



**UNIVERSIDADE ESTADUAL PAULISTA
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**Agonistas dos receptores GLP-1 e GIP no tratamento da doença
de Parkinson: Revisão sistemática translacional e meta-análise de
estudos clínicos e pré-clínicos**

Tese apresentada à Faculdade de
Medicina, Universidade Estadual
Paulista “Júlio de Mesquita Filho”,
Campus de Botucatu, para obtenção
do título de Doutora em Ciências –
Área de concentração Patologia.

Orientador: Prof. Dr. João Lauro Viana de Camargo
Coorientadora: Profa. Dra. Luciane Cruz Lopes

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Chorei muito. Sorri muito. Caí muito. Levantei muito. Comemorei muito. Cresci muito.

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Resumo

A Doença de Parkinson (DP) é uma condição neurodegenerativa progressiva e heterogênea na qual são observados, em intensidades variáveis, sinais motores e não motores. Apesar de por muito tempo ter sido essencialmente associada à morte de neurônios dopaminérgicos da *substantia nigra pars compacta* e diminuição dos níveis de dopamina estriatais, a DP têm se mostrado um distúrbio mais disseminado, com o envolvimento de outros neurotransmissores e interação entre o acúmulo de uma proteína anômala, a α -sinucleína, e o sistema imunológico. Independente do seu subtipo, ainda não há cura para a DP. Levodopa e medicamentos dopaminérgicos são utilizados como tratamento sintomático e não como agentes modificadores da doença. Recentemente, o Diabetes Mellitus tipo II (DMT2) tem sido associado a sintomas mais graves e progressão acelerada da DP, sugerindo possível ligação entre essas duas condições. Medicamentos utilizados ou mapeados como tratamento para a DMT2 têm sido sugeridos como possíveis tratamentos da DP, a exemplo dos agonistas dos receptores GLP-1 e GIP. Além de importantes para o controle glicêmico, a ativação desses receptores no sistema nervoso central pode estar envolvida em processos como autofagia, apoptose, função mitocondrial e neuroinflamação, bem como no aprendizado, memória e modulação da plasticidade sináptica. Este trabalho objetivou avaliar a força e o peso das evidências sobre a eficácia e segurança dos agonistas desses receptores no tratamento da DP por meio de revisão sistemática e metanálise de estudos clínicos e pré-clínicos. O risco de viés dos estudos clínicos foi avaliado pela ferramenta Cochrane (RoB 2) e dos estudos pré-clínicos por uma versão adaptada das ferramentas SYRCLE e CAMARADES. O sistema GRADE foi usado para classificar o nível de confiança dos estudos clínicos. Possíveis efeitos anti-parkinsonianos (avaliados pela melhora na atividade locomotora espontânea, coordenação, equilíbrio e força muscular) foram detectados em roedores por diferentes modelos de DP e diferentes esquemas de tratamento com os agonistas dos receptores GLP-1 e GIP. Já em ensaios clínicos (considerados baixo nível de evidência pelo GRADE), houve melhora significativa na atividade locomotora de pacientes com DP após tratamento com a exenatida, um dos agonistas do receptor GLP-1, após a retirada do medicamento por algumas semanas (*washout*). Tanto os estudos pré-clínicos como os clínicos aqui analisados apresentam limitações e vieses importantes, impedindo definir se os efeitos dos agonistas estudados são neuroprotetores ou sintomáticos. Estudos experimentais mais bem delineados e ensaios clínicos com maior período de *washout* são necessários para avançarmos no esclarecimento da ação desses potenciais fármacos para a DP.

