTP model(Fig. G-J).

Evaluation of cytokines in the brain

Substantia Nigra

Cytokine concentrations within the substantia nigra were assessed on the 11th day following infusion, employing Cytometric Bead Array (CBA) methodology. Notably, IL-2, IL-4, IL-6, IL-17, TNF, and IFN concentrations exhibited an increase across all experimental groups as compared to the control group. Moreover, it is pertinent to emphasize that the administered treatments increased the levels of IL-2, IL-4, IL-6, TNF, and IFN when contrasted with the MPTP group. However, it warrants mention that only within the AVE0991 group increase in IL-17 concentration compared to the MPTP group. (Fig. 6 A-G)

In the context of the IFN/IL-4 ratio, it is of significance to observe that all experimental groups exhibited a decrease in this ratio when compared to the control group. Notably, it is crucial to underscore that only the AVE0991 group decreases in the IL-10 /TNF ratio, a finding of particular relevance within this experimental paradigm (Fig.6 H-I).

Striatum

Local analyses of cytokine concentrations in the striatum on the 11th day post-infusion by CBA. IL-4 and IL-6 concentrations were increased in the MPTP compared to the control group. However, the IL-2 and IL-4 concentrations were higher in the PE and TEL compared to the control groups. Furthermore, the AVE0991 group only increases the IL-4 concentration (Fig.7 A-G).

In the context of the TNF/IL-10 ratio, only the TEL group increased compared to the control and MPTP groups. However, the IFN/IL-4 ratio lower values were evident in the PE, TEL, and AVE0991 groups when compared to both the control and MPTP groups (Fig.7 H-I).

AVE0991 treatment prevents long-term loss of sensorimotor and muscle strength

The sensorimotor function was analyzed by the adhesive removal test on the 19th-day post-infusion. The MPTP mice spent more time removing the adhesive compared with the control. This behaviour shows impairment in fine sensorimotor function. Importantly, the AVE0991 and Telmisartan treatments wer able to prevent the impairment in sensorimotor behaviour. (Fig.8 a)

The Grip forced test was used to evaluate the muscle strength at 20th-day post-MPTP infusion. The MPTP mice presented a decrease in the forelimb force compared with controls receiving saline. However, the AVE0991, Telmizartan and Perindopril prevented the muscle strength impairment following MPTP administration. (Fig. 8b)

Discussion

The pathogenesis of PD remains a complex and incompletely unclear, and multifactorial contributors such as neuroinflammation and oxidative stress can be involved (McDowell e Chesselet 2012). In addition to neuroinflammation and oxidative

stress, recent studies have highlighted the involvement of RAS in PD pathology (Claflin et al., 2017).It has been suggested that the RAS may interact with neuroinflammatory processes and contribute to the neurodegenerative cascade in PD (Claflin et al., 2017). Interestingly, some research has shown that inhibiting the classical axis of the RAS may confer neuroprotection in both PD patients and animal models of neurodegenerative disorders, including PD and Alzheimer's disease (Rocha et al., 2018b). However, it is important to note that these findings have not been without controversy, and further studies are needed to better understand the role of the RAS in PD and its potential as a therapeutic target (Rocha et al., 2018b). However, in the present study, the researchers found that activation of the alternative axis of the RAS through treatment with AVE0991, a Mas receptor agonist, led to an increase in neuroprotection, suggesting that the alternative axis is crucial for neuroprotection.

Non-motor symptoms, such as anxiety, hyposmia, and depression, often manifest years before the onset of motor symptoms in PD, and their physiological mechanisms remain unclear (Poewe 2008) . Animal models have been used to study the mechanisms and physiology of PD (Blesa e Przedborski 2014).

Remarkably, Predger et al. (2010) demonstrated that a single intranasal infusion of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) in mice led to developed non-motor symptoms similar to those observed in PD patients. These symptoms included olfactory and memory impairments, anxiety, and compulsive-like behaviors (Predger et al., 2010; Oliveira et al., 2023). Notably, these non-motor symptoms in PD may be underpinned by alterations within the dopaminergic system and dopaminergic receptor signaling pathways (Mishra, Singh, e Shukla 2018).

Indeed the relationship between increased dopamine receptors and PD is not clear. It is well-established that PD is characterized by the degeneration of dopaminergic

neurons in the substantia nigra, which ultimately leads to a significant reduction in dopamine levels within the nigrostriatal pathway (Feany e Bender 2000). The observed increase in dopamine receptor expression, such as Ddr1 and Ddr5, may indeed function as a compensatory mechanism in response to the progressive loss of dopaminergic neurons during the course of PD. As dopaminergic neurons diminish in number, the remaining neurons may attempt to compensate by increasing the expression of dopamine receptors (Feany e Bender 2000).

Concordant with this perspective, our present study, found upregulation in the expression of Ddr1, Ddr5, and the DAT within the substantia nigra of the MPTP group in compared to controls. Furthermore, another hallmark feature of PD pathology is the degeneration of dopaminergic neurons (Hirsch 1994), another phenomenon that corroborated with our findings, which demonstrated a decrease in the expression of Tyrosine Hydroxylase, a pivotal precursor enzyme in dopamine synthesis, within the MPTP group.

Neuroinflammation is currently considered to be involved in the PD pathophysiology (Araújo et al. 2022). It is characterized by the activation of microglia and astrocytes leading to the production of pro-inflammatory cytokines, TNF, IFN-β1, IL1β and IL-6, and chemokines such as chemoattractant-1 (CINC-1), monocyte chemoattractant protein-1 (MCP-1), and macrophage inflammatory protein-1α (MIP-1α) (Brydon et al. 2008; Kempuraj et al. 2017; Koziorowski et al. 2012; Heidari, Yazdanpanah, e Rezaei 2022). Corroborating our result, we found an increase in pro-inflammatory cytokines, IL-2, IL-6, IL-17, TNF, and IFN in the substantia nigra and the IL-6 in the striatum striatum of the MPTP group.

Indeed, within the spectrum of motor symptoms in PD, fine motor impairment and the loss of muscular strength are frequently encountered clinical manifestations. The

underpinning mechanism behind these motor deficits can be attributed to the progressive degeneration of dopaminergic neurons located in the substantia nigra, a hallmark feature of PD pathology(Jankovic 2008).

It is essential to emphasize that this neurodegenerative process, characterized by the specific loss of dopaminergic neurons, is a distinctive pathological hallmark that sets PD apart from other neurodegenerative disorders (Hirsch 1994; Jankovic 2008; Feany e Bender 2000). While PD shares certain clinical features with other conditions, the selective vulnerability of substantia nigra dopaminergic neurons is a defining characteristic of this disease. In this present study, the animals showed fine motor impairment and loss of and loss of strength in the MPTP group. These collective findings substantiate the intranasal infusion of MPTP as a pertinent and effective model for replicating PD pathology in preclinical research.

Analogous to the relationship between PD and the aging process, it is relevant to note that the RAS is also closely involved in age-related changes (Caslake et al. 2013; Diaz-Ruiz et al. 2018). Specifically, researchers hypothesize that the activation of the classical RAS axis contributes to the establishment of chronic neuroinflammation, a condition that has been linked to the development of neurodegenerative diseases (Abiodun e Ola 2020). As a result, several academic investigations have specifically sought to identify potential neuroprotective attributes associated with inhibiting the classical RAS axis (Sathiya et al. 2013; Perez-Lloret et al. 2017; Kurosaki et al. 2004). However, this has been shown to be controversial (Reardon et al. 2000), and more studies are necessary. In our current study, the treatment with AT1 inhibitors, specifically Telmisartan, had protective effects, in terms of mitigating social memory loss and enhancing overall muscle strength compared to the MPTP group. Concerning the dopaminergic receptor system, Telmisartan treatment effectively prevented only the

elevated expression of Ddr4, Ddr5, and DAT and the loss of dopaminergic neurons in the substantia nigra. Costa-Besada M.A. and collaborators (2018) propose that blocking the classical axis of the RAS leads to an increase in ACE2 levels and a shift towards the alternative axis, thereby promoting neuroprotection. Our findings support this hypothesis, as we observed an increase in the levels of Ang (1-7) and ACE2 in the substantia nigra compared with the MPTP group. Therefore, this might explain the neuroprotection because of the indirect activation of the alternative axis of RAS, not just because of the block of the classical axis.

Another way to modulate the RAS is by using the perindopril, an ACE inhibitor, which modulates the classical axis of the RAS. Messiha B.A.S. and collaborators demonstrated that the use of Perindopril attenuated the progression and improved cognitive deficits in rats with Alzheimer's disease (AD). Additionally, studies have shown positive effects of Perindopril on motor responses and dopamine levels in animal models and human patients with Parkinson's disease (PD) (Kurosaki et al. 2004). However, our results showed that Perindopril treatment did not prevent non-motor symptoms and fine motor impairment, despite preventing the increase of Ddr1, Ddr4, Ddr5, and DAT, and the decrease of Tyrosine Hydroxylase in the substantia nigra compared with the MPTP group.

EL-Shoura and collaborators (2018) demonstrated that Perindopril has the potential as a neuroprotective agent against lipopolysaccharide-induced brain damage by modulating signaling pathways related to the alternative RAS axis. This is consistent with our findings, where we observed an increase in Ang(1-7) levels in the substantia nigra compared to the MPTP group.

As previously indicated, alterations in the RAS could be directly implicated in neuroinflammatory processes, potentially playing a role in PD (Grammatopoulos et al.

2007; Gelders, Baekelandt, e Van der Perren 2018) . However, Perindopril treatment was not able to mitigate the imbalance between the pro and anti-inflammatory cytokines, where the PE group presented an increase in proinflammatory cytokines such as IL-2, IL6, TNF, and INF compared to the MPTP group, although it did not increase the anti-inflammatory cytokine IL-10. In summary, Perindopril, as an ACE inhibitor, may not have effectively shifted the equilibrium away from the classical to the alternative RAS axis, which could explain the observed outcomes.

Finally, in recent years, several new peptides involved in the RAS function were discovered. Ang(1-7) serving as a ligand for the Mas receptor, occupies a central role within the alternative RAS axis(Santos e Ferreira 2006). AVE0991, on the other hand, represents a non-peptide analog of Angiotensin (1-7) and acts by binding to the Mas receptor (Santos e Ferreira 2006). Extensive research has accumulated substantial evidence suggesting that the activation of the alternative RAS axis plays a pivotal role in promoting neuroprotection (see references for details).

Furthermore, research consistently supports the notion of an association between an imbalance in the classical and alternative RAS axes. This imbalance has been identified as a contributing factor in both the aging process and the initiation of neuroinflammatory responses (Arroja, Reid, & McCabe, 2016). In the present study, activation of the alternative axis of RAS, by AVE0991 treatment, prevented the loss of discriminatory olfactory memory, loss of social memory, anxiety, and compulsive-like behaviors. The RAS components are notably abundant in the nigrostriatal dopaminergic circuit, and the excessive activation of AT1 receptors has been shown to heighten dopaminergic vulnerability, leading to dopaminergic cell demise via NADPH production from ROD activation (Labandeira-García et al., 2014; Garrido-Gil et al., 2017). This phenomenon contributes to increased neuroinflammation (Cabrera, Baiardi, and

Bregonzio 2022) . In our study, activation of the alternative RAS axis effectively countered the elevation of dopamine receptors in the substantia nigra (SN) and prevented the decrease in Tyrosine Hydroxylase levels, indicative of reduced dopaminergic neuron loss.

Neuroinflammation is now recognized as a pivotal component of PD pathophysiology (Araújo et al., 2022). Some studies indicate can be initiated through the overactivation of AT1 receptors, leading to an increase in the production of pro-inflammatory mediators and oxidative stress, contributing to dopaminergic neuron degeneration and PD progression (Jackson et al., 2018).

