UNIVERSIDADE FEDERAL DO CEARÁ
FACULDADE DE MEDICINA
DEPARTAMENTO DE MORFOLOGIA
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MORFOFUNCIONAIS

JÚLIO CÉSAR CLAUDINO DOS SANTOS

O PAPEL DA GLIA ENTÉRICA E DO EIXO MICROBIOTA-INTESTINO-CÉREBRO NA PATOGÊNESE DA DOENÇA DE PARKINSON

FORTALEZA
2022

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Dissertação apresentada ao Curso de Mestrado Acadêmico em Ciências Morfofuncionais, do Departamento de Morfologia, da Faculdade de Medicina, na Universidade Federal do Ceará, como requisito para obtenção do título de Mestre em Ciências Morfofuncionais. Área de concentração: Neurociências.

Orientadora: Profa. Dra. Glauce Socorro de Barros Viana

Coorientadora: Profa. Dra. Gerly Anne de Castro Brito

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2022

Dados Internacionais de Catalogação na Publicação
Universidade Federal do Ceará
Sistema de Bibliotecas
Gerada automaticamente pelo módulo Catalog, mediante os dados fornecidos pelo(a) autor(a)

S235p Santos, Júlio César Claudino dos.
O papel da glia entérica e do eixo microbiota intestino-cérebro na patogênese da doença de Parkinson / Júlio César Claudino dos Santos. – 2022.
105 f. : il. color.

Tese (doutorado) – Universidade Federal do Ceará, Faculdade de Medicina, Programa de Pós-Graduação em Ciências Morfofuncionais, Fortaleza, 2022.
Orientação: Prof. Dr. Glauce Socorro de Barros Viana.
Coorientação: Prof. Dr. Gerly Anne de Castro Brito.

1. Doença de Parkinson. 2. Rotenona. 3. Eixo microbiota-intestino-cérebro. 4. Glia entérica. I. Título.
CDD 611

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À minha avó materna Marinete Princesa de Araújo (*in memorian*), pelo cuidado no transcorrer da minha vida, pela minha educação e formação enquanto pessoa; ainda que sem estudos, foi a minha maior professora na vida e a quem dedico e devo todos os meus êxitos.

AGRADECIMENTOS

Aos meus pais biológicos Rosinete Araújo e Jaciro Santos, pelo zelo em minha educação, amor, e pelos princípios éticos.

Ao meu irmão João Paulo, que sempre foi um grande incentivador dos meus sonhos e esteve ao meu lado ininterruptamente acreditando que eu poderia alçar voos maiores.

Aos meus pais adotivos Erivan Façanha e Jackeline Osterno, pelo acolhimento, apoio, ensinamentos e, principalmente, ombro amigo em Terras alencarina, desde o primeiro encontro.

À Profa. Dra. Glauce Viana, meus sinceros agradecimentos pelo acolhimento, ensinamentos e, sobretudo, por ter aceitado ser minha orientadora de modo que, com sua ampla experiência, me permitiu caminhar ao seu lado sob sua orientação nesse universo da pós-graduação acreditando que eu poderia me aperfeiçoar a cada novo desafio.

À Profa. Dra. Gerly Brito, meus sinceros agradecimentos pelo acolhimento, ensinamentos e, sobretudo, por ter aceitado ser minha orientadora de modo que, com sua ampla experiência, me permitiu caminhar ao seu lado sob sua orientação nesse universo da pós-graduação acreditando que eu poderia me aperfeiçoar a cada novo desafio.

À Profa. Dra. Laís Damasceno, por aceitar participar da banca de qualificação desta dissertação, proporcionando discussões e sugestões que servirão para um maior crescimento, aprendizado e incentivo pessoal na minha carreira como pesquisador.

Ao Prof. Dr. Leandro Freitas, por aceitar participar da banca de defesa desta dissertação, proporcionando discussões e sugestões que servirão para um maior crescimento, aprendizado e incentivo pessoal na minha carreira como pesquisador.

Ao amigo Prof. Dr. Howard Lopes Ribeiro Júnior, meus sinceros agradecimentos por sua amizade, pela cooperação e por seus inestimáveis ensinamentos estatísticos e metodológicos nesse trabalho.

Aos amigos Camila Carvalho, José Romero, Emanuel Inácio e Túlio Xavier pela amizade, apoio, compreensão. Meus sinceros agradecimentos!

Meus sinceros agradecimentos a todos do Programa de Pós-Graduação em Ciências Morfofuncionais, por apoiar continuamente a evolução da qualidade formativa deste programa.

Destaco também meus sinceros agradecimentos a cada um que compõe o Núcleo de Estudos em Microscopia e Processamento de Imagens (NEMPI) e o Núcleo de Pesquisa e Desenvolvimentos de Medicamentos (NPDM).

Ao CNPq e a CAPES, pelo auxílio financeiro às pesquisas.

“Eu, sou eu e minha circunstância, e se não a salvo a ela, não me salvo a mim” (José Ortega y Gasset).

RESUMO

O eixo microbiota-intestino-cérebro ou eixo intestino-cérebro simples (GBA) é uma rede de comunicação bidirecional complexa e interativa que liga o intestino ao cérebro. Alterações na composição do microbioma intestinal têm sido associadas à disfunção do GBA, inflamação do sistema nervoso central (SNC) e degeneração dopaminérgica, como as que ocorrem na doença de Parkinson (DP). Além da inflamação, sabe-se que a ativação da microglia cerebral desempenha um papel central no dano aos neurônios dopaminérgicos. A inflamação é atribuída ao efeito tóxico da α-sinucleína agregada, no cérebro de pacientes com DP. Foi sugerido que o desdobramento incorreto da α-sinucleína pode começar no intestino e se espalhar "semelhante ao príon", através do nervo vago para o tronco cerebral inferior e, finalmente, para o mesencéfalo, conhecido como hipótese de Braak. Nesta revisão, discutimos como o eixo microbiota-intestino-cérebro e as influências ambientais interagem com o sistema imunológico para promover um estado pró-inflamatório que está envolvido na iniciação e progressão de proteínas α-sinucleína mal dobradas e no início do desenvolvimento precoce não - sintomas motores da DP. Além disso, descrevemos um modelo bidirecional especulativo que explica como a glia entérica está envolvida na iniciação e disseminação da inflamação, ruptura da barreira epitelial e desdobramento incorreto da α-sinucleína, finalmente atingindo o sistema nervoso central e contribuindo para os processos neuroinflamatórios envolvidos com o não - sintomas motores da DP. Palavras-chave: doença de Parkinson; eixo intestino-cérebro; glia entérica; neuroinflamação.

Palavras-chave: Doença de Parkinson. Rotenona; Eixo microbiota-intestino-cérebro; Glia entérica.

ABSTRACT

The microbiota-gut-brain axis or simple gut-brain axis (GBA) is a complex and interactive bidirectional communication network linking the gut to the brain. Alterations in the composition of the gut microbiome have been linked to GBA dysfunction, central nervous system (CNS) inflammation, and dopaminergic degeneration, as those occurring in Parkinson's disease (PD). Besides inflammation, the activation of brain microglia is known to play a central role in the damage of dopaminergic neurons. Inflammation is attributed to the toxic effect of aggregated α-synuclein, in the brain of PD patients. It has been suggested that the α-synuclein misfolding might begin in the gut and spread "prion-like", via the vagus nerve into the lower brainstem and ultimately to the midbrain, known as the Braak hypothesis. In this review, we discuss how the microbiota-gut-brain axis and environmental influences interact with the immune system to promote a pro-inflammatory state that is involved in the initiation and progression of misfolded α-synuclein proteins and the beginning of the early non-motor symptoms of PD. Furthermore, we describe a speculative bidirectional model that explains how the enteric glia is involved in the initiation and spreading of inflammation, epithelial barrier disruption, and α-synuclein misfolding, finally reaching the central nervous system and contributing to neuroinflammatory processes involved with the initial non-motor symptoms of PD.

Keywords: Parkinson's disease. Gut-brain axis. Enteric glia. Neuroinflammation.

LISTA DE FIGURAS

ARTIGO 1

ARTIGO 2

LISTA DE ABREVIATURAS

Abx	Depleção bacteriana induzida por antibióticos
Ach	Acetilcolina
AMS	Atrofia de múltiplos sistemas
ATP	Adenosina trifosfato
BBB	Barreira hematoencefálica
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEE	Células enterocromafins
CEUA	Comissão de Ética no Uso de Animal
CNPq	Conselho Nacional Científico e Tecnológico
CNS	Central nervous system
Cx43	Connexina 43
DA	Dopamina
DCL	Demência com corpos de Lewy
DP	Doença de Parkinson
Dr.(a)	Doutor/Doutora
ECI	Eixo cérebro-intestino
EECs	Células enteroendócrinas
EEG	Eletroencefalograma
EGC	Glia entérica
ENS	Sistema Nervoso Entérico
GABA	Ácido gama-aminobutírico
GBA	Glucocerebrosidade (eixo cérebro-intestino)
GFAP	Proteína glial do ácido fibrilar
GI	Trato gastrointestinal
GM	Microbioma gastrointestinal
GSNO	s-nitrosoglutationa
HE	Hematoxilina-Eosina
HIV	Imunodeficiência humana
HLA	Humano antígeno leucocitário
HSV1	Vírus do herpes humano
IBD	Doença intestinal
IEB	Barreira epitelial intestinal

LCE	Líquido cerebroespinal
LPS	Lipopolissacarídeo
LRRK2	Quinase de repetição rica em leucina 2
M-CSF	Fator estimulante da colônia de macrófagos
MHC II	Complexo de histocompatibilidade II
MPTP	1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine
mRNA	RNA mensageiro
NEMPI	Núcleo de Estudos em Microscopia e Processamento de Imagens
NKA	Neuroquinina A
NO	Óxido nítrico
NPDM	Núcleo de Pesquisa e Desenvolvimento de Medicamentos
OS	Estresse Oxidativo
PINK1	Quinase 1 induzida por PTEN
P-LPS	Lipopolissacarídeos patológicos
Prof.(a)	Professor/Professora
REM	Movimento rápido dos olhos durante o sono
ROT	Rotenona
SCFA	Ácido graxo de cadeia curta
SNC	Sistema Nervoso Central
SNCA	Gene produtor de alfasinucleina
SNP	Sistema Nervoso Periférico
SNPc	Substância nigra compacta
SP	Substância P
SPECT	Osteoprotegerina
TC	Traumatismo craniano
TNF-α	Fator de necrose tumoral
UCB	Universidade Católica de Brasília
UFC	Universidade Federal do Ceará
UNIFESP	Universidade Federal de São Paulo
VEGF	Fator de crescimento endotelial vascular

SUMÁRIO

1 INTRODUÇÃO

1.1 Alfasinucleinopatias

A doença de Parkinson (DP), a atrofia de múltiplos sistemas (AMS) e a demência com corpos de Lewy (DCL) estão incluídas em um grupo de doenças neurológicas denominada alfasinucleinopatias, que são caracterizadas como distúrbios marcados clinicamente por parkinsonismo e patologicamente pela deposição de alfasinucleína em neurônios e células da glia (MCCANN *et al*., 2014). A patologia subjacente a esses distúrbios começa anos antes que as síndromes clínicas que constituem os critérios diagnósticos atuais estejam totalmente presentes. Esta fase pode se manifestar com vários sinais ou sintomas (PALERMO *et al*., 2020). Indivíduos com uma suposta alfasinucleinopatia subjacente e sinais ou sintomas presumivelmente atribuíveis a isso são denominados prodrômicos (BERG *et al.*, 2021). Além dos indivíduos na fase prodrômica, alguns indivíduos são assintomáticos, mas correm risco de desenvolver alfasinucleinopatias devido à predisposição genética ou outros fatores de risco (NUSSBAUM, 2018). Ainda nesse sentido, entre as alfasinucleinopatias, as características dos indivíduos que eventualmente desenvolverão a DP são mais bem caracterizadas, essas características podem ser consideradas em três grandes categorias: fatores de risco, sinais e sintomas e biomarcadores.

Em se tratando de fatores de risco, há duas categorias principais: fatores de risco ambientais e fatores de risco biológicos. Os fatores de risco ambientais para DP incluem o traumatismo craniano (TC) e exposição constante a pesticidas e solventes orgânicos (PEZZOLI; CEREDA, 2013; ASCHERIO; SCHWARZSCHILD, 2016). Ainda nesse sentido, a vida rural, o trabalho no campo, assim como o consumo de água de poço também estão correlacionados com o aumento do risco de DP (NOYCE *et al.*, 2012). Por outro lado, os principais fatores de risco biológicos para alfasinucleinopatias incluem sexo masculino – os homens têm um risco duas vezes maior em comparação com as mulheres – e genótipo, em que fatores genéticos explicam aproximadamente 30% do risco de DP (BERG *et al*., 2015; ASCHERIO; SCHWARZSCHILD, 2016).

Os sinais e sintomas prodrômicos da alfasinucleinopatia pode ser conceituado como uma síndrome com uma ampla gama de manifestações predominantemente não motoras (NOYCE *et al*., 2012), muitas vezes semelhantes, mas de menor gravidade, àquelas observadas na doença totalmente manifestada. Os sintomas de disautonomia pode estar entre as primeiras manifestações de alfasinucleinopatia (POSTUMA *et al*., 2013), possivelmente devido ao

envolvimento precoce do nervo vago e intestinos com a patologia de alfasinucleina (BERG *et al*., 2021; HORSAGER *et al*., 2020). A constipação, que é definida por uma frequência de evacuações espontâneas de menos de uma vez a cada 2 dias, é uma característica do pródromo de todas as alfasinucleinopatias e um dos sintomas não motores mais frequentes da doença de Parkinson, presente em 61,4% dos pacientes (YU *et al.*, 2018). Em pesquisas realizadas anteriormente, constatou-se que a constipação estava correlacionada com a duração e a gravidade da DP (KROGH *et al.*, 2008) e a frequência e a gravidade da constipação aumentaram à medida que a DP progredia (EDWARDS *et al*., 1993).

Por fim, os biomarcadores constituem um componente central nos critérios de pesquisa para os sinais e sintomas prodrômicos da DP. Recentemente, surgiram vários biomarcadores que provavelmente serão de grande utilidade para diagnósticas precocemente os pródromos da alfasinucleinopatia, são eles: tomografia computadorizada por emissão de fóton único de transportador de dopamina (SPECT), alfasinucleina no líquido cerebroespinal (LCE) ou tecidos periféricos, aumento da atividade muscular durante o sono com movimento rápido dos olhos (REM) e alentecimento da atividade de fundo no eletroencefalograma (EEG) (CHAHINE, 2022).

1.2 Doença de Parkinson

A doença de Parkinson (DP) consiste em uma doença neurogenerativa, crônica, progressiva e incapacitante, caracterizada pela perda progressiva de neurônios dopaminérgicos na parte compacta da substância nigra (SNpc), pela depleção do neurotransmissor dopamina (DA) no núcleo estriado (YU *et al.*, 2018) e pela presença de corpos de Lewy no tronco encefálico. Os sintomas motores tornam-se evidentes quando 60% a 80% dos neurônios SNpc são perdidos (HIRSCH; GRAYBIEL; AGID, 1988). A doença afeta milhões de pessoas em todo o mundo e é a segunda condição neurodegenerativa mais prevalente (HIRTZ *et al*., 2007) depois da doença de Alzheimer. Anteriormente, acreditava-se que a DP era caracterizada apenas por sintomas motores extrapiramidais, que incluíam bradicinesia, rigidez, tremor de repouso e instabilidade postural.

Atualmente, a DP afeta milhões de pessoas em todo o mundo e o número de indivíduos afetados pode dobrar até 2030. Estima-se que a doença de Parkinson afete 0,3% da população em países industrializados e pode variar de acordo com a faixa etária, de modo que, em pessoas com menor de 60 anos, varia de 0,13% a 1,6% e atinge o máximo de 9% para indivíduos com mais de 80 anos (NERIUS; FINK; DOBLHAMMER, 2017). Ainda nesse

sentido, há uma predominância em homens, correspondendo a 2%, e em mulheres, correspondendo a 1,3%, com 40 anos ou mais, quando contabilizados os riscos concorrentes (ELBAZ *et al.*, 2002). Estima-se que a incidência seja de 15 a 17 casos por 100.000 pessoas por ano (TYSNES; STORSTEIN, 2017; TWELVES; PERKINS, COUNSELL, 2013). Nenhuma área do mundo está imune à doença de Parkinson.

No que diz respeito aos achados clínicos da DP, podemos organizá-los em sinais e sintomas motores, não-motores e pré-motores. Os quatro sintomas motores cardinais da doença de Parkinson são tremor de repouso, rigidez, bradicinesia e instabilidade. O tremor diz respeito a uma oscilação rítmica em torno de uma articulação em repouso, assimétrico, contralateral, e, frequentemente, consiste no primeiro sintoma motor da DP e acomete 90% dos pacientes em algum momento do curso da doença (OBESO *et al*., 2017). A rigidez refere-se à resistência da movimentação de um segmento do corpo quando deslocado passivamente, coativando músculos agonistas e antagonistas, referido, clinicamente, como sinal da roda denteada. A bradicinesia está relacionada à redução na amplitude e na velocidade dos movimentos que pode ocorrer durante o início e a continuação movimento, consistindo no sinal mais importante no diagnóstico de uma síndrome parkinsoniana (BERARDELLI et al, 2001). Por fim, a instabilidade postural ocorre com a evolução do curso da doença, em torno de uma década após o diagnóstico inicial.

Ainda nesse sentido, os sintomas não motores da DP incluem:

a) **sintomas não-motores neuropsiquiátricos** (depressão, ansiedade, apatia, psicose, anedonia alucinações, abulia, transtorno do déficit de atenção e ataques de pânico);
b) **sintomas não-motores cognitivos** (disfunção executiva, perda de memória e demência), sintomas não-motores autonômicos (hipotensão ortostática, constipação, incontinência fecal, náusea, vômito, sialorreia, incontinência urinária, disfunção sexual, alteração dos reflexos cardíacos, disfunção olfatória, disfunção gastrointestinal e disfagia);
c) **sintomas não-motores do sono** (insônia, sonolência, sonolência diurna excessiva, síndrome das pernas inquietas, ataques de sono, transtorno comportamental do sono REM e sonhos vívidos);
d) **sintomas não-motores sensoriais** (anosmia, dor, ageusia, dormência e parestesia) (ZESIEWICZ, 2019).

Por sua vez, os sintomas pré-motores são definidos como sintomas que antecedem os sintomas motores da doença de Parkinson e incluem constipação, anosmia, transtorno comportamental do sono REM e depressão.

1.3 Etiologia da Doença de Parkinson

A doença de Parkinson é caracterizada por um distúrbio heterogêneo, multifatorial, com fatores genéticos e ambientais participando da sua fisiopatogenia (LAES; HARDY; REVESZ, 2009).

1.3.1 Genética da Doença de Parkinson

Embora a DP seja geralmente uma doença idiopática, há uma minoria de casos (10-15%) que relatam um histórico família, em que cerca de 5-10% têm herança mendeliana (DENG; WANG; JANKOVIC, 2017). Os genes que foram encontrados potencialmente causando DP recebem um nome "PARK" na ordem em que foram identificados. Até o momento, 23 genes PARK foram ligados à DP. As mutações nos genes PARK demonstram herança autossômica dominante (por exemplo, SCNA, LRRK2 e VPS32) ou herança autossômica recessiva (por exemplo, PRKN, PINK1 e DJ-1) (SCHULTE; GASSER, 2011) são resumidas na Tabela 1. O avanço da pesquisa genética com sequenciamento genético de todo o exoma deve fornecer direções futuras nas causas genéticas da doença de Parkinson, incluindo uma maior compreensão das etiologias patogenéticas (MAZZULLI *et al*., 2011).

Tabela 1. Genes PARK envolvidos na doença de Parkinson Familiar

PARK	GENE	HERANÇA	DESCRIÇÃO	CARACTERÍSTICAS CLÍNICAS
PARK1 PARK4	SNCA	AD	Alfasinucleína	Variando de DP clássica a casos de início precoce com demência, disfunção autonômica e progressão rápida
PARK2	PRKN	AR	Parkin RBR E3 ubiquitina proteína ligase	DP de início precoce, progressão lenta, muitas vezes com características de distonia
PARK5	UCHL1	AD	Ubiquitina C-terminal hidrolase L1	DP clássico - apenas uma família, achados não replicados desde então

PARK	GENE	HERANÇA	DESCRIÇÃO	CARACTERÍSTICAS CLÍNICAS
PARK6	PINK1	AR	PTEN induzida quinase putativa 1	DP de início precoce, progressão lenta
PARK7	DJ-1	AR	Desglicase associada ao parkinsonismo	DP de início precoce, progressão lenta
PARK8	LRRK2	AD	Quinase rica em leucina de repetição 2	DP clássica com demência menos frequente e progressão mais lenta
PARK9	ATP13A2	AR	ATPase 13A2 transportadora de cátions	Início precoce (adolescência), parkinsonismo atípico com demência, espasticidade e paralisia supranuclear (síndrome de Kufor-Rakeb)
PARK11	GIGYF2	AD	GRB10 interagindo com proteína GYF 2	DP Clássica
PARK13	HTRA2	AR	HtrA serina peptidase 2	DP Clássica
PARK14	PLA2G6	AR	Enzima fosfolipase A2 independente de cálcio	Início precoce com características atípicas (parkinsonismo de distonia)
PARK15	FBX07	AR	Proteína F-box 7	Início precoce com características atípicas (síndrome palido-piramidal)
PARK17	VPS35	AD	Proteína 35 associada à classificação de proteína vacuolar	DP Clássica
PARK18	EIF4G1	AD	Fator de iniciação de tradução eucariótica 4 gama 1	PD Clássica
PARK19	DNAJC6	AR	HSP40 Auxilina	DP de início precoce, progressão rápida
PARK20	SYNJ1	AR	Sinaptojanina 1	Parkinsonismo com distonia e declínio cognitivo
PARK21	DNAJC13	AD	Endocitose 8 mediada por receptor (RME-8)	DP Clássica
PARK23	VPS13C	AR	Proteína 13C associada à classificação de proteína vacuolar	DP de início precoce, progressão rápida

AD: autossômica dominante, AR: autossômica recessiva, DP: doença de Parkinson.

Fonte**:** (adaptado de Blauwendraat, 2020).

1.3.2 Fatores Ambientais na Doença de Parkinson

Acredita-se amplamente que as exposições ambientais contribuem em grande parte para a maioria da DP esporádica de início tardio, sozinha ou por meio de interações com fatores genéticos. A pesquisa sobre os gatilhos e modificadores ambientais para o desenvolvimento da DP é de suma importância, pois a DP esporádica de início tardio tem um curso arrastado que pode levar décadas para se desenvolver e, no momento do diagnóstico, as alterações neurodegenerativas são muito avançadas para desacelerar, parar ou até mesmo reverter. Ainda nesse sentido, apesar dos avanços genéticos no tocante às bases genéticas para a DP esporádica de início tardio, os achados genéticos explicam apenas parcialmente os casos de DP e não podem se estender facilmente para a prevenção dessa doença neurológica (CHEN, 2018).

O interesse em saber se tais exposições podem contribuir para o desenvolvimento da doença de Parkinson foi despertado pela associação entre 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) e parkinsonismo durante a década de 1980 (MAZZULLI *et al*., 2011). Nas últimas duas décadas, os cientista identificaram inúmeros fatores ambientais associados ao risco de desenvolver DP (CHEN, 2018; ASCHERIO, 2016), alguns dos fatores ambientais e exposições tóxicas que podem estar associados à doença de Parkinson incluem pesticidas (rotenona e paraquat) (TANNER *et al.*, 2011; COSTELLO *et al*., 2009); metais pesados (manganês, chumbo e cobre); água de poço; marcenaria; traumatismo craniano (FANG *et al*., 2012); outras substâncias, incluindo bifenilos policlorados, tricloroetileno, percloroetileno e tetracloreto de carbono; e vida rural. A exposição a toxinas, incluindo monóxido de carbono, vestígios de metais, solventes orgânicos e cianeto também foi apontada como fator de risco ambiental. Alternativamente, acredita-se que o fumo, o uso de ibuprofeno e a ingestão de cafeína reduzam o risco de doenças, embora mais estudos estejam em andamento (TAYLOR; COOK; COUNSELL, 2007; OBESO, 2017).

Para a maioria dessas associações, hipóteses biológicas plausíveis foram propostas. Entretanto, a inferência causal para esses achados epidemiológicos tem sido muito difícil. Além de dados experimentais inconsistentes e, por vezes, limitados, para a maioria das observações epidemiológicas, a causalidade reversa é uma explicação potencial viável, ou seja, que o desenvolvimento da DP antes do diagnóstico clínico muda o estilo de vida e o comportamento, em vez do contrário, com exceção para certos pesticidas. Por exemplo, achados epidemiológicos sobre a rotenona e o paraquat (TANNER *et al*., 2011; COSTELLO *et al*., 2009) são apoiados por fortes evidência experimentais, por essa razão esses produtos químicos são amplamente utilizados para gerar modelos experimentais de roedores para pesquisas pré-

clínicas e terapêuticas de DP (LE; SAYANA; JANKOVIC, 2014). Por essa razão, apesar da sua importância e do acúmulo de evidências científicas, a compreensão da contribuição dos fatores ambientais para a DP ainda está em fase inicial.

1.4 Eixo Microbiota-Cérebro-Intestino na Doença de Parkinson

A inflamação aguda, infecção ou desafios metabólicos podem induzir gliose reativa que pode ter efeitos sobre a neurodegeneração e mudanças na função neuromuscular. Isto mostra que a glia entérica (EGC) reage ao ambiente nocivo para proteger o sistema nervoso de danos, geralmente aumentando a expressão de GFAP e S100B, associados à resposta neuroinflamatória mediada pela glia (SEGUELLA *et al*., 2019). Além disso, a gliose entérica reativa também pode ser desencadeada na ausência de estímulos inflamatórios, por neurônios sensoriais e neurônios entéricos (DEVALLE *et al*., 2018).

Neste contexto, a gliose reativa induz um processo de neuroinflamação que leva à plasticidade neuronal, perturbando a motilidade intestinal e produzindo dor visceral em distúrbios gastrointestinais funcionais e orgânicos (Spencer; Hu, 2020), bem como, pela liberação de óxido nítrico (NO), contribuindo para a disbiose intestinal (TURCO *et al*., 2014; SEGUELLA; SARNELLI, ESPOSITO, 2020). Entretanto, o sistema nervoso entérico (ENS) em pacientes com DP ainda não demonstrou estar correlacionado com perda neuronal nem neurodegeneração (Litvak; Byndloss; Bäumler, 2018) e isto poderia ser devido à interação de mecanismos intrínsecos de neuroproteção, em células intestinais dentro do ENS, ou ao fato de que a inflamação induzida pelos loops fisiopatológicos propostos não são suficientes para gerar perda neuronal nem para superar o mecanismo neuroprotetor, como os efeitos anti-inflamatórios do nervo vago, que é mediado pelos EGCs (LANGNESS *et al*., 2017). Além disso, essa causalidade específica precisa ser investigada mais detalhadamente em pacientes com DP.

Os EGCs podem desempenhar um papel importante nos distúrbios gastrointestinais relacionados à DP, assim como no desenvolvimento e progressão da doença central. Além de suas funções tróficas e estruturais, os AECTs são cruciais para o controle homeostático de uma ampla gama de atividades gastrointestinais (BENVENUTI *et al*., 2020). Os AECT são o tipo celular equivalente de astrocitos no SNC e compartilham com eles muitas propriedades neurotróficas e neuroimunomoduladoras (CAPPOCCIA *et al*., 2016).

Vários estudos mostraram que o EGC expressa o complexo de histocompatibilidade II (MHC II), associado à apresentação de antígenos a células imunes inatas e adaptativas, respondendo a estímulos prejudiciais através de TLR-2 e TLR-4, protegendo o hospedeiro

contra patógenos e controlando o eixo neuroimune (CHOW; GRUBIŠIĆ; GULBRANSEN, 2021). Curiosamente, células T periféricas CD4+ que adquirem um fenótipo pró-inflamatório (como Th1 e Th17) por estimulação da glia entérica, podem cruzar o BBB e dar origem a inflamação central, que é um achado comum em distúrbios neurodegenerativos no SNC (SOLLEIRO-VILLAVICENCIO; RIVAS-ARANCIBIA, 2018). Também foi demonstrado que o EGC inibe diretamente as atividades anti-inflamatórias de Treg e Th2, demonstrando seu potencial para induzir infecções crônicas (SOLLEIRO-VILLAVICENCIO; RIVAS-ARANCIBIA, 2018).

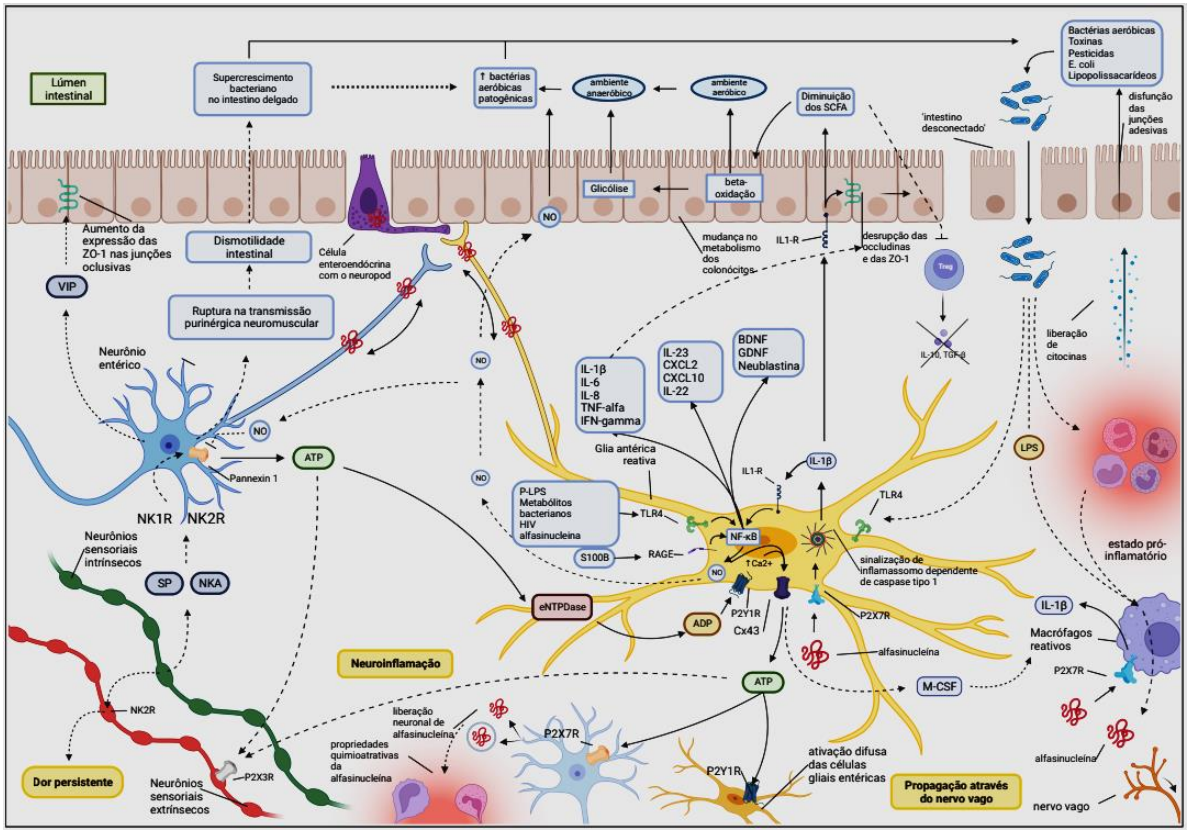
A ativação da glia entérica por uma resposta inflamatória aguda começa com a liberação de ATP por canais de panexina 1, de neurônios entéricos estimulados por mediadores neuronais, de outros neurônios ativados, como a neuroquinina A (NKA), substância P (SP), ou ATP. A ativação de P2Y1Rs, em EGC, por ATP, induz a produção de connexina 43 (Cx43) e ATP, que ativa mais EGC, perpetuando o processo de gliose entérica reativa. Além disso, o ATP, liberado pela glia entérica, liga-se ao receptor neuronal P2X7, promovendo morte neuronal e neuroinflamação (BROWN *et al*., 2016).