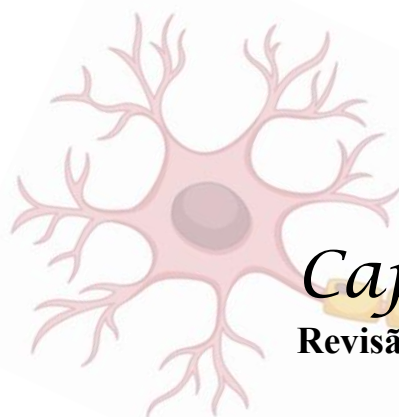
Abstract

Parkinson's Disease (PD) is a progressive and heterogeneous neurodegenerative condition in which motor and non-motor signs are observed at varying intensities. Although for a long time it has been primarily associated with the death of *substantia nigra pars compacta* dopaminergic neurons and decreased striatal dopamine levels, PD has been shown to be a more widespread disorder with the involvement of other neurotransmitters and an interaction between the accumulation of an anomalous protein, α -synuclein, and the immune system. Regardless of the subtype, there is still no cure for PD. Levodopa and dopaminergic drugs are used as symptomatic treatment rather than disease modifying agents. More recently, type II Diabetes Mellitus (DMT2) has been associated with more severe symptoms and accelerated progression of PD, revealing a possible link between these two. Medications used or mapped as a treatment for T2DM have been suggested as possible treatments for PD, such as GLP-1 and GIP receptor agonists. In addition to being important for glycemic control, the activation of these receptors in the central nervous system may be involved in processes such as autophagy, apoptosis, mitochondrial function and neuroinflammation, as well as in learning, memory and modulation of synaptic plasticity. This study aimed to evaluate the efficacy and safety of these receptor agonists in the treatment of PD through a systematic review and meta-analysis of clinical and preclinical studies. The risk of bias of clinical studies was assessed by the Cochrane tool (RoB 2) and the preclinical studies by an adapted version of the SYRCLE and CAMARADES tools; GRADE was used for the certainty of the evidence of clinical studies. Possible anti-parkinsonian effects (improvement in spontaneous locomotor activity, coordination, balance, and muscle strength) were detected in rodents by different PD lesion models and different treatment regimens with GLP-1 and GIP receptor agonists. In clinical trials (ranked as low levels of certainty evidence by GRADE), there was significant improvement in locomotor activity in PD patients treated with exenatide, one of the GLP-1 receptor agonists, after drug withdrawal for a few weeks (washout). Both the preclinical and clinical studies analyzed here have important limitations and/or biases, preventing us from defining whether the effects of the agonists are neuroprotective or symptomatic. Better-designed experimental studies and clinical trials with a longer washout period are necessary to advance in the clarification on the action of these potential drugs for PD.

Sumário

Agradecimentos.....	4
Resumo.....	5
Abstract	6
Sumário	7
1. Capítulo 1. Revisão da literatura	9
1.1. Doença de Parkinson.....	10
1.2. Patogenia da doença de Parkinson	11
1.2.1. α -sinucleínas e os corpos de Lewy	12
1.2.2. Disfunção mitocondrial e estresse oxidativo	13
1.2.3. Sistemas de depuração de proteínas disfuncionais	14
1.2.4. Neuroinflamação	15
1.3. Modelos animais da DP.....	16
1.3.1. Modelos de DP induzido por neurotoxinas	16
1.3.1.1. 6-Hidroxidopamina (6-OHDA)	16
1.3.1.2. 1-metil-4-fenil-1, 2, 3, 6-tetrahidropiridina (MPTP).....	19
1.3.1.3. Pesticidas.....	21
1.3.2. Modelos genéticos.....	22
1.4. Tratamento farmacológico da DP.....	23
1.5. DP e o diabetes mellitus tipo II	25
1.5.1. GLP-1	26
1.5.2. GIP	30
1.6. Revisão sistemática e metanálise	31
1.7. Referências	33
2. Capítulo 2. Artigo científico.....	42
Abstract	43
Background	45
Methods.....	46
Standards	45
Protocol registration and publication number	46
Eligibility criteria	46
For clinical studies.....	45
For preclinical studies	48
Search methods for primary studies	49