Our results showed that the AVE0991 treatment did not prevent the increase of pro-inflammatory cytokines, such as IL-2, IL-6, IL-17, TNF, and INF. The treatment led to the upregulation of anti-inflammatory molecules, specifically IL-4 and IL-10, compared to the MPTP group. It communicates the idea of a compensatory response to the inflammatory environment induced by MPTP. In line with our findings, Jiang and colleagues in 2018 reported that AVE0991 treatment ameliorated aging-related neuroinflammation in SAMP8 mice, an accelerated aging model, leading to a reduction in the expression of pro-inflammatory markers such as TNF, IL-1β, and IL-6.

The relationship between neuroinflammation and motor symptoms in PD is complex and involves various factors. One study suggests that there is a cross-talk between neurotransmitters and neuroinflammation in the striatum and cerebellum, which play a role in mediating motor behavior (Wahab et al., 2019). However, AVE0991 treatment prevented fine motor impairments and loss of muscle strength, both key indicators of PD, on the 20th-day post-infusion, thus confirming its potential for neuroprotection.

In conclusion, the present study demonstrated that the activation of the alternative axis of the RAS has a pivotal role in the neuroprotection against MPTP-induced neurodegeneration in C57BL/6 mice.

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Figures Legends

Figure 1: Behavioral Effects of RAS Modulator

(A) Experimental Design: Overview of the experimental desing for assessing the effects of the RAS modulator on animal behaviors**. (B) Olfactory Memory Recognition (5th Day Post-Infusion):** Percentage of time spent in each compartment, evaluating discriminatory olfactory memory recognition. n = 8/9 animals per group.(**C) Social Memory Recognition (6th Day Post-Infusion):** Percentage of time spent in each compartment during social memory recognition on the 6th day post-infusion. n = 6/7 animals per group**.(D) Open Field Activity (6th Day Post-Infusion):** Total distance traveled in the open field on the 6th day post-infusion. n = 8/9 animals per group.(**E) Proportion of Time in Open Field Center (6th Day Post-Infusion):** Proportion of time spent in the center of the open field on the 6th day post-infusion. n = 8/9 animals per group.(**F) Open Field Activity (11th Day Post-Infusion):** Total distance traveled in the open field on the 11th day post-infusion. n = 8/9 animals per group**.(G) Proportion of Time in Open Field Center (11th Day Post-Infusion):** Proportion of time spent in the center of the open field on the 11th day post-infusion. n = 8/9 animals per group.

(H) Marble Buried Test (8th Day Post-Infusion): Number of marbles buried in the Marble Buried Test on the 8th day post-infusion. Statistical significance is denoted * as $P \leq 0.05$ compared to the same group (t student test), * $P \leq 0.05$ compared to Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figure 2: Effects of RAS Modulators on Dopaminergic Receptor Expression in the *Substantia Nigra* (11th Day Post-Infusion)

(A) Dopamine receptor 1(Ddr1) mRNA expression; **(B)** Dopamine receptor 2(Ddr2) mRNA expression; **(C)** Dopamine receptor 4(Ddr4) mRNA expression**;(D)** Dopamine recptors 5(Ddr5) mRNA expression; **(E)** Dopamine Transporter (DAT) mRNA expression;(F) Tyrosine Hydroxylase (TH) mRNA expression; The entire experiment was conducted with a sample size of n = 5/6 animals per group. Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figure 3: Figure: Effects of RAS Modulators on the expression Dopaminergic Receptors in the *Striatum* (11th Day Post-Infusion)

(A) Dopamine receptor 1(Ddr1) mRNA expression; **(B)** Dopamine receptor 2(Ddr2) mRNA expression; **(C)** Dopamine receptor 4(Ddr4) mRNA expression**;(D)** Dopamine recptors 5(Ddr5) mRNA expression; **(E)** Dopamine Transporter (DAT) mRNA expression**;(F)** Tyrosine Hydroxylase (TH) mRNA expression; The entire experiment was conducted with a sample size of n = 5/6 animals per group. Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figure 4: Effects of RAS Modulator Treatments on RAS Component Concentrations in the *Substantia Nigra* (11th Day Post-Infusion):

(A) Angiotensin II(AngII) concentration; **(B)** Angiotensin Converting Enzyme (ACE) concentration; **(C)** ACE1/ACE2 Ratio: Calculation of the ratio of ACE and ACE2 in the substantia nigra. **(D)** Angiotensin(1-7)(Ang(1-7)) concentration; **(E)** Angiotensin Converting Enzyme 2(ACE2) concentration; **(F)** AngII/Ang (1-7) Ratio: Calculation of the ratio between Angiotensin II and Angiotensin(1-7) in the substantia nigra. **(G)**

Angiotensin Receptor Type 1a (AT1a) expression; **(H)** Angiotensin Receptors Type 1b (AT1b) expression;**(I)** Angiotensin Receptor Type 2 (AT2) expression; **(J)** Mas Receptor expression; The entire experiment was conducted with a sample size of n = 5/6 animals per group. Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figure 5: Effects of RAS Modulator Treatments on RAS Component Concentrations and Receptor Expression in the *Striatum* (11th Day Post-Infusion): **(A)** Angiotensin II(AngII) concentration; **(B)** Angiotensin Converting Enzyme (ACE) concentration; **(C)** ACE1/ACE2 Ratio: Calculation of the ratio of ACE and ACE2 in the substantia nigra. **(D)** Angiotensin(1-7)(Ang(1-7)) concentration; **(E)** Angiotensin Converting Enzyme 2(ACE2) concentration; **(F)** AngII/Ang (1-7) Ratio: Calculation of the ratio between Angiotensin II and Angiotensin(1-7) in the substantia nigra. **(G)** Angiotensin Receptor Type 1a (AT1a) expression; **(H)** Angiotensin Receptors Type 1b (AT1b) expression;**(I)** Angiotensin Receptor Type 2 (AT2) expression; **(J)** Mas Receptor expression; The entire experiment was conducted with a sample size of n = 5/6 animals per group. Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figure 6: Local Analysis of Cytokine Concentrations in the *Substantia Nigra* (11th Day Post-Infusion):

(A) Interleukin-2 (IL-2) concentration; **(B)** Interleukin-4 (IL-4) concentration**; (C)** Interleukin-17 (IL-17) concentration; **(D)** Tumor Necrosis Factor (TNF) concentration; (E) Interleukin-6 (IL-6) concentration; (F) Interleukin-10 (IL-10) concentration;**(G)** Interferon (IFN) concentration;**(H)** Ratio TNF/IL-10: Calculation of the ratio of TNF to IL-10 **(I)** Ratio IFN/IL-4 Calculation of the ratio of IFN to IL-4. The entire experiment was conducted with a sample size of n = 5/6 animals per group Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figure7: Local Analysis of Cytokine Concentrations in the Striatum (11th Day Post-Infusion):

(A) Interleukin-2 (IL-2) concentration; (B) Interleukin-4 (IL-4) concentration; (C) Interleukin-17 (IL-17) concentration; (D) Tumor Necrosis Factor (TNF) concentration; (E) Interleukin-6 (IL-6) concentration; (F) Interleukin-10 (IL-10) concentration;(G) Interferon (IFN) concentration;(H) Ratio TNF/IL-10: Calculation of the ratio of TNF to IL-10 (I) Ratio IFN/IL-4 Calculation of the ratio of IFN to IL-4. The entire experiment was conducted with a sample size of n = 5/6 animals per group Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figure 8: Effects of RAS Modulator Treatments on Sensory-Motor Function and Muscle Strength

(A) Adhesive Removal Test (19th Day Post-Infusion): Evaluation of sensory-motor function using the Adhesive Removal Test, measuring the time taken to remove the adhesive on the 19th day post-infusion. Data collected from n = 5/6 animals per group.

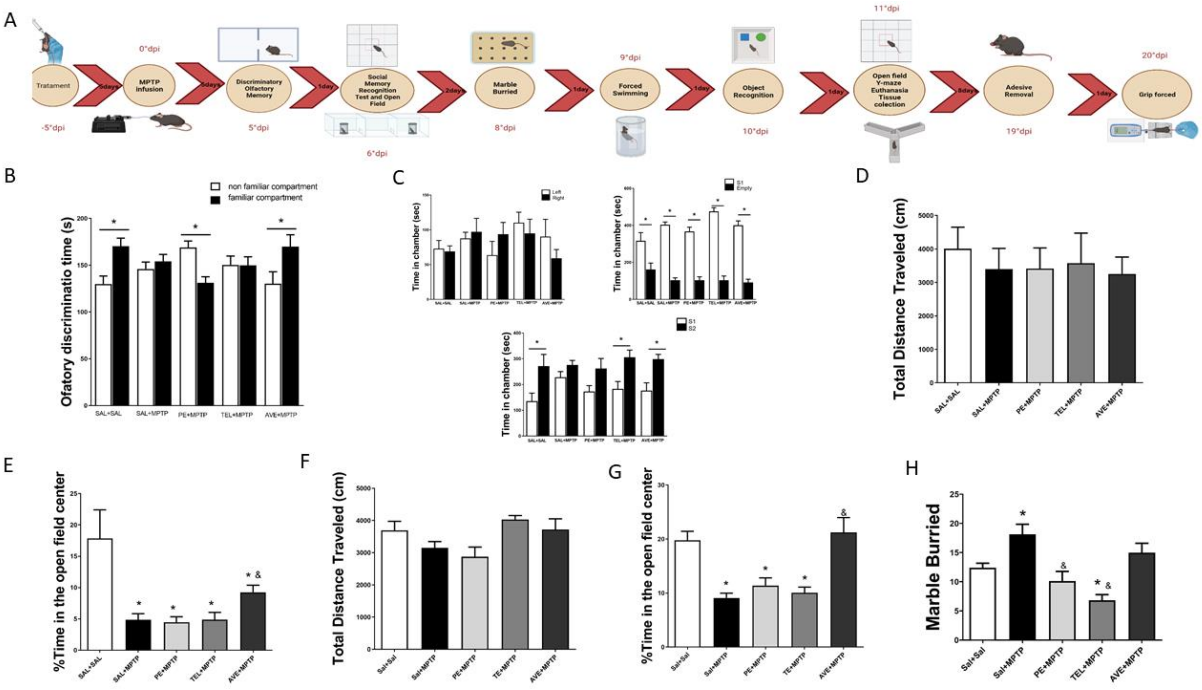
(B) Grip Force Test (20th Day Post-Infusion): Assessment of muscle strength through the Grip Force Test conducted on the 20th day post-infusion, measuring force exertion. Data obtained from n = 5/6 animals per group. Statistical significance is indicated * as *P ≤ 0.05 compared to the Sal+Sal Group*, and *& P ≤ 0.05 compared to the MPTP group (One-way ANOVA followed by t student test).*

Supplementary Figure 1: Effects of RAS Modulator Treatments on Working Memory and Depression-Like Behaviors

(A) Forced Swimming Test (9th Day Post-Infusion - dpi): Evaluation of depression-like behavior using the Forced Swimming Test, with analysis of the time of immobility. Data collected from n = 6/7 animals per group.**(B) Y Maze Test (11th Day post infusion):** Assessment of immediate working memory through the Y Maze Test, presenting the percentage of spontaneous alternation. Summary of spontaneous alternation data from n = 7/8 animals per group.**(C) Novel Recognition Test (10th dpi):** Evaluation of working memory behavior using the Novel Recognition Test, with analysis of the percentage of time spent exploring the new object. n = 7/8 animals per group.Statistical significance is indicated * as *P ≤ 0.05 compared to the Sal+Sal Group* and *& P ≤ 0.05 compared to the MPTP group* using One-way ANOVA followed by t student test.