Além disso, a suprarregulação do óxido nítrico sintase, aumentando a produção de NO, foi observada nesta condição de inflamação, tendo-se mostrado interromper a transmissão neuromuscular purinérgica no cólon inflamado, que ressoa com o fenótipo glial reativo e a dismotilidade PD (ROBERTS *et al*., 2013). Entretanto, NO também desempenha um papel essencial na fisiologia GI (BROWN *et al*., 2016), apresentando um importante desafio sobre como reduzir as consequências prejudiciais da gliose reativa na ENS, sem comprometer as funções fisiológicas. Além disso, no CNS, foi demonstrado que o α-synuclein aumenta o estresse oxidativo (OS), desencadeando a apoptose neuronal e aumentando o OS, o que intensifica ainda mais a agregação anormal da alfasinucleina (TRAVAGLI; BROWNING; CAMILLERI, 2020).

A liberação de fatores de crescimento pró-inflamatórios de citocinas e outras moléculas de sinal imunomodulador, como óxido nítrico (NO), pelos EGCs reativos, amplifica o ambiente pró-inflamatório (que ocorre quando a homeostase é perturbada), aumenta os danos da barreira intestinal e induz mudanças neuroquímicas (CHOW; GULBRANSEN, 2017). Além disso, essa neuroinflamação foi correlacionada com o início de um estado inflamatório, que envolve o desenvolvimento de gliose entérica reativa, neuroplasticidade, disfunções sinápticas e, como mostraram estudos clínicos, o desenvolvimento de dismotilidade intestinal e dor nos estágios iniciais da DP (SEGUELLA, GULBRANSEN, 2021).

Sobre o papel imunomodulador da glia entérica, foi especificamente demonstrado que citocinas gliais, tais como IL-1β e IL-6, aumentam a excitabilidade neuronal, e CXCL2, CXCL10, IL-22 e IL-23 podem afetar a sensibilidade neuronal, induzindo respostas imunológicas locais. Além disso, também através da sinalização dependente de Cx-43, há uma produção do fator estimulante da colônia de macrófagos glial (M-CSF), que estabelece um fenótipo pró-inflamatório nos macrófagos musculares, contribuindo para a sensibilidade neuronal e dor persistente, como ilustrado na Figura 1 (MURAKAMI; OHTA; ITO, 2008).

Figura 1. Alças patológicas bidirecionais gliais envolvidas no desdobramento incorreto da alfasinucleína. Disbiose intestinal dinâmica, translocação bacteriana, ativação de macrófagos, recrutamento de linfócitos, imunossupressão indireta (pela diminuição de bactérias SCFA) e a própria disbiose, bem como outros fatores ambientais, produzem um estado pró-inflamatório, que pode induzir o desdobramento incorreto da alfasinucleína. Quando ativadas pela inflamação ou pelas cepas de alfasinucleína mal dobradas, as células gliais entéricas reativas iniciam e propagam um estado pró-inflamatório que promove a ruptura das junções estreitas, sintomas prodrômicos da DP e, ao longo dos anos, a ascensão e disseminação do nervo vago pelo SNC, promovendo uma ativação difusa da microglia do SNC. Vale ressaltar que esse processo também é mediado por outros processos de regulação que, ao tentar manter a homeostase, previnem a inflamação exacerbada e a perda neuronal, diferente do que ocorre no SNC, que avançam para a neurodegeneração.



Fonte: adaptado de Seguella (2021), usando BioRender. https://app.biorender.com/biorender-templates.

Neste contexto, corpos de Lewy, neurite e a suraprregulação da proteína LRRK-2, uma enzima cinase associada a um risco aumentado de DP, foram encontrados na glia entérica com marcadores pró-inflamatórios (CLAIREMBAULT *et al*., 2015). Além disso, a ativação glial pela agregação de alfasinucleina e a indução de alfasinucleina mal dobrada, pela glia entérica ativada, indica um dos "loops patológicos" criados pela inflamação na DP (DEVOS *et al*., 2013). Curiosamente, um estudo observou que, no cérebro e antes da neurodegeneração, a inflamação dos neurônios é mediada pela microgliose, em locais distantes de inclusões de alfasinucleina, sendo ativada de forma difusível, algo correlato à capacidade dos EGCs, não apenas para induzir a neuroinflamação, mas também para induzir um estado reativo em outras células da glial, independentemente da ativação da alfasinucleina. Como resultado do aumento da expressão da LRRK-2 e do desdobramento da alfasinucleina no intestino, a alfasinucleina migra, via transporte associado aos microtúbulos, em neurônios até o nervo vago, para o núcleo motor dorsal no tronco cerebral (Houser; Tansey, 2017), acumulando-se na área nigrostriatal e predispondo a neurodegeneração PD (SEGUELLA; SARNELLI; ESPOSITO, 2020).

Curiosamente, sugere-se que uma resposta glial inicial, percebida pelos marcadores pró-inflamatórios relacionados à glial entérica no cólon de pacientes com DP, está presente no início da patologia da DP (Clairembault *et al*., 2015), sendo inversamente ativada durante a progressão da doença, diminuindo ao longo do curso do tempo da patologia, como no cérebro, a ativação microglial tende a aumentar. Além disso, foi feita uma ligação direta entre as influências ambientais (toxinas) e alfasinucleina dobrando e espalhando para os neurônios intestinais. As células enteroendócrinas (EECs) são células quimiossensoriais eletricamente excitáveis, localizadas no revestimento da mucosa intestinal, responsáveis pela produção de hormônios, imunidade GI, motilidade, função de barreira, hormônio de crescimento e secreção de insulina (YU *et al*., 2020). As células enterocromafins, um tipo de CEE, secretam 5-HT em reação a forças mecânicas, sendo responsáveis por 95% do corpo 5-HT (BANSKOTA; GHIA; KHAN, 2019).

As EECs têm uma borda de escova apical e projeções de membrana basal tipo axônio chamadas neuropodes, que são responsáveis pela comunicação neuronal com os neurônios (sinapse) e a glia (contato direto). Curiosamente, os EECs têm semelhanças com células neuronais, tais como receptores de neurotrofina, e proteínas pré e pós-sinápticas, incluindo α-sinucleína, que implicam ECG como um nicho para a proteinopatia em resposta a sinais luminosos (BOHÓRQUEZ *et al*., 2014; BOHÓRQUEZ *et al*., 2015).

A alfasinucleína expressa em EECs foi descoberta como sendo mais abundante no intestino delgado do que no cólon, provavelmente devido à maior exposição a toxinas e patógenos no primeiro, e não no segundo. Além disso, os EECs estão em contato direto com os neurônios e o EGC, via conexão neuropodular, espalhando o alfasinucleina dobrável, via contato célula a célula e ativação Rab35 (Rodrigues *et al*., 2022) diretamente aos neurônios intestinais e, em seguida, ao nervo vago 110 (Chandra *et al*., 2017), estabelecendo uma conexão entre o conteúdo luminal e o nervo vago. A difusão do alfasinucleina dos neurônios para os EECs também foi demonstrada, como sendo ainda mais eficiente do que a via oposta. Entretanto, ainda não é conhecida a importância deste fluxo bidirecional de α-synuclein na DP, como foi descrito como o mecanismo da alfasinucleina mal dobrada, começando nas EECs e avançando para ENS e CNS, mas também o contrário (RODRIGUES *et al*., 2022; CHANDRA *et al*., 2017; SHERWIN *et al.*, 2019). Além disso, as condições inflamatórias que alteram a homeostase intestinal, aumentam a permeabilidade intestinal, induzindo a síndrome do "vazamento intestinal". Além disso, a taquicinina, a substância P e a neuroquinina A também demonstraram aumentar a permeabilidade paracelular, contribuindo para o "escape intestinal" apresentado nos distúrbios inflamatórios (RENZI *et al*., 2000; O'CONNOR *et al*., 2004).

1.5 Papel da glia entérica na inflamação e na fisiopatologia gastrointestinal na Doença de Parkinson

Os processos inflamatórios e a ativação da microglia cerebral são conhecidos por desempenharem um papel central nos danos dos neurônios dopaminérgicos e estão implicados na patogênese da DP. Estes processos inflamatórios têm sido atribuídos ao efeito tóxico do agregado de alfasinucleína, no cérebro de pacientes com DP. Entretanto, as evidências também implicam em disbiose que pode levar ao aumento da permeabilidade das barreiras intestinais e hemato-encefálicas, aumentando a entrada de substâncias produzidas por microbiota no SNC (BAIZABAL-CARVALHO; ALONSO-JUAREZ, 2020; ELFIL *et al*., 2020; TOLEDO *et al*., 2022; TILOCCA *et al*., 2020).

As evidências sugerem que os danos oxidativos derivados da inflamação e a toxicidade induzida pela citocina podem desempenhar um papel significativo nos danos neuronais associados à DP. Além disso, foram relatadas alterações inflamatórias no ENS, no nervo vago e nas células da glial. É notável que a presença de sintomas gastrointestinais (constipação, disfagia e hipersalivação), disbiose e escape intestinal têm sido observados em pacientes com DP, vários anos antes do início clínico da doença. A restauração do microbioma

intestinal em pacientes com DP pode alterar a progressão clínica da DP (DUTTA *et al*., 2019; PAVAN *et al*., 2022).

Alterações no microbioma gastrointestinal (GM) provocam a má administração e agregação anormal de alfasinucleína no intestino. A sinucleína anormal α não é eliminada através de mecanismos fisiológicos e é transportada para o sistema nervoso central (SNC), através do nervo vago. Os níveis anormais do agregado de alfasinucleína na substância *nigra pars* compacta, não só levam à formação de corpos Lewis eosinofílicos, no citoplasma, e disfunção mitocondrial, nos neurônios dopaminérgicos, mas também levam à estimulação de uma resposta inflamatória na micróglia.

Estas mudanças patológicas aumentam o estresse oxidativo (OS), o que desencadeia a apoptose das células nervosas, uma característica da DP. Este aumento no OS oxida e intensifica ainda mais a agregação anormal de alfasinucleína, eventualmente formando um *loop* de *feedback* positivo (LEI *et al*., 2021). Inflamação intestinal crônica e integridade da barreira intestinal prejudicada têm sido observadas em pacientes humanos com DP e modelos de camundongos de DP. Estas observações levaram à hipótese de que um microambiente intestinal alterado é um potencial desencadeador do processo de DP, em um hospedeiro geneticamente suscetível (CHEN; LIN, 2022).

A microbiota intestinal de pacientes com DP mostra mudanças únicas, que podem ser usadas como primeiros biomarcadores de doenças. A microbiota intestinal e seus metabólitos foram sugeridos para serem envolvidos na patogênese da DP, regulando a neuroinflamação, função de barreira e atividade de neurotransmissores (WANG *et al*., 2021). Estudos recentes encontraram as alterações da microbiota intestinal em pacientes com DP e a relevância clínica dessas alterações, indicando a relação subjacente entre o microbioma gastrointestinal (GM) e a DP. A disbiose intestinal pode influenciar a progressão e o início da DP, através do aumento da permeabilidade intestinal, agravando a neuroinflamação, agregando níveis anormais de alfasinucleína, aumentando o estresse oxidativo e diminuindo a produção de neurotransmissores. Além disso, respostas inflamatórias elevadas, originadas do intestino, desempenham um papel crucial no início e progressão da DP, que está intimamente associada à GM (GUO; CHEN, 2022; ZHU *et al*., 2022).

O trato gastrointestinal (GI) é dotado de uma rede nervosa peculiar o ENS e é dedicado ao controle fino das funções digestivas. O trato gastrointestinal desempenha um papel essencial na digestão, absorção e sistema imunológico da mucosa dos alimentos, e abriga um grande número de micro-organismos. É estimulado por milhões de neurônios que

compreendem o ENS, que interage com o microbioma intestinal, o sistema imunológico intestinal e o sistema endócrino (NATALE *et al*., 2021; GENG *et al*., 2022).

O ENS controla as principais funções gastrointestinais e é caracterizado principalmente por dois plexos ganglionares localizados na parede intestinal: o plexo mioentérico e o plexo submucoso. O ENS abriga um alto número e diversidade de neurônios entéricos e células gliais, que geram circuitos neuronais para regular a fisiologia intestinal (GRUNDMANN *et al*., 2019).

O pé cruzado entre esta unidade neuronal-endotelial e a microbiota desempenha um papel fundamental na modulação da permeabilidade da barreira epitelial, no desenvolvimento intestinal e na resposta imune (LUO *et al*., 2021). Embora o ENS funcione de forma amplamente autônoma, como parte do sistema nervoso periférico (SNP), ele está conectado ao SNC, através do GBA. Tanto no SNC quanto no SNP, o fator de crescimento endotelial vascular (VEGF) medeia os efeitos neuroprotetores e neurodegenerativos (HECKING *et al*., 2022).

Assim, o circuito entérico desempenha um papel central na homeostase intestinal (FUNG; VANDEN BERGHE, 2020). O ENS é uma porta de comunicação bidirecional entre o cérebro e o intestino, principalmente através do nervo vago. A exposição ambiental desempenha um papel central tanto na composição quanto na função do microbioma intestinal e pode contribuir para a suscetibilidade a distúrbios neurodegenerativos, tais como a DP. A marca neuropatológica da DP é o aparecimento generalizado de agregados de alfasinucleína, tanto no sistema nervoso central quanto no periférico, incluindo o ENS (SANTOS *et al*., 2019).

Os EGCs são necessários para a manutenção da homeostase do tecido intestinal. Além disso, a proteína glial do ácido fibrilar (GFAP) (um astrócito e biomarcador de EGC) e os EGC regulam a saúde da barreira epitelial intestinal e produzem moléculas imunorreguladoras que regulam o reparo do tecido e a defesa do hospedeiro. Tanto os EGCs quanto à barreira epitelial intestinal (IEB) mostraram ser alterados na PD (PROGATZKY; PACHNIS *et al*., 2022; THOMASI *et al*., 2022).

Com o avanço da idade, a composição da microbiota no intestino muda, em comparação com os controles (YATSUNENKO *et al*., 2012), uma condição chamada inflamação, que é uma inflamação crônica de baixo grau associada com o envelhecimento. É caracterizada pela presença de infecções latentes com vírus (por exemplo: citomegalovírus) e aumento dos níveis circulantes de marcadores inflamatórios, como TNF-α, IL-6 e proteína C reativa (FRASCA; BLOMBERG, 2018). Dessa forma, o fenótipo inflamatório da microbiota idosa pode participar da patogênese da DP, que também está associada às mudanças dietéticas e aos efeitos cumulativos dos pesticidas, contribuindo para a ativação de várias cascatas

inflamatórias no ENS, CNS e nervo vago, e resultando no acúmulo de alfasinucleína (YATSUNENKO *et al.*, 2012; FRASCA; BLOMBERG, 2016; DUTTA *et al.*, 2019).

A disbiose do microbioma está associada ao aumento de bactérias potencialmente nocivas, que podem comprometer a integridade da barreira intestinal e o BBB, através da produção bacteriana de endotoxinas (por exemplo, lipopolissacarídeo ou LPS) que podem danificar as células epiteliais intestinais, alterar a resposta imunológica e iniciar vias pró-inflamatórias. Curiosamente, em ratos, foi demonstrado que o LPS induziu uma cepa estruturalmente distinta de fibrilas de auto-renovação alfasinucleina, que é capaz de iniciar um padrão de sinucleinopatia semelhante às formas observadas na DP (BANKS *et al*., 2015; GHOSH *et al*., 2020; KIM *et al*., 2018).

Neste contexto, como já mencionado, uma grande meta-análise correlacionou um padrão específico de disbiose intestinal, na DP, como disbiose, sendo a alteração na configuração estrutural e/ou funcional da microbiota intestinal que perturba a homeostase intestinal. Esta condição poderia ser causada não apenas por alterações na dieta, mas também pela exposição a antibióticos, toxinas, crescimento excessivo de bactérias, estados de doença e envelhecimento em si, surgindo tanto como causa da agregação de alfasinucleína, quanto como consequência disso. Portanto, infecções GI crônicas poderiam induzir um aumento de α-synuclein, suficiente para provocar a patologia PD (SOMMER; BÄCKHED, 2013; STOLZENBERG, 2018).

Estudos recentes consideram a glia entérica como uma população de neuroglia periférica que está associada aos neurônios entéricos, em todo o trato digestivo, estando envolvida na regulação dos reflexos entéricos, através da comunicação bidirecional entre a glia entérica e os neurônios, e na comunicação entre os neurônios extrínsecos e intrínsecos que inervam o trato digestivo (GRUBIŠIĆ *et al.*, 2018; SEGUELLA; GULBRANSEN, 2021). Embora os EGCs compartilhem a mesma origem genética (das células progenitoras bipotenciais da crista neural), as células gliais são fenotípicamente diferentes, pois adaptam suas funções, devido aos sinais no microambiente (por exemplo, microbiota diferente). Para manter a homeostase local, elas adquirem certos subtipos fenotípicos, como a glia da mucosa (BOESMANS *et al*., 2022).

O principal papel da glia entérica é manter a homeostase do ENS, especialmente no que diz respeito à ação nos reflexos entéricos neuronais e na neuroinflamação. Esta forte interação é demonstrada nas junções neurônio-glia, onde a comunicação bidirecional é estabelecida. Foi comprovado que, além de neuroglia recebendo neurotransmissores de neurônios entéricos, eles também são capazes de produzir gliotransmissores, tais como ATP e

GABA, e mediadores como PGE2, capazes de ativar vias ascendentes de excitação ou suprimir vias descendentes inibitórias, através da interação de receptores (BROWN *et al*., 2016).

Os ECGs formam uma rede que comunica o plexo e todas as camadas da parede do intestino. Similar aos astrocíticos, eles atuam como um sincício funcional, promovendo a propagação de ondas de cálcio, em resposta a uma variedade de estímulos neurotransmissores, tais como ATP, serotonina (5-HT), ou Acetilcolina (Ach), devido às junções de fendas hemicanal connexin-43 (BOESMANS *et al*., 2015). Acrescentando a esta noção, o EGC não só desempenha um papel na neurotransmissão neuronal, através da degradação ou sequestro de neurotransmissores liberados por sinapses, mas também produz precursores neurotransmissores, por exemplo, L-arginina ou glutamina (VALÈS; TOUVRON; LANDEGHEM, 2018).

Outros experimentos mostram os efeitos protetores da glial derivada da s-nitrosoglutationa (GSNO), como importante modulador da homeostase intestinal, após a ablação da glia entérica em camundongos transgênicos. Ele tem o potencial de inibir a permeabilidade intestinal pela expressão da actina prejuncional e proteínas associadas à função *tight-junction*, mantendo assim a função de barreira mucosa. GSNO também esteve envolvido no retardo da inflamação intestinal pela diminuição do acúmulo de TNF-α mRNA intestinal, após a deleção da glia entérica, como observado após a ablação da glia entérica em ratos transgênicos (SAVIDGE *et al*., 2007).

Ao abordar o papel da glia entérica na neurogênese, estudos que testam a proliferação epitelial intestinal, após danos induzidos pela mucosa, sugerem o papel protetor da glia entérica. Em um cenário pós-colite, a glia expressou fatores de proliferação, tais como PLP1- e Sox2, que estimulam o crescimento do neurônio entérico e sua capacidade de conduzir processos de cura. Além disso, a influência da glia entérica na arborização axonal dos neurônios também foi observada, especialmente em relação à sinalização do receptor purinérgico P2Y1 (P2Y1R). Assim, os neurônios cultivados sem células gliais entéricas mostraram uma diminuição considerável tanto na estrutura axonal quanto na densidade de sinapse, o que implica na necessidade de fatores derivados da glial para a modulação do circuito neuronal (BELKIND-GERSON *et al*., 2017; BERRE-SCOUL *et al*., 2017).

Finalmente, a interação astrocítico-glia bidirecional contribui para a sensibilidade à dor abdominal e medeia as respostas pró-inflamatórias. Este mecanismo envolve a produção de glial connexin-43 e fator estimulante da colônia de macrófagos (M-CSF), modificando assim o fenótipo do macrófago sobre a camada muscular. Tal processo estimula a migração e a atividade

durante a colite e possibilita a hipersensibilidade visceral na inflamação intestinal crônica (GRUBIŠIĆ *et al.*, 2020).

Como a glia entérica contribui para a manutenção da homeostase intestinal, estas células estão intrinsecamente envolvidas em mecanismos que regulam a neuroplasticidade e as respostas imunes (ROSEMBERG; RAO, 2021). Assim, elas estão em constante "sensoriamento" do ambiente extracelular, por receptores que disparam cascatas de transdução de sinal intracelular, mediadas por Ca+2 e cAMP (WANG *et al*., 2021). Diferente da ativação constante normal dos EGC, sempre que ocorre uma perturbação fisiopatológica, eles ativam um "estado reativo", alterando sua composição molecular, estrutura e/ou função. Esta função depende do tipo, da gravidade da lesão, do subtipo glial e dos sinais específicos recebidos.

Além disso, mudanças no EGC podem alterar a atividade glial pelo ganho e perda de funções, que podem ser benéficas ou prejudiciais para o ambiente ao redor e células não neuronais (SEGUELLA; GULBRANSEN, 2021). A gliose entérica reativa pode ser induzida sempre que houver uma perturbação fisiopatológica, o que resulta em aumento da função pró-inflamatória e leva à hipertrofia e proliferação celular progressiva (SEGUELLA; GULBRANSEN, 2021), sendo muitas vezes considerada uma resposta deletéria, como resultado de uma tentativa de manter a homeostase (DELVALLE *et al*., 2018).

1.6 Perfil da Microbiota e Disbiose na Doença de Parkinson

A DP é uma doença neurodegenerativa crônica caracterizada pela deposição da proteína alfasinucleína (corpos de Lewy) em neurônios dopaminérgicos da substância nigra que contribuem para o desenvolvimento de sintomas motores (bradicinesia, tremores, rigidez, marcha anormal) e não motores (complicações gastrointestinais e urinogenitais, disfunção olfativa e comprometimento cognitivo). A disfunção olfativa (hiposmia) é comum na DP e muitas vezes prediz o diagnóstico por anos, refletindo o depósito precoce de corpos Lewy, a marca histológica da DP, no bulbo olfativo (Fullard; Morley; Duda, 2017), e é considerado um preditor útil da progressão da DP (HE *et al*., 2020). Além disso, as manifestações de IG também precedem o aparecimento dos sintomas motores e o diagnóstico de doenças, dando suporte ao papel potencial que o microbioma-GBA pode desempenhar nos mecanismos patológicos subjacentes à DP (KLANN; DISSANAYAKE; GURRALA, 2022; TAN; LIM; LANG, 2022; CHAKRABARTI *et al*., 2022; SCHAEFFER *et al*., 2020).

A DP mostra um processo neurodegenerativo multifocal que influencia várias estruturas neuronais além da substância nigra, uma das quais é a ENS. A presença de disfunção gastrointestinal, mesmo antes do início dos sintomas motores, indica que o depósito de salfainucleína pode começar no ENS e depois se propagar para o SNC (MENG *et al*., 2019).

A microbiota intestinal regula a fisiologia da IG em parte através de interações com o ENS. As alterações no microbioma intestinal ocorrem com distúrbios no controle neural entérico em condições fisiopatológicas. Trabalhos recentes (Vicentini *et al*., 2021) demonstraram o papel da microbiota intestinal na regulação da estrutura e função do trato gastrointestinal, em um modelo de depleção bacteriana induzida por antibióticos (Abx) em camundongos. Estes resultados mostraram que a microbiota é essencial para a manutenção da integridade do ENS, regulando a sobrevivência neuronal entérica e promovendo a neurogênese. Os determinantes moleculares da microbiota, LPS e ácido graxo de cadeia curta (SCFA), regulam a sobrevivência neuronal entérica, enquanto o SCFA também estimula a neurogênese.

Pode haver mais de 10 milhões de casos confirmados de DP no mundo todo, até 2040, e estudos recentes descobriram que a microbiota intestinal pode desempenhar papéis-chave na progressão de uma ampla gama de doenças, incluindo a DP (FAN; SHENG; ZHANG, 2022). Assim, uma meta-análise recente foi realizada para avaliar as diferenças da microbiota intestinal entre pacientes com DP e controles saudáveis (HCs), através de diferentes regiões geográficas. Os resultados mostraram níveis significativamente menores de abundância de *Prevotellaceae*, *Faecalibacterium* e *Lachnospiraceae,* em pacientes com DP, em comparação com os controles. Níveis significativamente mais altos de abundância de *Bifidobacteriaceae*, *Ruminococcaceae*, *Verrucomicro-biaceae* e *Christensenellaceae* também foram encontrados em pacientes com DP. Esta microbiose intestinal relacionada à DP pode levar ao comprometimento do processo de produção de SCFAs, metabolismo lipídico, função imunorregulatória e permeabilidade intestinal, que contribuem para a patogênese da DP (SHEN *et al*., 2021).

Outra meta-análise recente reanalisou os dez conjuntos de dados microbiológicos de 16S atualmente disponíveis para investigar se existem alterações comuns na microbiota intestinal de pacientes com DP em coortes. Foram encontradas alterações significativas no microbioma associado à DP, enriquecimento dos gêneros *Lactobacillus, Akkermansia* e *Bifidobacterium* e depleção de bactérias pertencentes à família *Lachnospiraceae* e ao gênero *Faecalibacterium,* ambos importantes produtores de SCFAs, surgiram como as alterações mais consistentes no microbioma intestinal da DP. Esta disbiose pode resultar em um status pró-

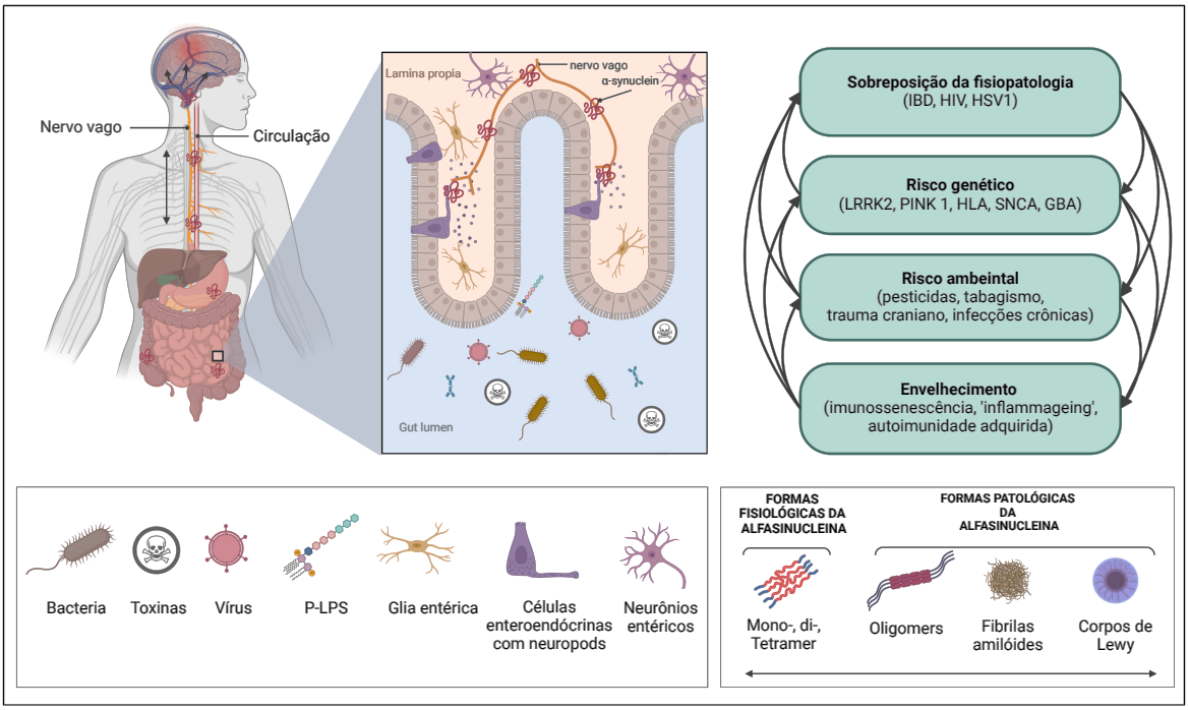
inflamatório que pode estar ligado aos sintomas recorrentes da IG que afetam os pacientes com DP (ROMANO *et al*., 2021).

Outro trabalho observou que quinze gêneros estavam associados à DP, em nível de significância microbiológica, representando três grupos de micro-organismos co-ocorrentes. O cluster 1 foi composto de patógenos oportunistas, e todos foram elevados na DP. O grupo 2 era de bactérias produtoras de SCFA, e todos foram reduzidos na DP. O grupo 3 era de probióticos carboidratos-metabolizadores, e foi elevado na DP (WALLEN *et al*., 2020). Os metabólitos derivados de microbiota e seus receptores, além do sistema imunológico, mantêm a homeostase metabólica, essencial para equilibrar a utilização e a ingestão de nutrientes. SCFAs, tais como butirato, acetato e succinato, são produzidos devido ao processo de fermentação de bactérias no intestino (MIRZAEI *et al*., 2021).

Além disso, uma redução significativa foi encontrada em butirato, acetato e propionato, nas fezes dos pacientes com DP, bem como uma redução significativa nas bactérias produtoras de SCFAs. Pensa-se que uma diminuição no SCFA pode induzir mudanças no ENS e levar a distúrbios gastrointestinais, na DP. O impacto da microbiota intestinal não está limitado ao intestino, mas sua interação com o hospedeiro produz metabólitos ativos, que podem ser transportados pela circulação sanguínea para desempenhar papéis importantes em outros lugares. SCFAs, como importantes produtos ativos das bactérias intestinais, têm demonstrado exercer efeitos anti-inflamatórios e imunomoduladores e podem desempenhar papéis ativos como moléculas sinalizadoras no desenvolvimento de várias doenças intestinais e extraintestinais. Desta forma, a modulação da microbiota intestinal e de substâncias ativas do metabolismo tornou-se gradualmente um método terapêutico popular para muitas doenças de órgãos além do intestino, incluindo a DP.

Como resultado, o perfil da disbiose DP poderia contribuir para um estado pró-inflamatório, que reforça a progressão da doença com a inflamação intestinal, justifica, em parte, os sintomas gastrointestinais e se correlaciona com o erro de sinucleína α. Desta forma, é possível que as mudanças na microbiota sejam tanto um produto da patologia da DP, quanto um fator potencial para o aparecimento e progressão da doença, como representado na Figura 2 (SCHEPERJANS *et al*., 2015; KESHAVARZIA *et al*., 2015; LIN *et al*., 2019).

Figura 2. Modelo bidirecional da iniciação e progressão do desdobramento incorreto da alfasinucleína. O surgimento complexo do desdobramento incorreto da alfasinucleína no intestino parece ocorrer em uma variedade de processos: influências ambientais (pesticidas, tabagismo, traumatismo craniano, infecções crônicas), interações epitelial-mucosa (agravadas por DII, HIV e HSV1), neuroglia interações imunes (agravadas por imunosenescência, inflamação e autoimunidade adquirida com a idade) e predisposição genética (como em LRRK2, PINK1, HLA, SNCA e GBA). Além disso, propõe-se que essas interações possam ocorrer de forma bidirecional, mediadas ambiental e geneticamente, não sendo claras as especificidades de cada uma, pois seus componentes fisiopatológicos tendem a se fundir e progredir em 'loops' moleculares.



Fonte: Próprio autor, 2022.