Electronic searches	49
Search strategy	50
Eligibility determination	50
Data extraction	50
Assessment of internal validity of individual studies.....	51
Data synthesis and statistical analysis.....	52
Heterogeneity assessment.....	53
Subgroup and meta-regression analyses.....	53
Funnel plot asymmetry	53
GRADE	54
Results.....	54
Literature search.....	55
Study characteristics.....	57
Clinical studies	57
Preclinical studies.....	58
Risk of bias – clinical studies.....	74
Risk of bias – preclinical studies.....	76
Metanalyses.....	81
Clinical studies - Primary outcomes.....	81
Clinical studies - Secondary outcomes.....	83
Preclinical studies - Primary outcomes	84
Preclinical studies - Secondary outcomes	88
Subgroup analyses.....	89
Meta-regression.....	95
Funnel Plot asymmetry.....	96
GRADE	97
Discussion	98
Conclusion.....	104
Appendix.....	105
References.....	162



Capítulo 1.

Revisão da Literatura



1. Capítulo 1. Revisão da literatura

1.1. Doença de Parkinson

O circuito motor responsável pelo controle dos movimentos realizados pela musculatura estriada esquelética envolve áreas do córtex motor e dos núcleos da base encefálica¹. As conexões que partem dos núcleos da base e alcançam as áreas corticais podem tanto as estimular como as inibir, dependendo do tipo de via ativada. Enquanto a via direta é capaz de ativar as atividades do córtex motor, estimulando os movimentos, a via indireta as reduz².

A dopamina, neurotransmissor derivado do aminoácido tirosina, é sintetizada principalmente em uma região do mesencéfalo conhecida por Substância Negra *Pars Compacta* (SNpc) e tem papel neuromodulador no circuito motor. Ela facilita a via direta ou inibe a via indireta pela ativação de receptores específicos³. A desregulação da dopamina pode levar a distúrbios do movimento caracterizados pelas hipocinesias (redução dos movimentos) ou hipercinesias (exacerbação dos movimentos)³.

A Doença de Parkinson (DP) é o tipo de hipocinesia mais frequente. Nesta condição, a falta de dopamina acarreta a não-excitação do córtex motor, o que causa as manifestações motoras clássicas: bradicinesia comumente associada à rigidez muscular e/ou tremor de repouso⁴.

Características secundárias observadas durante o exame clínico do paciente contribuem para o diagnóstico da DP, como expressão facial apática, redução do piscar de olhos, disartria (fala monótona, suave e, por vezes, “enrolada”), disfagia (dificuldade de deglutição), micrografia (diminuição gradual na amplitude das letras na escrita), marcha arrastada, diminuição no balanço do braço durante a marcha, festinação (aceleração involuntária da marcha devido a deslocamento anterior do centro de gravidade), súbita parada e incapacidade de recomeçar movimentos (“congelamento”, sensação de “pés colados no chão”), distonia e reflexos glabulares^{5, 6}.

Sintomas não-motores envolvem déficits cognitivos, ansiedade, depressão, distúrbios do sono REM (*rapid eye movement*), constipação, bexiga urinária neurogênica, perda olfativa, disfunção erétil, entre outros⁴. De fato, constipação, distúrbios do sono e perda olfativa podem preceder o aparecimento dos sinais motores em mais de 10 anos, constituindo o que se sugere ser a fase prodromal da doença⁷.

O conjunto de sinais e sintomas é variável em frequência e intensidade, o que caracteriza a DP como uma doença complexa e heterogênea. Muitos autores têm proposto a existência de subtipos da doença, baseados, por exemplo, na predominância de determinado sinal motor, características cognitivas, idade de início da doença, progressão da doença ou ainda uma combinação dessas características^{4, 8, 9}. Independentemente do subtipo, o tratamento permanece primordialmente sintomático, pois não há tratamento efetivo para frear a progressão da doença⁴.

Estima-se que há 6 milhões de pessoas com DP no mundo e que a doença afete 1% da população acima de 60 anos¹⁰. Com base no crescimento esperado da população com mais de 65 anos, a previsão é de que até 2030 esse número tenha dobrado. Melhores métodos para detecção e diagnóstico da doença, maior consciência da doença, envelhecimento das populações, maior expectativa de vida e, possivelmente, maior exposição a contaminantes ambientais podem explicar o aumento da prevalência de DP no mundo todo¹¹.