FIGURAS

Figure 1:



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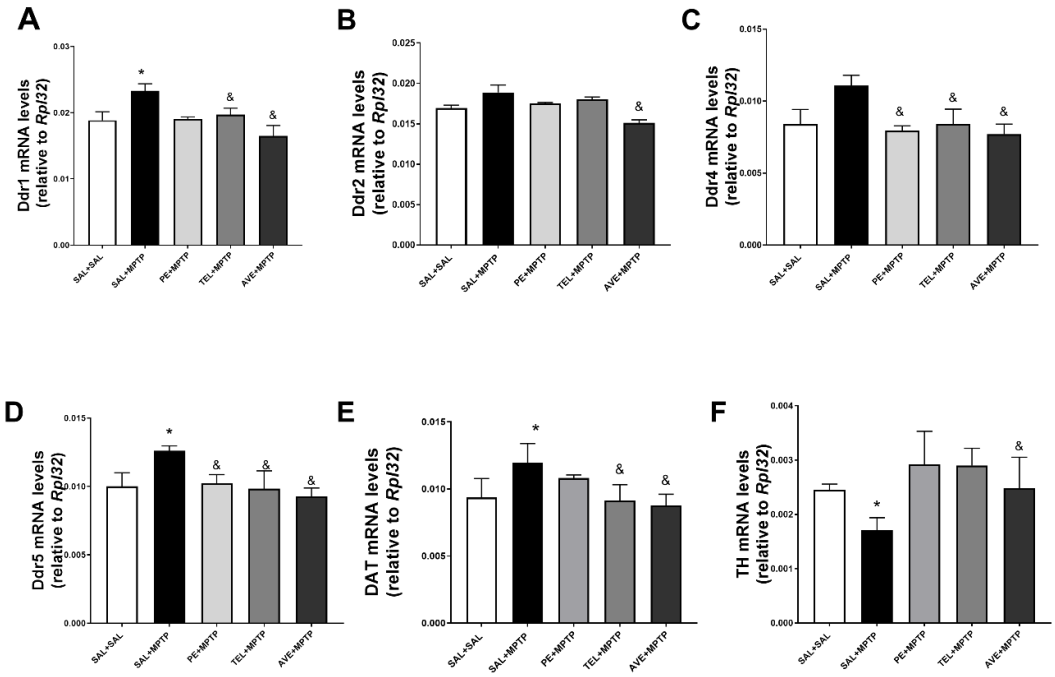

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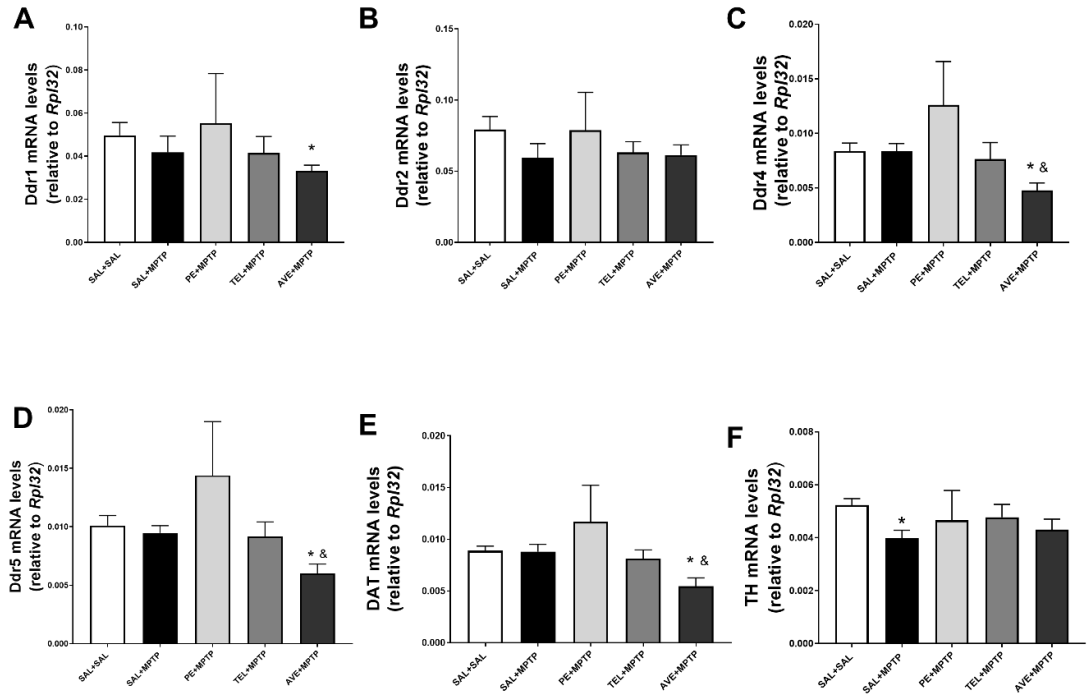

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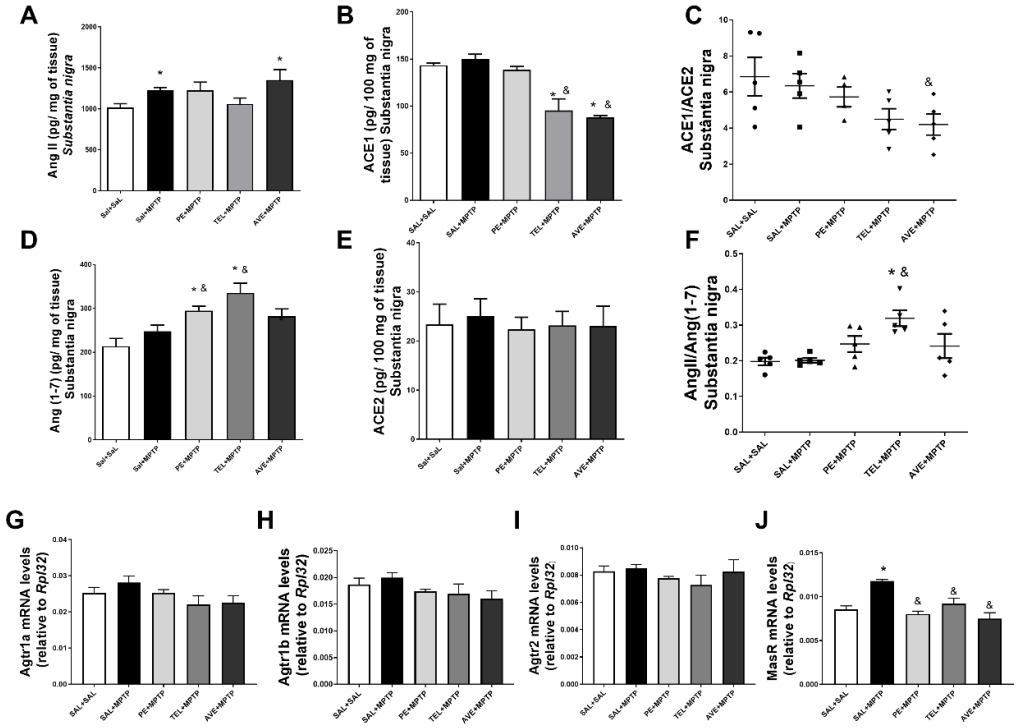

A
Ang II (pg/ mg of tissue) Substantia nigra
Sal+Sal
Sal+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
B
ACE1 (pg/ 100 mg of tissue) Substantia nigra
SAL+SAL
SAL+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
C
ACE1/ACE2 Substantia nigra
D
Ang (1-7) (pg/ mg of tissue) Substantia nigra
E
ACE2 (pg/ 100 mg of tissue) Substantia nigra
F
AngII/Ang(1-7) Substantia nigra
G
Agtr1a mRNA levels (relative to Rpl32)
H
Agtr1b mRNA levels (relative to Rpl32)
I
Agtr2 mRNA levels (relative to Rpl32)
J
MasR mRNA levels (relative to Rpl32)

Figure 5:

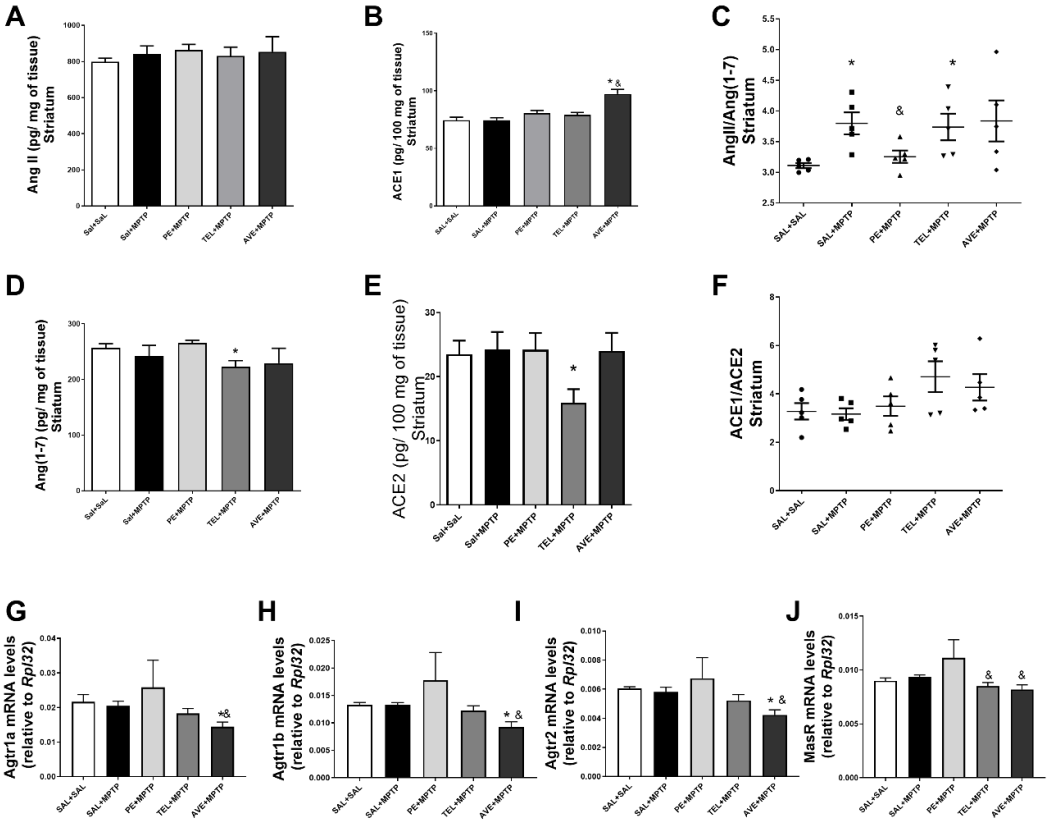

A
Ang II (pg/ mg of tissue) Striatum
B
ACE1 (pg/ 100 mg of tissue) Striatum
C
AngII/Ang(1-7) Striatum
D
Ang(1-7) (pg/ mg of tissue) Striatum
E
ACE2 (pg/ 100 mg of tissue) Striatum
F
ACE1/ACE2 Striatum
G
Agtr1a mRNA levels (relative to Rpl32)
H
Agtr1b mRNA levels (relative to Rpl32)
I
Agtr2 mRNA levels (relative to Rpl32)
J
MasR mRNA levels (relative to Rpl32)
Sal+SAL
Sal+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
SAL+SAL
SAL+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP

Figure 6:

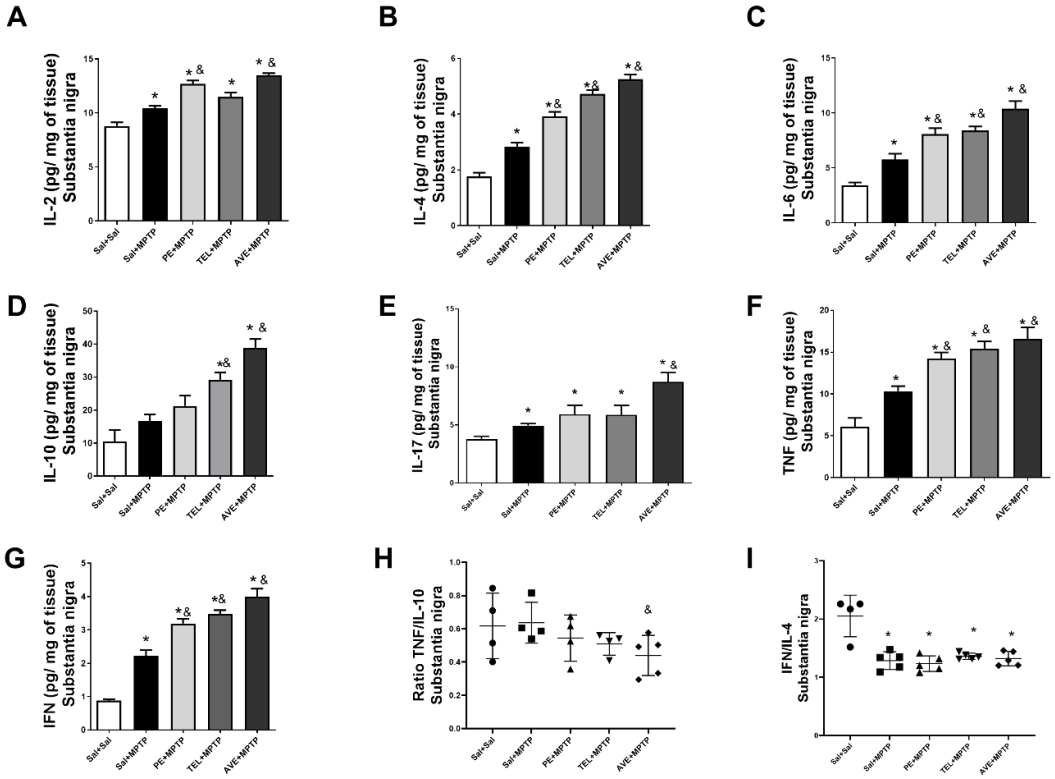

A
IL-2 (pg/ mg of tissue) Substantia nigra
B
IL-4 (pg/ mg of tissue) Substantia nigra
C
IL-6 (pg/ mg of tissue) Substantia nigra
D
IL-10 (pg/ mg of tissue) Substantia nigra
E
IL-17 (pg/ mg of tissue) Substantia nigra
F
TNF (pg/ mg of tissue) Substantia nigra
G
IFN (pg/ mg of tissue) Substantia nigra
H
Ratio TNF/IL-10 Substantia nigra
I
IFN/IL-4 Substantia nigra
Sal+Sal
Sal+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP

Figure 7:

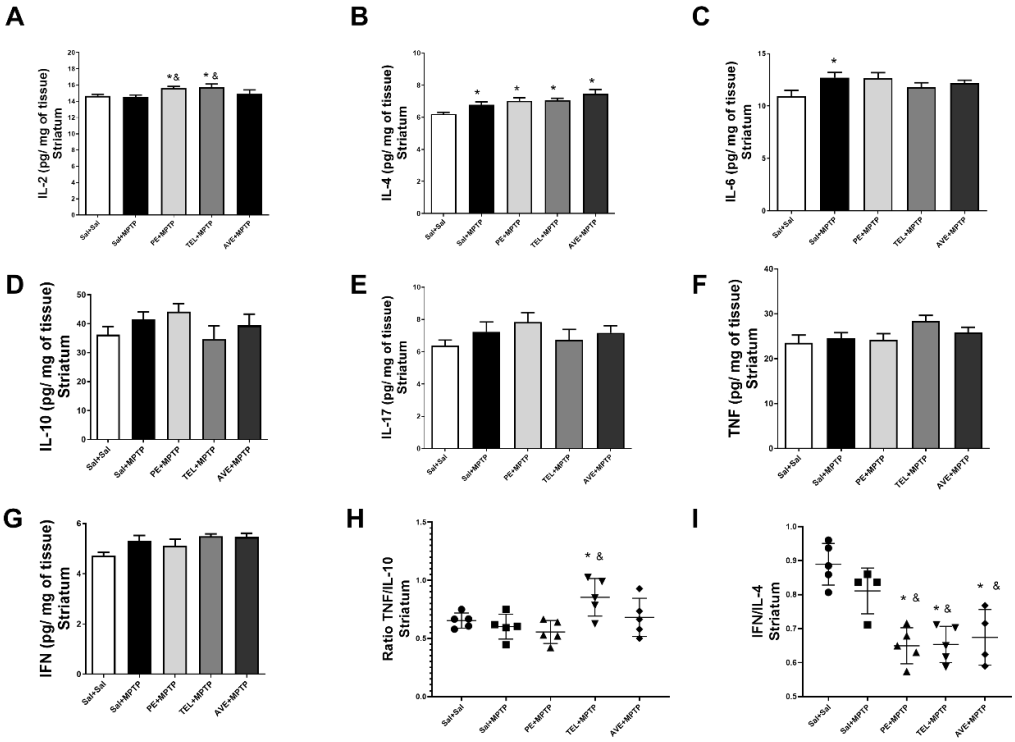
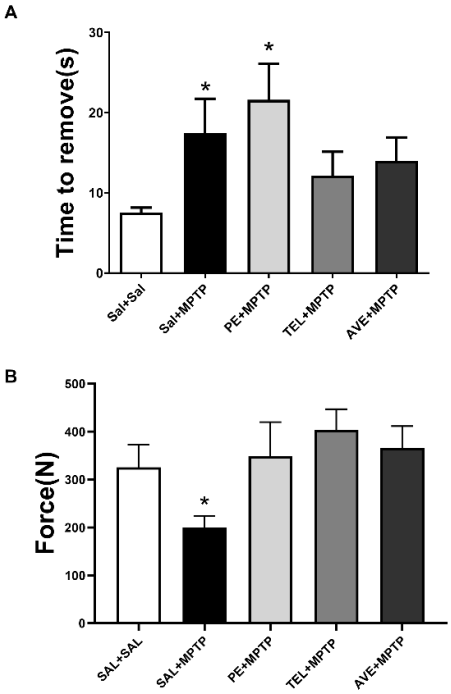
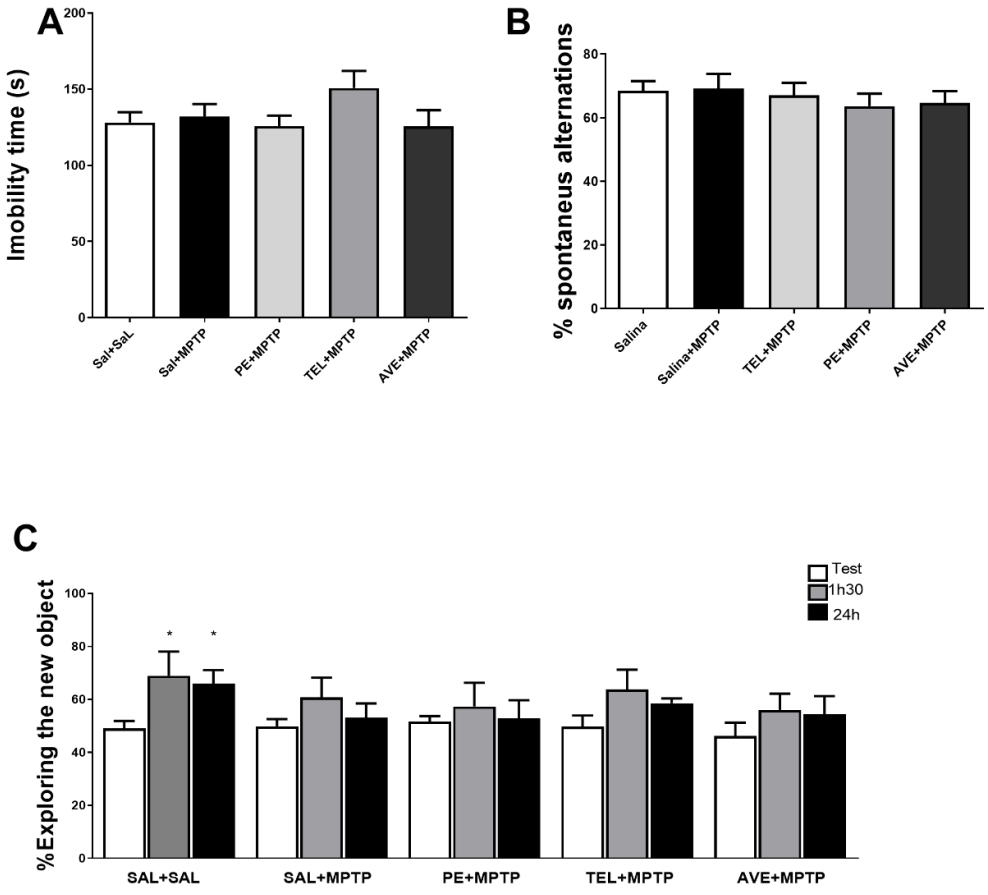


Figure 8:



Suplementar figure 1:



5.3 Resultados produzidos no doutorado que não entraram nos artigos:

5.3.1 Avaliação do sistema dopaminérgico no Sistema Nervoso Central

5.3.1.1 Hipocampo:

A expressão de mRNA dos receptores dopaminérgicos foi analisada no hipocampo no 11º dia após a infusão de MPTP por PCR em tempo real. Os níveis de mRNA de Ddr4 foram significativamente mais elevados no grupo MPTP, mas o tratamento com moduladores do RAS não previniu essa alteração. Já os níveis de Ddr5 foram elevados nos grupos tratados com o Perindopril e o Telmisartan quando comparados com o controle. Os níveis de mRNA de Ddr1, Ddr2 and DAT não apresentaram diferença significativa entre os grupos (Fig.1 A-E).

5.3.1.2 Córtex pré-frontal

A expressão de mRNA dos receptores dopaminérgicos foi mensurada no córtex pré-frontal no 11º dia após a infusão de MPTP por PCR em tempo real. Os níveis de mRNA de Ddr4 foram significativamente mais elevados no grupo MPTP comparado com o controle, mas o tratamento com o Telmisartan and AVE0991 não foi capaz de prevenir essa alteração. Já os níveis de Ddr5 foram mais elevados no grupo do MPT, mas o tratamento com moduladores do SRA preveniu essa alteração, restaurando-a ao padrão de controle. No entanto, os níveis de mRNA de Ddr1, Ddr2 and DAT não apresentaram diferença significativa entre os grupos (Fig. 2 A-E).

5.3.2 Avaliação dos Componentes do Sistema Renina-Angiotensina

5.3.2.1 Hipocampo

As concentrações locais dos componentes do sistema renina-angiotensina (RAS) foram analisada no hipocampo no 11º dia após a infusão de MPTP por ELISA. No grupo MPTP, os níveis de Ang II e Ang (1-7) foram reduzidos em comparação com o grupo de controle, entretanto apenas o tratamento com o Telmisartan abaixou ainda mais a concentração da Ang (1-7) em comparação com o grupo MPTP. Já a razão entre AngII/Ang(1-7) apresentou aumentada apenas no grupo tratdo com o AVE0991 em comparação com os grupos MPTP e controle. Por outro lado, os níveis de ECA, ECA2 e a relação ECA/ECA2 juntamente com a expressão dos receptores AT1a, AT1b, AT2 e Mas não apresentam diferenças significativas entre os grupos (Fig.3 A-J).