Criada usando BioRender)(P-LPS: lipopolissacarídeos patológicos; IBD: doença intestinal; HIV: imunodeficiência humana; HSV1: vírus do herpes humano; LRKK2: quinase de repetição rica em leucina 2; PINK-1: quinase 1 induzida por PTEN; HLA: humano antígeno leucocitário; SNCA: gene produtor de alfasinucleína; GBA: gene produtor de beta-glicocerebrosidase).

2 OBJETIVOS

2.1 Geral

Avaliar as alterações morfológicas, neuroquímicas e funcionais, periférica e central, no eixo cérebro-intestino (ECI), bem como alterações comportamentais em um modelo de doença de Parkinson induzido pela injeção intraperitoneal de rotenona em ratos Wistar.

2.2 Específicos

- Induzir a doença de Parkinson por meio de injeção intraperitoneal de rotenona em ratos Wistar machos;
- avaliar as alterações comportamentais em um modelo de doença de Parkinson induzido por injeção intraperitoneal de rotenona;
- avaliar as alterações histopatológicas no intestino delgado proximal, nervo vago e cérebro de ratos Wistar submetidos ao modelo experimental de DP induzido por rotenona;
- avaliar a expressão gênica e proteica de marcadores de células gliais centrais e periféricas (GFAP) através de imuno-histoquímica;
- avaliar a expressão gênica e proteica de marcadores neuronais centrais e periféricos (S100) através de imuno-histoquímica;
- verificar a expressão de alfasinucleína no tecido do intestino e fazer colocalização (dupla marcação) com GFAP e um marcador neuronal (S100);
- correlacionar as alterações encontradas no sistema nervoso entérico com as alterações encontrados no sistema nervoso central.

3 DESENVOLVIMENTO

Por se tratar de pesquisa envolvendo animais, o projeto de pesquisa referente a esta dissertação foi submetido à apreciação da Comissão de Ética no Uso de Animal (CEUA) do Núcleo de Pesquisa de Desenvolvimento de Medicamentos (NPDM) da Universidade Federal do Ceará, tendo sido aprovado sob número de protocolo 48/2017 (Anexo A).

Esta dissertação de Mestrado baseia-se no Artigo 37º parágrafo 7 do Regimento Interno de 21 de março de 2018 do Programa de Pós-Graduação em Ciências Morfofuncionais da Universidade Federal do Ceará, que regulamenta o formato alternativo para dissertações de Mestrado e teses de Doutorado. Este capítulo consta de uma cópia do artigo científico de autoria do candidato, redigido de acordo com as normas da revista científica escolhida para publicação ("**Ageing Research Reviews**") Impact Factor (2017-2018 = **11.788**) (Anexo B).

ARTIGO 1

Artigo Científico submetido na Revista Ageing Research Reviews (FI 11.788).

ROLE OF ENTERIC GLIA AND MICROBIOTA-GUT-BRAIN AXIS IN PARKINSON DISEASE PATHOGENESIS

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Conflict of interest
The authors declare that they have no conflicts of interest. All authors read and approved the final manuscript.

Acknowledgments
Federal University of Ceará - UFC and Medical School of the Christus University Center - UNICHRISTUS.

ABSTRACT

The microbiota-gut-brain axis or simple gut-brain axis (GBA) is a complex and interactive bidirectional communication network linking the gut to the brain. Alterations in the composition of the gut microbiome have been linked to GBA dysfunction, *central nervous system* (CNS) inflammation, and dopaminergic degeneration, as those occurring in Parkinson's disease (PD). Besides inflammation, the activation of brain microglia is known to play a central role in the damage of dopaminergic neurons. Inflammation is attributed to the toxic effect of aggregated α-synuclein, in the brain of PD patients. It has been suggested that the α-synuclein misfolding might begin in the gut and spread "prion-like", via the vagus nerve into the lower brainstem and ultimately to the midbrain, known as the Braak hypothesis. In this review, we discuss how the microbiota-gut-brain axis and environmental influences interact with the immune system to promote a pro-inflammatory state that is involved in the initiation and progression of misfolded α-synuclein proteins and the beginning of the early non-motor symptoms of PD. Furthermore, we describe a speculative bidirectional model that explains how the enteric glia is involved in the initiation and spreading of inflammation, epithelial barrier disruption, and α-synuclein misfolding, finally reaching the central nervous system and

contributing to neuroinflammatory processes involved with the initial non-motor symptoms of PD.

Keywords: Parkinson's disease; gut-brain axis; enteric glia; neuroinflammation.

INTRODUCTION

The gut-brain axis (GBA) is an interactive network, linking the gut to the brain and involves bidirectional communication between the gastrointestinal system (GIS) and the central nervous system (CNS). The gut microbiome is a pivotal component of the GBA, and alterations in its composition have been linked to GIS dysfunction, CNS inflammation, and dopaminergic degeneration. The gut microbiome might influence CNS homeostasis through the modulation of the immune system (Ghezzi *et al*., 2022, Gubbert *et al*., 2022, Soni *et al*., 2022).

The afferent vagus nerve is the main retrograde signaling system, bringing information from the gut to the brain. The efferent vagus nerve, however, has a cholinergic anti-inflammatory activity that promotes the balance between the tumor necrosis factor-α (TNF-α) and other cytokines secreted by macrophages in response to stress signals in the gut (Moustafa *et al*., 2021). Also, it has been described that GBA is regulated by the microbiome, through immunological, neuroendocrine, and neural mechanisms. And disturbances in this axis are responsible for gastrointestinal manifestations, subsequent motor symptoms, and PD pathogenesis itself (Dogra *et al*., 2022).

The GBA describes a bidirectional interplay within the enteric environment, between the intestinal epithelium, the mucosal immune system, and the microbiota, with the enteric nervous system (ENS) (Dowling *et al*., 2022). The ENS, the intrinsic innervation of the GIS is a vast network of neurons and glia. Through its complex circuitry and neuronal diversity, the ENS is capable of functioning autonomously, but it is modulated by inputs from the CNS. The communication between the ENS and CNS is bidirectional, participates together in the crosstalk of the gut microbiota and the GBA (Chanpong *et al*., 2021). The enteric glial cells (EGCs) are one of the major cell types of neural crest lineage, distributed in the gastrointestinal tract. EGCs represent an integral part of the ENS and significantly outnumber ENS neurons. Recent evidence revealed that EGCs could possess multiple immune functions and, thereby, may participate in the immune homeostasis of the gut (Liu; Yang, 2022). The ENS is involved in the control of local gastrointestinal functions, through neuronal circuits (Gulbransen and Sharken, 2012).

The EGC is a population of peripheric neuroglia in association with the bodies and neurites of enteric neurons, in all extensions of the gastrointestinal tract. In general, neuroglia has homeostatic functions in the CNS, which is consistent with the known functions of EGCs on the ENS. Recent studies demonstrated that EGCs are the most dynamic components of ENS signalization due to their bidirectional and rapid transit that regulates enteric reflexes and the communication between intrinsic and extrinsic groups of neurons that innervate the GI tract (Yu and Li, 2014).

Inside the ENS, neurons and glial cells are strongly compacted in ganglia that are interconnected by nervous fibers, containing axons of intrinsic (enteric) and extrinsic (sympathetic and parasympathetic) neurons. Enteric glia surrounds the enteric synapses and shares a lot of morphological, molecular, and electrophysiological characteristics with the astrocytes (Devos *et al*., 2013).

Furthermore, the function of ENS is mediated by two important cellular groups: the enteric neurons and the enteric glial cells (EGC). The enteric neurons are involved in intestine contractility (Furness *et al*., 2014), while the EGC, have specific transcriptional features (Rao

et al., 2015; Ibiza *et al*., 2016). EGC display, besides mechanical support, neurotransmitter, immune and homeostatic functions in the gut and are also involved in epithelial barrier integrity, proliferation and differentiation of epithelial cells, and intestinal defense (Capoccia *et al*., 2015). However, despite those important functions, their impacts on the development and progression of PD were not well investigated (Bassoti *et al*., 2007; Liu *et al*., 2021).

The pathophysiology of PD consists of the aggregation of intracellular amyloid inclusions in neuronal bodies (Lewy bodies – LB) and neurites (Lewy neuritis – LN), of aggregated α-synuclein. Many factors are responsible for structural changes of α-synuclein, such as lipids interaction and membranes (Drobny *et al*., 2021). Also, modifications such as phosphorylation, oxidation, and nitration were demonstrated to accelerate α-synuclein aggregation (Zhang *et al*., 2019). Despite continuous progress in investigating the disease, the exact mechanisms responsible for its initiation and progression remain unclear. However, the bidirectional communication of the commensal bacteria that influence neurodevelopment was demonstrated to influence susceptibility, the onset of the non-motor symptoms, and the progress of PD (Mennozi *et al*., 2021; Chao *et al*., 2020).

The gastrointestinal symptoms occur in the prodromal phase, in almost all PD patients, at a given moment, due to the deposition of Lewy bodies in the ENS (misfolded α-synuclein proteins) and are described as being one of the most debilitating non-motor symptoms of this disease.

In the last years, a growing body of evidence proposed that the oxidative stress derived from the inflammation process and the toxicity derived from cytokines could influence the degeneration of the *substantia nigra* and the progression of PD (Helander and Fandricks, 2014). The role of inflammation in the development of the physiopathology of PD is sustained by the occurrence of activated microglia infiltration and T lymphocytes, and by the presence of pro-inflammatory cytokines, like tumor necrosis factor (TNF-α), interferon-gamma (IFN-gamma), interleukin-6 (IL-6) and interleukin-1 beta (IL1-beta), in the brain and the cerebrospinal fluid of PD patients. As many of the cytokines involved in PD are expressed in high levels in glial cells, probably, glial dysregulation is critically involved in neuroinflammation in PD patients (Yoo and Mazmanian, 2017).

Inflammatory processes that sustain conditions in the intestine could promote systemic inflammation and neuroinflammation (Kwon and Koh, 2020). Studies demonstrated that PD patients presented inflammation and oxidative stress in the intestine. In this context, biochemistry alterations that suggest glial dysregulation were shown to occur with enteric cells in PD (Devos *et al*., 2013; Trichka and Zou, 2021).

In this review, we discuss the neuron-glia interactions, the role of ENS focusing on EGC, and microbiota, and how these complex factors interact with the immune system to promote a pro-inflammatory state that is involved in the initiation and progression of misfolded α-synuclein proteins and the beginning of the early non-motor symptoms of PD, directly correlating to the gut-brain axis. Furthermore, we describe a speculative bidirectional model that explains how the enteric glia may be involved in gut inflammation, epithelial barrier disruption, and α-synuclein misfolding, contributing to the initial pathogenesis of PD.

MICROBIOTA PROFILE AND DYSBIOSE IN PARKINSON DISEASE (PD)

PD is a chronic neurodegenerative disease characterized by the deposition of misfolded alpha-synuclein protein (Lewy bodies) in dopaminergic neurons of the *substantia nigra* which contribute to the development of motor (bradykinesia, tremors, stiffness, abnormal gait) and non-motor symptoms (gastrointestinal and urinogenital complications, olfactory dysfunction, and cognitive impairment). Olfactory dysfunction (hyposmia) is common in PD and often predates the diagnosis by years, reflecting early deposition of Lewy bodies, the histologic

hallmark of PD, in the olfactory bulb (Fullard *et al*., 2017), and is considered a useful predictor of PD progression (He *et al*., 2020). In addition, GI manifestations also precede the onset of motor symptoms and disease diagnosis, lending support to the potential role that the microbiome–GBA might play in the underlying pathological mechanisms of PD (Klann *et al*., 2022; Tan *et al*., 2022; Chakrabarti *et al*., 2022; Peterson *et al*., 2020; Schaeffer *et al*., 2020).

PD shows a multifocal neurodegenerative process that influences several neuronal structures aside from the substantia nigra, one of which is the ENS. The presence of GI dysfunction, even before the onset of motor symptoms, indicates that α-synuclein deposition may start in the ENS and then propagates to the CNS (Meng *et al*., 2019).

The intestinal microbiota regulates GI physiology in part through interactions with the ENS. Alterations in the gut microbiome occur with disturbances in enteric neural control in pathophysiological conditions. Recent work (Vicentini *et al*., 2021) demonstrated the role for the gut microbiota in regulating the structure and function of the GI tract, in a mouse model of antibiotic (Abx)-induced bacterial depletion. These results showed that the microbiota is essential for the maintenance of ENS integrity, by regulating enteric neuronal survival and promoting neurogenesis. Molecular determinants of the microbiota, LPS, and short-chain fatty acid (SCFA), regulate enteric neuronal survival, while SCFA also stimulates neurogenesis.

There might be more than 10 million confirmed cases of PD worldwide, by 2040, and recent studies have found that gut microbiota may play key roles in the progression of a wide range of diseases, including PD (Fan *et al*., 2022). Thus, a recent meta-analysis was performed for evaluating the gut microbiota differences between patients with PD and healthy controls (HCs), across different geographical regions. The results showed significantly lower abundance levels of *Prevotellaceae, Faecalibacterium, and Lachnospiraceae,* in patients with PD, compared to controls. Significantly higher abundance levels of *Bifidobacteriaceae, Ruminococcaceae, Verrucomicro-biaceae,* and *Christensenellaceae* were also found in patients with PD. These PD-related gut microbiota dysbiosis might lead to the impairment of the SCFAs-producing process, lipid metabolism, immunoregulatory function, and intestinal permeability, which contribute to the pathogenesis of PD (Shen *et al*., 2021).

Another recent meta-analysis re-analyzed the ten currently available 16S microbiome datasets to investigate whether common alterations in the gut microbiota of PD patients exist across cohorts. Significant alterations were found in the PD-associated microbiome, enrichment of the genera *Lactobacillus*, *Akkermansia,* and *Bifidobacterium* and depletion of bacteria belonging to the Lachnospiraceae family and the *Faecalibacterium* genus, both important SCFAs producers, emerged as the most consistent PD gut microbiome alterations. This dysbiosis might result in a pro-inflammatory status which could be linked to the recurrent GI symptoms affecting PD patients (Romano *et al*., 2021).

Another work observed that fifteen genera were associated with PD, at a microbiome-wide significance level, representing three clusters of co-occurring microorganisms. Cluster 1 was composed of opportunistic pathogens, and all were elevated in PD. Cluster 2 was SCFA-producing bacteria, and all were reduced in PD. Cluster 3 was carbohydrate-metabolizing probiotics, and was elevated in PD (Wallen *et al*., 2020). Microbiota-derived metabolites and their receptors, beyond the immune system, maintain metabolic homeostasis, essential for balancing the utilization and intake of nutrients. SCFAs, such as butyrate, acetate, and succinate, are produced due to the fermentation process of bacteria in the gut (Mirzaei *et al*., 2021).

Furthermore, a significant decrease has been found in butyrate, acetate, and propionate, in the stools of PD patients, as well as a significant reduction in SCFAs-producing bacteria. It is thought that a decrease in SCFA may induce changes in ENS and lead to gastrointestinal disorders, in PD. The impact of the gut microbiota is not limited to the intestine, but its interaction with the host produces active metabolites, which can be transported by the blood

circulation to play important roles elsewhere. SCFAs, as important active products of gut bacteria, have been shown to exert anti-inflammatory and immunomodulatory effects and can play active roles as signaling molecules in the development of various intestinal and extraintestinal diseases. In this way, modulation of the intestinal microbiota and metabolism-active substances has gradually become a popular therapeutic method for many diseases of organs beyond the gut, including PD (see Cao *et al*., 2022, for a review).

As a result, the PD profile of dysbiosis could contribute to a pro-inflammatory state, which reinforces the progression of the disease with intestinal inflammation, justifies, in part, the gastrointestinal symptoms and correlates with the α-synuclein misfolding. In this way, it is possible that the changes in the microbiota are both a product of PD pathology, as well as a potential factor for the onset and progression of the disease, as represented in figure 1 (Scheperjans *et al*., 2015; Keshavarzia, 2015; Lin *et al*., 2019).

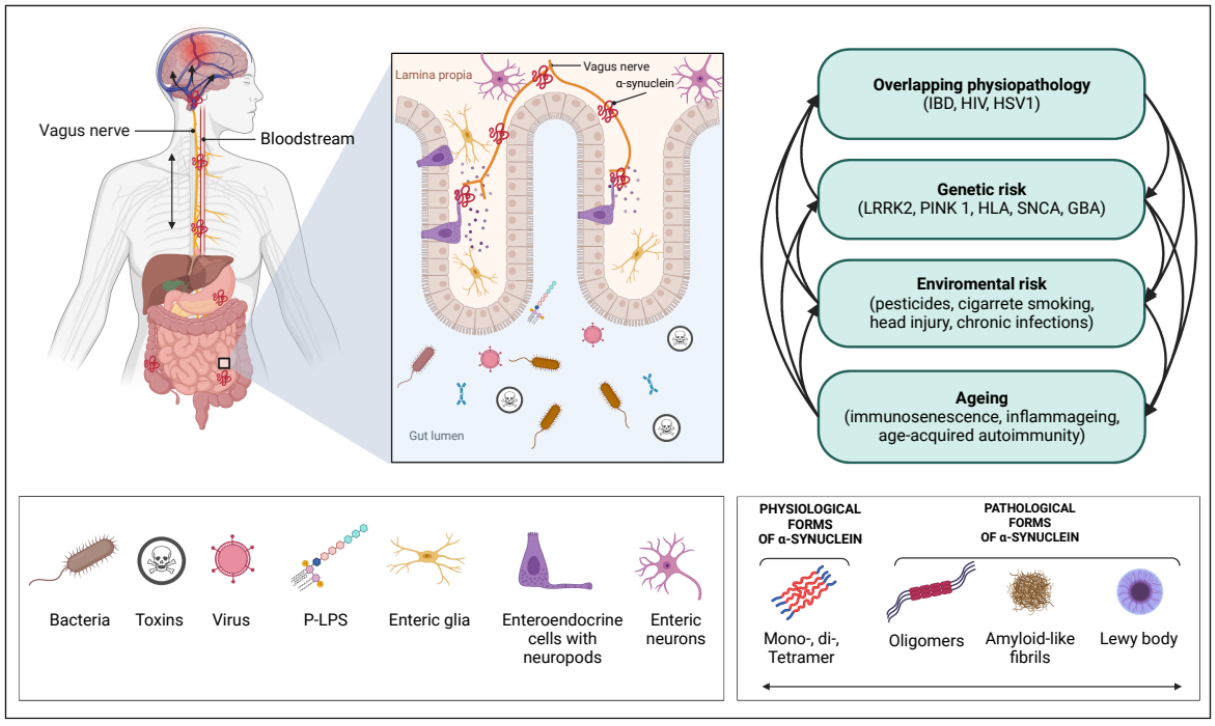

Figure 1. Bidirectional model of α-synuclein misfolding initiation and progression. The complex emergence of α-synuclein misfolding in the gut seems to occur in a variety of processes: environmental influences (pesticides, cigarrete smoking, head injury, chronic infections), epithelial-mucosa interactions (worsened by IBD, HIV and HSV1), neuro-glia immune interactions (worsened by immunosenescence, inflammaging and age-acquired autoimmunity) and genetic predisposition (such as in LRRK2, PINK1, HLA, SNCA and GBA). Furthermore, it is proposed that these interactions can occur in a bidirectional way, environmentally and genetically mediated, not being clear the specificities of each, as their physio-pathological components tend to merge and progress in molecular 'loops'. (P-LPS: pathological lipopolissacarides; IBD: intestinal bowel disease; HIV: human immunodeficiency; HSV1: human herpes virus; LRKK2: leucine-rich repeat kinase 2; PINK-1: PTEN-induced kinase 1; HLA: human leukocyte antigen; SNCA: α-synuclein producing gene; GBA: beta-glucocerebrosidase producing gene). (Figure created by the authors using BioRender (https://app.biorender.com/biorender-templates).

ROLE OF ENTERIC GLIA AND INFLAMMATION IN PD GASTROINTESTINAL PATHOPHYSIOLOGY

Inflammatory processes and the activation of brain microglia are known to play a central role in the damage of dopaminergic neurons and are implicated in the pathogenesis of PD. These inflammatory processes have been attributed to the toxic effect of aggregated α-synuclein, in

the brain of PD patients. However, evidence also implicates dysbiosis that may lead to increased permeability of the intestinal and blood-brain barriers, enhancing the entrance of microbiota-produced substances into the CNS (Baizabal-Carvallo *et al*., 2020; Elfil *et al*., 2020; Toledo *et al*., 2022; Tilocca *et al*., 2020).

Evidence suggests that inflammation-derived oxidative damage and cytokine-induced toxicity may play a significant role in the neuronal damage associated with PD. Furthermore, inflammatory changes have been reported in the ENS, the vagus nerve, and glial cells. It is noteworthy that the presence of GI symptoms (constipation, dysphagia, and hypersalivation), dysbiosis, and leaky gut have been observed in PD patients, several years before the clinical onset of the disease. The restoration of the gut microbiome in patients with PD may alter the clinical progression of PD. (Dutta *et al*., 2019; Pavan *et al*., 2022).

Changes in the gastrointestinal microbiome (GM) cause misfolding and abnormal aggregation of α-synuclein in the intestine. Abnormal α-synuclein is not eliminated via physiological mechanisms and is transported into the central nervous system (CNS), via the vagus nerve. The abnormal levels of α-synuclein aggregate in the *substantia nigra pars compacta*, not only lead to the formation of eosinophilic Lewis Bodies, in the cytoplasm, and mitochondrial dysfunction, in dopaminergic neurons, but also lead to the stimulation of an inflammatory response in the microglia.

These pathological changes increase oxidative stress, which triggers nerve cell apoptosis, a characteristic of PD. This increase in OS further oxidizes and intensifies abnormal aggregation of α-syn, eventually forming a positive feedback loop (Lei *et al*., 2021). Chronic gut inflammation and impaired intestinal barrier integrity have been observed in human PD patients and mouse models of PD. These observations led to the hypothesis that an altered gut microenvironment is a potential trigger of the PD process, in a genetically susceptible host (Chen and Lin, 2022).

The gut microbiota of patients with PD shows unique changes, which may be used as early biomarkers of disease. The gut microbiota and its metabolites have been suggested to be involved in the pathogenesis of PD, by regulating neuroinflammation, barrier function, and neurotransmitter activity (Wang *et al*., 2021). Recent studies have found the gut microbiota alterations in PD patients and the clinical relevance of these changes, indicating the underlying relationship between GM and PD. Gut dysbiosis can influence the progression and onset of PD, via increasing intestinal permeability, aggravating neuroinflammation, aggregating abnormal levels of α-synuclein fibrils, increasing oxidative stress, and decreasing neurotransmitter production. Additionally, elevated inflammatory responses, originating from the gut, play a crucial role in the initiation and progression of PD, which is closely associated with GM (Guo *et al*., 2022; Zhu *et al*., 2022).

The gastrointestinal (GI) tract is provided with a peculiar nervous network the ENS and is dedicated to the fine control of digestive functions. The GI tract plays an essential role in food digestion, absorption, and the mucosal immune system, and is home to a huge number of microorganisms. It is innervated by millions of neurons that comprise the ENS, which interacts with the gut microbiome, the intestinal immune system, and the endocrine system (Natale *et al*., 2021; Geng *et al*., 2022).

The ENS controls gastrointestinal key functions and is mainly characterized by two ganglionated plexus located in the gut wall: the myenteric plexus and the submucous plexus. The ENS harbors a high number and diversity of enteric neurons and glial cells, which generate neuronal circuitry to regulate intestinal physiology (Grundmann *et al*., 2019).

The crosstalk between this neuronal-glial–endothelial unit and microbiota plays a pivotal role in modulating the epithelial barrier's permeability, intestinal development, and immune response (Luo *et al*., 2021). Although the ENS functions largely autonomously, as part of the peripheral nervous system (PNS), it is connected to the CNS, via the GBA. In both

the CNS and PNS, vascular endothelial growth factor (VEGF) mediates neuroprotective and neurodegenerative effects (Hecking *et al*., 2022).

Thus, the enteric circuitry plays a central role in intestinal homeostasis (Fung & Vanden Berghe, 2020). The ENS is a gateway for bidirectional communication between the brain and the gut, mostly through the vagus nerve. Environmental exposure plays a pivotal role in both the composition and function of the gut microbiome and may contribute to susceptibility to neurodegenerative disorders, such as PD. The neuropathological hallmark of PD is the widespread appearance of α-synuclein aggregates, in both the central and peripheral nervous systems, including the ENS (Santos *et al*., 2019).

EGCs are required for the maintenance of gut tissue homeostasis. In addition, glial fibrillar acid protein (GFAP) (an astrocyte and EGC biomarker) and EGCs regulate the health of the intestinal epithelial barrier and produce immunoregulatory molecules that regulate tissue repair and host defense Both EGCs and IEB were shown to be altered in PD (Progatzky *et al*. 2022; Thomasi *et al*., 2022).

With advancing age, microbiota composition in the gut changes, compared with controls (Yatsunenko *et al*., 2012), a condition named inflammaging, which is a low-grade chronic inflammation associated with aging. It's characterized by the presence of latent infections with viruses (e.g.: cytomegalovirus) and increased circulating levels of inflammatory markers, like TNF-α, IL-6, and C-reactive protein (Frasca and Blomberg, 2018). In that way, the inflammatory phenotype of elderly microbiota may participate in the pathogenesis of PD, which is also associated with dietary changes and the cumulative effects of pesticides, contributing to the activation of various inflammatory cascades in the ENS, CNS, and vagus nerve, and resulting in the accumulation of α-synuclein (Yatsunenko, 2012; Frasca & Blomberg, 2016; Dutta *et al*., 2019).

Dysbiosis of the microbiome is associated with the increase of potentially harmful bacteria, which can compromise the gut barrier integrity and the BBB, through bacterial production of endotoxins (e.g., lipopolysaccharide or LPS) that can damage the intestinal epithelial cells, alter the immune response and initiate proinflammatory pathways. Interestingly, in mice, LPS was demonstrated to induce a structurally distinct strain of self-renewing α-synuclein fibrils, which is capable of initiating a pattern of synucleinopathy similar to the forms observed in PD (Banks *et al*., 2015; Ghosh *et al*., 2020; Kim *et al*., 2018).

In this context, as already mentioned, a large meta-analysis correlated a specific pattern of gut dysbiosis, in PD, as dysbiosis, being the alteration in structural and/or functional configuration of the gut microbiota that disrupts gut homeostasis. This condition could be caused not only by dietary alterations, but also by antibiotic exposure, toxins, bacteria overgrowth, disease states, and aging itself, emerging both as the cause of α-synuclein aggregation and as a consequence of this. Therefore, chronic GI infections could induce an increase of α-synuclein, sufficient to provoke PD pathology (Sommer & Bäckhed, 2013; Kroemer & Wong, 2018).

Recent studies consider enteric glia as a population of peripheral neuroglia that is associated with enteric neurons, throughout the digestive tract, being involved in the regulation of enteric reflexes, through bidirectional communication between enteric glia and neurons, and in the communication between extrinsic and intrinsic neurons that innervates the digestive tract (Grubišić *et al*., 2018; Seguella and Gulbransen, 2021). Although EGCs share the same genetic origin (from the bipotential progenitor cells of the neural crest), glial cells are phenotypically different, as they adapt their functions, due to cues in the microenvironment (e.g. different microbiota). To maintain local homeostasis, they acquire certain phenotype subtypes, such as the mucosal glia (Boesmans *et al*., 2022).

The major role of enteric glia is to maintain ENS homeostasis, especially regarding the action in neuronal enteric reflexes and neuroinflammation. This strong interaction is

demonstrated in neuron-glia junctions, where bidirectional communication is established. It was proven that, besides neuroglia receiving neurotransmitters from enteric neurons, they are also able to produce gliotransmitters, such as ATP and GABA, and mediators like PGE2, capable of either activation of ascending excitatory pathways or suppression of inhibitory descendant pathways, through receptor interaction (Brown *et al*., 2016).

ECGs form a network that communicates the plexus and all the layers of the gut wall. Similar to astrocytes, they act as a functional syncytium, promoting the propagation of calcium waves, in response to a variety of neurotransmitters stimuli, such as ATP, serotonin (5-HT), or Acetylcholine (Ach), due to connexin-43 hemichannel gap junctions (Boesmans *et al*., 2015). Adding to this notion, EGC not only plays a role in neuronal neurotransmission, through the degradation or sequestration of neurotransmitters released by synapses but also produces neurotransmitter precursors, e.g., L-arginine or glutamine (Valès *et al*., 2018).

Other experiments show the protective effects of the glial-derived *s*-nitrosoglutathione (GSNO), as an important modulator of intestinal homeostasis, after the ablation of enteric glia in transgenic mice. It has the potential to inhibit intestinal permeability by the expression of prejunctional actin and tight-junction–associated proteins (zonula occludens-1 and occludin), therefore maintaining the mucosal barrier function. GSNO was also involved in delaying intestinal inflammation by decreasing intestinal TNF-α mRNA accumulation, after the deletion of enteric glia, as observed after the ablation of enteric glia in transgenic mice (Savidge *et al*., 2007).

When addressing the role of enteric glia on neurogenesis, studies testing intestinal epithelial proliferation, after induced mucosal damage, suggest the protective role of enteric glia. In a post-colitis scenario, glia expressed proliferation factors, such as PLP1- and Sox2, which stimulate enteric neuron growth and its capability of conducting healing processes. Moreover, the influence of enteric glia on axonal arborization of neurons was also observed, especially regarding purinergic P2Y1 receptor (P2Y1R) signaling. Hence, neurons cultivated without enteric glial cells showed a considerable decrease in both axonal structure and synapse density, which implies the necessity of glial-derived factors for neuronal circuitry modulation (Belkind-Gerson *et al*., 2017; Le Berre-Scoul *et al*., 2017).

Lastly, the astrocyte-glia bidirectional interaction contributes to abdominal pain sensitivity and mediates proinflammatory responses. This mechanism involves the production of glial connexin-43 and macrophage colony-stimulating factor (M-CSF), hence modifying the macrophage's phenotype over the muscularis layer. Such a process stimulates migration and activity during colitis and enables visceral hypersensitivity in chronic intestinal inflammation (Grubišić *et al*., 2020).

As enteric glia contributes to gut homeostasis maintenance, these cells are intrinsically involved in mechanisms that regulate neuroplasticity and immune responses (Rosemberg and Rao, 2021). Thus, they are in constant ''sensing'' of the extracellular environment, by receptors that trigger intracellular signal transduction cascades, mediated by Ca^{+2} and cAMP (Wang *et al*., 2022). Different from the normal constant activation of EGCs, whenever a pathophysiological perturbation occurs, they activate a ''reactive status'', altering their molecular composition, structure, and/or function. This function depends on the type, severity of the injury, the glial subtype, and the specific signals received.

Also, changes in EGC can alter the glial activity by gain and loss of functions, which can be beneficial or detrimental to the surrounding environment and non-neuronal cells (Seguella, & Gulbransen, 2021). Reactive enteric gliosis may be induced whenever there is a pathophysiological perturbation, which results in proinflammatory function increases and leads to progressive cellular hypertrophy and proliferation (Seguella, & Gulbransen, 2021), often being considered a deleterious response, as a result of an attempt to maintain homeostasis (Delvalle *et al*., 2018).

GUT-BRAIN AXIS IN PD

Acute inflammation, infection, or metabolic challenges can induce reactive gliosis that can have effects on neurodegeneration and changes in neuromuscular function. This shows that the enteric glia reacts to the harmful environment to protect the nervous system from damage, usually increasing the expression of GFAP and S100B, associated with glia-mediated neuroinflammatory response (Seguella *et al*., 2019). Furthermore, reactive enteric gliosis can also be triggered in absence of inflammatory stimuli, by sensory neurons and enteric neurons (Delvalle *et al*., 2018). In this context, reactive gliosis induces a process of neuroinflammation that leads to neuronal plasticity, disturbing intestinal motility and producing visceral pain in functional and organic gastrointestinal disorders (Spencer *et al*., 2020), as well as, by the release of NO, contributing to gut dysbiosis (Turco *et al*., 2014; Seguella *et al*., 2020).