1.2. Patogenia da doença de Parkinson

A DP é uma doença neurodegenerativa progressiva comumente associada à morte de neurônios dopaminérgicos na SNpc e redução dos níveis de dopamina estriatais⁴. No entanto, a neurodegeneração não é limitada aos neurônios dopaminérgicos nigrais: a perda de neurônios dopaminérgicos que se projetam da área tegmental ventral (VTA) para áreas límbicas e o córtex pré-frontal¹² pode explicar a grande heterogeneidade dos sinais e sintomas da DP, especialmente os não-motores.

1.2.1. α -sinucleínas e os corpos de Lewy

Histologicamente, a assinatura da DP (embora não patognomônica) é a presença de depósitos citoplasmáticos anormais imunorreativos para a proteína α -sinucleína dentro dos corpos celulares neuronais, os chamados corpos de Lewy (Fig. 1)¹³.

As α -sinucleínas são proteínas desdobradas que desempenham papel na reciclagem, armazenamento e compartimentação de neurotransmissores¹⁴. No entanto, quando adquirem estrutura filamentosa com conformação em folhas β , tornam-se propensas a formar agregados e acúmulos no interior de neurônios, causando alterações nas comunicações neuronais, disfunção neuronal e, eventualmente, morte celular¹⁵.

A relação entre a α -sinucleína e a DP se fortaleceu pela identificação de mutações no gene que codifica a α -sinucleína, SNCA, e induz uma forma autossômica dominante da DP^{16, 17}. No entanto, o mecanismo exato pelo qual a α -sinucleína causa toxicidade e contribui para a morte neuronal permanece obscuro¹³.

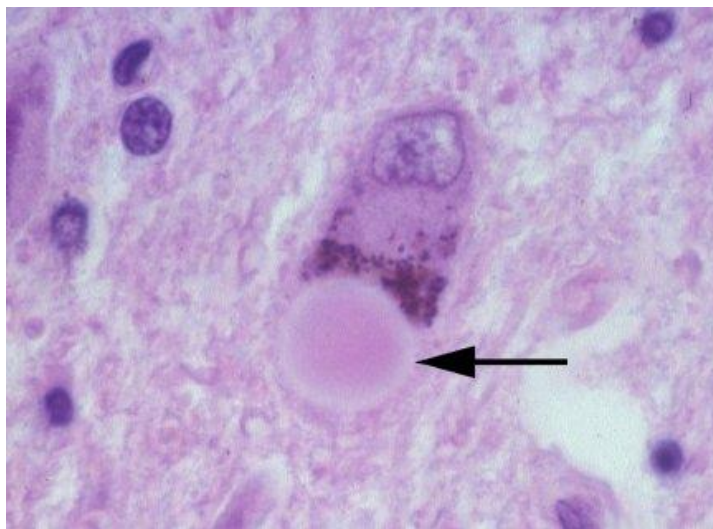


Figura 1. Corpo de Lewy¹⁸.

Em 2003, Braak et al. criaram um sistema de estadiamento para a DP, descrevendo a conexão entre a disseminação da α -sinucleína malformada e a

gravidade da doença (“estágios de Braak”)¹⁹. Nas fases iniciais do Parkinson, as inclusões de α -sinucleína seriam encontradas em duas regiões cerebrais distintas: no núcleo motor dorsal do nervo vago e no núcleo olfatório anterior. Os autores propõem que a α -sinucleinopatia possa ser iniciada na mucosa nasal e intestinal, especificamente no bulbo olfatório e nos plexos celulares entéricos, pela ação de possíveis substâncias neurotóxicas que adentram o organismo pela via oral e/ou inalatória^{20, 21}.