5.3.3 Avaliação de citocinas no Sistema Nervoso Central

5.3.3.1 Hipocampo

As concentrações de citocinas no hipocampo foram avaliadas no 11º dia após a infusão de MPTP, utilizando a técnica de Cytometric Bead Array (CBA). É importante destacar que as concentrações de IL-2, IL-4, IL-6, IL-17, TNFα e IFNγ não apresentaram diferenças significativa no grupo MPTP em comparação com o grupo controle. Entretanto, o tratamento com o Perindopril apresentou um aumento das citocinas IL-4 e IL-6 em comparação com o grupo MPTP e controle. Já o tratamento com o Telmisartan apresentou um aumento nas citocinas IL-4 e TNFα em comparação com o grupo controle. Por fim, o tratamento com o AVE0991 aumentou os níveis das citocinas IL4, IL-6, IL-17 e TNFα em comparação aos grupos MPTP e controle (Fig. 4 A-G).

No contexto da razão IL-10/TNFα, não foi encontrada diferença significativa entre os grupos. É crucial destacar que o tratamento com os moduladores do RAS apresentaram uma diminuição na razão IFNγ /IL-4, em comparação com o grupo controle (Fig. 4 H-I).

5.3.3.2 Córtex Pré-Frontal

As concentrações de citocinas no córtex pré-frontal foram avaliadas no 11º dia após a infusão de MPTP, utilizando a técnica de Cytometric Bead Array (CBA). As concentrações de IL-2, IL-4, IL-6, IL-17, TNFα e IFNγ não apresentaram diferença significativas no grupo MPTP comparadas com o grupo controle. Entretanto o tratamento com o perindopril diminuiu as concentrações das citocinas IL-2, IL-4, IL-6, TNF e IFN em comparação com o grupo MPTP e controle. Já o tratamento com o Telmisartan diminuíram as concentrações das citocinas IL-2, IL-4, IL6, IL-17, TNF e IFNγ comparado ao grupo MPTP e controle. Por fim o tratamento com o AVE0991 não apresentou nenhuma diferença comparado aos grupos MPTP e controle (Fig.5 A-G).

No contexto da razão TNFα/IL-10, o grupo MPTP apresentou uma diminuição em relação ao grupo controle. O tratamento com moduladores do SRA preveniu essa alteração, restaurando-a ao padrão de controle. Não foram encontradas diferenças significativas na razão IFNγ /IL-4 (Fig.5 H-i).

5.3.4 Avaliação dos Fatores Neurotróficos

5.3.4.1 Substância Negra

As concentrações locais dos Fatores Neurotróficos foram avaliadas na substância negra no 11º dia após a infusão de MPTP por ELISA. Não se observou diferença significativa nos grupos MPTP nas concentrações de BDNF, NGF e GDNF em comparação com o grupo

controle. Apenas o tratamento com o AVE0991 diminuiu a concentração do NGF em comparação aos grupos MPTP e controle (Fig.6 A-C).

Além disso, avaliamos a expressão de mRNA dos receptores Tirosina Quinase 1 do Fator Neurotrófico (NRKT1) e Receptor Tirosina Quinase 2 do Fator Neurotrófico (NRKT2) na Ssubstância negra. Os animais do grupo MPTP mostraram uma diminuição nos níveis de NRKT1 em comparação com os do grupo controle. No entanto, apenas os animais tratados com Telmisartan e Perindopril conseguiram prevenir essa alteração, restaurando os níveis de expressão aos patamares dos animais controle. Não encontramos diferenças significativas nos níveis de expressão de NRKT2 em nenhum dos grupos (Fig.6 D-E).

5.3.4.2 Estriado

As concentrações locais dos fatores neurotróficos foram mensuradas no estriado no 11º dia após a infusão de MPTP por ELISA. Não se observou diferença significativa nos grupos MPTP nas concentrações de BDNF, NGF e GDNF em comparação com o grupo controle. Apenas o tratamento com o Telmisartan diminuiu a concentração do GDNF em comparação com os grupos MPTP e controle (Fig.7 A-C).

Além disso, avaliamos a expressão dos mRNA dos receptores Tirosina Quinase 1 do Fator Neurotrófico (NRKT1) e Receptor Tirosina Quinase 2 do Fator Neurotrófico (NRKT2) no estriado. Não encontramos diferenças significativas nos níveis de expressão de NRKT1 em nenhum dos grupos. Somente o tratamento com o Telmisartan e AVE0991 foram capazes de reduzir a expressão dos NRKT2 no estriado comparado aos grupos MPTP e controle (Fig.7 D-E).

5.3.4.3 Hipocampo

As concentrações locais dos fatores neurotróficos foram mensuradas no hipocampo no 11º dia após a infusão de MPTP por ELISA. O grupo MPTP apresentou um aumento nas concentrações do BDNF e GDNF comparado com o controle. O tratamento com o AVE0991 foi capaz de aumentar as concentrações do BDNF em comparação com o controle. (Fig. 8 A-C).

Além disso, avaliamos a expressão dos mRNA dos receptores dos Receptores Tirosina Quinase 2 do Fator Neurotrófico (NRKT2) no Hipocampo. Somente o tratamento com o Telmisartan e o AVE0991 foram capazes de reduzir a expressão dos NRKT2 no hipocampo comparado aos grupos MPTP e controle (Fig.8 D).

5.3.5 Legenda das figuras

Figura 1: Efeitos dos Moduladores do Sistema Renina-Angiotensina na Expressão de Receptores Dopaminérgicos no Hipocampo (11º Dia pós-Infusão MPTP):

(A) Dopamine receptor 1(Ddr1) mRNA expression; **(B)** Dopamine receptor 2(Ddr2) mRNA expression; **(C)** Dopamine receptor 4(Ddr4) mRNA expression**;(D)** Dopamine recptors 5(Ddr5) mRNA expression; **(E)** Dopamine Transporter (DAT) mRNA expression. The entire experiment was conducted with a sample size of n = 5/6 animals per group. Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figura 2: Efeitos dos Moduladores do Sistema Renina-Angiotensina na Expressão de Receptores Dopaminérgicos no Cortex Pré - Frontal (11º Dia pós-Infusão MPTP):

(A) Dopamine receptor 1(Ddr1) mRNA expression; **(B)** Dopamine receptor 2(Ddr2) mRNA expression; **(C)** Dopamine receptor 4(Ddr4) mRNA expression**;(D)** Dopamine recptors 5(Ddr5) mRNA expression; **(E)** Dopamine Transporter (DAT) mRNA expression. The entire experiment was conducted with a sample size of n = 5/6 animals per group. Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figura 3: Efeitos dos Tratamentos com Moduladores do Sistema Renina-Angiotensina nas Concentrações de Componentes do RAS no Hipocampo (11º Dia pós-Infusão MPTP):

(A) Angiotensin II(AngII) concentration; **(B)** Angiotensin Converting Enzyme (ACE) concentration; **(C)** ACE1/ACE2 Ratio: Calculation of the ratio of ACE and ACE2 in the substantia nigra. **(D)** Angiotensin(1-7)(Ang(1-7)) concentration; **(E)** Angiotensin Converting Enzyme 2(ACE2) concentration; **(F)** AngII/Ang (1-7) Ratio: Calculation of the ratio between Angiotensin II and Angiotensin(1-7) in the substantia nigra. **(G)** Angiotensin Receptor Type 1a (AT1a) expression; **(H)** Angiotensin Receptors Type 1b (AT1b) expression;**(I)** Angiotensin Receptor Type 2 (AT2) expression; **(J)** Mas Receptor expression; The entire experiment was conducted with a sample size of n = 5/6 animals per group. Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figura 4: Análise Local das Concentrações de Citocinas no Hipocampo (11º Dia pós-Infusão MPTP):

(A) Interleukin-2 (IL-2) concentration; **(B)** Interleukin-4 (IL-4) concentration**; (C)** Interleukin-17 (IL-17) concentration; **(D)** Tumor Necrosis Factor (TNF) concentration; (E)

Interleukin-6 (IL-6) concentration; **(**F) Interleukin-10 (IL-10) concentration;**(G)** Interferon (IFN) concentration;**(H)** Ratio TNF/IL-10: Calculation of the ratio of TNF to IL-10 **(I)** Ratio IFN/IL-4 Calculation of the ratio of IFN to IL-4. The entire experiment was conducted with a sample size of n = 5/6 animals per group Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figura 5: Análise Local das Concentrações de Citocinas no Cortex Pré-Frontal (11º Dia pós-Infusão MPTP):

(A) Interleukin-2 (IL-2) concentration; **(B)** Interleukin-4 (IL-4) concentration**; (C)** Interleukin-17 (IL-17) concentration; **(D)** Tumor Necrosis Factor (TNF) concentration; (E) Interleukin-6 (IL-6) concentration; **(**F) Interleukin-10 (IL-10) concentration;**(G)** Interferon (IFN) concentration;**(H)** Ratio TNF/IL-10: Calculation of the ratio of TNF to IL-10 **(I)** Ratio IFN/IL-4 Calculation of the ratio of IFN to IL-4. The entire experiment was conducted with a sample size of n = 5/6 animals per group. Statistical significance is indicated * as $P \leq 0.05$ compared to the Sal+Sal Group, and & $P \leq 0.05$ compared to the MPTP mice group (One-way ANOVA followed by t student test).

Figura 6: Efeitos dos Moduladores do Sistema Renina-Angiotensina nos Fatores Neurotróficos na *Substância Nigra* (11º Dia pós-Infusão MPTP):

(A) Fator Neurotrófico Derivado do Cérebro (BDNF) concentration; **(B)** Fator de Crescimento Neural (NGF) concentration; **(C)** Fator de Crescimento Derivado das Gliais (GDNF) concentration; **(D)** Expressão mRNA do Receptores Tirosina Quinase 1 do Fator Neurotrófico (NRKT1); **(E)** Expressão mRNA dos Receptores Tirosina Quinase 2 do Fator Neurotrófico (NRKT2); The entire experiment was conducted with a sample size of n = 5/6

animals per group. A significância estatística é indicada por * para P ≤ 0,05 em comparação com o Grupo Sal+Sal e & para P ≤ 0,05 em comparação com o grupo de camundongos MPTP (One way ANOVA seguida pelo teste t de Student).

Figura 7: Efeitos dos Moduladores do Sistema Renina-Angiotensina nos Fatores Neurotróficos no Estriado (11º Dia pós-Infusão MPTP)

(A) Fator Neurotrófico Derivado do Cérebro (BDNF) concentration; **(B)** Fator de Crescimento Neural (NGF) concentration; **(C)** Fator de Crescimento Derivado das Gliais (GDNF) concentration; **(D)** Expressão mRNA do Receptores Tirosina Quinase 1 do Fator Neurotrófico (NRKT1); **(E)** Expressão mRNA dos Receptores Tirosina Quinase 2 do Fator Neurotrófico (NRKT2); The entire experiment was conducted with a sample size of n = 5/6 animals per group. A significância estatística é indicada por * para P ≤ 0,05 em comparação com o Grupo Sal+Sal e & para P ≤ 0,05 em comparação com o grupo de camundongos MPTP (One way ANOVA seguida pelo teste t de Student).