However, as discussed before, ENS in PD patients was yet not shown to be correlated with neuronal loss nor neurodegeneration (Litvak *et al*., 2018) and this could be due to the interaction of intrinsic mechanisms of neuroprotection, in gut cells within the ENS, or to the fact that the inflammation induced by the proposed physiopathology loops are not sufficient to generate neuronal loss nor to overcome neuroprotective mechanism, like the anti-inflammatory effects of the vagus nerve, that is mediated by EGCs (Langness *et al*., 2017). Furthermore, this specific causality needs to be further investigated in PD patients.

EGCs may play a major role in PD-related gastrointestinal disturbances, as well as in the development and progression of the central disease. In addition to their trophic and structural functions, EGCs are crucial for the homeostatic control of a wide range of gastrointestinal activities (Benvenuti *et al*., 2020). EGCs are the equivalent cell type of astrocytes in the CNS and share with them many neurotrophic and neuro-immunomodulatory properties (Cappoccia *et al.*, 2016).

Several studies had shown that EGC expresses the major histocompatibility complex II (MHC II), associated with antigen presentation to innate and adaptive immune cells, responding to harmful stimulations through TLR-2 and TLR-4, protecting the host against pathogens and controlling the neuro-immune axis (Chow *et al*., 2021). Interestingly, peripheral CD4+ T cells that acquire a pro-inflammatory phenotype (such as Th1 and Th17) by enteric glia stimulation, can cross the BBB and give rise to central inflammation, which is a common finding in neurodegenerative disorders in the CNS (Solleiro-Villavicencio *et al*., 2018). Also, EGC was shown to directly inhibit Treg and Th2 anti-inflammatory activities, demonstrating its potential to induce chronic infections (Solleiro-Villavicencio & Rivas-Arancibia, 2018).

The activation of enteric glia by an acute inflammatory response begins with the release of ATP by pannexin 1 channels, from enteric neurons stimulated by neuronal mediators, from other activated neurons, like neurokinin A (NKA), substance P (SP), or ATP. The activation of P2Y1Rs, in EGC, by ATP, induces the production of connexin 43 (Cx43) and ATP, which activates more EGC, perpetuating the enteric reactive gliosis process. Also, the ATP, released by the enteric glia, bonds to the neuronal P2X7 receptor, promoting neuronal death and neuroinflammation (Brown *et al*., 2016).

Furthermore, the upregulation of nitric oxide synthase, increasing the production of NO, was observed in this condition of inflammation, having been shown to disrupt purinergic neuromuscular transmission in the inflamed colon, which resonates with reactive glial phenotype and PD dysmotility (Roberts *et al*., 2013). However, NO also plays an essential role in GI physiology (Brown et al., 2016), presenting an important challenge on how to reduce detrimental consequences of reactive gliosis in the ENS, without compromising physiological functions. In addition, in the CNS, α-synuclein was demonstrated to increase oxidative stress (OS), triggering neuronal apoptosis and increasing the OS, which further intensifies abnormal aggregation of α-synuclein (Travagli *et al*., 2020).

The release of proinflammatory cytokines growth factors and other immunomodulatory signal molecules, like nitric oxide (NO), by the reactive EGCs, amplifies the pro-inflammatory environment (that occurs when homeostasis is disturbed), increases the intestinal barrier damage, and induces neurochemical changes (Chow and Gulbransen, 2016). Furthermore, this neuroinflammation was correlated with the initiation of an inflammatory state, that involves the development of enteric reactive gliosis, neuroplasticity, synaptic dysfunctions, and, as clinical studies have shown, the development of intestinal dysmotility and pain in the early stages of PD (Seguella & Gulbransen, 2021).

About the immunomodulatory role of enteric glia, it was specifically shown that glial cytokines, such as IL-1β and IL-6, enhance neuronal excitability, and CXCL2, CXCL10, IL-22, and IL-23 may affect neuronal sensitivity, inducing local immune responses. In addition, also through Cx-43 dependent signaling, there is a production of the glial macrophage colony-stimulating factor (M-CSF), which establishes a pro-inflammatory phenotype in muscularis macrophages, contributing to neuronal sensitivity and persistent pain, as illustrated in figure 2 (Murakami *et al*., 2008).

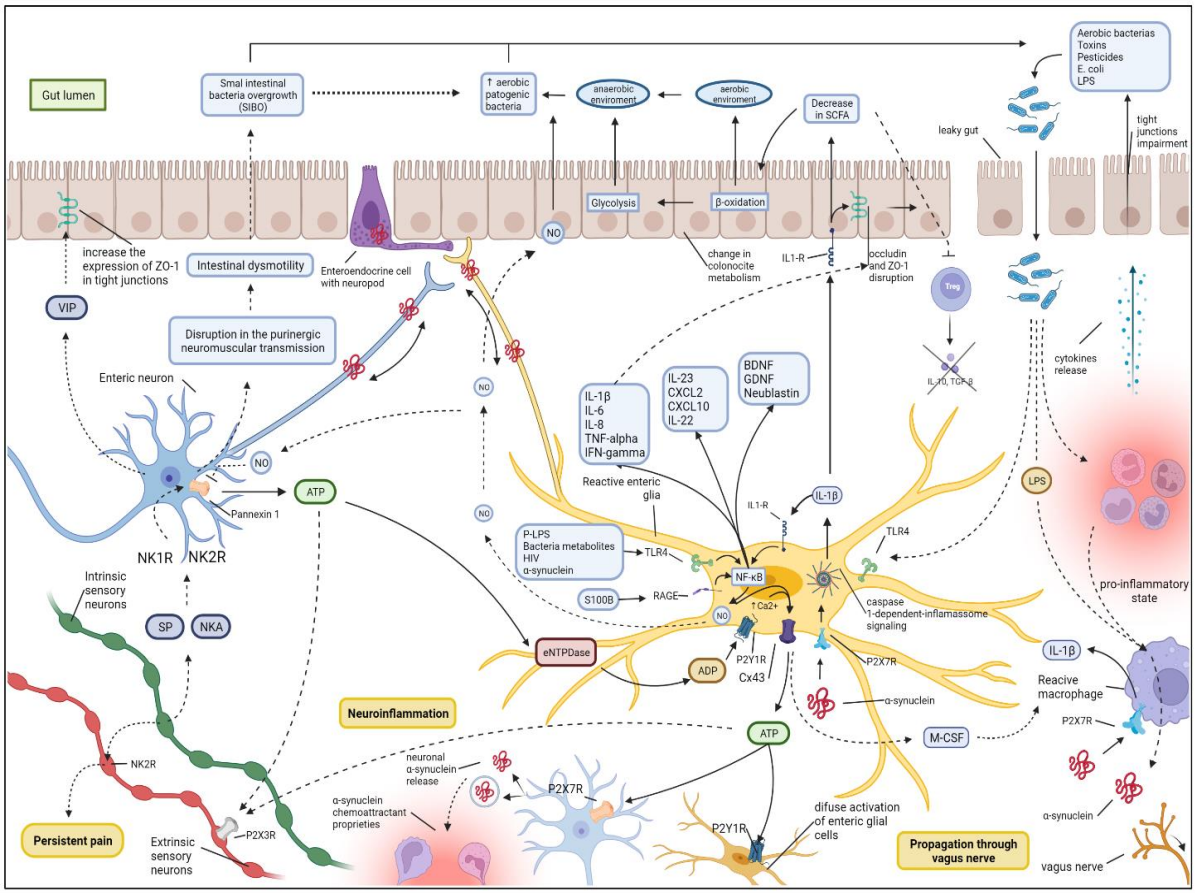

Figure 2. Glial bidirectional pathological loops involved in α-synuclein misfolding.
Dynamic gut dysbiosis, bacterial translocation, macrophage activation, lymphocyte recruitment, indirect immunosuppression (by the decrease in SCFA bacteria), and dysbiosis itself, as well as other environmental factors, produce a pro-inflammatory state, which can induce the misfolding of α-synuclein. When activated by inflammation or by the misfolded α-synuclein strains, reactive enteric glial cells initiate and propagate a pro-inflammatory state that promotes tight junctions' disruption, prodromal PD symptoms, and, along the years, the vagus nerve ascension and spreading through the SNC, promoting a diffuse activation of SNC microglia. Of note, this process is also mediated by other regulation processes that, in trying to maintain homeostasis, prevent exacerbated inflammation and neuronal loss, which is different from what happens in the CNS, which advance to neurodegeneration.
(Figure adapted from Seguella *et al*., 2021, using BioRender. https://app.biorender.com/biorender-templates)

In this context, Lewy bodies, neuritis, and the upregulation of the LRRK-2 protein, a kinase enzyme associated with an increased risk of PD, were found in enteric glia with proinflammatory markers (Clairembault *et al*., 2015). Furthermore, the glial activation by the aggregation of α-synuclein and the induction of α-synuclein misfolding, by the activated enteric glia, indicates one of the ‘‘pathological loops’’ created by inflammation in PD (Devos *et al*., 2013). Interestingly, a study observed that, in the brain and before neurodegeneration, the inflammation of neurons is mediated by microgliosis, in distant sites of α-synuclein inclusions, being activated in a diffusible seeding/spreading way, something correlating to EGCs' capacity, not only to induce neuroinflammation but also to induce a reactive state in other glial cells, independently from α-synuclein activation. As a result of increased expression of LRRK-2 and misfolded α-synuclein in the gut, α-synuclein migrates, via microtubule-associated transport, in neurons up to the vagus nerve, to the dorsal motor nucleus in the brainstem (House *et al*., 2017), accumulating in the nigrostriatal area and predisposing PD neurodegeneration (Seguella *et al*., 2020).

Interestingly, it is suggested that an initial glial response, perceived by enteric glial-related pro-inflammatory markers in the colon of PD patients, is present in the onset of PD pathology (Clairembault *et al*., 2015), being inversely activated during the progression of the disease, declining over the pathology time course, as in the brain, microglial activation tends to increase. Additionally, a direct link between environmental influences (toxins) and α-synuclein folding and spreading to gut neurons was made. Enteroendocrine cells (EECs) are electrically excitable chemosensory cells, located in the gut mucosal lining, responsible for hormone production, GI immunity, motility, barrier function, growth hormone, and insulin secretion (Yu *et al*., 2020). Enterochromaffin cells, a type of EECs, secretes 5-HT in reaction to mechanical forces, being responsible for 95% of body 5-HT (Banskota *et al*., 2019).

EECs have an apical brush border and axon-like basal membrane projections called neuropods, that are responsible for neuronal communication with neurons (synapse) and the glia (direct contact). Interestingly, EECs have similarities with neuronal cells, such as neurotrophin receptors, and pre-and post-synaptic proteins including α-synuclein, that imply ECG as a niche for proteinopathy in response to luminal signals (Bohórquez *et al*., 2014; Bohorquez *et al*., 2015).

The α-synuclein expressed in EECs was discovered to be more abundant in the small intestine than the colon, probably due to greater exposure to toxins and pathogens in the first, rather than the latter. Also, EECs are in direct contact with the neurons and the EGC, via neuropod connection, spreading the α-synuclein folding, via cell-to-cell contact and Rab35 activation (Rodrigues *et al*., 2022) directly to gut neurons and, then, to the vagus nerve (Chandra *et al*., 2017), establishing a connection between the luminal content and the vagus nerve. The spreading of α-synuclein from neurons to EECs was also demonstrated, as being even more efficient than the opposite route. However, it is still not known the importance of this bidirectional flow of α-synuclein in PD, as it was described as the mechanism of the α-synuclein misfolding, starting in the EECs and progressing to ENS and CNS, but also the contrary (Rodrigues *et al*., 2022; Chandra *et al*., 2017; Sherwinet *et al*., 2019). Moreover, inflammatory conditions that alter gut homeostasis, increase intestinal permeability. by inducing the ‘‘leaky gut’’ syndrome. Furthermore, tachykinin, substance P, and neurokinin A were also shown to increase paracellular permeability, contributing to the ‘‘leaky gut’’ presented in inflammatory disorders (Renzi *et al*., 2000; O'Connor *et al*., 2004).

ENVIRONMENTAL INFLUENCE IN PD

The gut microbiota is considered a potent modulator of intestinal, systemic, and CNS-resident immune cells function, suggesting that gut-brain interactions may involve the host

immune system (Fung *et al*., 2020). Activations of the immune system in the gut and the brain are implicated in neuroinflammation responses and brain injury, as well as in neurogenesis and plasticity (Salvo-Romero *et al*., 2020). Dysbiosis represents the most common non-motor symptoms in Parkinson's disease (PD). Thus, changes in gut microbiota, impairments of the intestinal epithelial barrier (IEB), bowel inflammation, and neuroplasticity rearrangements of the ENS could be involved in the pathophysiology of intestinal disturbances in PD. Evidence from translational studies shows that morphological changes in the intestinal barrier and ENS contribute to the pathophysiology of dysbiosis occurring in PD (Pellegrini *et al*., 2021).

Bowel inflammation, impaired intestinal epithelial barrier (IEB), and gut dysbiosis could represent early Parkinson's disease (PD) events. Descriptively, a recent study examined the correlation among enteric α-synuclein, bowel inflammation, impairments of IEB, and alterations of enteric bacteria, in a transgenic (Tg) model of PD, before brain pathology. Taken together, early enteric α-synuclein accumulation contributes to compromise IEB, through the direct activation of canonical caspase-1-dependent inflammasome signaling. These changes could contribute both to bowel symptoms, as well as central pathology (Pellegrini *et al*., 2022).

The intestinal epithelial barrier (IEB) is crucial for gut homeostasis, promoting the absorption of nutrients, while at the same time preventing the passage of pathogens and toxins. IEB regulation is modulated by the external environment (microbiota) and by the internal environment (immune cells, fibroblasts, or the enteric nervous system – ENS) (Neunlist *et al*., 2013). In this context, ENS (neurons and glia) work to promote IEB homeostasis, after infectious, chemical, or mechanical injuries, through regulation of paracellular or transcellular permeabilities, intestinal epithelial cell proliferation (important for epithelial restitution), and wound healing (Blikslager *et al*., 2007).

The paracellular permeability of the IEB is regulated by adherent junctions, desmosome, and mainly by tight junctions, which are formed by transmembrane proteins, like claudin, occludin, and tricellulin, connected to an actin cytoskeleton, via a high molecular weight protein called the *zona occludens* (ZO-1, ZO-2, and ZO-3) (Clairembault *et al*., 2015). Two pathways are involved in paracellular permeability. The first regulates the passage of small-sized solutes (less than 4 Å), which passes through an electrostatic selective filter, and the second regulates the passage of large solutes, with no selective filtration, the ''leaky'' pathway (Watson *et al*., 2005).

In this context, the increase in permeability of the IEB was also shown to be a common feature, in a variety of inflammatory digestive diseases or inflammatory bowel diseases (Gassler *et al*., 2001) and irritable bowel syndromes (Bertiaux-Vandaële *et al*., 2011) and also reduced expression and distribution of occluding, in the intestinal tissue (Clairembault *et al*., 2015). Interestingly, there are associations between diseases of the GI tract and the susceptibility to develop PD. For example, the CARD15 gene, associated with Crohn's disease, is overexpressed in PD patients (Bialecka *et al*., 2007) and, in contrast, the LRRK2 gene, responsible for PD mutation, was correlated as a major susceptibility gene for Crohn's disease (Barrett et al., 2008). Also, patients with irritable bowel syndrome have almost 50% more chances to develop PD than people that do not have IBS (Lai *et al*., 2014).

Enteric neuron activation has been shown to lead to the reinforcement of IEB functions. As a result, enteric neuromediators can produce different effects on IEB functions. Acetylcholine can increase both paracellular and transcellular permeability (Cameron *et al*., 2007), having this effect be prevented by administering muscarinic and nicotinic antagonists in an animal model of maternal separation. Acetylcholine was also associated with stimulating intestinal epithelial cell proliferation (Hirota *et al*, 2006). Substance P (SP), although playing a role in increasing paracellular permeability [112], can also stimulate intestinal epithelial cell proliferation, participating in the mucosa healing process (Turner *et al*., 2007).

Finally, vasoactive intestinal peptide (VIP) is recognized as one of the main neuromodulators involved in IEB maintenance (Neunlist *et al*., 2013). VIP reduces paracellular permeability in a variety of intestinal epithelial cells, through the increased expression of ZO-1 (Neunlist *et al*., 2003) and reduces the increased paracellular permeability induced by SP or other inflammatory mediators (Conlin *et al*., 2009). In addition, VIP showed antiproliferative effects on human intestinal epithelial cells. This result indicates the role of the ENS in the homeostasis of IEB, in physiological and pathological states, explaining its importance in wound healing and the harmful of extensive glial-reactive phenotype activation (by gut dysbiosis, infections, and α-synuclein misfolding), that could induce neuronal inflammation and neuronal loss. However, this type of inflammation seems not to be too strong in PD patients, because it was not correlated with neuronal loss nor neurodegeneration, which could be due to physiological neuroprotective programs, as that present in muscularis macrophages, aiming to limit infection-induced neuronal loss.

EGCs might play an important role in IEB integrity, as shown by *in vivo* ablation of EGCs induction of jejunoileitis and by the increase in paracellular permeability, in moderate loss of enteric glial cells (Aubé *et al*., 2006). These effects are partially explained by the glia role in the upregulation of protein expression, on the tight junctions, and by nitrosylation-dependent pathways (Rao *et al*., 2017). The protective effect of S-nitrosoglutathione (GSNO), a bioavailable source of NO, is from the prevention of bacterial invasion in intestinal epithelial cells, however, its high concentration might induce barrier damage, as it acts as a NO donor (Dijkstra *et al*., 2004). Glia also shows the release of barrier-enhancing factors *in vitro*, like glial-cell-derived neurotrophic factor (GDNF), also present in the smooth muscle, which prevents TNF-induced intestinal epithelial cell death and promotes neurogenesis and neuron survival (Steinkamp *et al*., 2003).

However, recent findings indicate that mice can tolerate a large loss of enteric glia, decreasing the notion of the crucial role of the enteric glial cells for IEB homeostasis. Although the influence of intestinal epithelial cells in culture systems is clear, the essential role of EGC for homeostatic function *in vivo* is still controversial (Seguella & Gulbransen, 2021).

In a recent study (Pellegrini *et al*., 2022) it was demonstrated that, in a genetic mouse model (human A53T α-synuclein transgenic mice) of α-synuclein aggregation, the co-treatment with LPS decreases SCFAs, increases mucosal glial activation and IL-1β production by macrophage caspase-1-dependent inflammasome. The IL-1β decreases colonic ZO-1 and occludin expressions, and induces neutrophil recruitment and IFN-γ release, activating the macrophage system, which cyclically produces more IL-1β. As a result, in the early stages of genetic PD, enteric α-synuclein, by the activation of innate immune cells, impairs IEB and is responsible for the increase of plasma levels of LPS.

Additionally, an important function was given to TLR-2, as it is expressed in glial cells, enteric neurons, intestinal epithelial cells, and immune/inflammatory cells being involved in the maintenance of the IEB integrity and the composition of the microbiota, as TLR-2 influence ZO-1 distribution, increasing transepithelial resistance (Cario *et al*., 2004) and bacterial translocation.

In such a way, it appears that the IEB, in PD patients, is impaired in a bidirectional way: by the environmental factors that can cause inflammation and dysbiosis, altering the expression of the tight junction, and by the production of IL-1β in the gut macrophages, induced by α-synuclein. Recent reports show that these factors decrease ZO-1 and occluding, in PD patient's brain (Van Ijzendoorn & Derkinderen, 2019), which resonates with other work (Pellegrini *et al*., 2022) findings showing that peripheral immune/inflammatory responses, in α-synuclein transgenic mouse models, induce inflammation in the gut and the brain.

CONCLUSION

Although other pathogenic processes that involve enteric glia in the progression of intestinal diseases are proposed to be associated with PD initiation and progression, no conclusive finding on the critical role of the enteric glia is described in the literature. This review showed how external mechanisms and genetically induced processes contribute to generating and propagating α-synuclein misfolding, involved in enteric glia, and its capacity to propagate inflammation, by activating other enteric glial cells and by contributing to neuronal plasticity and pain, correlated to the early non-motor symptoms of PD. However, studies demonstrating the importance of EGC to IEB homeostasis were not conclusive and further research on *in vivo* IEB, in the EGC ablation model of induced PD, should be made to test the strength of the proposed mechanisms.

Due to the evolutionary contact with pathogens, the gut is increasingly showing ways how not only to tolerate external microbes but to thrive with them, for its neurodevelopment and protection against external pathogens. In comparison to the brain, a sterile site, it seems to have evolved better on how to prevent inflammatory damage and neuronal loss, at least to about the observed α-synuclein inflammation that did not provoke neuronal loss in the gut. As a result, the intrinsic neuroprotective mechanism in the gut may contain pathways that could also induce neuroprotection in the brain, when in contact with external pathogens, and, as many of the symptoms of PD and other neurodegenerative diseases arise due to neuroinflammation, research on this intrinsic neuroprotective mechanism should be further stimulated. Therefore, speculatively, it is logical to say that we can learn, with the gut, how to heal the brain and most importantly, the gut microbiota can be considered a promising and therapeutic target for neurodegenerative diseases such as PD.

References

Aho VTE, Houser MC, Pereira PAB, *et al*. Relationships of gut microbiota, short-chain fatty acids, inflammation and the gut barrier in Parkinson's disease. *Mol Neurodegener*. 2021 Feb 8;16(1):6.

Aubé AC, Cabarrocas J, Bauer J, *et al*. Changes in enteric neurone phenotype and intestinal functions in a transgenic mouse model of enteric glia disruption. *Gut*. 2006 May;55(5):630-7.

Bach-Ngohou K, Mahé MM, Aubert P, *et al*. Enteric glia modulate epithelial cell proliferation and differentiation through 15-deoxy-12,14-prostaglandin J2. *J Physiol*. 2010 Jul 15;588(Pt 14):2533-44.

Baizabal-Carvallo JF, Alonso-Juarez M. The Link between Gut Dysbiosis and Neuroinflammation in Parkinson's Disease. *Neuroscience*. 2020 Apr 15;432:160-173.

Banks WA, Gray AM, Erickson MA, *et al*. Lipopolysaccharide-induced blood-brain barrier disruption: roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. *J Neuroinflammation*. 2015 Nov 25;12:223.

Banskota S, Ghia JE, Khan WI. Serotonin in the gut: Blessing or a curse. *Biochimie*, 2019 161:56-64.

Barbara G, Barbaro MR, Fuschi D, *et al*., Inflammatory and Microbiota-Related Regulation of the Intestinal Epithelial Barrier. *Front Nutr*. 2021 Sep 13;8:718356.

Barrett JC, Hansoul S, Nicolae DL, *et al*. Genome-wide association defines more than 30 distinct susceptibility loci for Crohn's disease. *Nat Genet*. 2008 Aug;40(8):955-62.

Bassotti G, Villanacci V, Antonelli E, *et al*. Enteric glial cells: new players in gastrointestinal motility? *Lab Invest.* 2007 Jul;87(7):628-32.

Belkind-Gerson J, Graham HK, Reynolds J. *et al.* Colitis promotes neuronal differentiation of Sox2+ and PLP1+ enteric cells. *Sci Rep.* 2017 7:2525.

Belkind-Gerson J, Hotta R, Nagy N, *et al*. Colitis induces enteric neurogenesis through a 5-HT4-dependent mechanism. *Inflamm Bowel Dis*. 2015 Apr;21(4):870-8.

Benvenuti L, D'Antongiovanni V, Pellegrini C, *et al*. Enteric Glia at the Crossroads between Intestinal Immune System and Epithelial Barrier: Implications for Parkinson Disease. *Int J Mol Sci*. 2020 Dec 2;21(23):9199.

Bertiaux-Vandaële N, Youmba SB, Belmonte L, *et al*. The expression and the cellular distribution of the tight junction proteins are altered in irritable bowel syndrome patients with differences according to the disease subtype. *Am J Gastroenterol*. 2011 Dec;106(12):2165-73.

Bialecka M, Kurzawski M, Klodowska-Duda G, *et al*. CARD15 variants in patients with sporadic Parkinson's disease. *Neurosci Res*. 2007 Mar;57(3):473-6.

Bienenstock J, Kunze W, Forsythe P. Microbiota and the gut-brain axis. *Nutr. Rev.* 2015 73(Suppl 1):28-31.

Blikslager AT, Moeser AJ, Gookin JL, *et al*. Restoration of barrier function in the injured intestinal mucosa. *Physiol. Rev.* 2007 87:545-564.

Boesmans W, Lasrado R, Vanden Berghe P, Pachnis V. Heterogeneity and phenotypic plasticity of glial cells in the mammalian enteric nervous system. *Glia* 2015 63:229-241.

Boesmans W, Nash A, Tasnády KR, *et al*. Development, Diversity, and Neurogenic Capacity of Enteric Glia. *Front Cell Dev Biol*. 2022 Jan 17;9:775102.

Bohórquez DV, Samsa LA, Roholt A, *et al*. An enteroendocrine cell-enteric glia connection revealed by 3D electron microscopy. *PLoS One*. 2014 Feb 26;9(2):e89881.

Bohórquez DV, Shahid RA, Erdmann A, *et al*. Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. *J Clin Invest*. 2015 Feb;125(2):782-6.

Brown IAM, McClain JL, Watson RE, *et al*. Enteric glia mediate neuron death in colitis through purinergic pathways that require connexin-43 and nitric oxide. *Cell. Mol. Gastroenterol. Hepatol.* 2016 2:77-91.

Burgueño JF, Abreu MT. Epithelial Toll-like receptors and their role in gut homeostasis and disease. *Nat. Rev. Gastroenterol. Hepatol.* 2020 17:263-278.

Cameron HL, Perdue MH. Muscarinic acetylcholine receptor activation increases transcellular transport of macromolecules across mouse and human intestinal epithelium in vitro. *Neurogastroenterol. Motil. Off. J. Eur. Gastrointest. Motil. Soc.* 2007 19:47-56.

Cao RY, Zeng YQ, Li SH, *et al*. Short-Chain Fatty Acids-A Healthy Bus between Gut Microbiota and Organs beyond the Gut. *Advances in Bioscience and Biotechnology*, 2022 13: 362-387.

Capoccia E, Cirillo C, Gigli S, *et al*. Enteric glia: A new player in inflammatory bowel diseases. *Int J Immunopathol Pharmacol*. 2015 Dec;28(4):443-451.

Cario E, Gerken G, Podolsky DK. Toll-like receptor 2 enhances ZO-1-associated intestinal epithelial barrier integrity via protein kinase C. *Gastroenterology* 2004 127:224-238.

Cersosimo MG, Raina GB, Pecci C, Pellene A, *et al*. Gastrointestinal manifestations in Parkinson's disease: prevalence and occurrence before motor symptoms. *J Neurol*. 2013 May;260(5):1332-8.

Chakrabarti A, Geurts L, Hoyles L, *et al*. The microbiota-gut-brain axis: pathways to better brain health. Perspectives on what we know, what we need to investigate and how to put knowledge into practice. *Cell Mol Life Sci*. 2022 Jan 19;79(2):80.

Chandra R, Hiniker A, Kuo YM, *et al*. α-Synuclein in gut endocrine cells and its implications for Parkinson's disease. *JCI Insight* 2017 2:1-13.

Chanpong A, Borrelli O, Thapar N. Recent advances in understanding the roles of the enteric nervous system. *Fac Rev*. 2022 Mar 24;11:7.

Chao YX, Gulam MY, Chia NSJ, *et al*. Gut-Brain Axis: Potential Factors Involved in the Pathogenesis of Parkinson's Disease. *Front Neurol*. 2020 Aug 25;11:849.

Chen QQ, Haikal C, Li W, *et al*. Gut Inflammation in Association With Pathogenesis of Parkinson's Disease. *Front Mol Neurosci*. 2019 Sep 13;12:218.

Chen SJ, Chen CC, Liao HY, *et al*. Association of Fecal and Plasma Levels of Short-Chain Fatty Acids With Gut Microbiota and Clinical Severity in Patients With Parkinson Disease. *Neurology*. 2022 Feb 22;98(8):e848-e858.

Chen SJ, Lin CH. Gut microenvironmental changes as a potential trigger in Parkinson's disease through the gut–brain axis. *J Biomed Sci,* 2022 29:54.

Cheng WY, Ho YS, Chang RC. Linking circadian rhythms to microbiome-gut-brain axis in aging-associated neurodegenerative diseases. *Ageing Res Rev*. 2022 Jun;78:101620.

Chow AK, Grubišić V, Gulbransen BD. Enteric Glia Regulate Lymphocyte Activation via Autophagy-Mediated MHC-II Expression. *Cell Mol Gastroenterol Hepatol*. 2021 12(4):1215-1237.

Chow AK, Gulbransen BD. Potential roles of enteric glia in bridging neuroimmune communication in the gut. *Am J Physiol Gastrointest Liver Physiol*. 2017 312(2):G145-G152.

Clairembault T, Leclair-Visonneau L, Coron E, *et al*. Structural alterations of the intestinal epithelial barrier in Parkinson's disease. *Acta Neuropathol Commun*. 2015 Mar 10;3:12.

Clairembault T, Leclair-Visonneau L, Neunlist M, Derkinderen P. Enteric glial cells: new players in Parkinson's disease? *Mov Disord*. 2015 Apr;30(4):494-8. doi: 10.1002/mds.25979.

Conlin VS, Wu X, Nguyen C, *et al*. Vasoactive intestinal peptide ameliorates intestinal barrier disruption associated with Citrobacter rodentium-induced colitis. *Am J Physiol Gastrointest Liver Physiol*. 2009 Oct;297(4):G735-50.

Costantini TW, Bansal V, Krzyzaniak M, *et al*. Vagal nerve stimulation protects against burn-induced intestinal injury through activation of enteric glia cells. *Am J Physiol Gastrointest Liver Physiol*. 2010 Dec;299(6):G1308-18.

De Giorgio R, Giancola F, Boschetti E, *et al*. Enteric glia and neuroprotection: basic and clinical aspects. *Am J Physiol Gastrointest Liver Physiol*. 2012 Oct 15;303(8):G887-93. doi: 10.1152/ajpgi.00096.2012.

Delenclos M, Burgess JD, Lamprokostopoulou A, *et al*. Cellular models of alpha-synuclein toxicity and aggregation. *J Neurochem*. 2019 Sep;150(5):566-576.

Delvalle NM, Dharshika C, Morales-Soto W, *et al*., Communication Between Enteric Neurons, Glia, and Nociceptors Underlies the Effects of Tachykinins on Neuroinflammation. *Cell Mol Gastroenterol Hepatol*. 2018 May 29;6(3):321-344.

Devos D, Lebouvier T, Lardeux B, *et al*. Colonic inflammation in Parkinson's disease. *Neurobiol Dis*. 2013 Feb;50:42-48.

Dijkstra G, van Goor H, Jansen PLM, Moshage H. Targeting nitric oxide in the gastrointestinal tract. *Curr. Opin. Investig. Drugs*, 2004 5:529-536.

Dogra N, Mani RJ, Katare DP. The Gut-Brain Axis: Two Ways Signaling in Parkinson's Disease. *Cell. Mol. Neurobiol.* 2022 42:315-332.

Dong M, Li L, Chen M, Kusalik A, Xu W. Predictive analysis methods for human microbiome data with application to Parkinson's disease. *PLoS One*. 2020 Aug 24;15(8):e0237779.

Dowling LR, Strazzari MR, Keely S, Kaiko GE. Enteric nervous system and intestinal epithelial regulation of the gut-brain axis. *J Allergy Clin Immunol*. 2022 Sep;150(3):513-522.