A α -sinucleína agiria como um príon (proteína infecciosa), com disseminação célula-a-célula^{22, 23}, e, pelo nervo vago ou olfatório, ascenderia ao SNC, afetando primeiramente as estruturas inferiores do tronco cerebral antes de envolver a SNpc. Essa teoria explicaria a presença de perda olfativa, constipação e distúrbios do sono REM em pacientes com DP antes mesmo do aparecimento dos sinais motores.

No entanto, o esquema proposto por Braak não explica todos os casos de DP. Os padrões de deposição da α -sinucleína descritos por Braak muitas vezes não são observados, principalmente na demência por corpos de Lewy (parkinsonismo atípico) ou quando a α -sinucleinopatia ocorre na ausência de manifestações neurológicas^{24, 25}. Há relatos de indivíduos com α -sinucleinopatia em estágios de Braak iniciais, que apresentam parkinsonismo clínico, assim como há indivíduos em estágios de Braak mais avançados que não manifestam sinais clínicos. Portanto, ainda não é possível assumir que há relação entre os estágios de Braak e a gravidade clínica da DP.

1.2.2. Disfunção mitocondrial e estresse oxidativo

Um dos mecanismos da neurodegeneração mais reconhecidos na DP é a disfunção mitocondrial²⁶. Os primeiros estudos *post-mortem* de cérebros humanos com DP relataram deficiência do complexo mitocondrial I (NADH desidrogenase), componente vital da cadeia de transporte de elétrons.

A transferência de elétrons por entre os complexos gera espécies reativas de oxigênio (EROS) principalmente quando essas proteínas integrais de membrana

estão carregadas o suficiente com elétrons e a energia potencial de transferência é elevada. Em situações fisiológicas, as células são capazes de desintoxicar as EROS geradas no processo respiratório devido à extensa ação de enzimas antioxidantes^{26, 27}. No entanto, devido a inibição do complexo 1 e a interrupção do transporte de elétrons para os complexos subsequentes, há acúmulo de elétrons no complexo 1 e geração excessiva de EROS, que provocam dano mitocondrial. Na presença de dano mitocondrial, as enzimas antioxidantes não conseguem neutralizar eficientemente as EROS em excesso, culminando em estresse oxidativo. Neste momento, instala-se um ciclo vicioso: quanto maior o estresse oxidativo, maior o dano mitocondrial e quanto maior o dano mitocondrial, maior o estresse oxidativo.

A disfunção mitocondrial pode também gerar desregulação da homeostase celular de Ca^{2+} ²⁸. O fluxo aumentado de Ca^{2+} desencadeia perda do potencial de membrana mitocondrial (MMP), abertura do poro de transição de permeabilidade mitocondrial (MPTP), liberação de citocromo c no citoplasma e, conseqüentemente, ativação da via intrínseca da apoptose^{29, 30}.

Estudos experimentais mostram que naturalmente ocorre considerável geração de EROS no SNC, pela grande taxa de oxigênio consumida pelo cérebro, pela baixa atividade de enzimas antioxidantes e pelo próprio mecanismo de auto-oxidação da dopamina e de seus produtos de metabolização³¹⁻³³. Portanto, os neurônios dopaminérgicos podem ser mais suscetíveis a um quadro de estresse oxidativo, principalmente quando se adiciona disfunção mitocondrial na equação.

1.2.3. Sistemas de depuração de proteínas disfuncionais

Existem dois sistemas centrais dentro das células responsáveis pela remoção de proteínas disfuncionais e renovação de organelas: o sistema ubiquitina-proteassoma (UPS) e a via autofagia/lisossoma³⁴.

O sistema UPS marca as proteínas anormais com ubiquitina e as transporta para o proteassoma para degradação. Já a autofagia pode ocorrer de três maneiras: macroautofagia, microautofagia e autofagia mediada por chaperonas (*chaperone-mediated autophagy*, CMA)³⁵. Resumidamente, na macroautofagia, componentes

intracelulares, incluindo proteínas citosólicas, são engolfados pelo autofagossomo, que então se funde com o lisossomo, levando à quebra de seu conteúdo. Por outro lado, na microautofagia, o lisossomo sozinho engolfa e destrói os componentes citoplasmáticos. Já a CMA é um processo mais seletivo, pelo qual chaperonas moleculares têm como alvo proteínas específicas, que são transportadas para o lisossomo para degradação^{35, 36}.