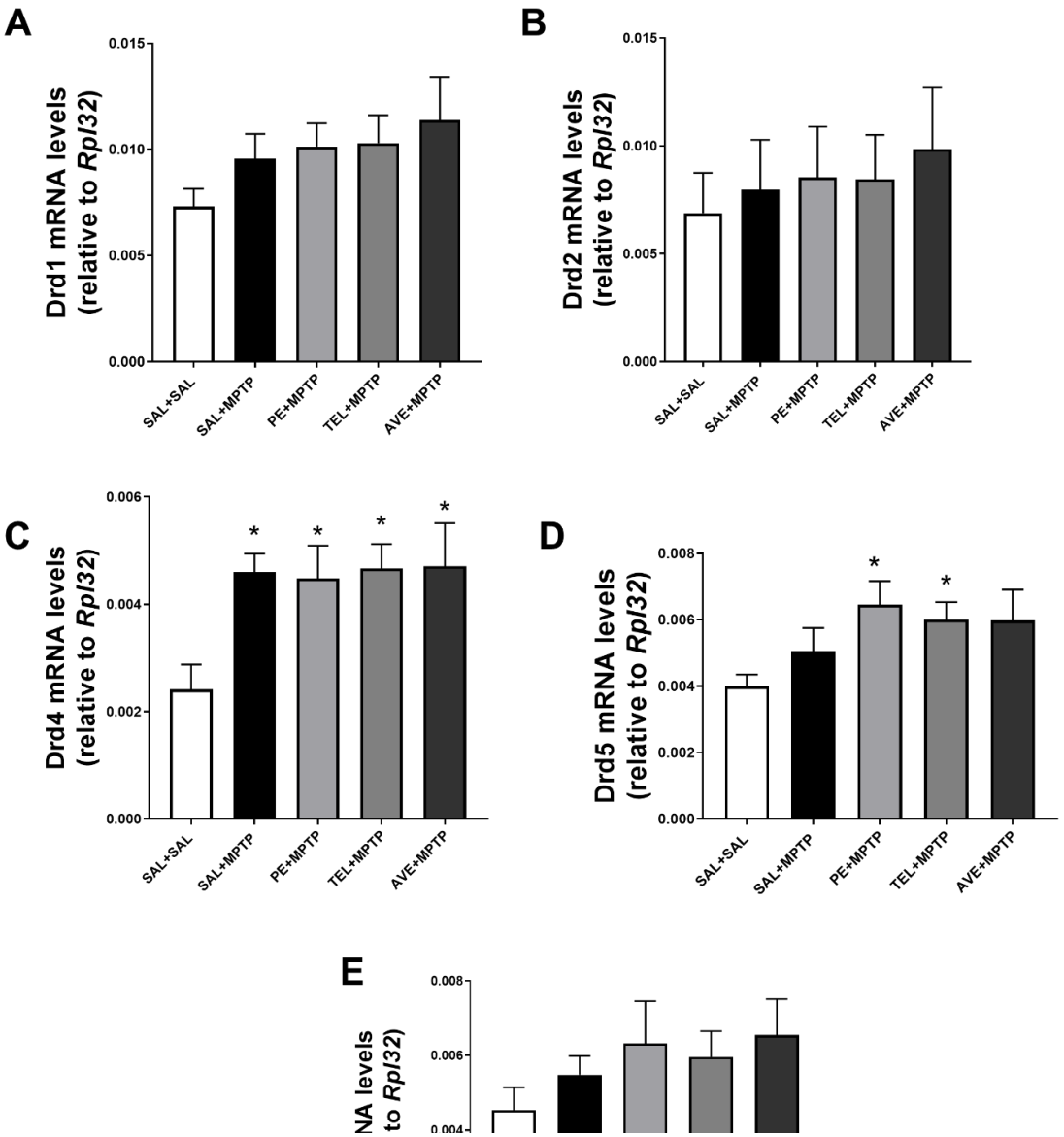
Figura 8: Efeitos dos Moduladores do Sistema Renina-Angiotensina nos Fatores Neurotróficos no Hipocampo (11º Dia pós-Infusão MPTP)

(A) Fator Neurotrófico Derivado do Cérebro (BDNF) concentration; **(B)** Fator de Crescimento Neural (NGF) concentration; **(C)** Fator de Crescimento Derivado das Gliais (GDNF) concentration; **(D)** Expressão mRNA do Receptores Tirosina Quinase 1 do Fator Neurotrófico (NRKT1); **(E)** Expressão mRNA dos Receptores Tirosina Quinase 2 do Fator Neurotrófico (NRKT2); The entire experiment was conducted with a sample size of n = 5/6 animals per group. A significância estatística é indicada por * para P ≤ 0,05 em comparação

com o Grupo Sal+Sal e & para P ≤ 0,05 em comparação com o grupo de camundongos MPTP (One way ANOVA seguida pelo teste t de Student).

Figuras:

Figura 1:



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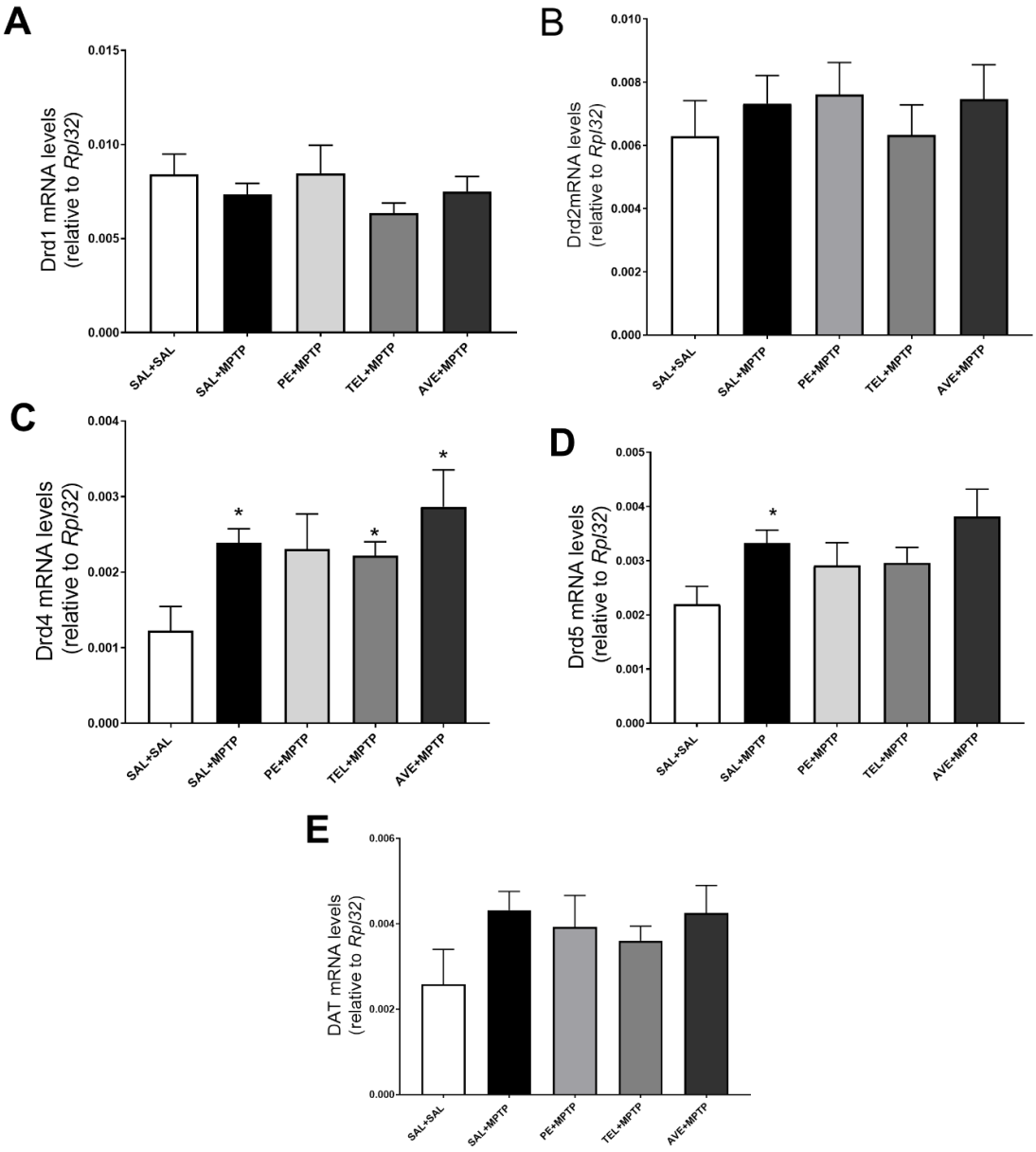

A
Drd1 mRNA levels (relative to Rpl32)
0.015
0.010
0.005
0.000
SAL+SAL
SAL+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
B
Drd2mRNA levels (relative to Rpl32)
0.010
0.008
0.006
0.004
0.002
0.000
SAL+SAL
SAL+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
C
Drd4 mRNA levels (relative to Rpl32)
0.004
0.003
0.002
0.001
0.000
*
*
*
SAL+SAL
SAL+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
D
Drd5 mRNA levels (relative to Rpl32)
0.005
0.004
0.003
0.002
0.001
0.000
*
SAL+SAL
SAL+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
E
DAT mRNA levels (relative to Rpl32)
0.006
0.004
0.002
0.000
SAL+SAL
SAL+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP

Figura 3:

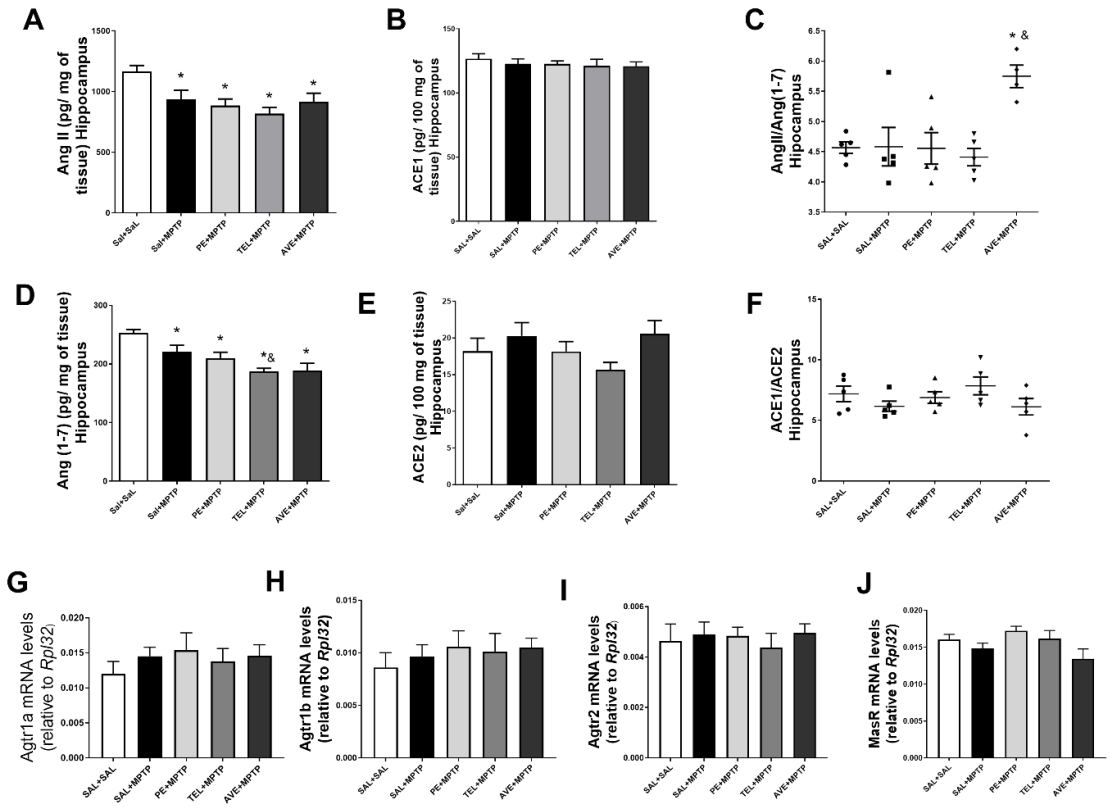

A
Ang II (pg/ mg of tissue) Hippocampus
B
ACE1 (pg/ 100 mg of tissue) Hippocampus
C
AngII/Ang(1-7) Hippocampus
D
Ang (1-7) (pg/ mg of tissue) Hippocampus
E
ACE2 (pg/ 100 mg of tissue) Hippocampus
F
ACE1/ACE2 Hippocampus
G
Agtr1a mRNA levels (relative to Rpl32)
H
Agtr1b mRNA levels (relative to Rpl32)
I
Agtr2 mRNA levels (relative to Rpl32)
J
MasR mRNA levels (relative to Rpl32)
Sal+SAL
Sal+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
SAL+SAL
SAL+MPTP