Drobny A, Ngo PA, Neurath MF, *et al*. Molecular Communication Between Neuronal Networks and Intestinal Epithelial Cells in Gut Inflammation and Parkinson's Disease. *Front Med (Lausanne)*. 2021 Jul 22;8:655123.

Dutta SK, Verma S, Jain V, *et al*. The Emerging Role of Gut Dysbiosis, Antibiotics, Probiotics, and Fecal Microbiota Transplantation. *J Neurogastroenterol Motil*. 2019 Jul 1;25(3):363-376.

Elfil M, Kamel S, Kandil M, Koo BB, Schaefer SM. Implications of the Gut Microbiome in Parkinson's Disease. *Mov Disord*. 2020 Jun;35(6):921-933.

Fan HX, Sheng S, Zhang F. New hope for Parkinson's disease treatment: Targeting gut microbiota. *CNS Neurosci Ther*. 2022 28:1675-1688.

Fasano A, Visanji NP, Liu LWC, *et al*. Gastrointestinal dysfunction in Parkinson's disease. *Lancet Neurol.* 2015 14:625-639.

Fellner L, Irschick R, Schanda K, *et al*. Toll-like receptor 4 is required for α-synuclein dependent activation of microglia and astroglia. *Glia*. 2013 Mar;61(3):349-60.

Fleming MA, Ehsan L, Moore SR, Levin DE. The Enteric Nervous System and Its Emerging Role as a Therapeutic Target. *Gastroenterology research and practice*, 2020 v.8024171.

Fletcher EL, Clark MJ, Furness JB. Neuronal and glial localization of GABA transporter immunoreactivity in the myenteric plexus. *Cell Tissue Res.* 2002 308:339-346.

Frasca D, Blomberg BB. Inflammaging decreases adaptive and innate immune responses in mice and humans. *Biogerontology* 2016 17:7-19.

Frost G, Sleeth ML, Sahuri-Arisoylu M, *et al*. The short-chain fatty acid acetate reduces appetite via a central homeostatic mechanism. *Nat Commun.* 2014 Apr 29;5:3611.

Fullard ME, Morley JF, Duda JE. Olfactory Dysfunction as an Early Biomarker in Parkinson's Disease. *Neurosci Bull*. 2017 33(5):515-525.

Fung TC. The microbiota-immune axis as a central mediator of gut-brain communication. *Neurobiol Dis*. 2020 Mar;136:104714.

Fung C, Vanden Berghe P. Functional circuits and signal processing in the enteric nervous system. *Cell. Mol. Life Sci.* 2020 77:4505-4522.

Furness JB, Callaghan BP, Rivera LR, Cho HJ. The enteric nervous system and gastrointestinal innervation: integrated local and central control. *Adv. Exp. Med. Biol.* 2014 817:39-71.

Gassler N, Rohr C, Schneider A, *et al*. Inflammatory bowel disease is associated with changes of enterocytic junctions. *Am J Physiol Gastrointest Liver Physiol*. 2001 Jul;281(1):G216-228.

Geng ZH, Zhu Y, Li QL, *et al*. Enteric Nervous System: The Bridge Between the Gut Microbiota and Neurological Disorders. *Frontiers in Aging Neuroscience*, 2022 14:810483.

Ghezzi L, Cantoni C, Rotondo E, Galimberti D. The Gut Microbiome-Brain Crosstalk in Neurodegenerative Diseases. *Biomedicines*. 2022 Jun 23;10(7):1486.

Ghosh SS, Wang J, Yannie PJ, Ghosh S. Intestinal Barrier Dysfunction, LPS Translocation, and Disease Development. *J. Endocr. Soc.* 2020 4:bvz039.

Gorecki AM, Anyaegbu CC, Anderton RS. TLR2 and TLR4 in Parkinson's disease pathogenesis: the environment takes a toll on the gut. *Transl.Neurodegener.* 202110:1-19.

Grenn FP, Kim JJ, Makarious MB, *et al*. International Parkinson's Disease Genomics Consortium (IPDGC). The Parkinson's Disease Genome-Wide Association Study Locus Browser. *Mov Disord*. 2020 Nov;35(11):2056-2067.

Grubišić V, McClain JL, Fried DE, *et al*., Enteric Glia Modulate Macrophage Phenotype and Visceral Sensitivity following Inflammation. *Cell Rep*. 2020 Sep 8;32(10):108100.

Grubišić V, Verkhratsky A, Zorec R, Parpura V. Enteric glia regulate gut motility in health and disease. *Brain Res Bull*. 2018 136:109-117.

Grundmann D, Loris E, Maas-Omlor S, Schäfer KH. Enteric Neurogenesis During Life Span Under Physiological and Pathophysiological Conditions. *Anat Rec (Hoboken)*. 2019 Aug;302(8):1345-1353.

Gubert C, Gasparotto J, Morais L. Convergent pathways of the gut microbiota-brain axis and neurodegenerative disorders. *Gastroenterology Report*, 2022 10:goac017.

Gulbransen BD, Sharkey KA. Novel functional roles for enteric glia in the gastrointestinal tract. *Nat. Rev. Gastroenterol. Hepatol.* 2012 9:625-632.

Gulbransen BD, Bains JS, Sharkey KA. Enteric glia are targets of the sympathetic innervation of the myenteric plexus in the guinea pig distal colon. *J. Neurosci.* 2010 30:6801-6809.

Guo T, Chen L. Gut microbiota and inflammation in Parkinson's disease: Pathogenetic and therapeutic insights. *European Journal of Inflammation*. 2022 20.

Hallett PJ, McLean JR, Kartunen A, *et al*. α-Synuclein overexpressing transgenic mice show internal organ pathology and autonomic deficits. *Neurobiol. Dis.* 2012 47:258-267.

He R, Zhao Y, He Y, *et al*. Olfactory Dysfunction Predicts Disease Progression in Parkinson's Disease: A Longitudinal Study. *Front Neurosci*. 2020 Dec.14;14:569777.

Hecking I, Stegemann LN, Theis V, Vorgerd M, Matschke V, Stahlke S, Theiss C. Neuroprotective Effects of VEGF in the Enteric Nervous System. *Int J Mol Sci.* 2022 Jun 17;23(12):6756.

Helander HF, Fändriks L. Surface area of the digestive tract - revisited. *Scand. J. Gastroenterol.* 2014 49:681-689.

Hirota CL, McKay DM. Cholinergic regulation of epithelial ion transport in the mammalian intestine. *Br J Pharmacol*. 2006;149(5):463-479.

Houser MC, Tansey MG. The gut-brain axis: Is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? *npj Park. Dis.* 2017 3.

Huang Y, Liao J, Liu X, *et al*. Review: The Role of Intestinal Dysbiosis in Parkinson's Disease. *Front. Cell. Infect. Microbiol.* 2021 11:615075. doi: 10.3389/fcimb.2021.615075

Ibiza S, García-Cassani B, Ribeiro H, *et al*. Glial-cell-derived neuroregulators control type 3 innate lymphoid cells and gut defence. *Nature*. 2016 Jul 21;535(7612):440-443.

Joseph NM, He S, Quintana E, *et al*. Enteric glia are multipotent in culture but primarily form glia in the adult rodent gut. *J Clin Invest.* 2011 Sep;121(9):3398-411.

Kabouridis PS, Lasrado R, McCallum S, *et al*. Microbiota controls the homeostasis of glial cells in the gut lamina propria. *Neuron.* 2015 Jan 21;85(2):289-95.

Keshavarzian A, Green SJ, Engen PA, *et al*. Colonic bacterial composition in Parkinson's disease. *Mov Disord.* 2015 Sep;30(10):1351-60.

Kim R, Kim HJ, Kim A, *et al*. Peripheral blood inflammatory markers in early Parkinson's disease. *J Clin Neurosci.* 2018 Dec;58:30-33.

Kincaid HJ, Nagpal R, Yadav H. Diet-Microbiota-Brain Axis in Alzheimer's Disease. Ann Nutr Metab. 2021;77 Suppl 2:21-27.

Klann EM, Dissanayake U, Gurrala A, *et al*. The Gut-Brain Axis and Its Relation to Parkinson's Disease: A Review. *Front Aging Neurosci*. 2022 Jan 7;13:782082.

Kroemer A, Wong GCL. A role for neuronal alpha-synuclein in gastrointestinal immunity. *Journal of Innate Immunity,* 2017 9:456-463.

Kumari U, Tan EK. LRRK2 in Parkinson's disease: genetic and clinical studies from patients. *FEBS J.* 2009 276:6455-6463.

Kwon HS, Koh SH. Neuroinflammation in neurodegenerative disorders: the roles of microglia and astrocytes. *Transl Neurodegener,* 2020 9:42.

Lai SW, Liao KF, Lin CL, Sung FC. Irritable bowel syndrome correlates with increased risk of Parkinson's disease in Taiwan. *Eur. J. Epidemiol.* 2014 29:57-62.

Langness S, Kojima M, Coimbra R, et al. Enteric glia cells are critical to limiting the intestinal inflammatory response after injury. *Am. J. Physiol. - Gastrointest. Liver Physiol.* 2017 312:G274-G282.

Larraufie P, Doré J, Lapaque N, Blottière HM. TLR ligands and butyrate increase Pyy expression through two distinct but inter-regulated pathways. *Cell. Microbiol.* 2017 19.

Lasrado R, Boesmans W, Kleinjung J, *et al*. Lineage-dependent spatial and functional organization of the mammalian enteric nervous system. *Science*. 2017 May 19;356(6339):722-726.

Le Berre-Scoul C, Chevalier J, Oleynikova E, *et al*. A novel enteric neuron-glia coculture system reveals the role of glia in neuronal development. *J Physiol*. 2017 Jan 15;595(2):583-598.

Lei Q, Wu T, Wu J, *et al*. Roles of α-synuclein in gastrointestinal microbiome dysbiosis-related Parkinson's disease progression (Review). *Mol Med Rep*., 2021 Oct;24(4):734.

Litvak Y, Byndloss MX, Bäumler AJ. Colonocyte metabolism shapes the gut microbiota. *Science,* 2018 80:362.

Liu C, Yang J. Enteric Glial Cells in Immunological Disorders of the Gut. *Front Cell Neurosci*. 2022 Apr 28;16:895871.

Liu L, Huh JR, Shah K. Microbiota and the gut-brain-axis: Implications for new therapeutic design in the CNS. *E Bio Medicine*. 2022 Mar;77:103908.

Luo S, Zhu H, Zhang J, Wan D. The Pivotal Role of Microbiota in Modulating the Neuronal–Glial–Epithelial Unit. *Infect Drug Resist*. 2021 14:5613-5628.

McClain JL, Fried DE, Gulbransen BD. Agonist-Evoked Ca2+ Signaling in Enteric Glia Drives Neural Programs That Regulate Intestinal Motility in Mice. *Cmgh,* 2015 1:631-645.

McCormack AL, Mak SK, Di Monte DA. Increased α-synuclein phosphorylation and nitration in the aging primate substantia nigra. *Cell Death Dis.* 2012, 3, e315–e315 (2012).

Meng L, Yuan X, Cao X, Zhang Z. The gut-brain axis in the pathogenesis of Parkinson's disease. *Brain Science Advances,* 2019 5:73-81.

Menozzi E, Macnaughtan J, Schapira AHV. The gut-brain axis and Parkinson disease: clinical and pathogenetic relevance. *Ann Med.* 2021 Dec;53(1):611-625. doi:10.1080/07853890.2021.1890330.

Mirzaei R, Bouzari B, Hosseini-Fard SR, *et al*. Role of microbiota-derived short-chain fatty acids in nervous system disorders. *Biomed Pharmacother*. 2021 Jul;139:111661.

Moustafa SA, Mohamed S, Dawood A, *et al*. Gut brain axis: an insight into microbiota role in Parkinson's disease. *Metab Brain Dis*. 2021 Oct;36(7):1545-1557.

Murakami M, Ohta T, Ito S. Interleukin-1β enhances the action of bradykinin in rat myenteric neurons through up-regulation of glial B1 receptor expression. *Neuroscience*, 2008 151:222-231.

Natale G, Ryskalin L, Morucci G, *et al*. The Baseline Structure of the Enteric Nervous System and Its Role in Parkinson's Disease. *Life (Basel)*. 2021 Jul 22;11(8):732.

Neunlist M, Toumi F, Oreschkova T, *et al*. Human ENS regulates the intestinal epithelial barrier permeability and a tight junction-associated protein ZO-1 via VIPergic pathways. *Am J Physiol Gastrointest Liver Physiol*. 2003 Nov;285(5):G1028-36.

Neunlist M, Van Landeghem L, Mahé MM, *et al*. The digestive neuronal-glial-epithelial unit: a new actor in gut health and disease. *Nat Rev Gastroenterol Hepatol*, 2013 Feb;10(2):90-100.

Nielsen SD, Pearson NM, Seidler K. The link between the gut microbiota and Parkinson's Disease: A systematic mechanism review with focus on α-synuclein transport. *Brain Res*, 2021 Oct 15;1769:147609.

O'Connor TM, O'Connell J, O'Brien DI, *et al*. The role of substance P in inflammatory disease. *J Cell Physiol*. 2004 Nov;201(2):167-80.

Pavan S, Prabhu AN, Prasad Gorthi S, *et al*. Exploring the multifactorial aspects of Gut Microbiome in Parkinson's Disease. *Folia Microbiol (Praha)*. 2022 May 18.

Pellegrini C, D'Antongiovanni V, Ippolito C, *et al*. From the intestinal mucosal barrier to the enteric neuromuscular compartment: an integrated overview on the morphological changes in Parkinson's disease. *European journal of histochemistry: EJH*, 2021 65;(s1):3278.

Pellegrini C, D'Antongiovanni V, Miraglia F, *et al*. Enteric α-synuclein impairs intestinal epithelial barrier through caspase-1-inflammasome signaling in Parkinson's disease before brain pathology. *NPJ Parkinson's disease*, 8(1):9.

Peng L, Li ZR, Green RS, Holzman IR, Lin J. Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. *J. Nutr.*, 2009 139:1619-1625.

Postuma RB, Berg D, Stern M, *et al*. MDS clinical diagnostic criteria for Parkinson's disease. Mov Disord. 2015 Oct;30(12):1591-601.

Progatzky F, Pachnis V. The role of enteric glia in intestinal immunity. *Curr Opin Immunol*, 2022 Aug;77:102183.

Rao M, Nelms BD, Dong L, *et al*. Enteric glia express proteolipid protein 1 and are a transcriptionally unique population of glia in the mammalian nervous system. *Glia*, 2015 Nov;63(11):2040-2057.

Rao M, Rastelli D, Dong L, *et al*. Enteric Glia Regulate Gastrointestinal Motility but Are Not Required for Maintenance of the Epithelium in Mice. *Gastroenterology*, 2017 153(4):1068-1081.e7.

Rao M, Gershon M. The bowel and beyond: the enteric nervous system in neurological disorders. *Nat Rev Gastroenterol Hepatol,* 2016 13:517-528.

Reigstad CS, Salmonson CE, Rainey JF, *et al*. Gut microbes promote colonic serotonin production through an effect of short-chain fatty acids on enterochromaffin cells. *FASEB J.* 2015 Apr;29(4):1395-403.

Renzi D, Pellegrini B, Tonelli F, *et al*. Substance P (neurokinin-1) and neurokinin A (neurokinin-2) receptor gene and protein expression in the healthy and inflamed human intestine. *Am J Pathol*, 2000;157(5):1511-1522.

Roberts JA, Durnin L, Sharkey KA, *et al*. Oxidative stress disrupts purinergic neuromuscular transmission in the inflamed colon. *J. Physiol.*, 2013 591:3725-3737.

Rodrigues PV, de Godoy JVP, Bosque BPA, *et al*. Transcellular propagation of fibrillar α-synuclein from enteroendocrine to neuronal cells requires cell-to-cell contact and is Rab35-dependent. *Sci Rep.* 2022 Mar 9;12(1):4168.

Romano S, Savva GM, Bedarf JR, *et al.* Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. *npj Parkinsons Dis*, 2021 7:27.

Rosenberg HJ, Rao M. Enteric glia in homeostasis and disease: From fundamental biology to human pathology. *iScience*. 2021 Jul 15;24(8):102863.

Rutsch A, Kantsjö JB, Ronchi F. The Gut-Brain Axis: How Microbiota and Host Inflammasome Influence Brain Physiology and Pathology. *Front. Immunol.*, 2020 11:1-24.

Salvo-Romero E, Stokes P, Gareau MG. Microbiota-immune interactions: from gut to brain. *LymphoSign Journal*, 2020 7(1):1-23.

Santos SF, Oliveira HL de, Yamada ES, *et al*. The Gut and Parkinson's Disease-A Bidirectional Pathway. *Front Neurol*, 2019 Jun 4;10:574.

Savidge TC, Newman P, Pothoulakis C, *et al*. Enteric glia regulate intestinal barrier function and inflammation via release of S-nitrosoglutathione. *Gastroenterology*, 2007 Apr;132(4):1344-1358.

Schaeffer E, Kluge A, Böttner M, *et al*. Alpha Synuclein Connects the Gut-Brain Axis in Parkinson's Disease Patients - A View on Clinical Aspects, Cellular Pathology and Analytical Methodology. *Frontiers in cell and developmental biology*, 2020 Sep 8,8:573696.

Schaffernicht G, Shang Q, Stievenard A, *et al*. Pathophysiological Changes in the Enteric Nervous System of Rotenone-Exposed Mice as Early Radiological Markers for Parkinson's Disease. *Front Neurol*., 2021 Mar 22;12:642604.

Schapira AHV, Chaudhuri KR, Jenner P. Non-motor features of Parkinson disease. *Nat. Rev. Neurosci.*, 2017 18:435-450.

Scheperjans F, Aho V, Pereira PA, *et al*. Gut microbiota avre related to Parkinson's disease and clinical phenotype. *Mov Disord*. 2015 Mar;30(3):350-8.

Seguella L, Capuano R, Sarnelli G, Esposito G. Play in advance against neurodegeneration: exploring enteric glial cells in gut-brain axis during neurodegenerative diseases. *Expert Rev*

Clin Pharmacol, 2019 Jun;12(6):555-564.

Seguella L, Gulbransen BD. Enteric glial biology, intercellular signalling and roles in gastrointestinal disease. *Nat Rev Gastroenterol Hepatol*, 2021 18(8):571-587.

Seguella L, Sarnelli G, Esposito G. Leaky gut, dysbiosis, and enteric glia activation: The trilogy behind the intestinal origin of Parkinson's disease. *Neural Regen. Res.* 2020 15:1037-1038.

Shen T, Yue Y, He T, *et al*. The Association Between the Gut Microbiota and Parkinson's Disease, a Meta-Analysis. *Front Aging Neurosci*, 2021 Feb 12;13:636545.

Sherwin E, Bordenstein SR, Quinn JL, *et al*. Microbiota and the social brain. *Science,* 2019 80:366.

Smith PM, Howitt MR, Panikov N, *et al*. The microbial metabolites, short-chain fatty acids, regulate colonic Treg cell homeostasis. *Science*, 2013 Aug 2;341(6145):569-73.

Solleiro-Villavicencio H, Rivas-Arancibia S. Effect of Chronic Oxidative Stress on Neuroinflammatory Response Mediated by CD4+T Cells in Neurodegenerative Diseases. *Front. Cell. Neurosci*, 2018 12.

Sommer F, Bäckhed F. The gut microbiota--masters of host development and physiology. *Nat. Rev. Microbiol.*, 2013 11:227-238.

Soni A, Verma A, Gupta P. Microbiota–Gut–Brain Axis and Neurodegenerative Disorder. In: Tripathi AK, Kotak M. (eds) Gut Microbiome in Neurological Health and Disorders. Nutritional Neurosciences. Springer, Singapore. 2022.

Spencer NJ, Hu H. Enteric nervous system: sensory transduction, neural circuits and gastrointestinal motility. *Nat Rev Gastroenterol Hepatol*, 2020 17:338-351.

Stefanova N, Fellner L, Reindl M, *et al*. Toll-like receptor 4 promotes α-synuclein clearance and survival of nigral dopaminergic neurons. *Am J Pathol*, 2011 Aug;179(2):954-63.

Steinkamp M, Geerling I, Seufferlein T, *et al*. Glial-derived neurotrophic factor regulates apoptosis in colonic epithelial cells. *Gastroenterology*, 2003 Jun;124(7):1748-1757.

Tan AH, Lim SY, Lang AE. The microbiome–gut–brain axis in Parkinson disease — from basic research to the clinic. *Nat Rev Neurol*, 2022.

Tansey MG, Wallings RL, Houser MC, *et al.* Inflammation and immune dysfunction in Parkinson disease. *Nat Rev Immunol*, 2022.

Thomasi BBM, Valdetaro L, Ricciardi MCG, *et al*. Enteric glial cell reactivity in colonic layers and mucosal modulation in a mouse model of Parkinson's disease induced by 6-hydroxydopamine. *Brain Res Bull*., 2022 Sep;187:111-121.

Tilocca B, Pieroni L, Soggiu A, *et al*. Gut-Brain Axis and Neurodegeneration: State-of-the-Art of Meta-Omics Sciences for Microbiota Characterization. *Int J Mol Sci*. 2020 Jun 5;21(11):4045.

Toledo ARL, Monroy GR, Salazar FE, *et al*. Gut-Brain Axis as a Pathological and Therapeutic Target for Neurodegenerative Disorders. *Int J Mol Sci*, 2022 Jan 21;23(3):1184.

Travagli RA, Browning KN, Camilleri M. Parkinson disease and the gut: new insights into pathogenesis and clinical relevance. *Nat. Rev. Gastroenterol. Hepatol.,* 2020 17:673-685.

Turco F, Sarnelli G, Cirillo C, *et al*. Enteroglial-derived S100B protein integrates bacteria-induced Toll-like receptor signalling in human enteric glial cells. *Gut*, 2014 Jan;63(1):105-115. DOI: 10.1136/gutjnl-2012-302090.

Turner DJ, Martin PC, Rao JN, *et al*. Substance P regulates migration in rat intestinal epithelial cells. *Ann Surg*. 2007 245(3):408-414.

Valès S, Touvron M, Van Landeghem L. Enteric glia: Diversity or plasticity? *Brain Res,* 2018 1693:140-145.

Van Ijzendoorn SCD, Derkinderen P. The Intestinal Barrier in Parkinson's Disease: Current State of Knowledge. *J. Parkinsons. Dis.,* 2019 9:S323-S329.

Vicentini FA, Keenan CM, Wallace LE, *et al*. Intestinal microbiota shapes gut physiology and regulates enteric neurons and glia. *Microbiome* 2021 9:210.

Visanji NP, Marras C, Hazrati LN, *et al*. Alimentary, my dear Watson? The challenges of enteric α-synuclein as a Parkinson's disease biomarker. *Mov. Disord.*, 2014 29:444-450.

Wallen ZD, Appah M, Dean MN, *et al*. Characterizing dysbiosis of gut microbiome in PD: evidence for overabundance of opportunistic pathogens. *NPJ Parkinson's disease*, 2020 6:11.

Wang Q, Luo Y, Ray Chaudhuri K, *et al*. The role of gut dysbiosis in Parkinson's disease: mechanistic insights and therapeutic options. Brain, 2021 Oct 22;144(9):2571-2593.

Watson CJ, Hoare CJ, Garrod DR, *et al*. Interferon-gamma selectively increases epithelial permeability to large molecules by activating different populations of paracellular pores. *J. Cell Sci.,* 2005 118:5221-5230.

Yatsunenko T, Rey F, Manary M. *et al.* Human gut microbiome viewed across age and geography. *Nature,* 2012 486:222-227.

Yoo BB, Mazmanian SK. The Enteric Network: Interactions between the Immune and Nervous Systems of the Gut. *Immunity*, 2017 46:910-926.

Yu YB, Li YQ. Enteric glial cells and their role in the intestinal epithelial barrier. *World journal of gastroenterology,* 2014 20(32):11273-11280.

Yu Y, Yang W, Li Y, Cong Y. Enteroendocrine Cells: Sensing Gut Microbiota and Regulating Inflammatory Bowel Diseases. *Inflamm. Bowel Dis*, 2020 26:11-20.

Zhang DK, He FQ, Li TK, *et al*. Glial-derived neurotrophic factor regulates intestinal epithelial barrier function and inflammation and is therapeutic for murine colitis. *J Pathol*, 2010 222:213-222.

Zhu M, Liu X, Ye Y, *et al*. Gut Microbiota: A Novel Therapeutic Target for Parkinson's Disease. *Front Immunol*., 2022 Jun 24;13:937555. doi: 10.3389/fimmu.2022.937555.

ARTIGO 2

Artigo Científico para Submissão na Revista: Ageing Research Reviews (FI 11.788)

CHARACTERIZATION OF PARKINSON'S DISEASE RAT MODEL USING ROTENONE AND THE ROLE OF ENTERIC GLIA AND MICROBIOTA-GUT-BRAIN AXIS IN IT'S PATHOGENESIS

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Conflict of interest
The authors declare that they have no conflicts of interest. All authors read and approved the final manuscript.

Acknowledgments
We would like to thank Medical School of the Christus University Center (UNICHRISTUS), Federal University of Ceará (UFC), and Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP; grants 2017/16443-0 and 2021/06473-4).

1. Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disease (Wirdefeldt *et al*., 2011), affecting more than 6.1 million people worldwide in 2016 (GBD Global Collaborators, 2016), characterized by the progressive loss of dopaminergic neurons in the compact part of the substantia nigra (SNpc), by depletion of the neurotransmitter dopamine (DA) in the striatum nucleus (Yu *et al*., 2018) and by the presence of Lewy bodies in the brainstem (Seidel *et al*., 2015), resulting in motor, premotor and non-motor symptoms. The cardinal motor symptoms of bradykinesia, rest tremor, rigidity, and postural instability become evident when 60% to 80% of SNpc neurons are lost (Hirsch *et al*., 1988; Rumesh *et al*., 2022); however, in the new diagnostic criteria, there is integration of non-motor symptoms, which include autonomic, affective, cognitive, and sleep impairments (Thaler *et al*., 2022).

Environmental exposures are widely believed to contribute largely to most sporadic late onset PD, alone or through interactions with genetic factors. Interest in whether such exposures may contribute to the development of Parkinson's disease was sparked by the association between 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) and parkinsonism during the 1980s (Mazzulli *et al*., 2011). Over the past two decades, scientists have identified numerous environmental factors associated with the risk of developing PD (Chen *et al*., 2018; Ascherio *et al*., 2016), some of the environmental factors and toxic exposures that may be associated with Parkinson's disease include pesticides (rotenone and paraquat) (Tanner *et al*., 2011; Costello *et al*., 2009); heavy metals (manganese, lead, and copper); well water; carpentry; head trauma (Fang *et al*., 2012); other substances, including polychlorinated biphenyls, trichloroethylene, perchloroethylene, and carbon tetrachloride; and rural living. Exposure to toxins, including carbon monoxide, trace metals, organic solvents, and cyanide has also been noted as an environmental risk factor. Alternatively, smoking, ibuprofen use (Chen *et al*., 2005), and caffeine intake are believed to reduce disease risk, although more studies are ongoing (Taylor *et al*., 2007; Obeso *et al*., 2017).

Animal models are essential for experimental research and extensive efforts have been made to establish such models that reproduce the main clinical features of PD in order to gain a greater understanding of the pathophysiology of the disease and to trial new therapeutic strategies (Lama *et al*., 2018). Currently, animal models of PD that are used in preclinical research are obtained through the administration of neurotoxins such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), rotenone, and paraquat, or through the overexpression of genetically modified PD-related genes such as alphasynuclein (Vingill *et al*., 2018). Neurotoxin models are well known to produce prominent neurodegeneration of the nigrostriatal pathway open to examination of possible disease-modifying therapeutic strategies (Fornai *et al*., 2005). The model using rotenone to reproduce the PD model in animals became of great interest after the paper by the Greenamyre group in 2000 (Betarbet *et al*., 2000). This paper reported for the first time that systemic rotenone was able to reproduce the two main neuropathological hallmarks of PD, the degeneration of dopaminergic neurons of the SNpc and the presence of Lewy bodies, as well as certain parkinsonian motor deficits. From this research, many research groups have actively used the rotenone model worldwide.

Here, we developed a model of PD in rats with systemic, continuous, and chronic exposure to rotenone, a well-characterized, high-affinity, and specific inhibitor insecticide of complex I, one of five inner mitochondrial membrane enzyme complexes involved in phosphorylation oxidation that readily crosses biological membranes, and does not depend on the dopamine transporter for access to the cytoplasm.

2. Materials and Methods

2.1. Animals

Twenty male Wistar rats, weighing 100 to 240 g were used in this study. The colony room was maintained at 21 ± 2 °C with a 12 h light/ dark schedule (light: 7 am until 7 pm), and food and water were provided *ad libitum* throughout the experimental period.

2.2. Ethical Statement

All experimental protocols were approved by the ethics committee of the Universidade Federal do Ceará (UFC) (CEUA: nº 9680310818) and all efforts were made to minimize animal

suffering in accordance with the proposals of the International Ethical Guideline for Biomedical Research (CIOMS, 1985).

2.3. Drugs

The following drugs were used: rotenone (Sigma-Aldrich®-USA), dimethyl sulfoxide (DMSO), Sunflower Oil (Cargill Agrícola SA®-BR), apomorphine (Sigma- Aldrich®-USA), Ketamine (König, Argentina) and Xylazine (König, Argentina). All other reagents used were of analytical grade.

Rotenone was prepared daily, protected from light, being initially diluted in 100% DMSO and then diluted in sunflower oil to reach a solution of 2.75 mg/mL in 98% sunflower oil and 2% DMSO. The suspensions were passed through the vortex at each stage of the preparation, such as when separating the doses and before administration to each rat.

2.4. Experimental Protocol

The rats were randomly divided into 2 groups. The control group (CTL; n=10) received vehicle (98% sunflower oil and 2% DMSO, 1ml/kg) intraperitoneally (i.p.) and another received rotenone (2.75mg/kg, i.p.), (ROT; n=48). Both groups were treated weekly (Monday to Friday.) for 21 days. This protocol is an adaptation of the model proposed by Cannon and colleagues (2009), in which intraperitoneal administration of rotenone during 21 consecutive days causes low or no peripheral toxicity. The adaptation to a model with intervals between applications aimed to decrease the mortality generated by rotenone.

Concomitant with the administration of rotenone, behavioral tests were performed on days 0, 3, 7, 14 and 21 after the beginning of drug administration, in order to establish the timeline of occurrence of such findings. For this, the rats were submitted to the open field test and apomorphine challenge (rotarod) (Figure 1).

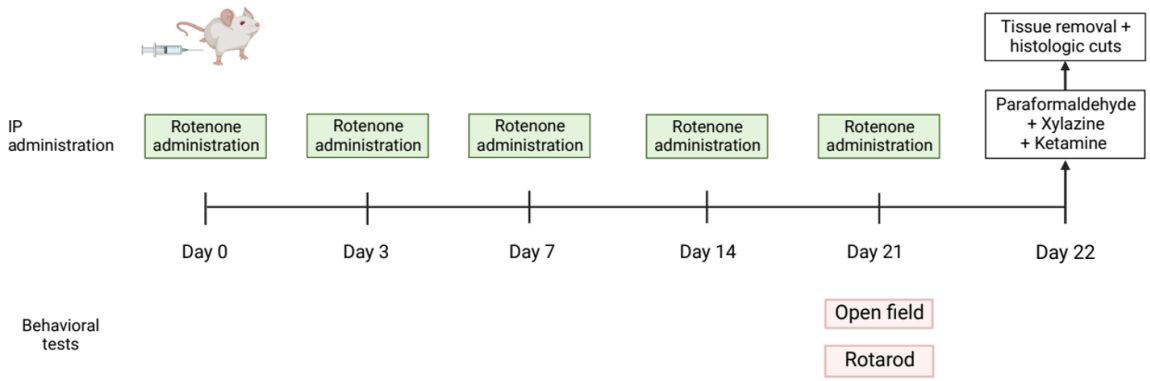

Figure 1. Experimental design. Male Wistar rats from the control (CTL; n = 10), and rotenone (ROT; *n*=10) groups were submitted to open field and rotarod tests. Twenty-four hours after the last rotenone administration and the behavioral test session, the rats were euthanized and the brains were removed for further analysis.

For the immunohistochemical analyses (n=10 per group), the rats were anesthetized with Ketamine (100 mg/kg) and Xylazine (10mg/ kg) and perfused with 4% paraformaldehyde in PBS (0.1 M phosphate buffer containing 0.9% saline, pH 7.4). The brains were removed, fixed in 10% buffered formalin for 24 h and then cryoprotected in 30% sucrose in PBS at 4oC, subsequently histological sections were made on the cryostat (10μm) mounted on silanized slides for analysis of brain, vagal nerve and proximal bowel damages (TH, GFAP and iba-1).

2.5. Behavioral tests

2.5.1. Open field

The exploratory activity of the animals was assessed by open field test on the day before the start of rotenone treatment (day 0) and on days 3, 7, 14, and 21 after the start of rotenone administration. The floor of the arena was divided into 9 equal quadrants. The rats were gently placed in the center of the arena to explore for 5 min. The animals were returned to their home cage immediately after the experiment finished. Crossings of quadrant lines and rearings were counted and used as measures of locomotion and exploration.

2.5.2. Rotarod Test

To evaluate motor coordination, balance, grip strength, and motor learning ability for smoothly walking, the rotarod test was performed using a rotarod apparatus MK-630B (Muromachi Kikai, Tokyo, Japan) according to the instructions. Briefly, animals were placed on a rotating cylinder, and a training session was performed at a constant speed of 4 rpm for 1 min. Mice were then subjected to the walking test on the accelerating spindle (4–20 rpm over 180 s), and the latency for rat falling off the cylinder was recorded. The rats that rotated passively were recorded as having fallen. The trial was performed three times with a 30-min interval between consecutive trials. Mean times of the test were recorded for each animal.

2.6. Body Weight

The animals were weighed daily until the 21th day of the experimental protocol and the variation in body mass was determined, as previously described. The values obtained were expressed as a weight variable in relation to the initial weight (Costa ***et al***., 2020).

2.7. Immunohistochemical Analyses

Immunohistochemistry for GFAP, S100, IBA 1 was performed using the streptavidin-biotin-peroxidase method (HSU; RAINE, 1981). Next, the tissues were dehydrated in alcohol and then embedded in paraffin. After this procedure, serial cuts of 4 μm were made using an appropriate microtome and placed on L-polylysine slides, suitable for performing immunohistochemistry. The cuts were deparaffinized, hydrated in xylene and alcohol and immersed in 0.1 M citrate buffer (pH 6.0), and (9.0. under heating in a water bath, for 30 minutes for antigen recovery at 95ºC. After cooling, obtained at room temperature for 20 minutes, washings were performed with phosphate buffered solution (PBS), interspersed with endogenous peroxidase blocking with 3% H2O2 solution (20 minutes). Then protein blocking was performed with 5% BSA (bovine albumin) for 20 minutes. The sections were incubated or even night with goat primary antibody GFAP (DAKO), S100 (DAKO) anti-IBA1 (Santa Cruz) 1:100 diluted in antibody diluent. After washing in PBS, an incubation with polymer HRP (Dako) for 30 minutes. After washing again with PBS, staining with the chromogen 3,3'diaminobenzidine-peroxide (DAB) followed by counterstaining with Mayer's hematoxylin. dehydration of samples and mounting of slides. were processed simultaneously as described above, with the primary antibody being replaced with PBS-BSA 5.

2.8. Morphological Analyses

After euthanasia, on day 23, the entire proximal intestine, brain and vagal nerve were removed, washed with 0.9% saline. A portion of the segment was fixed in 10% buffered formaldehyde, dehydrated and embedded in paraffin. Sections (5 µm thick) were obtained for hematoxylin and eosin (HE) staining and *subsequent* evaluation *using light microscope* (Olympus BH-2) at 200 × magnification, by an experienced observer, in a blinded manner.

2.9. Statistical analysis

Analyzes were performed using GraphPad Prism 6.0 Version for Windows (San Diego, CA, USA). All results are expressed as means ± SEM (standard errors of the mean). Behavioral, weight gain and histological analyzes were analyzed using the non-parametric Mann-Whitney test. The Spearman's correlation test was used for obtaining the *r* and the r-square (*r2*) values, that demonstrates the influence of protein expression level (S100, GFAP and IBA1) of a given protein in control and rotenone group, independently. For all analyses, the significance level was set at $P \leq 0.05$.

3. Results

3.1. Effect of rotenone on body weight in rats

Rotenone administration significantly decreased daily body weight on day 3 (CTL: 327.5±19.0g, ROT: 293.0±36.3g) and from day 5 to day 19 compared to rats in the control group (CTL 5: 336.5±21.4g, ROT 5: 296.5±22.7g; CTL 6: 346.5±19.8g, ROT 6: 310.0 ± 26.4g; CTL 7: 340.5±21.2g, ROT 7: 299.5±26.5g; CTL 8: 348.5±35.3g, ROT 8: 303.5±29.5g; CTL 9: 352.0±21.1g, ROT 9: 283.5±27.7g; CTL 10: 355.5±21.2g, ROT 10: 297.5±34.3g; CTL 11: 362.0±22.5g, ROT 11: 304.0±32.7g; CTL 12: 373.0±22.7g, ROT 12: 314.0±30.1g; CTL 13: 369.0±24.4g, ROT 13: 313.5±28.2g; CTL 14: 375.5±28.1g, ROT 14: 319.0±26.1g; CTL 15: 384.5±24.8g, ROT 15: 325.0±25.4g; CTL 16: 389.0±23.7g, ROT 16: 331.5±26.7g; CTL 17: 391.5±24.4g, ROT 17: 328.5±28.4g; CTL 18: 393.5±24.1g, ROT 18: 336.5±29.8g; CTL 19: 400.5±26.6g, ROT 19: 343.5±28.4g; $p<0.05$) (Figure 2). Taken together, these data showed that rotenone administration reduced the body weight of the rats.

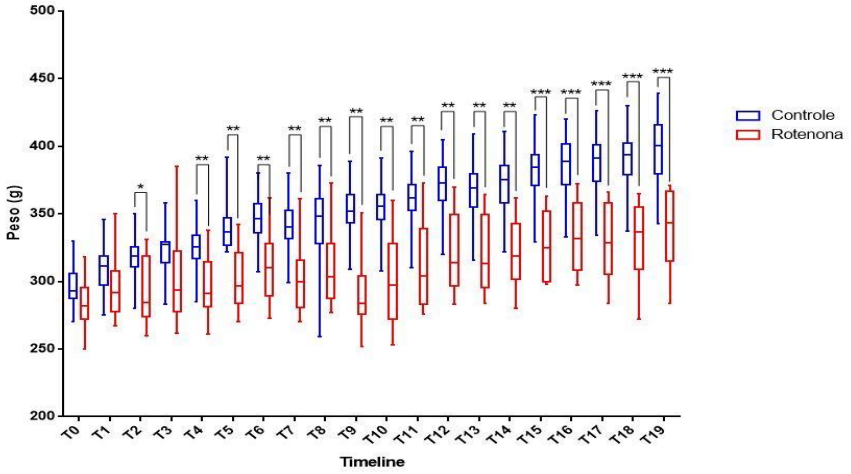

Figure 2. Time-dependent changes in body weight show the difference between PD rotetone-injected rat and non-rotenone injected rat. The rotenone administration reduced the body weight of the rats (*p < 0.05; **p < 0.01; ***p < 0.001; Mann-Whitney test).

3.2. Effect of rotenone on behavioral performance in rats

3.2.1. Open field test

Exploratory activity was measured in an open field apparatus on the 20th day of the experiment. Our data showed that the animals from the rotenone group exhibited reduced exploratory activity, compared to the control group [number of crossings (p=0.004; Figure 3B) and number of rearings (p=0.004; Figure 3C).

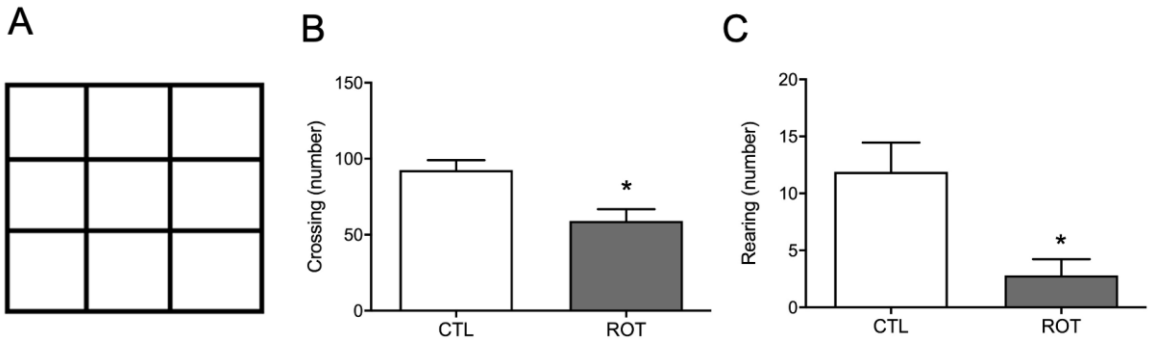

Figure 3. Open field quadrants (left; A) and exploratory activity (B and C) from control (CTL; *n*=10) and rotenone (ROT; *n*=10) groups. Exploratory activity is expressed as number of quadrants crossed (locomotion units) and number of rearings. Animals that received rotenone application exhibited reduced exploratory activity compared to the control group (*) (*p*<0,05; Mann Whitney).

3.2.2. Rotarod test

Motor activity was measured in the rotarod apparatus on the 20th day of the experiment. Our data showed that the rats from the rotenone group exhibited a lower latency (p<0.001) and a higher number of falls (p=0.004), compared to the control group (Figure 4). Taken together, these data show that rotenone reduces the exploratory and motor activity in rats.

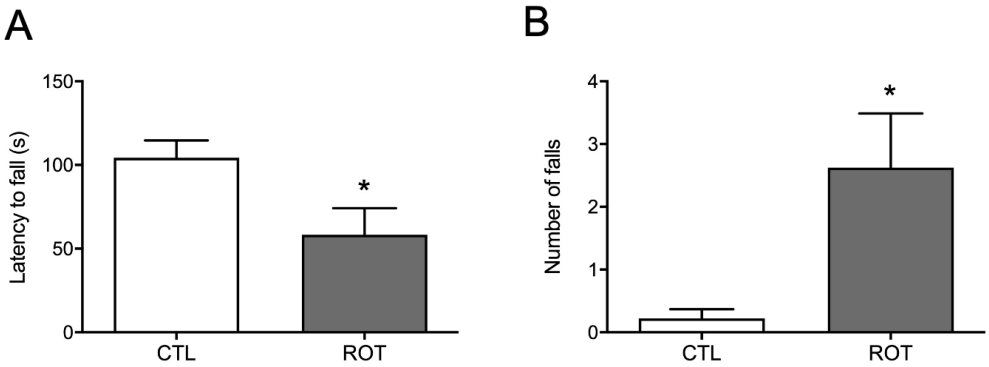

Figure 4. Latency to fall and number of falls in the rotarod test from control (CTL; *n*=10) and rotenone (ROT; *n*=10) groups. Rats that received rotenone application exhibited reduced motor activity, compared to the control group (*) (*p*<0,05; Mann Whitney).

3.3. Effect of rotenone on immunoreactivity for IBA-1 in rats

Our data showed that the rats from the rotenone group exhibited a significant increase in microgliosis, observed by iba-1 immunolabeling in the striatum (p=0.004) and segments of intestines (p=0.019).

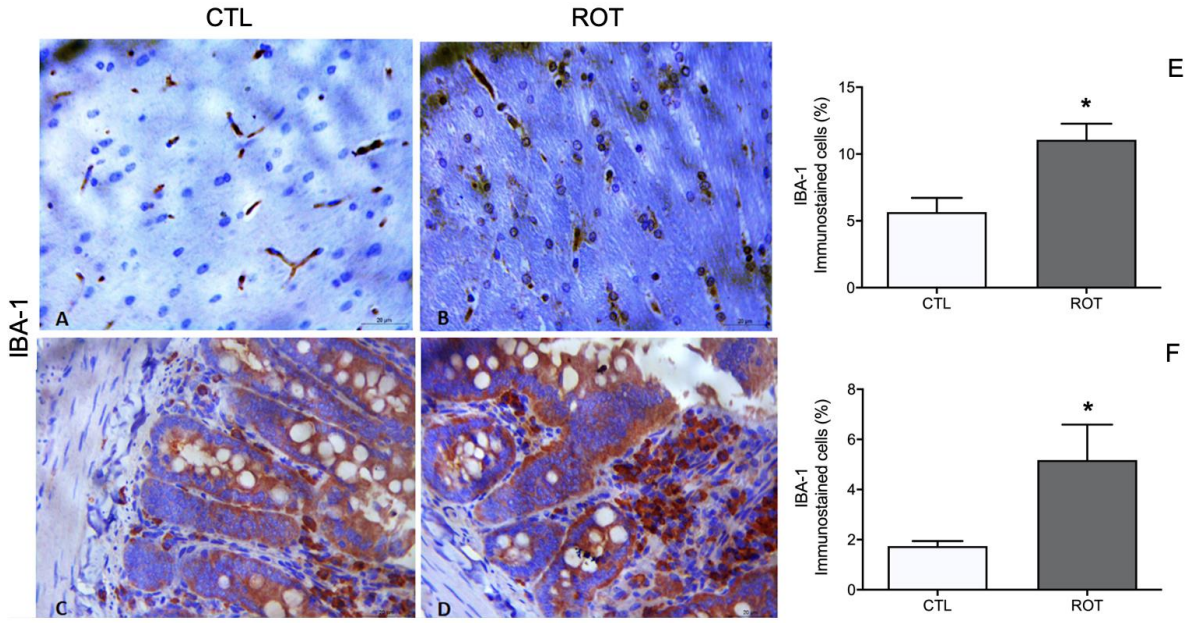

Figure 5. Immunoexpression of IBA 1 proteins in brain (A and B) and intestine segment (C and D). Segments of the intestines and brain were collected and processed to perform immunohistochemistry for IBA-1. Worksheet of the immunoexpression of IBA-1 proteins in the 2 groups of the experiment. The graphs represent the mean ± SEM of the percentage of the immunopositive area for IBA-1 in brain (E) and intestine (F) relation to the total area (p<0,05; Mann Whitney).

3.4. Effect of rotenone on immunoreactivity for GFAP in rats

The results showed that animals receiving rotenone showed increased immunoreactivity for GFAP, characteristic for astrogliosis, in the striatum (p=0.009) and intestinal segments (p=0.015).

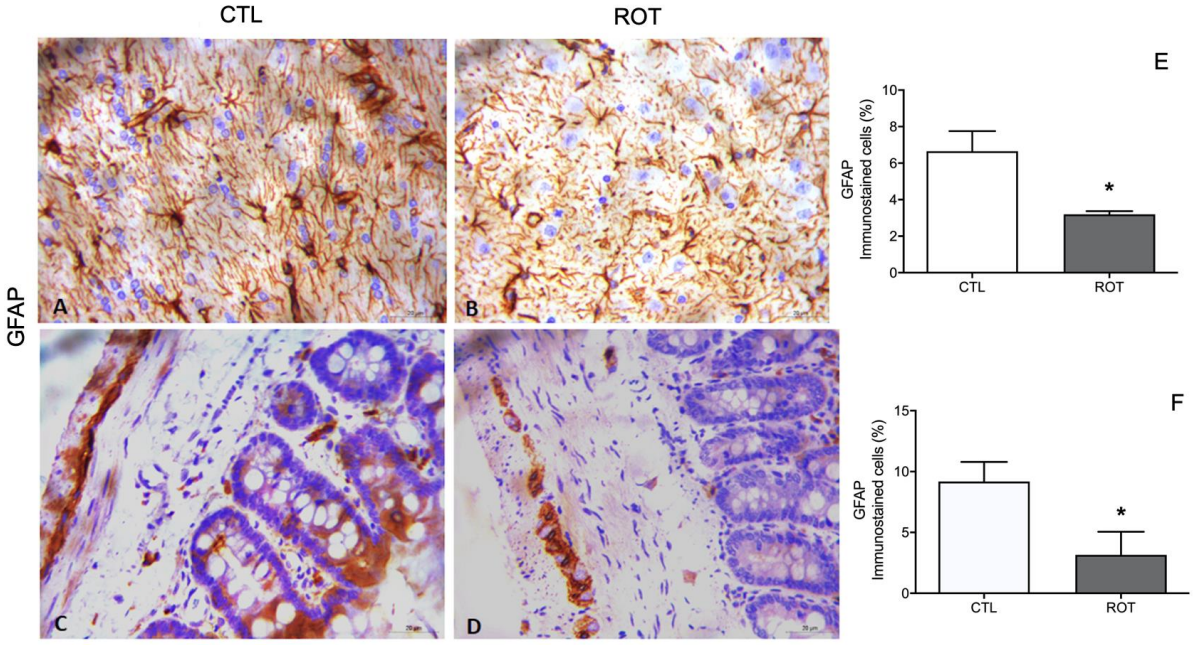

Figure 6. Immunoexpression of GFAP proteins in brain (A and B) and intestine segment (C and D). Segments of the intestines and brain were collected and processed to perform immunohistochemistry for GFAP. Worksheet of the immunoexpression of GFAP (Fibrillar acid protein) proteins in the 2 groups of the experiment. The graphs represent the mean ± SEM of the percentage of the immunopositive area for GFAP in brain (E) and intestine segment (F) relation to the total area (p<0,05; Mann Whitney).

3.5. Effect of rotenone on immunoreactivity for S100 in rats

Animals receiving rotenone showed a significant decrease in immunolabeling for S100 when compared to the control group, in the striatum ($p<0.001$) and intestinal segments ($p=0.040$).

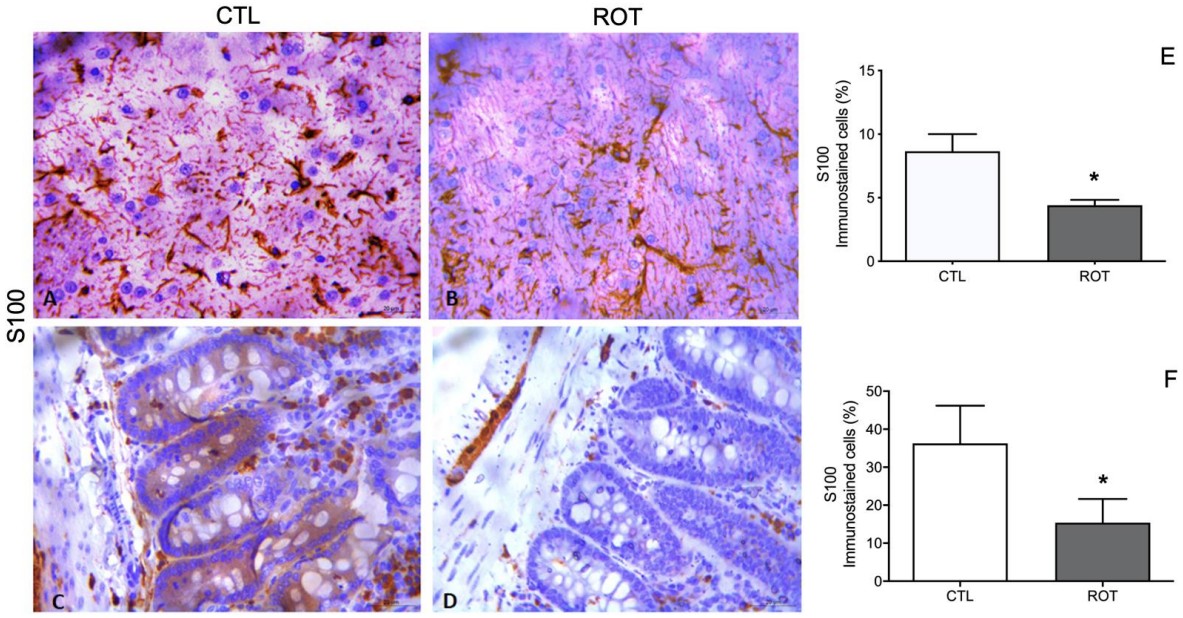

Figure 7. Immunoexpression of S100 proteins in brain (A and B) and intestine segment (C and D). Segments of the intestines and brain were collected and processed to perform immunohistochemistry for S100. Worksheet of the immunoexpression of S100 proteins in the 2 groups of the experiment. The graphs represent the mean ± SEM of the percentage of the immunopositive area for S100 in brain (E) and intestine segment (F) relation to the total area ($p<0,05$; Mann Whitney).

3.6. Correlations of the S100, GFAP and IBA1 immuno expressions in control and rotenone rat groups

By Spearman's correlation analysis, we observed significant and positive correlations to GFAP immunoexpression between brain and intestine segments in control mice group ($r = 0.771$; $r2 = 0.745$; $p = 0.003$). It is also verified that the expression of GFAP in the brain influences the expression of this protein in the intestine by 74.5% ($r2 = 0.745$). This result reinforces that GFAP work in a dependent manner as a cascade of events in health mice. However, this association was not identified in the rotenone group ($r = -0.371$; $r2 = 0.062$; $p = 0.235$), showing the effect of this drug on the brain-intestine axis.

We did not identify significant correlations between S100 (Control: $r = -0.018$; $p = 0.951$ / Rotenone: $r = 0.000$; $p = 1.000$) and IBA1 (Control: $r = 0.116$; $p = 0.720$ / Rotenone: $r = -0.029$; $p = 0.930$) immunostaining between brain and intestinal segments, either in the control or rotenone group.

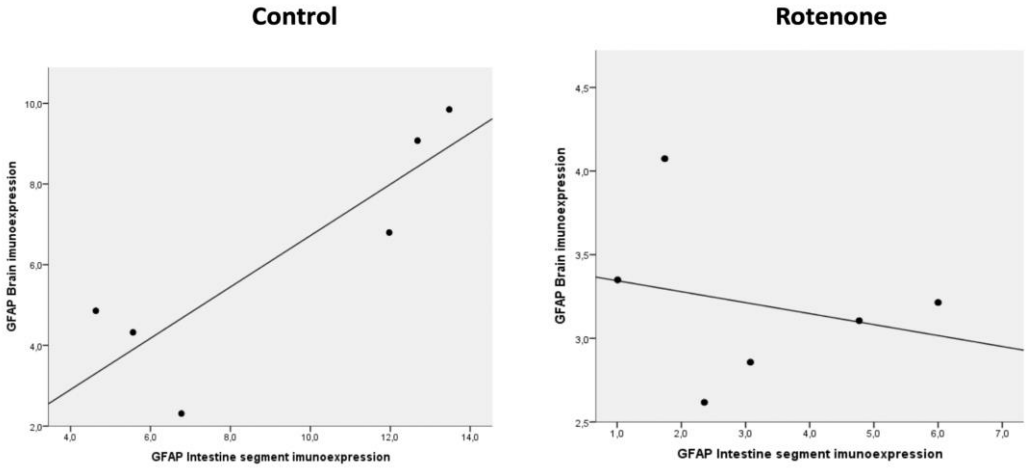

Figure 8. Spearman's correlation plot of GFAP immunoexpression in brain-intestine axis in control (A) and rotenone (B) groups, independently. Correlation plots represent the correlation between the density of positive GFAP cells in traditional chromogenic immunohistochemistry assay. Correlation analysis between the GFAP cell densities showed significant positive correlations in brain and intestine segments in control grupo (A). There was no evidence of GFAP correlation between brain and intestine in animals from the rotenone group (B). Data are Spearman's correlation values with $p < 0.05$.

4. Discussion

There are no models that optimally reproduce PD, so each one has its benefits and limitations when it comes to cellular, molecular and clinical aspects. Therefore, updating these current models to better approximate the pathophysiology of the disease is fundamental. Here, we describe an updated version of the rat model of PD (Betarbet *et al.*, 2000; Cannon *et al.*, 2009) induced by chronic, continuous, systemic intraperitoneal rotenone administration (Figure 1) with involvement of the gut and brain.

Using animal models of environmental exposure, rats subjected to systemic administration for 21 days of rotenone (2.75mg/kg) recapitulate the behavioral, cellular, and molecular features of PD, including L-DOPA-responsive motor deficits, accumulation and aggregation of alphasynuclein with Lewy body formation, systemic mitochondrial impairment, oxidative stress, microglial activation (Alam *et al.*, 2004; Betarbet *et al.*, 2006; Sakai *et al.*, 1994), neurodegeneration, astrogliosis, and microgliosis. It is commonly proposed that the cardinal signs of PD appear, the loss of dopaminergic neurons of the SNpc is around 50-70% (Dauer *et al.*, 2003; Ross *et al.*, 2004).

PD is widely recognized as a hypokinetic dysfunction included in movement disorders with slow and continuous loss of dopaminergic neurons in the SNpc (Wang *et al.*, 2015). However, numerous recent studies suggest that PD constitutes a heterogeneous systemic disorder and PD patients present with both motor and non-motor symptoms (Hou *et al.*, 2019; Pfeiffer, 2016). Constipation is one of the most frequent non-motor symptoms in Parkinson's disease (Sakakibara *et al.*, 2003) with about 50% (Ashraf *et al.*, 1997) to 80% (Verbaan *et al.*, 2007) of patients being affected with this condition. In a study by Savica et al. it was reported that constipation may precede motor symptoms by up to 20 years and people with constipation may have a relatively increased risk of developing PD (Lin *et al.*, 2014). In previous studies, it was evidenced that constipation was associated with the duration and severity of PD (Krogh *et al.*, 2008) and the frequency and severity of constipation increased as PD progressed (Edwards *et al.*, 1993). In our study, rotenone-treated animals had lower expression of neurons in the enteric nervous system and the striatum nucleus (Figure 8). Studies highlight a relationship between degeneration of neurons in the enteric nervous system and changes in intestinal

contractility (Tasselli *et al.*, 2013). In the gut, pro-inflammatory stimuli, including IL-1, IL-6, and LPS, cause a pathological activation of enteric glia. As a consequence, the expression of glial markers such as GFAP and S100 increase significantly triggering the release of pro-inflammatory cytokines, glial cell-derived neurotrophic factors and other immunomodulatory signaling molecules (Rühl *et al.*, 2001) that may underlie the impaired gastrointestinal functionality in PD.

There are no models that optimally reproduce PD, so each one has its benefits and limitations when it comes to cellular, molecular and clinical aspects. Therefore, updating these current models to better approximate the pathophysiology of the disease is fundamental. Here, we describe an updated version of the rat model of PD (Betarbet *et al.*, 2000; Cannon *et al.*, 2009) induced by chronic, continuous, systemic intraperitoneal rotenone administration (Figure 1) with involvement of the gut and brain.

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Weight loss consists of a relatively common clinical finding among patients in all stages of Parkinson's disease and it has been proposed that it may be a prodromal feature of PD (Kistner *et al.*, 2014; Logroscino *et al.*, 2007; Chen *et al.*, 2003). This weight loss has been related mainly to female sex (Lorefält *et al.*, 2004), dopaminergic therapy (Bachmann *et al.*, 2009), dysautonomia (Umehara *et al.*, 2017), and olfactory dysfunction (Sharma *et al.*, 2012), as well as greater severity of motor features (Sharma *et al.*, 2014). In our study, rotenone-treated animals had a severe reduction in body weight, especially from the fourth day of treatment when compared to the control group, corroborating with the model induced by Alam *et al.* (2002). The mechanisms underlying rotenone-induced weight loss are mainly related to decreased intestinal transit and motility (Betarbet *et al.*, 2000; Bu *et al.*, 2019).

The motor symptoms of PD are attributed to the selective loss of striatal dopaminergic neurons and are characterized by bradykinesia, rigidity, resting tremor, and postural instability (DeMaagd *et al.*, 2015). The onset of motor symptoms is usually unilateral and asymmetry persists throughout the disease (Poewe *et al.*, 2017). In our study, we observed significantly reduced locomotor activity and postural changes in behavioral tests represented by exploratory activity and rearing of the animals after 21 days of rotenone treatment when compared to the control group. Similar findings are found in the work of Rocha *et al.* (2022) that showed that chronic exposure to rotenone in mice reduced locomotor activity.

In our murine model of PD induced by systemic rotenone, histopathological examination of treated animals revealed progressive activation of glial cells in the striatum and gut. This activation was associated with neuronal loss, astrogliosis and microgliosis. Reactive and dystrophic microglia appear before neurodegeneration in the context of PD (Doorn *et al.*, 2014). Microglial cells act as innate immune cells resident in the CNS, after which they encounter pathogens or toxins, they rapidly convert from a resting M2 state to a pro-inflammatory M1 state. These innate inflammatory immune responses have been found in PD patients as increased levels of IL-1 beta, IL-6, and reactive microglia (Lecours *et al.*, 2018). A study employing chronic exposure of C547Bl/6 mice to low levels of rotenone in the diet reported increases in astrocyte-derived cytokine (Rocha *et al.*, 2022). Similar to the responses observed in microglia, there was an increase in astrocyte expression in the intestine and striatum of rotenone-treated animals.

5. Conclusion

In this exploratory study, the experimental model of Parkinson's disease induced by systemic, chronic, continuous (21 days) intraperitoneal administration of rotenone (2.75 mg/kg) likely reproduces motor deficits, neurodegeneration, and neuroinflammation. In this sense, the model reported here reproduces cellular, biochemical and behavioral features similar to PD, representing a significant tool to investigate the interaction between the brain and gut and the multiple mechanisms involved in the neurodegeneration underlying this neurological disease.

References

Alam M, Schmidt WJ. L-DOPA reverses the hypokinetic behaviour and rigidity in rotenone-treated rats. *Behav Brain Res.* 2004 Aug 31;153(2):439-46. doi: 10.1016/j.bbr.2003.12.021. PMID: 15265640.

Alam M, Schmidt WJ. Rotenone destroys dopaminergic neurons and induces parkinsonian symptoms in rats. *Behav Brain Res*. 2002 Oct 17;136(1):317-24. doi: 10.1016/s0166-4328(02)00180-8. PMID: 12385818.

Ashraf W, Pfeiffer RF, Park F, Lof J, Quigley EM. Constipation in Parkinson's disease: objective assessment and response to psyllium. *Mov Disord.* 1997 Nov;12(6):946-51. doi: 10.1002/mds.870120617. PMID: 9399219.

Bachmann CG, Zapf A, Brunner E, Trenkwalder C. Dopaminergic treatment is associated with decreased body weight in patients with Parkinson's disease and dyskinesias. *Eur J Neurol*. 2009 Aug;16(8):895-901. doi: 10.1111/j.1468-1331.2009.02617.x. Epub 2009 Apr 3. PMID: 19374662.

Betarbet R, Sherer TB, MacKenzie G, Garcia-Osuna M, Panov AV, Greenamyre JT. Chronic systemic pesticide exposure reproduces features of Parkinson's disease. *Nat Neurosci*. 2000 Dec;3(12):1301-6. doi: 10.1038/81834. PMID: 11100151.

Betarbet R, Canet-Aviles RM, Sherer TB, Mastroberardino PG, McLendon C, Kim JH, Lund S, Na HM, Taylor G, Bence NF, Kopito R, Seo BB, Yagi T, Yagi A, Klinefelter G, Cookson MR, Greenamyre JT. Intersecting pathways to neurodegeneration in Parkinson's disease: effects of the pesticide rotenone on DJ-1, alpha-synuclein, and the ubiquitin-proteasome system. *Neurobiol Dis*. 2006 May;22(2):404-20. doi: 10.1016/j.nbd.2005.12.003. Epub 2006 Jan 24. PMID: 16439141.