Figura 4:

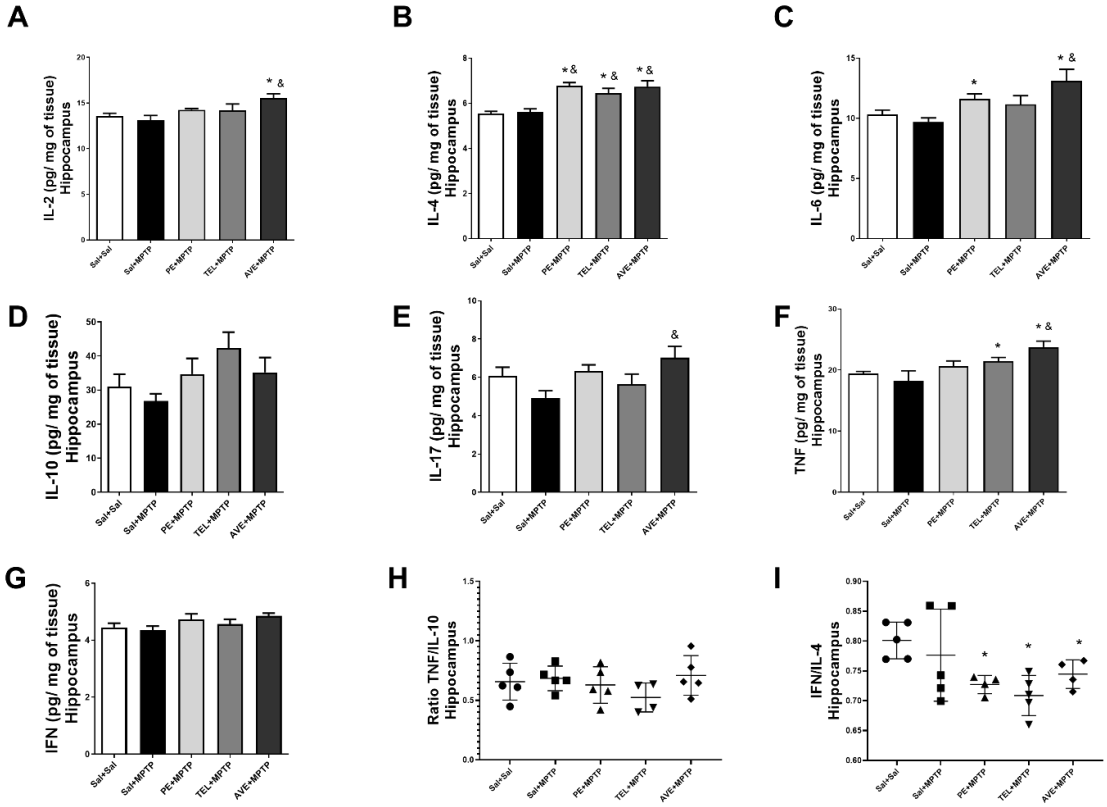

A
IL-2 (pg/ mg of tissue) Hippocampus
B
IL-4 (pg/ mg of tissue) Hippocampus
C
IL-6 (pg/ mg of tissue) Hippocampus
D
IL-10 (pg/ mg of tissue) Hippocampus
E
IL-17 (pg/ mg of tissue) Hippocampus
F
TNF (pg/ mg of tissue) Hippocampus
G
IFN (pg/ mg of tissue) Hippocampus
H
Ratio TNF/IL-10 Hippocampus
I
IFN/IL-4 Hippocampus
Sal+Sal
Sal+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP

Figura 5:

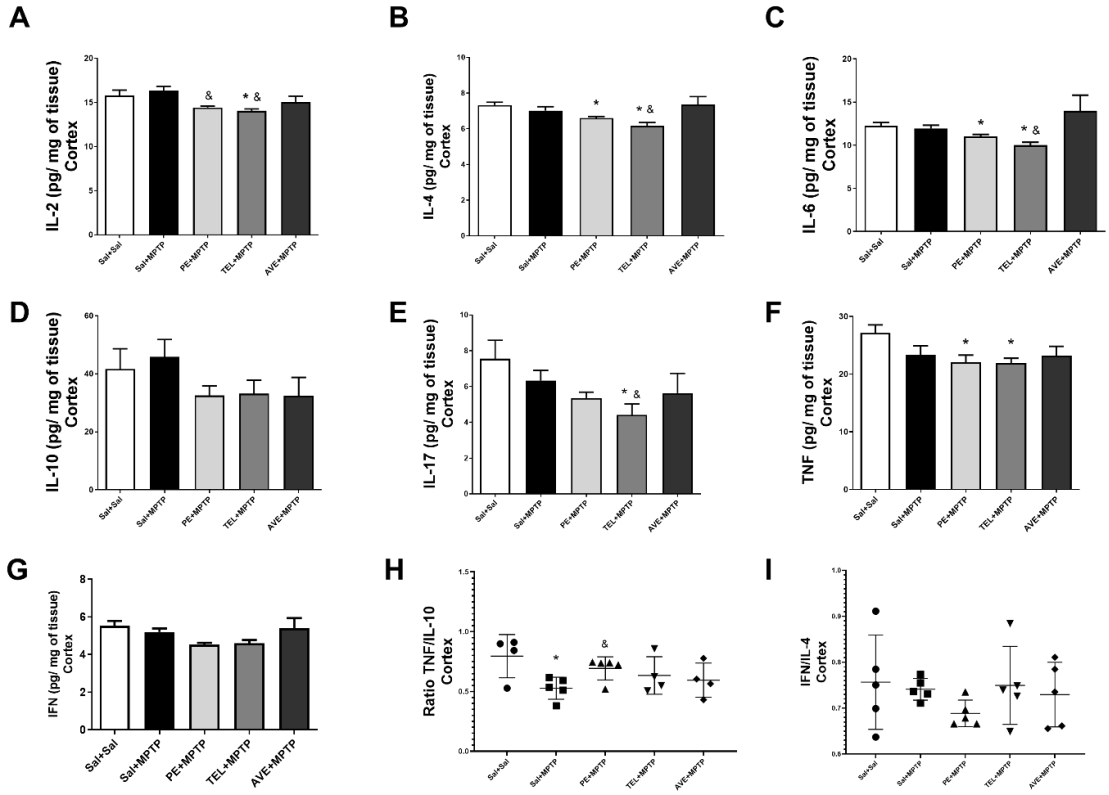
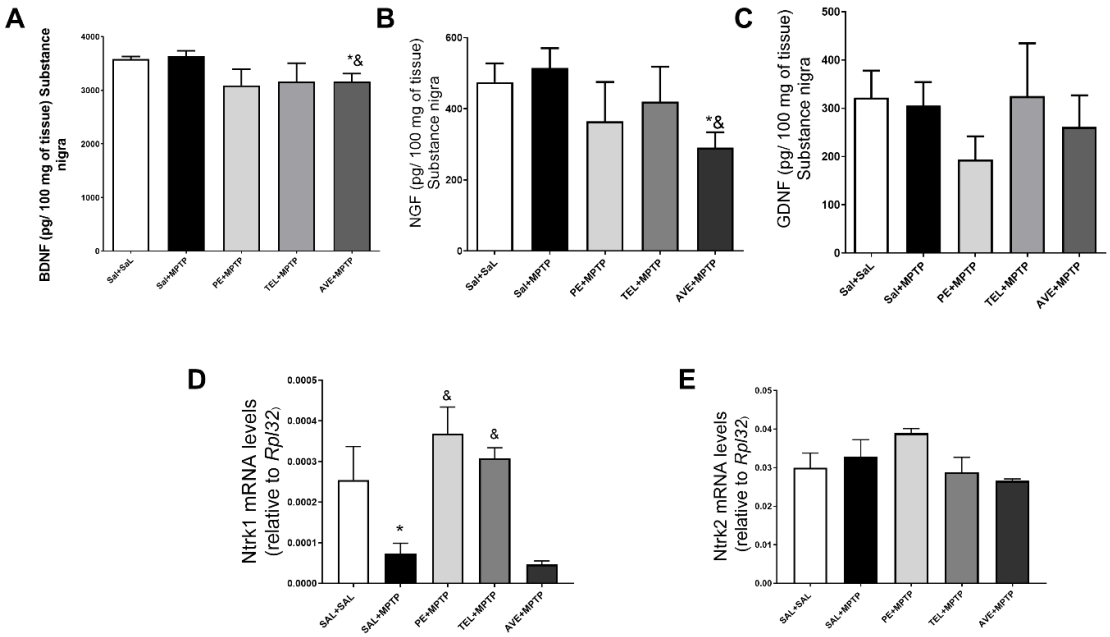

A
IL-2 (pg/ mg of tissue) Cortex
B
IL-4 (pg/ mg of tissue) Cortex
C
IL-6 (pg/ mg of tissue) Cortex
D
IL-10 (pg/ mg of tissue) Cortex
E
IL-17 (pg/ mg of tissue) Cortex
F
TNF (pg/ mg of tissue) Cortex
G
IFN (pg/ mg of tissue) Cortex
H
Ratio TNF/IL-10 Cortex
I
IFN/IL-4 Cortex
Sal+Sal
Sal+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP

Figura 6:



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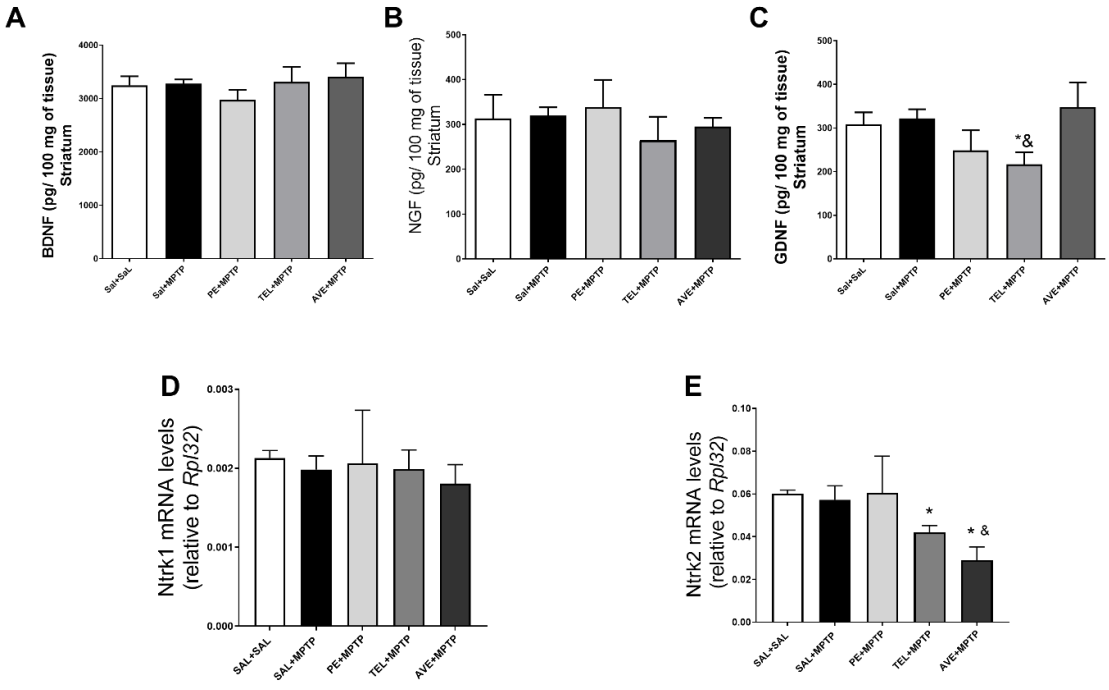

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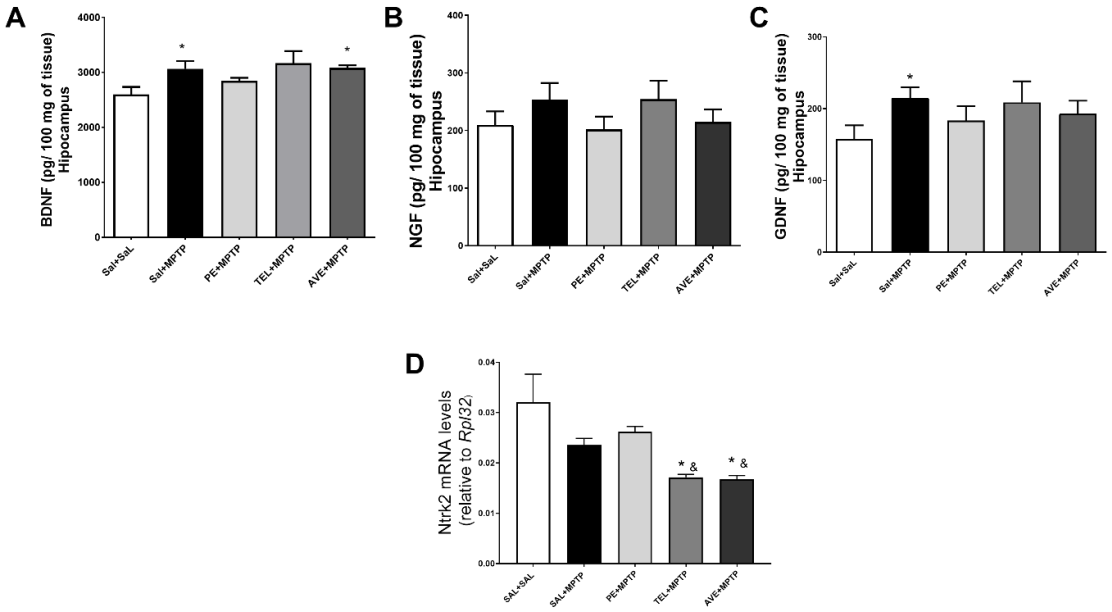
A
BDNF (pg/ 100 mg of tissue)
Hipocampus
4000
3000
2000
1000
0
*
*
Sal+SaL
Sal+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
B
NGF (pg/ 100 mg of tissue)
Hipocampus
400
300
200
100
0
Sal+SaL
Sal+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
C
GDNF (pg/ 100 mg of tissue)
Hipocampus
300
200
100
0
*
Sal+SaL
Sal+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP
D
Ntrk2 mRNA levels
(relative to Rpl32)
0.04
0.03
0.02
0.01
0.00
* &
* &
SAL+SAL
SAL+MPTP
PE+MPTP
TEL+MPTP
AVE+MPTP

6. CONSIDERAÇÕES FINAIS

A análise das alterações comportamentais, neuroinflamatórias e neuroquímicas demonstra a relevância do eixo alternativo do RAS como um promissor alvo terapêutico, em especial, relacionado aos sintomas não motores da DP.

É importante observar que os resultados referentes à quantificação das micróglias, astrócitos e a quantificação dos neurônios dopaminérgicos através de imunofluorescência e imunohistoquímica estão atualmente em análise.

No entanto, é relevante destacar que este estudo apresenta suas limitações, como a compreensão dos mecanismos envolvidos na neuroproteção proferida pela estimulação do eixo alternativado SRA Estudos futuros são urgentemente necessários para investigar de forma sistemática esses mecanismos.

Em resumo, esta tese buscou investigar processos inflamatórios e neuroquímicos na fase prodrômica da Doença de Parkinson em camundongos e examinar o efeito de estratégias farmacológicas que modulam o Sistema Renina Angiotensina na prevenção de alterações cognitivas e comportamentais. Os resultados obtidos são promissores e podem impactar positivamente o desenvolvimento de tratamentos focados em prevenir ou minimizar os sintomas da DP.

7. CONCLUSÕES

✔ A infusão única (1mg por narina de MPTP) pode induzir redução dos neurônios dopaminérgicos na SNpc e neuroinflamação, causando uma série de alterações cognitivas e comportamentais no estágio inicial da doença de Parkinson experimental.

✔ A infusão de (1mg por narina de MPTP) por via intranasal representa um modelo válido de sintomas não motores da DP.

✔ A ativação do eixo contra-regulatório do SRA pode ter um papel crucial na neuroproteção da DP

8. REFERÊNCIAS

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SILVA SENRA; **OLIVEIRA, BRUNA DA SILVA**; CAMPOS, MARCO ANTÔNIO; FONSECA, FLÁVIO GUIMARÃES DA; CAMARGOS, QUEZYA MENDES; SOUSA, GABRIELA FERREIRA DE; CALIARI, MARCELO VIDIGAL; TEIXEIRA, ANTÔNIO LÚCIO; MIRANDA, ALINE SILVA DE; RACHID, MILENE ALVARENGA Brain-derived neurotrophic factor is down regulated after bovine alpha-herpesvirus 5 infection in both wild-type and TLR3/7/9 deficient mice. Journal of Veterinary Medical Science (Online). Fator de Impacto(2022 JCR): 1,2000, v.83, p.180 - 186, 2021.(Anexo 6)

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11 - RACHID, M. A.; CARMAGOS, E. S.R.; MARZANO, L.; **OLIVEIRA, B. S**.; FERREIRA, R. N.; MARTINELLI, P. M.; TEIXEIRA, A. L.; SILVA, A. C. S. E.; MIRANDA, A. S. Effect of blockade of nitric oxide in heart tissue levels of Renin Angiotensin System components in acute experimental Chagas disease. LIFE SCIENCES. Fator de Impacto(2022 JCR): 6,1000, p.336 - 342, 2019.

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13 - MIRANDA, ALINE SILVA; RACHID, MILENE ALVARENGA; SOUZA, CÁSSIO FERRAZ; **OLIVEIRA, BRUNA DA SILVA;** FERREIRA, RODRIGO NOVAES;

MARTINELLI, PATRÍCIA MASSARA; TEIXEIRA, ANTÔNIO LÚCIO; CAMARGOS, ELIZABETH R.S.; SIMÕES E SILVA, ANA CRISTINA Interactions between local renin angiotensin system and nitric oxide in the brain of Trypanosoma cruzi infected rats. ACTA TROPICA. Fator de Impacto(2022 JCR): 2,7000, v.194, p.36 - 40, 2019.

14 - ALVES, RAFAEL LEITE; CARDOSO, BÁRBARA RAMALHO LADEIRA; RAMOS, ISALIRA PEROBA REZENDE**; DA SILVA OLIVEIRA, BRUNA**; DOS SANTOS, MARA LÍVIA; DE MIRANDA, ALINE SILVA; DE ALMEIDA, TATIANE CRISTINE SILVA; VIEIRA, MARIA APARECIDA RIBEIRO; MACHADO, FABIANA SIMÃO; FERREIRA, ANDERSON JOSÉ; DE AVELAR, GLEIDE FERNANDES Physical training improves exercise tolerance, cardiac function and promotes changes in neurotrophins levels in chagasic mice. LIFE SCIENCES. Fator de Impacto(2022 JCR): 6,1000, v.232, p.116629 - , 2019.

15 - MIRANDA, ALINE SILVA; CAMARGOS, ELIZABETH R. S.; MARZANO, LUCAS ALEXANDRE SANTOS; MARZANO, ALESSANDRA CRISTINA SANTOS; **DA SILVA OLIVEIRA, BRUNA;** FERREIRA, RODRIGO NOVAES; MARTINELLI, PATRÍCIA MASSARA; TEIXEIRA, ANTÔNIO LÚCIO; RACHID, MILENE ALVARENGA; SIMÕES E SILVA, ANA CRISTINA Renin angiotensin system molecules and nitric oxide local interactions in the adrenal gland of Trypanosoma cruzi infected rats. PARASITOLOGY RESEARCH. Fator de Impacto (2022 JCR): 2,0000, v.119, p.333 - 337, 2019

Prêmios

2023 Travel award, International Astrocyte School, Bertinoro Italy

Apresentação orais em Eventos

2023: Internation Astrocyte School 2023 (Bertinoro, Italy) – Apresentação Oral

2019: XLII Reunião Anual da Sociedade Brasileira de Neurociências e Comportamento (Campos do Jordão, SP) – apresentação de pôster

2019: XII International Symposium on Vasoactive Peptides (Nova Lima, MG) – apresentação oral e de pôster

Atividades representativas e de administração

2019 – Atual Representante discente do Colegiado do Programa de Pós-Graduação em Biologia Celular

2019 – Atual Coordenadora discente do Canal de divulgação cientifica "Fala aí Cientista" do Programa de Pós-Graduação em Biologia Celular

2020 – 2023 Membro discente do Núcleo de Apoio a Pós-Graduação (NAPG) do instituto de ciências biológicas.

Organização de eventos

1 – **VIII Simpósio de Integração dos Programas de Pós-graduação em Biologia Celular**, 2023, (coordenadora discente, Organização de evento)

2 - **AULA MAGNA DA PÓS-GRADUAÇÃO DO ICB 2022**, sobre o tema "PARASITOS INTRACELULARES, LISOSSOMOSEREPARODE MEMBRANAS: UMA AVENTURA INESPERADA EM BIOLOGIA CELULAR", 2022. (Outro, Organização de evento)

3 - **DARWIN DAY** , 2022. (Outro, Organização de evento)

4 - **SEMINARIO NAPG :** “Impactos do fogo no Brasil: novas ferramentas, tecnologias e análisesparaenfrentarmos um desafio crescente”, 2022. (Outro, Organização de evento)

5 - **AULA MAGNA DA PÓS-GRADUAÇÃO DO ICB 2021**: “Epidemiologia de viroses em ergentes, com ênfase na disseminação do SARS-CoV-2 e suas variantes no mundo.”, 2021. (Outro, Organização de evento)

6 -.**I Simposio de Neurobiologia da UFMG**, 2021. (Outro, Organização de evento)

7 - **Mesaredonda: “COVID-19 em Foco**”,, 2021. (Outro, Organização de evento)

8 - **MOSTRA do ICB**, 2021. (Outro, Organização de evento)

9 - **Seminário NAPG**: “As ações do centro de tecnologia em vacinas da UFMG no enfrentamento à pandemia de SARS-COV2: um exemplo da resposta da Universidade em tempo de crise.”, 2021. (Outro, Organização de evento)

10 - **SEMINÁRIO NAPG:** sobre o tema “Danos neuromusculares e declínio motor na Doença de Huntington”, 2021. (Outro, Organização de evento)

11. **SEMINÁRIO NAPG**: sobre o tema “Diagnóstico, pesquisa e formação de recursos humanos no contexto da pandemia de Covid-19: a experiência do Laboratório de Biologia Integrativas”, 2021. (Outro, Organização de evento)

12. **SEMINÁRIO NAPG**: sobre o tema “Empreendedorismo e Inovação para as Ciências Biológicas”, 2021. (Outro, Organização de evento)

13.**Mostra do ICB**, 2020. (Outro, Organização de evento)

14. “**VIII Curso de Férias do PPGBIOCEL** – COVID-19: Do vírus a pandemia, o que sabemos?”, 2020. (Outro, Organização de evento)