Bu J, Qiao X, He Y, Liu J. Colonic electrical stimulation improves colonic transit in rotenone-induced Parkinson's disease model through affecting enteric neurons. *Life Sci*. 2019 Aug 15;231:116581. doi: 10.1016/j.lfs.2019.116581. Epub 2019 Jun 17. PMID: 31220524.

Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT. A highly reproducible rotenone model of Parkinson's disease. *Neurobiol Dis*. 2009 May;34(2):279-90. doi: 10.1016/j.nbd.2009.01.016. PMID: 19385059; PMCID: PMC2757935.

Chen H, Zhang SM, Hernán MA, Willett WC, Ascherio A. Weight loss in Parkinson's disease. *Ann Neurol*. 2003 May;53(5):676-9. doi: 10.1002/ana.10577. PMID: 12731005.

CIOMS *International Guiding Principles for Biomedical Research Involving Animals.* Altern Lab Anim. 1985;12: ii.

Costa DVS, Costa DVS, Sousa CNS, Silva AMHP, Medeiros IS, Martins DS, Martins CS, Pequeno ALV, Lima-Júnior RCP, Soares PMG, Vasconcelos SMM, Brito GAC, Souza EP. The Alpha-Lipoic Acid Improves Survival and Prevents Irinotecan-Induced Inflammation and Intestinal Dysmotility in Mice. *Pharmaceuticals (Basel)*. 2020 Nov 3;13(11):361. doi: 10.3390/ph13110361. PMID: 33152996; PMCID: PMC7692584.

Dauer W, Przedborski S. Parkinson's disease: mechanisms and models. *Neuron*. 2003 Sep 11;39(6):889-909. doi: 10.1016/s0896-6273(03)00568-3. PMID: 12971891.

Doorn KJ, Moors T, Drukarch B, van de Berg WDj, Lucassen PJ, van Dam AM. Microglial phenotypes and toll-like receptor 2 in the substantia nigra and hippocampus of incidental Lewy body disease cases and Parkinson's disease patients. *Acta Neuropathol Commun*. 2014 Aug 7;2:90. doi: 10.1186/s40478-014-0090-1. PMID: 25099483; PMCID: PMC4224021.

Edwards L, Quigley EM, Hofman R, Pfeiffer RF. Gastrointestinal symptoms in Parkinson disease: 18-month follow-up study. *Mov Disord*. 1993;8(1):83-6. doi: 10.1002/mds.870080115. PMID: 8093549.

Fornai F, Schlüter OM, Lenzi P, Gesi M, Ruffoli R, Ferrucci M, Lazzeri G, Busceti CL, Pontarelli F, Battaglia G, Pellegrini A, Nicoletti F, Ruggieri S, Paparelli A, Südhof TC. Parkinson-like syndrome induced by continuous MPTP infusion: convergent roles of the ubiquitin-proteasome system and alpha-synuclein. *Proc Natl Acad Sci USA*. 2005 Mar 1;102(9):3413-8. doi: 10.1073/pnas.0409713102. Epub 2005 Feb 16. PMID: 15716361; PMCID: PMC552938.

GBD 2016 Neurology Collaborators. Global, regional, and national burden of neurological disorders, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. *Lancet Neurol* 2019;18(5):459-480. doi:10.1016/S1474- 4422(18)30499-X

Hirsch E, Graybiel AM, Agid YA. Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. *Nature*. 1988 Jul 28;334(6180):345-8. doi: 10.1038/334345a0. PMID: 2899295.

Hou L, Sun F, Huang R, Sun W, Zhang D, Wang Q. Inhibition of NADPH oxidase by apocynin prevents learning and memory deficits in a mouse Parkinson's disease model. *Redox Biol*. 2019 Apr;22:101134. doi: 10.1016/j.redox.2019.101134. Epub 2019 Feb 8. PMID: 30798073; PMCID: PMC6389731.

Kistner A, Lhommée E, Krack P. Mechanisms of body weight fluctuations in Parkinson's disease. *Front Neurol*. 2014 Jun 2;5:84. doi: 10.3389/fneur.2014.00084. PMID: 24917848; PMCID: PMC4040467.

Krogh K, Ostergaard K, Sabroe S, Laurberg S. Clinical aspects of bowel symptoms in Parkinson's disease. *Acta Neurol Scand*. 2008 Jan;117(1):60-4. doi: 10.1111/j.1600-0404.2007.00900.x. PMID: 18095955.

Lama J, Buhidma Y, Fletcher EJR, Duty S. Animal models of Parkinson's disease: a guide to selecting the optimal model for your research. *Neuronal Signal*. 2021 Dec 8;5(4):NS20210026. doi: 10.1042/NS20210026. PMID: 34956652; PMCID: PMC8661507.

Lecours C, Bordeleau M, Cantin L, Parent M, Paolo TD, Tremblay MÈ. Microglial Implication in Parkinson's Disease: Loss of Beneficial Physiological Roles or Gain of Inflammatory Functions? *Front Cell Neurosci*. 2018 Aug 30;12:282. doi: 10.3389/fncel.2018.00282. PMID: 30214398; PMCID: PMC6125334.

Lin CH, Lin JW, Liu YC, Chang CH, Wu RM. Risk of Parkinson's disease following severe constipation: a nationwide population-based cohort study. *Parkinsonism Relat Disord.* 2014 Dec;20(12):1371-5. doi: 10.1016/j.parkreldis.2014.09.026. Epub 2014 Sep 28. PMID: 25293395.

Logroscino G, Sesso HD, Paffenbarger RS Jr, Lee IM. Body mass index and risk of Parkinson's disease: a prospective cohort study. *Am J Epidemiol*. 2007 Nov 15;166(10):1186-90. doi: 10.1093/aje/kwm211. Epub 2007 Aug 20. PMID: 17709328.

Lorefält B, Ganowiak W, Pålhagen S, Toss G, Unosson M, Granérus AK. Factors of importance for weight loss in elderly patients with Parkinson's disease. *Acta Neurol Scand*. 2004 Sep;110(3):180-7. doi: 10.1111/j.1600-0404.2004.00307.x. PMID: 15285776.

Pfeiffer RF. Non-motor symptoms in Parkinson's disease. *Parkinsonism Relat Disord*. 2016 Jan;22 Suppl 1:S119-22. doi: 10.1016/j.parkreldis.2015.09.004. Epub 2015 Sep 3. PMID: 26372623.

Poewe W, Seppi K, Tanner CM, Halliday GM, Brundin P, Volkmann J, Schrag AE, Lang AE. Parkinson disease. *Nat Rev Dis Primers*. 2017 Mar 23;3:17013. doi: 10.1038/nrdp.2017.13. PMID: 28332488.

Ramesh V, Bilal E. Detecting motor symptom fluctuations in Parkinson's disease with generative adversarial networks. *NPJ Digit Med*. 2022 Sep 9;5(1):138. doi: 10.1038/s41746-022-00674-x. PMID: 36085350; PMCID: PMC9463161.

Rocha SM, Bantle CM, Aboellail T, Chatterjee D, Smeyne RJ, Tjalkens RB. Rotenone induces regionally distinct α-synuclein protein aggregation and activation of glia prior to loss of dopaminergic neurons in C57Bl/6 mice. *Neurobiol Dis*. 2022 Jun 1;167:105685. doi: 10.1016/j.nbd.2022.105685. Epub 2022 Mar 5. PMID: 35257879; PMCID: PMC9615439.

Ross GW, Petrovitch H, Abbott RD, Nelson J, Markesbery W, Davis D, Hardman J, Launer L, Masaki K, Tanner CM, White LR. Parkinsonian signs and substantia nigra neuron density in decendents elders without PD. *Ann Neurol*. 2004 Oct;56(4):532-9. doi: 10.1002/ana.20226. PMID: 15389895.

Rühl A, Franzke S, Collins SM, Stremmel W. Interleukin-6 expression and regulation in rat enteric glial cells. *Am J Physiol Gastrointest Liver Physiol*. 2001 Jun;280(6):G1163-71. doi: 10.1152/ajpgi.2001.280.6.G1163. PMID: 11352809.

Sharma JC, Vassallo M. Prognostic significance of weight changes in Parkinson's disease: the Park-weight phenotype. *Neurodegener Dis Manag*. 2014;4(4):309-16. doi: 10.2217/nmt.14.25. PMID: 25313987.

Sharma JC, Turton J. Olfaction, dyskinesia and profile of weight change in Parkinson's disease: identifying neurodegenerative phenotypes. *Parkinsonism Relat Disord*. 2012 Sep;18(8):964-70. doi: 10.1016/j.parkreldis.2012.05.004. Epub 2012 Jun 8. PMID: 22682755.

Sakai K, Gash DM. Effect of bilateral 6-OHDA lesions of the substantia nigra on locomotor activity in the rat. *Brain Res*. 1994 Jan 7;633(1-2):144-50. doi: 10.1016/0006-8993(94)91533-4. PMID: 7907930.

Sakakibara R, Odaka T, Uchiyama T, Asahina M, Yamaguchi K, Yamaguchi T, Yamanishi T, Hattori T. Colonic transit time and rectoanal videomanometry in Parkinson's disease. *J Neurol Neurosurg Psychiatry*. 2003 Feb;74(2):268-72. doi: 10.1136/jnnp.74.2.268. PMID: 12531969; PMCID: PMC1738286.

Seidel K, Mahlke J, Siswanto S, Krüger R, Heinsen H, Auburger G, Bouzrou M, Grinberg LT, Wicht H, Korf HW, den Dunnen W, Rüb U. The brainstem pathologies of Parkinson's disease and dementia with Lewy bodies. *Brain Pathol*. 2015 Mar;25(2):121-35. doi: 10.1111/bpa.12168. Epub 2014 Sep 12. PMID: 24995389; PMCID: PMC4397912.

Sharma JC, Turton J. Olfaction, dyskinesia and profile of weight change in Parkinson's disease: identifying neurodegenerative phenotypes. *Parkinsonism Relat Disord*. 2012 Sep;18(8):964-70. doi: 10.1016/j.parkreldis.2012.05.004. Epub 2012 Jun 8. PMID: 22682755.

Tasselli M, Chaumette T, Paillusson S, Monnet Y, Lafoux A, Huchet-Cadiou C, Aubert P, Hunot S, Derkinderen P, Neunlist M. Effects of oral administration of rotenone on gastrointestinal functions in mice. *Neurogastroenterol Motil*. 2013 Mar;25(3):e183-93. doi: 10.1111/nmo.12070. Epub 2013 Jan 3. PMID: 23281940.

Thaler A, Alcalay RN. Diagnosis and Medical Management of Parkinson Disease. *Continuum (Minneap Minn).* 2022 Oct 1;28(5):1281-1300. doi: 10.1212/CON.0000000000001152. PMID: 36222765.

Umehara T, Nakahara A, Matsuno H, Toyoda C, Oka H. Body weight and dysautonomia in early Parkinson's disease. *Acta Neurol Scand*. 2017 May;135(5):560-567. doi: 10.1111/ane.12633. Epub 2016 Jun 30. PMID: 27357472.

Verbaan D, Marinus J, Visser M, van Rooden SM, Stiggelbout AM, van Hilten JJ. Patient-reported autonomic symptoms in Parkinson disease. *Neurology*. 2007 Jul 24;69(4):333-41. doi: 10.1212/01.wnl.0000266593.50534.e8. PMID: 17646625.

Vingill S, Connor-Robson N, Wade-Martins R. Are rodent models of Parkinson's disease behaving as they should? *Behav Brain Res.* 2018 Oct 15;352:133-141. doi: 10.1016/j.bbr.2017.10.021. Epub 2017 Oct 24. PMID: 29074404.

Wang Q, Qian L, Chen SH, Chu CH, Wilson B, Oyarzabal E, Ali S, Robinson B, Rao D, Hong JS. Post-treatment with an ultra-low dose of NADPH oxidase inhibitor diphenyleneiodonium attenuates disease progression in multiple Parkinson's disease models. *Brain*. 2015 May;138(Pt 5):1247-62. doi: 10.1093/brain/awv034. Epub 2015 Feb 25. PMID: 25716193; PMCID: PMC4407187.

Wirdefeldt K, Adami HO, Philip C, Trichopoulos D, Mandel J. Epidemiology and etiology of Parkinson’s disease: a review of the evidence. *Eur J Epidemiol* 2011;26(suppl 1):S1-S58. doi:10.1007/s10654-011-9581-6

4 CONCLUSÕES GERAIS

Dentro dos limites deste estudo, pode-se concluir que:

1. A rotenona promove a redução da atividade exploratória dos animais induzidos com o modelo de Parkinson;
2. A rotenona promove a baixa latência e alto número de quedas dos animais induzidos com o modelo de Parkinson;
3. A rotenona aumentou a imunomarcação de IBA-1 nos animais com o modelo de Parkinson;
4. A rotenona aumentou a imunomarcação de GFAP nos animais com o modelo de Parkinson.
5. A rotenona diminui a imunomarcação de S100 nos animais com o modelo de Parkinson.

REFERÊNCIAS

ASCHERIO, A.; SCHWARZSCHILD, M.A. The epidemiology of Parkinson's disease: risk factors and prevention. **Lancet Neurol**, v.15, n.12, p.1257-1272, Nov. 2016. https://doi.org./10.1016/S1474-4422(16)30230-7

BAIZABAL-CARVALHO, J.F.; ALONSO-JUAREZ, M. The Link between Gut Dysbiosis and Neuroinflammation in Parkinson's Disease. **Neuroscience**., v.15, n.432, p.160-173, Apr. 2020. https://doi.org./10.1016/j.neuroscience.2020.02.030.

BANKS, W.A.; GRAY, A.M.; ERICKSON, M.A.; SALAMEH, T.S. *et al*. Lipopolysaccharide-induced blood-brain barrier disruption: roles of cyclooxygenase, oxidative stress, neuroinflammation, and elements of the neurovascular unit. **J Neuroinflammation,** v.12, n.223, p.1-15, Nov. 2015. https://doi.org.q10.1186/s12974-015-0434-1.

BANSKOTA, S.; GHIA, J.E.; KHAN, W.I. Serotonin in the gut: Blessing or a curse. **Biochimie**, v.161, p.56-64, Jun. 2019. https://doi.org./10.1016/j.biochi.2018.06.008.

BELKIND-GERSON, J.; GRAHAM, H.K.; REYNOLDS, J.; HOTTA, R.; NAGY, N. *et al.* Colitis promotes neuronal differentiation of Sox2+ and PLP1+ enteric cells. **Sci Rep**, v.7, n.1, p.1-15, 2017. https://doi.org./10.1038/s41598-017-02890-y.

BENVENUTI, L.; D'ANTONGIOVANNI, V.; PELLEGRINI, C.; ANTONIOLI, L.; BERNARDINI, N.; BLANDIZZI, C.; FORNAI, M. Enteric Glia at the Crossroads between Intestinal Immune System and Epithelial Barrier: Implications for Parkinson Disease. **Int J Mol Sci**., v.21, n.23, p.2-16, Dec. 2020. https://doi.org./10.3390/ijms21239199

BERARDELLI, A.; ROTHWELL, J.C.; THOMPSON, P.D.; HALLET, M. Pathophysiology of bradykinesia in Parkinson's disease. Brain 2001;124(pt 11):2131–2146. https://doi.org./10.1093/brain/124.11.2131.

BERG, D.; BORGHAMMER, P.; FERESHTEHNEJAD, S.M.; HEINZEL, S.; HORSAGER, J.; SCHAEFFER, E.; POSTUMA, R.B. Prodromal Parkinson disease subtypes - key to understanding heterogeneity. **Nat Rev Neurol**. 2021 Jun;v.17, n.6, p.349-361, Jun. 2021. https://doi.org./10.1038/s41582-021-00486-9.

BERG, D.; POSTUMA, R.B.; ADLER, C.H.; BLOEM, B.R.; CHAN, P. *et al*. MDS research criteria for prodromal Parkinson's disease. **Movement Disorders**, v.30, n.12, p.1600-1611, Oct. 2015. https://doi.org./10.1002/mds.26431

BERRE-SCOUL, L.C.; CHEVALIER, J.; OLEYNIKOVA, E.; COSSAIS, F.; TALON, S.; NEUNLIST, M.; BOUDIN, H. A novel enteric neuron-glia coculture system reveals the role of glia in neuronal development. **J Physiol**., v.595, n.2, p.583-598, Jan. 2017. https://doi.org./10.1113/JP271989.

BOESMANS, W.; LASRADO, R.; BERGHE, P.V.; PACHNIS, V. Heterogeneity and phenotypic plasticity of glial cells in the mammalian enteric nervous system. **Glia,** v.63, n.2, p.229-241, 2015. https://doi.org./10.1002/glia.22746.

BOESMANS, W.; NASH, A.; TASNÁDY, K.R.; YANG, W.; STAMP, L.A.; HAO, M.M. Development, Diversity, and Neurogenic Capacity of Enteric Glia. **Front Cell Dev Biol.,** v.9, n.775102, p.1-20. Jan. 2022. https://doi.org./10.3389/fcell.2021.775102.

BOHÓRQUEZ, D.V.; SAMSA, L.A.; ROHOLT, A.; MEDICETTY, S.; CHANDRA, R.; LIDDLE, R.A. An enteroendocrine cell-enteric glia connection revealed by 3D electron microscopy. **PLoS One**., v.9, n.2, p.e89881. Fev. 2014. https://doi.org./10.1371/journal.pone.0089881.

BOHÓRQUEZ, D.V.; SHAHID, R.A.; ERDMANN, A.; KREGER, A.M.; WANG, Y.; CALAKOS, N.; WANG, F.; LIDDLE, R.A. Neuroepithelial circuit formed by innervation of sensory enteroendocrine cells. **J Clin Invest**., v.125, n.2, p.782-786, Feb. 2015. https://doi.org./10.1172/JCI78361.

BROWN, I.A.M.; McCLAIN, J.L.; WATSON, R.E.; PATEL, B.A.; GULBRANSEN, B.D. Enteric glia mediate neuron death in colitis through purinergic pathways that require connexin-43 and nitric oxide. **Cell. Mol. Gastroenterol. Hepatol**., v.2, n.1, p.77-91, Jan. 2016. https://doi.org./101016/j.jcmgh.2015.08.007.

CAPOCCIA, E.; CIRILLO, C.; GIGLI, S.; PESCE, M.; D'ALESSANDRO; CUOMO, R.; SARNELLI, G.; STEARDO, L.; ESPOSITO, G. Enteric glia: A new player in inflammatory bowel diseases. **Int J Immunopathol Pharmacol**., v.28, n.4, p.443-451, Dec. 2015. https://doi.org./10.1177/0394632015599707.

CHAHINE, L.M. Prodromal α-Synucleinopathies. **Continuum**, v.28, n.5, p.1268-1280, Oct. 2022. https://doi.org./10.1212/CON.0000000000001153

CHAKRABARTI, A.; GEURTS, L.; HOYLES, L; LOZZO, P.; KRANEVELD, A.D. *et al*. The microbiota-gut-brain axis: pathways to better brain health. Perspectives on what we know, what we need to investigate and how to put knowledge into practice. **Cell Mol Life Sci**., v.79, n.80, p.1-15, Jan. 2022. https://doi.org./10.1007/s00018-021-04060-w.

CHANDRA, R.; HINIKER, A.; KUO, Y.M., NUSSBAUM, R.L.; LIDDLE, R.A. α-Synuclein in gut endocrine cells and its implications for Parkinson's disease. **JCI Insight**, v.2, n.12, p.1-13, Jun. 2017. https://doi.org./10.1172/jci.insight.92295.

CHEN, H. The Changing Landscape of Parkinson Epidemiologic Research. **J Parkinsons Dis**. v.8, n.1, p.1-12, 2018. https://doi.org./10.3233/JPD-171238.

CHEN, S.J.; LIN, C.H. Gut microenvironmental changes as a potential trigger in Parkinson's disease through the gut–brain axis. **J Biomed Sci,** v.29, n.54, p.1-18, July 2022. https://doi.org/10.1186/s12929-022-00839-6.

CHOW, A.K.; GRUBIŠIĆ, V.; GULBRANSEN, B.D. Enteric Glia Regulate Lymphocyte Activation via Autophagy-Mediated MHC-II Expression. **Cell Mol Gastroenterol Hepatol**., v.12, n.4, p.1215-1237, 2021. https://doi.org./10.1016/j.jcmgh.2021.06.008.

CHOW, A.K.; GULBRANSEN, B.D. Potential roles of enteric glia in bridging neuroimmune communication in the gut. **Am J Physiol Gastrointest Liver Physiol.**, v.312, n.2, p.G145-G152, Feb. 2017. https://doi.org./10.1152/ajpgi.00384.2016.

CLAIREMBAULT, T.; LECLAIR-VISONNEAU, L.; NEUNLIST, M.; DERKINDEREN, P. Enteric glial cells: new players in Parkinson's disease? **Mov Disord**., v.30, n.4, p.494-498, Apr. 2015. https://doi.org./10.1002/mds.25979.

COSTELLO, S.; COCKBURN, M.; BRONSTEIN, J.; ZHANG, X.; RITZ, B. Parkinson's disease and residential exposure to maneb and paraquat from agricultural applications in the central valley of California. **Am J Epidemiol**., v.169, n.8, p.919-926, Apr. 2009. https://doi.org./10.1093/aje/kwp006.

DELVALLE, N.M.; DHARSHIKA, C.; MORALES-SOTO, W.; FRIED, D.E.; GAUDETTE, L.; BULBRANSEN, B.D. Communication Between Enteric Neurons, Glia, and Nociceptors Underlies the Effects of Tachykinins on Neuroinflammation. **Cell Mol Gastroenterol Hepatol**., 2018 May v.6, n.3, p.321-344, May, 2018. https://doi.org./10.1016/j.jcmgh.2018.05.009.

DENG, H.; WANG, P.; JANKOVIC, J. The genetics of Parkinson disease. **Ageing Res Rev.**, v.42, p.72-85, Mar. 2018. https://doi.org./10.1016/j.arr.2017.12.007.

DEVOS, D.; LEBOUVIER, T.; LARDEUX, B.; BIRAUD, M.; ROUAUD, T. *et al.* Colonic inflammation in Parkinson's disease. **Neurobiol Dis**., v.50, p.42-48, Feb. 2013. https://doi.org./10.1016/j.nbd.2012.09.007

DUTTA, S.K.; VERMA, S.; JAIN, V.; SURAPANENI, B.K.; VINAYEK, R.; PHILLIPS, L.; NAIR, P.P. Parkinson’s Disease: The Emerging Role of Gut Dysbiosis, Antibiotics, Probiotics, and Fecal Microbiota Transplantation. **J Neurogastroenterol Motil**., v.25, n.3, p.363-376, Jul. 2019. https://doi.org./10.5056/jnm19044.

EDWARDS, L.; QUIGLEY, M.M.; HOFMAN, R.; PFEIFFER, R.F. Gastrointestinal symptoms in Parkinson disease: 18-month follow-up study. **Mov Disord**., v.8, n.1, p.83-86, 1993. https://doi.org/10.1002/mds.870080115 . PMID: 8093549.

ELBAZ, A.; BOWER, J.H.; MARAGANORE, D.M. *et al*. Risk tables for parkinsonism and Parkinson’s disease. **J Clin Epidemiol**, v.55, n.1, p.25-31, 2002. https://doi.org./10.1016/S0895-4356(01)00425-5.

ELFIL, M.; KAMEL, S.; KANDIL, M.; KOO, B.B.; SCHAEFER, S.M. Implications of the Gut Microbiome in Parkinson's Disease. **Mov Disord**., v.35, n.6, p.921-933, Jun. 2020. https://doi.org./10.1002/mds.28004.

FAN, H.X.; SHENG, S.; ZHANG, F. New hope for Parkinson's disease treatment: Targeting gut microbiota. **CNS Neurosci Ther**. v.28, n.11, p.1675-1688, Nov. 2022. https://doi.org./10.1111/cns.13916.

FANG, F.; CHEN, H.; FELDMAN, A.L.; KAMEL, F.; YE, W.; WIRDEFELDT, K. Head injury and Parkinson's disease: a population-based study. **Mov Disord**., v.27, n.13, p.1632-1635, Nov. 2012. https://doi.org./10.1002/mds.25143.

FRASCA, D.; BLOMBERG, B.B. Inflammaging decreases adaptive and innate immune responses in mice and humans. **Biogerontology,** v.17, n.1, p.7-19, Feb. 2016. https://doi.org./10.1007/s10522-015-9578-8.

FULLARD, M.E.; MORLEY, J.F.; DUDA, J.E. Olfactory Dysfunction as an Early Biomarker in Parkinson's Disease. **Neurosci Bull.**, v.33, n.5, p.515-525, Oct. 2017. https://doi.org./10.1007/s12264-017-0170-x

FUNG, C.; VANDEN BERGHE, P. Functional circuits and signal processing in the enteric nervous system. **Cell. Mol. Life Sci.**, v.77, n.22, p.4505-4522, Nov. 2020. https://doi.org./10.1007/s00018-020-03543-6.

GENG, Z.H.; ZHU, Y.; LI, Q.L.; ZHAO, C.; ZHOU, P.H. Enteric Nervous System: The Bridge Between the Gut Microbiota and Neurological Disorders. **Frontiers in Aging Neuroscience**, v.14, n.810483, p.1-11, Apr. 2022. https://doi.org./10.3389/fnagi.2022.810483.

GHOSH, S.S.; WANG, J.; YANNIE, P.J.; GHOSH, S. Intestinal Barrier Dysfunction, LPS Translocation, and Disease Development. **J. Endocr. Soc.**, v.4, n.2, p.1-15, Feb. 2020. https://doi.org./10.1210/jendso/bvz039

GRUBIŠIĆ, V.; McCLAIN, J.L.; FRIED, D.E.; GRANTS, I.; RAJASEKHAR, P.; CSIZMADIA, E. *et al*. Enteric Glia Modulate Macrophage Phenotype and Visceral Sensitivity following Inflammation. **Cell Rep.**, v.32, n.10, p.1-26, Sep. 2020. https://doi.org./10.1016/j.celrep.2020.108100.

GRUBIŠIĆ, V.; VERKHRATSKY, A.; ZOREC, R.; PARPURA, V. Enteric glia regulate gut motility in health and disease. **Brain Res Bull**, v.136, p.109-117, Jan. 2018. https://doi.org./10.1016/j.brainresbull.2017.03.011.

GRUNDMANN, D.; LORIS, E.; MAAS-OMLOR, S.; SCHÄFER, K.H. Enteric Neurogenesis During Life Span Under Physiological and Pathophysiological Conditions. **Anat Rec (Hoboken)**, v.302, n.8, p.1345-1353, Aug. 2019. https://doi.org./10.1002/ar24124.

GUO, T.; CHEN, L. Gut microbiota and inflammation in Parkinson’s disease: Pathogenetic and therapeutic insights. **European Journal of Inflammation,** v.20, p.1-15, March 2022. https://doi.org/10.1177/1721727X221083763

HE, R.; ZHAO, Y.; HE, Y.; ZHOU, Y.; YANG, J.; ZHOU, X. *et al*. Olfactory Dysfunction Predicts Disease Progression in Parkinson's Disease: A Longitudinal Study. **Front Neurosci.**, v.14, n.569777, p.1-9, Dec. 2020. https://doi.org./10.3389/fnins.2020.569777.

HECKING, I.; STEGEMANN, L.N.; THEIS, V.; VORGERD, M.; MATSCHKE, V.; STAHLKE, S.; THEISS, C. Neuroprotective Effects of VEGF in the Enteric Nervous System. **Int J Mol Sci**, v.23, n.12, p.1-14, Jun. 2022. https://doi.org./10.3390/ijms23126756.

HIRSCH, E.; GRAYBIEL, A.M.; AGID, Y.A. Melanized dopaminergic neurons are differentially susceptible to degeneration in Parkinson's disease. **Nature**, v.334, n.6180, p.345-348, Jul. 1988. https://doi.org./10.1038/334345a0

HIRTZ, D.; THURMAN, D.J.; GWINN-HARDY, K.; MOHAMED, M.; CHAUDHURI, A.R.; ZALUTSKY, R. How common are the “common” neurologic disorders? **Neurology,** v.68, n.5, p.326-327, Jan. 2007. https://doi.org./10.1212/01.wnl.0000252807.38124.a3.

HORSAGER, J.; KATRINE, B.A.; KNUDSEN, K.; SKJAERBAEK, C.; FEDOROVA, T.D. *et al*. Brain-first versus body-first Parkinson's disease: a multimodal imaging case-control study. **Brain,** .v.143, n.10, p.3077-3088, Oct. 2020. https://doi.org./10.1093/brain/awaa238 .

HOUSER, M.C.; TANSEY, M.G. The gut-brain axis: Is intestinal inflammation a silent driver of Parkinson's disease pathogenesis? **npj Park. Dis.**, v.3, n.3, p.1-9, 2017. https://doi.org/10.1038/s41531-016-0002-0.

KIM, R.; KIM, H.J.; KIM, A.; JANG, M.; KIM, A.; KIM, Y.; YOO, D.; IM, J.H.; CHOI, J.H.; JEON, B. Peripheral blood inflammatory markers in early Parkinson's disease. **J Clin Neurosci**, v.58, p.30-33, Dec. 2018. https://doi.org./10.1016/j.jocn.2018.10.079.

KLANN, E.M.; DISSANAYAKE, U.; GURRALA, A. *et al*., The Gut-Brain Axis and Its Relation to Parkinson's Disease: A Review. **Front Aging Neurosci**, v.13, n.782082, p.1-17, Jan. 2022. https://doi.org./10.3389/fnagi.2021.782082.

KROGH, K.; OSTERGAARD, K.; SABROE, S.; LAURBERG, S. Clinical aspects of bowel symptoms in Parkinson's disease. **Acta Neurol Scand**., v.117, n.1, p.60-64, Jan. 2008. https://doi.org./10.1111/j.1600-0404.2007.00900.x.

LANGNESS, S.; KOJIMA, M.; COIMBRA, R.; ELICEIRI, B.P.; COSTANTINI, T.W. Enteric glia cells are critical to limiting the intestinal inflammatory response after injury. **Am. J. Physiol. Gastrointest. Liver Physiol.**, v.312, n.3, p.G274-G282, Mar. 2017. https://doi.org./10.1152/ajpgi.00371.2016.

LE, W.; SAYANA, P.; JANKOVIC, J. Animal models of Parkinson's disease: a gateway to therapeutics? **Neurotherapeutics**, v.11, n.1, p.92-110, Jan. 2014. https://doi.org./10.1007/s13311-013-0234-1

LEI, Q.; WU, T.; WU, J.; HU, X.; GUAN, Y.; WANG, Y.; YAN, J.; SHI, G. Roles of α-synuclein in gastrointestinal microbiome dysbiosis-related Parkinson's disease progression (Review). **Mol Med Rep**., v.24, n.4, p.1-14. Oct. 2021. https://doi.org/10.3892/mmr.2021.12374.

LIN, C.H.; LIN, J.W.; LIU, Y.C.; CHANG, C.H.; WU, R.M. Risk of Parkinson's disease following severe constipation: a nationwide population-based cohort study. **Parkinsonism Relat Disord**, 2014 Dec;v.20, n.12, p.1371-1375, Dec. 2014. https://doi.org.10.1016/j.parkreldis.2014.09.026.

LITVAK, Y.; BYNDLOSS, M.X.; BÄUMLER, A.J. Colonocyte metabolism shapes the gut microbiota. **Science,** v.362, n.6418, Nov. 2018. https://doi.org./10.1126/science.aat9076.

LUO, S.; ZHU, H.; ZHANG, J.; WAN, D. The Pivotal Role of Microbiota in Modulating the Neuronal–Glial–Epithelial Unit. **Infect Drug Resist.**, v.14, p.5613-5628, Dec. 2021. https://doi.org./10.2147/IDR.S342782.

MAZZULLI, J.R.; XU, Y.H.; SUN, Y.; KNIGHT, A.L.; McLEAN, P.J.; CALDWELL, G.A.; SIDRANSKY, E.; GRABOWSKI, G.A.; KRAINC, D. Gaucher disease glucocerebrosidase and α-synuclein form a bidirectional pathogenic loop in synucleinopathies. **Cell**, v.146, n.1, p.37-52, Jul. 2011. https://doi.org./10.1016/j.cell.2011.06.001.

McCANN, H.; STEVENS, C.H.; CARTWEIGHT, H.; HALLIDAY, G.M. α-Synucleinopathy phenotypes. Parkinsonism Relat Disord., v.20, n.Suppl 1, p.S62-S67, Jan. 2014. https://doi.org/10.1016/S1353-8020(13)70017-8

MENG, L.; YUAN, X.; CAO, X.; ZHANG, Z. The gut-brain axis in the pathogenesis of Parkinson's disease. **Brain Science Advances**, v.5, n.2, p.73-81, 2019. https://doi.org/10.26599/BSA.2019.9050009.

MIRZAEI, R.; BOUZARI, B.; HOSSEINI-FARD, S.R.; MAZAHERI, M. *et al*. Role of microbiota-derived short-chain fatty acids in nervous system disorders. **Biomed Pharmacother,** v.139, Jul. 2021. https://doi.org./10.1016/j.biopha.2021.111661.

MURAKAMI, M.; OHTA, T.; ITO, S. Interleukin-1β enhances the action of bradykinin in rat myenteric neurons through up-regulation of glial B1 receptor expression. **Neuroscience**, v.151, n.1, p.222-231, Jan. 2008. https://doi.org./10.1016/j.neuroscience.2007.09.065.

NATALE, G.; RYSKALIN, L.; MORUCCI, G.; LAZZERI, G.; FRATI, A.; FORNAI, F. The Baseline Structure of the Enteric Nervous System and Its Role in Parkinson's Disease. **Life (Basel)**, v.11, n.8, p.1-20, Jul. 2021. https://doi.org./10.3390/life11080732.

NERIUS, M.; FINK, A.; DOBLHAMMER, G. Parkinson's disease in Germany: prevalence and incidence based on health claims data. **Acta Neurol Scand**., v.136, n.5, p.386-392, Nov. 2017. https://doi.org./10.1111/ane.12694.

NOYCE, A.J.; BESTWICK, J.P.; SILVEIRA-MORIYAMA, L.; HAWKES, C.H.; GIOVANNONI, G. *et al*. Meta-analysis of early nonmotor features and risk factors for Parkinson disease. **Annals of Neurology**, v.72, n.6, p.893-901, Dec. 2012. https://doi.org./10.1002/ana.23687

NUSSBAUM, R.L. Genetics of Synucleinopathies. **Cold Spring Harb Perspect Med**., v.8, n.a024109, p.1-9, Jun. 2018. https://doi.org./10.1101/cshperspect.a024109.

O'CONNOR, T.M.; O'CONNELL, J.; O'BRIEND, D.I.; GOODE, T.; BREDIN, C.P.; SHANAHAN, F. The role of substance P in inflammatory disease. **J Cell Physiol.,** v.201, n.2, p.167-180, Nov. 2004. https://doi.org./10.1002/jcp.20061.

OBESO, J.; STAMELOU, M.; GEOTZ, C.G. *et al*. Past, present, and future of Parkinson's disease: a special essay on the 200th anniversary of the shaking palsy. **Mov Disord**, v.32, n.9, p.1264-1310, Sep. 2017. https://doi.org./10.1002/mds.27115.

PALERMO, G.; DEL PRETE, E.; BONUCCELLI, U.; CERAVOLO, R. Early autonomic and cognitive dysfunction in PD, DLB and MSA: blurring the boundaries between α-synucleinopathies. **J Neurol**., v.267, n.12, p.3444-3456, Dec. 2020. https://doi.org/10.1007/s00415-020-09985-z

PAVAN, S.; PRABHU, A.N.; GORTHI, S.P.; DAS, B.; MUTREJA, A.; SHETTY, V.; RAMAMURTHY, T.; BALLAL, M. Exploring the multifactorial aspects of Gut Microbiome in Parkinson's Disease. **Folia Microbiol (Praha),** v.67, n.5, p.693-706, May. 2022. https://doi.org./10.1007/s12223-022-00977-2.

PEZZOLI, G.; CEREDA, E. Exposure to pesticides or solvents and risk of Parkinson disease. **Neurology,** v.80, n.22, p.2035-2041, May, 2013. https://doi.org./10.1212/WNL.0b013e318294b3c8

POSTUMA, R.B.; GAGNON, J.F.; PELLETIER, A.; MONTPLAISIR, J. Prodromal autonomic symptoms and signs in Parkinson's disease and dementia with Lewy bodies. Movement Disorders, v.28, n.5, p.597-604 May. 2013. https://doi.org./10.1002/mds.25445

PROGATZKY, F.; PACHNIS, V. The role of enteric glia in intestinal immunity. **Curr Opin Immunol**, v.77, n.102183, p.1-6, Aug. 2022. https://doi.org/10.1016/j.coi.2022.102183

RENZI, D.; PELLEGRINI, B.; TONELLI, F.; SURRENTI, C.; CALABRÒ, A. Substance P (neurokinin-1) and neurokinin A (neurokinin-2) receptor gene and protein expression in the healthy and inflamed human intestine. **Am J Pathol.**, v.157, n.5, p.1511-1522, Nov. 2000. https://doi.org./10.1016/S0002-9440(10)64789-X

ROBERTS, J.A.; DURNIN, L.; SHARKEY, K.A; YAMBOLIEVA, V.N.M.; MAWE, G.M. Oxidative stress disrupts purinergic neuromuscular transmission in the inflamed colon. **J. Physiol**., v.591, n.15, p.3725-3737, Aug. 2013. https://doi.org./10.1113/jphysiol.2013.254136.

RODRIGUES, P.V.; GODOY, J.V.P.; BOSQUE, B.P.; AMORIM NETO, D.P.; TOSTES, K. *et al*. Transcellular propagation of fibrillar α-synuclein from enteroendocrine to neuronal cells requires cell-to-cell contact and is Rab35-dependent. **Sci Rep.**, v.12, n.1, p.1-19, Mar. 2022. https://doi.org./10.1038/s41598-022-08076-5.

ROMANO, S.; SAVVA, G.M.; BEDARF, J.R.; CHARLES, I.G.; HILDEBRAND, F.; NARBAD, A. Meta-analysis of the Parkinson's disease gut microbiome suggests alterations linked to intestinal inflammation. **npj Parkinsons Dis**, v.7, n.27, 2021. https://doi.org/10.1038/s41531-021-00156-z.

ROSENBERG, H.J.; RAO, M. Enteric glia in homeostasis and disease: From fundamental biology to human pathology. **iScience**, v.24, n.8, p.1-20, Aug. 2021. https://doi.org./10.1016/j.isci.2021.102863.

SANTOS, S.F.; OLIVEIRA, H.L.; YAMADA, E.S.; NEVES, B.C.; PEREIRA JÚNIOR, A. The Gut and Parkinson's Disease-A Bidirectional Pathway. **Front Neurol**, v.10, n.574, p.1-8, Jun. 2019. https://doi.org./10.3389/fneur.2019.00574.

SAVIDGE, T.C.; NEWMAN, P.; POTHOULAKIS, C.; NEUNLIST, A.R.M.; BOURREILLE, A.; HURST, R.; SOFRONIEW, M.V. Enteric glia regulate intestinal barrier function and inflammation via release of S-nitrosoglutathione. **Gastroenterology**, v.132, n.4, p.1344-1358, Apr. 2007. https://doi.org/10.1053/j.gastro.2007.01.051.

SCHAEFFER, E.; KLUGE, A.; BÖTTNER, M.; ZUNKE, F.; COSSAIS, F.; BERG, D.; ARNOLD, P. Alpha Synuclein Connects the Gut-Brain Axis in Parkinson's Disease Patients - A View on Clinical Aspects, Cellular Pathology and Analytical Methodology. **Frontiers in cell and developmental biology**, v.8, n.573696, p.1-11, Sep. 2020. https://doi.org./10.3389/fcell.2020.573696.

SCHEPERJANS, F.; AHO, V.; PEREIRA, P.A.B.; KOSKINEN, K.; PAULIN, L.; PEKKONEN, E. *et al*. Gut microbiota are related to Parkinson's disease and clinical phenotype. **Mov Disord.**, v.30, n.3, p.350-358, Mar. 2015. https://doi.org./10.1002/mds.26069.

SCHULTE, C.; GASSER, T. Genetic basis of Parkinson's disease: inheritance, penetrance, and expression. **Appl Clin Genet**., v.4, p.67-80, Jun. 2011. https://doi.org./10.2147/TACG.S11639.

SEGUELLA, L.; CAPUANO, R.; SARNELLI, G.; ESPOSITO, G. Play in advance against neurodegeneration: exploring enteric glial cells in gut-brain axis during neurodegenerative diseases. **Expert Rev Clin Pharmacol**., v.12, n.6, p.555-564, Jun. 2019. https://doi.org./10.1080/17512433.2019.1612744.

SEGUELLA, L.; GULBRANSEN, B.D. Enteric glial biology, intercellular signalling and roles in gastrointestinal disease. **Nat Rev Gastroenterol Hepatol.**, v.18, n.8, p.571-587, Aug. 2021. https://doi.org./10.1038/s41575-021-00423-7.

SEGUELLA, L.; SARNELLI. G.; ESPOSITO, G. Leaky gut, dysbiosis, and enteric glia activation: The trilogy behind the intestinal origin of Parkinson's disease. **Neural Regen. Res**, v.15, n.6, p.1037-1038, Dec. 2020. https://doi.org./10.4103/1673-5374.270308.

SHEN, T.; YUE, Y.; HE, T.; HUANG, C.; QU, B.; LV, W.; LAI, H.Y. The Association Between the Gut Microbiota and Parkinson's Disease, a Meta-Analysis. **Front Aging Neurosci**., v.13, n.636545, P.1-12, Fev. 2021. https://doi.org./10.3389/fnagi.2021.636545.

SHERWIN, E.; BORDENSTEIN, S.R.; QUINN, J.L.; DINAN, T.G.; CRYAN, J.F. Microbiota and the social brain. **Science,** v.366, n.6465, p.1-9, Nov. 2019. https://doi.org./10.1126/science.aar2016.

SOLLEIRO-VILLAVICENCIO, H.; RIVAS-ARANCIBIA, S. Effect of Chronic Oxidative Stress on Neuroinflammatory Response Mediated by CD4+T Cells in Neurodegenerative Diseases. **Front. Cell. Neurosci.**, v.12, n.114, 2018. https://doi.org./10.3389/fncel.2018.00114.

SOMMER, F.; BÄCKHED, F. The gut microbiota--masters of host development and physiology. **Nat. Rev. Microbiol**., v.11, n.4, p.227-238, Apr. 2013. https://doi.org./10.1038/nrmicro2974.

SPENCER, N.J.; HU, H. Enteric nervous system: sensory transduction, neural circuits and gastrointestinal motility. **Nat Rev Gastroenterol Hepatol,** v.17, p.338-351, March 2020.

STOLZENBERG, E.; BERRY, D.; YANG, D.; LEE, E.Y.; KROEMER, A. *et.al*. A role for neuronal alpha-synuclein in gastrointestinal immunity. **Journal of Innate Immunity**, v.9, n.5, p.456-463, 2017. https://doi.org./10.1159/000477990.

TAN, A.H.; LIM, S.Y.; LANG, A.E. The microbiome-gut-brain axis in Parkinson disease – from basic research to the clinic. **Nat Rev Neurol**, v.18, n.8, p.476-495, Aug. 2022. https://doi.org./10.1038/s41582-022-00681-2.

TANNER, C.M.; KAMEL, F.; ROSS, G.W.; HOPPIN, J.A.; GOLDMAN, S.M. *et al*. Rotenone, Paraquat, and Parkinson's disease. **Environ Health Perspect**., v.119, n.6, p.866-872, Jun. 2011. https://doi.org./10.1289/ehp.1002839.

TAYLOR, K.S.; COOK, J.A.; COUNSELL, C.E. Heterogeneity in male to female risk for Parkinson's disease. **J Neurol Neurosurg Psychiatry,** v.78, n.8, p.905-906, 2007. https://doi.org./10.1136/jnnp.2006.104695.

THOMASI, B.B.M.; VALDETARO, L.; RICCIARDI, M.C.G.; HAYASHIDE, L.; FERNANDES, A.C.M.N. *et al*. Enteric glial cell reactivity in colonic layers and mucosal modulation in a mouse model of Parkinson's disease induced by 6-hydroxydopamine. **Brain Res Bull**, v.187, p.111-121, Sep. 2022. https:// doi.org./10.1016/j.brainresbull.2022.06.013.

TILOCCA, B.; PIERONI, L.; SOGGIU, A.; BRITTI, D.; BONIZZI, L.; RONCADA, P.; GRECO, V. Gut-Brain Axis and Neurodegeneration: State-of-the-Art of Meta-Omics Sciences for Microbiota Characterization. **Int J Mol Sci**., v.21, n.11, p.4045, Jun. 2020. https://doi.org./10.3390/ijms21114045.

TOLEDO, A.R.L.; MONROY, G.R.; SALAZAR, F.E.. LEE, J.Y.; JAIN, S.; YADAV, H.; BORLONGAN, C.V. Gut-Brain Axis as a Pathological and Therapeutic Target for Neurodegenerative Disorders. **Int J Mol Sci**., v.23, n.3, p.1-15, Jan. 2022. https://doi.org./10.3390/ijms23031184.

TRAVAGLI, R.A.; BROWNING, K.N.; CAMILLERI, M. Parkinson disease and the gut: new insights into pathogenesis and clinical relevance. **Nat. Rev. Gastroenterol. Hepatol**., v.17, p.673-685, July 2020.

TURCO, F.; SARNELLI, G.; CIRILLO, C.; PALUMBO, I.; De GIORGI, F.; D'ALESSANDRO, A.; CAMMAROTA, M.; GIULIANO, M.; CUOMO, R. Enteroglial-derived S100B protein integrates bacteria-induced Toll-like receptor signalling in human enteric glial cells. **Gut**., v.63, n.1, p.105-115, Jan. 2014. https://doi.org./10.1136/gutjnl-2012-302090.

TWELVES, D.; PERKINS, K.S.M.; COUNSELL, C. Systematic review of incidence studies of Parkinson's disease. **Mov Disord.**, v.18, n.1, p.19-31, Jan. 2003. https://doi.org./10.1002/mds.10305.

TYSNES, O.B.; STORSTEIN, A. Epidemiology of Parkinson's disease. **J Neural Transm**. v.124, n.8, p.901-905, Aug. 2017. https://doi.org./10.1007/s00702-017-1686-y.

VALÈS, S.; TOUVRON, M.; LANDEGHEM, L.V. Enteric glia: Diversity or plasticity? **Brain Res.**, v.1693, p.140-145, Aug. 2018. https://doi.org./10.1016/j.brainres.2018.02.001.

WALLEN, Z.D.; APPAH, M.; DEAN, M.N.; SESLER, C.L.; FACTOR, S.A.; MOLHO, E.; ZABETIAN, C.P.; STANDAERT, D.G.; PAYAMI, H. Characterizing dysbiosis of gut microbiome in PD: evidence for overabundance of opportunistic pathogens. **NPJ Parkinson's disease**, v.6, n.11, p.1-12, Jun. 2020. https://doi.org./10.1038/s41531-020-0112-6.

WANG, Q.; LUO, Y.; CHAUDHURI, K.R.; REYNOLDS, R.; TAN, E.K.; PETTERSSON, S. The role of gut dysbiosis in Parkinson's disease: mechanistic insights and therapeutic options. **Brain**, v.144, n.9, p.2571-2593, Oct. 2021. https://doi.org./10.1093/brain/awab156.

YATSUNENKO, T.; REY, F.E.; MANARY, M.J.; TREHAN, I.; DOMINGUEZ-BELLO, M.G. *et al*. Human gut microbiome viewed across age and geography. **Nature**, v.486, n.7402, p.222-227, May 2012. https://doi.org./10.1038/nature11053.

YU, O.J.; YU, S.Y.; ZUO, L.J.; LIAN, T.H.; HU, Y.; WANG, R.D. *et al*. Parkinson disease with constipation: clinical features and relevant factors. **Scientific Reports**, v.8, n.567, p.1-9, Jan. 2018. https://doi.org./10.1038/s41598-017-16790-8

YU, Y.; YANG, W.; LI, Y.; CONG, Y. Enteroendocrine Cells: Sensing Gut Microbiota and Regulating Inflammatory Bowel Diseases. **Inflamm. Bowel Dis.**, v.26, n.1, p.11-20, Jan. 2020. https://doi.org./10.1093/ibd/izz217.

ZESIEWCZ, T.A. Parkinson Disease. **Continuum**, v.25, n.4, p.896-918, Aug. 2019. https://doi.org./10.1212/CON.0000000000000764.

ZHU, M.; LIU, X; YE, Y.; YAN, X.; CHENG, Y.; ZHAO, L.; CHEN, F.; LING, Z. Gut Microbiota: A Novel Therapeutic Target for Parkinson's Disease. **Front Immunol**., v.13, n.937555, p.1-18, June 2022. https://doi.org./10.3389/fimmu.2022.937555.

ANEXO A. Certificado de aprovação na Comissão de Ética no Uso de Animais (CEUA) do Núcleo de Pesquisa e Desenvolvimento de Medicamentos (NPDM).

UNIVERSIDADE FEDERAL DO CEARÁ
FACULDADE DE MEDICINA
NÚCLEO DE PESQUISA E DESENVOLVIMENTO DE MEDICAMENTOS - NPDM
COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

C E R T I F I C A D O

Certificamos que a proposta intitulada **"ANÁLISE DAS ALTERAÇÕES MORFOLÓGICAS E NEUROQUÍMICAS NO EIXO CÉREBRO-INTESTINO EM UM MODELO ANIMAL DE DOENÇA DE PARKINSON INDUZIDO POR ROTENONA"** registrada com o protocolo **21181021-0,** sob a responsabilidade de **Glauce Socorro de Barros Viana e Júlio César Claudino dos Santos**, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata* (exceto humanos), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794 de 8 de outubro de 2008, do Decreto nº 6.899 de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), foi **APROVADA** pela Comissão de Ética no Uso de Animais (CEUA) do Núcleo de Pesquisa e Desenvolvimento de Medicamentos (NPDM) da Universidade Federal do Ceará, na reunião de 17 /03/ 2022.

We hereby certify that the project entitled **"ANALYSIS OF MORPHOLOGICAL AND NEUROCHEMICAL CHANGES IN THE GUT-BRAIN AXIS IN A ROTENONE-INDUCED PARKINSON'S DISEASE ANIMAL MODEL"** identified by the protocol number **21181021-0**, and conducted by **Glauce Socorro de Barros Viana and Júlio César Claudino dos Santos**, which involves the production, maintenance or use of animals belonging to the filo *Chordata*, sub-vertebrate *Vertebrata* (except humans), for the purpose of scientific research - is in accordance with the provisions of the Law number 11,794, from October 8th, 2008, of Decree number 6,899, from July 15th, 2009, and with the regulations issued by the National Council for the Control of Animal Experimentation (CONCEA), was **APPROVED** by the Ethics Committee on Animal Use (CEUA) from the Center for Research and Development of Medicines (NPDM) of the Federal University of Ceará, in the meeting of 03 / 17 / 2022.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	Início: 18/03/2022 Fim: 10/02/2023
Espécie	Camundongo heterogênico
Linhagem	*Swiss*
Nº de animais autorizados	20
Peso	25 a 30 gramas
Idade	03 semanas
Sexo	Machos
Origem (fornecedor)	Biotério do NPDM
Local do experimento	Biotério do NPDM

Fortaleza, Ceará, 17 de março de 2022

Coordenação da Comissão de Ética e Uso de Animais
Núcleo de Pesquisas e Desenvolvimento de Medicamentos
Universidade Federal do Ceará

Rua Coronel Nunes de Melo, 1000, Rodolfo Teófilo – Fortaleza - CE - CEP 60430-275 - https://ceuanpdm.ufc.br/pt/
ceua-npdm@ufc.br

ANEXO B - Content of Author Guidelines

Introduction

As the average human life expectancy has increased, so too has the impact of ageing and age-related disease on our society. Ageing research is now the focus of thousands of laboratories that include leaders in the areas of genetics, molecular and cellular biology, biochemistry, and behaviour. Ageing Research Reviews (ARR) covers the trends in this field. It is designed to fill a large void, namely, a source for critical reviews and viewpoints on emerging findings on mechanisms of ageing and age-related disease. Rapid advances in understanding of mechanisms that control cellular proliferation, differentiation and survival are leading to new insight into the regulation of ageing. From telomerase to stem cells to energy and oxyradical metabolism, this is an exciting new era in the multidisciplinary field of ageing research. The cellular and molecular underpinnings of manipulations that extend lifespan, such as caloric restriction, are being identified and novel approaches for preventing age-related diseases are being developed. ARR publishes articles on focussed topics selected from the broad field of ageing research, with an emphasis on cellular and molecular mechanisms of the aging process and age-related diseases such as cancer, cardiovascular disease, diabetes and neurodegenerative disorders. Applications of basic ageing research to lifespan extension and disease prevention are also covered in this journal.

Types of article

Ageing Research Reviews (ARR) publishes critical reviews on emerging findings on mechanisms of ageing and age-related disease and authors can submit articles in three formats:

Review article - provides an in-depth review of topics of interest to the journal's broad readership.

Short review - focused on a timely aspect of a topic or review critical new findings. Submissions are typically around 2500 words.

View point - a forum for authors to provide their own views on a topic and their vision of future research directions. Submissions are typically 2000 word limit and no more than 3 Figures.

Contact details for submission

For enquiries relating to the submission of articles, please visit this journal's homepage at https://www.elsevier.com/locate/arr. You can track accepted articles at https://www.elsevier.com/trackarticle and set up e-mail alerts to inform you of when an article's status has changed, as well as copyright information, frequently asked questions and more.

Submission checklist

You can use this list to carry out a final check of your submission before you send it to the journal for review. Please check the relevant section in this Guide for Authors for more details.

Ensure that the following items are present:

One author has been designated as the corresponding author with contact details:

• E-mail address

• Full postal address

All necessary files have been uploaded:

Manuscript:

- Include keywords
- All figures (include relevant captions)
- All tables (including titles, description, footnotes)
- Ensure all figure and table citations in the text match the files provided
- Indicate clearly if color should be used for any figures in print *Graphical Abstracts / Highlights files* (where applicable) *Supplemental files* (where applicable)

Further considerations

- Manuscript has been 'spell checked' and 'grammar checked'
- All references mentioned in the Reference List are cited in the text, and vice versa
- Permission has been obtained for use of copyrighted material from other sources (including the Internet)
- A competing interests statement is provided, even if the authors have no competing interests to declare
- Journal policies detailed in this guide have been reviewed
- Referee suggestions and contact details provided, based on journal requirements

For further information, visit our Support Center.

Before You Begin

Ethics in publishing

Please see our information on Ethics in publishing.

Declaration of interest

All authors must disclose any financial and personal relationships with other people or organizations that could inappropriately influence (bias) their work. Examples of potential competing interests include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Authors must disclose any interests in two places: 1. A summary declaration of interest statement in the title page file (if double anonymized) or the manuscript file (if single anonymized). If there are no interests to declare then please state this: 'Declarations of interest: none'. 2. Detailed disclosures as part of a separate Declaration of Interest form, which forms part of the journal's official records. It is important for potential interests to be declared in both places and that the information matches. More information.

Submission declaration and verification

Submission of an article implies that the work described has not been published previously (except in the form of an abstract, a published lecture or academic thesis, see 'Multiple, redundant or concurrent publication' for more information), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if accepted, it will not be published elsewhere in the same form, in English or in any other language, including electronically without the written consent of the copyright-holder. To verify compliance, your article may be checked by Crossref Similarity Check and other originality or duplicate checking software.

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Author contributions

For transparency, we encourage authors to submit an author statement file outlining their individual contributions to the paper using the relevant CRediT roles: Conceptualization; Data curation; Formal analysis; Funding acquisition; Investigation; Methodology; Project administration; Resources; Software; Supervision; Validation; Visualization; Roles/Writing - original draft; Writing - review & editing. Authorship statements should be formatted with the names of authors first and CRediT role(s) following. More details and an example.

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Authors are expected to consider carefully the list and order of authors **before** submitting their manuscript and provide the definitive list of authors at the time of the original submission. Any addition, deletion or rearrangement of author names in the authorship list should be made only **before** the manuscript has been accepted and only if approved by the journal Editor. To request such a change, the Editor must receive the following from the **corresponding author**: (a) the reason for the change in author list and (b) written confirmation (e-mail, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed.

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It is important that the file be saved in the native format of the word processor used. The text should be in single-column format. Keep the layout of the text as simple as possible. Most formatting codes will be removed and replaced on processing the article. In particular, do not use the word processor's options to justify text or to hyphenate words. However, do use bold face, italics, subscripts, superscripts etc. When preparing tables, if you are using a table grid, use only one grid for each individual table and not a grid for each row. If no grid is used, use tabs, not spaces, to align columns. The electronic text should be prepared in a way very similar to that of conventional manuscripts (see also the Guide to Publishing with Elsevier). Note that source files of figures, tables and text graphics will be required whether or not you embed your figures in the text. See also the section on Electronic artwork.

To avoid unnecessary errors you are strongly advised to use the 'spell-check' and 'grammar-check' functions of your word processor.

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Divide your article into clearly defined and numbered sections. Subsections should be numbered 1.1 (then 1.1.1, 1.1.2, ...), 1.2, etc. (the abstract is not included in section numbering). Use this numbering also for internal cross-referencing: do not just refer to 'the text'. Any subsection may be given a brief heading. Each heading should appear on its own separate line.

Introduction

State the objectives of the work and provide an adequate background, avoiding a detailed literature survey or a summary of the results.

Discussion

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

Conclusions

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

Appendices

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

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- ***Author names and affiliations.*** Please clearly indicate the given name(s) and family name(s) of each author and check that all names are accurately spelled. You can add your name between parentheses in your own script behind the English transliteration. Present the authors' affiliation addresses (where the actual work was done) below the names. Indicate all affiliations with a lower-case superscript letter immediately after the author's name and

in front of the appropriate address. Provide the full postal address of each affiliation, including the country name and, if available, the e-mail address of each author.

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Highlights are mandatory for this journal as they help increase the discoverability of your article via search engines. They consist of a short collection of bullet points that capture the novel results of your research as well as new methods that were used during the study (if any). Please have a look at the examples here: example Highlights.

Highlights should be submitted in a separate editable file in the online submission system. Please use 'Highlights' in the file name and include 3 to 5 bullet points (maximum 85 characters, including spaces, per bullet point).

Abstract

A concise and factual abstract is required. The abstract should be approximately 100-200 words. The abstract should state briefly the purpose of the research, the principal results and major conclusions. An abstract is often presented separately from the article, so it must be able to stand alone. For this reason, References should be avoided, but if essential, then cite the author(s) and year(s). Also, non-standard or uncommon abbreviations should be avoided, but if essential they must be defined at their first mention in the abstract itself.

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Although a graphical abstract is optional, its use is encouraged as it draws more attention to the online article. The graphical abstract should summarize the contents of the article in a concise, pictorial form designed to capture the attention of a wide readership. Graphical abstracts should be submitted as a separate file in the online submission system. Image size: Please provide an

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Keywords

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

Acknowledgements

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

Formatting of funding sources

List funding sources in this standard way to facilitate compliance to funder's requirements:

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Electronic artwork

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- Embed the used fonts if the application provides that option.
- Aim to use the following fonts in your illustrations: Arial, Courier, Times New Roman, Symbol, or use fonts that look similar.
- Number the illustrations according to their sequence in the text.
- Use a logical naming convention for your artwork files.
- Provide captions to illustrations separately.
- Size the illustrations close to the desired dimensions of the published version.
- Submit each illustration as a separate file.
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If your electronic artwork is created in a Microsoft Office application (Word, PowerPoint, Excel) then please supply 'as is' in the native document format. Regardless of the application used other than Microsoft Office, when your electronic artwork is finalized, please 'Save as' or convert the images to one of the following formats (note the resolution requirements for line drawings, halftones, and line/halftone combinations given below):

EPS (or PDF): Vector drawings, embed all used fonts.

TIFF (or JPEG): Color or grayscale photographs (halftones), keep to a minimum of 300 dpi.

TIFF (or JPEG): Bitmapped (pure black & white pixels) line drawings, keep to a minimum of 1000 dpi.

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- Submit graphics that are disproportionately large for the content.

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Please submit tables as editable text and not as images. Tables can be placed either next to the relevant text in the article, or on separate page(s) at the end. Number tables consecutively in accordance with their appearance in the text and place any table notes below the table body. Be sparing in the use of tables and ensure that the data presented in them do not duplicate results described elsewhere in the article. Please avoid using vertical rules and shading in table cells.

References

Citation in text

Please ensure that every reference cited in the text is also present in the reference list (and vice versa). Any references cited in the abstract must be given in full. Unpublished results and personal communications are not recommended in the reference list, but may be mentioned in the text. If these references are included in the reference list they should follow the standard reference style of the journal and should include a substitution of the publication date with

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Web references

As a minimum, the full URL should be given and the date when the reference was last accessed. Any further information, if known (DOI, author names, dates, reference to a source publication, etc.), should also be given. Web references can be listed separately (e.g., after the reference list) under a different heading if desired, or can be included in the reference list.

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2. *Two authors:* both authors' names and the year of publication;
3. *Three or more authors:* first author's name followed by 'et al.' and the year of publication.

Citations may be made directly (or parenthetically). Groups of references can be listed either first alphabetically, then chronologically, or vice versa.

Examples: 'as demonstrated (Allan, 2000a, 2000b, 1999; Allan and Jones, 1999)…. Or, as demonstrated (Jones, 1999; Allan, 2000)… Kramer et al. (2010) have recently shown …'

List: References should be arranged first alphabetically and then further sorted chronologically if necessary. More than one reference from the same author(s) in the same year must be identified by the letters 'a', 'b', 'c', etc., placed after the year of publication.

Examples:

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Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2010. The art of writing a scientific article. J. Sci. Commun. 163, 51–59. https://doi.org/10.1016/j.Sc.2010.00372.

Reference to a journal publication with an article number:

Van der Geer, J., Hanraads, J.A.J., Lupton, R.A., 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205.

Reference to a book:

Strunk Jr., W., White, E.B., 2000. The Elements of Style, fourth ed. Longman, New York.

Reference to a chapter in an edited book:

Mettam, G.R., Adams, L.B., 2009. How to prepare an electronic version of your article, in:

Jones, B.S., Smith , R.Z. (Eds.), Introduction to the Electronic Age. E-Publishing Inc., New York, pp. 281–304.

Reference to a website:
Cancer Research UK, 1975. Cancer statistics reports for the UK. http://www.cancerresearchuk.org/aboutcancer/statistics/cancerstatsreport/ (accessed 13 March 2003).

Reference to a dataset:
[dataset] Oguro, M., Imahiro, S., Saito, S., Nakashizuka, T., 2015. Mortality data for Japanese oak wilt disease and surrounding forest compositions. Mendeley Data, v1. https://doi.org/10.17632/xwj98nb39r.1.

Reference to software:
Coon, E., Berndt, M., Jan, A., Svyatsky, D., Atchley, A., Kikinzon, E., Harp, D., Manzini, G., Shelef, E., Lipnikov, K., Garimella, R., Xu, C., Moulton, D., Karra, S., Painter, S., Jafarov, E., & Molins, S., 2020. Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88). Zenodo. https://doi.org/10.5281/zenodo.3727209.

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