Esses processos demandam alta energia e são muito suscetíveis ao estresse oxidativo. Na presença de disfunção mitocondrial, as funções UPS e autofágicas ficam comprometidas, resultando no aumento da agregação de proteínas, como a α -sinucleína, e no prejuízo da remoção dessas proteínas danificadas, que se acumulam³⁷⁻⁴⁰.

1.2.4. Neuroinflamação

O SNC é considerado um tecido imune privilegiado pela ausência de células dendríticas e pela barreira hematoencefálica separar o parênquima cerebral do sistema imunológico periférico^{41, 42}. A participação de células da medula óssea, como ocorre na inflamação periférica, é bem menos evidente. Apesar disso, o SNC pode iniciar resposta imune contra patógenos ou sinais de perigo endógeno (incluindo a presença da α -sinucleína malformada) pela ativação da micrógliia, que são os macrófagos teciduais residentes do SNC. Adicionalmente, a inflamação também pode ocorrer em resposta a moléculas secretadas de neurônios em degeneração⁴¹.

Toda a reação inflamatória deve ser interrompida em certo ponto para manter a homeostase tecidual. No entanto, se o insulto persistir ou os mecanismos anti-inflamatórios forem inadequados, pode surgir inflamação crônica⁴³.

Se a neuroinflamação é causa ou consequência da resposta a danos neuronais nas doenças neurodegenerativas, ainda não se sabe. De qualquer modo, as evidências mostram que na DP o envolvimento do sistema imunológico pode exacerbar a disfunção neuronal em um ciclo vicioso⁴¹.

1.3. Modelos animais da DP

A DP é uma condição humana específica não presente naturalmente em outras espécies. Portanto, diversos modelos animais foram criados para tentar induzir a doença e mimetizar sua biologia. Duas abordagens principais têm sido utilizadas: neurotoxinas e modificação genética^{44, 45}. As neurotoxinas geralmente induzem degeneração rápida e acentuada de células dopaminérgicas na região da SNpc associadas a sinais motores e alterações comportamentais, embora não se note a formação de corpos de Lewy – sinal histopatológico importante da DP⁴⁶. Em contraste, os modelos genéticos podem não induzir perdas neuronais, ao passo que ocasionam o acúmulo de α -sinucleína, dependendo do modelo específico⁴⁵.

1.3.1. Modelos de DP induzido por neurotoxinas

Uma das características da DP que todos os modelos animais induzidos por neurotoxinas mimetizam é a degeneração dos neurônios dopaminérgicos da SNpc⁴⁷. Cada modelo, no entanto, tem suas particularidades, dependendo das espécies envolvidas, das toxinas e, também, de suas doses utilizadas⁴⁶.

Se o objetivo for estudar estratégias de tratamento para a sintomatologia da DP, o mecanismo de ação da neurotoxina é irrelevante, uma vez que o foco é a consequência da morte celular dopaminérgica em si. No entanto, o cenário é outro se o objetivo for estudar as vias envolvidas na morte de células neuronais para desenvolvimento de estratégias neuroprotetoras.

1.3.1.1. 6-Hidroxidopamina (6-OHDA)

A 6-OHDA é um análogo hidroxilado da dopamina (Fig. 2) que, por apresentar semelhança estrutural a esse neurotransmissor, é captada na fenda sináptica pelos mesmos transportadores (DAT) e, uma vez no interior dos neurônios dopaminérgicos, é também oxidada pelas enzimas envolvidas no processo de metabolização da dopamina⁴⁸. Este processo, somado à auto-oxidação sofrida pela 6-OHDA, gera EROS, rápida depleção de enzimas antioxidantes, estresse oxidativo, disfunção mitocondrial e, consequentemente, lesão neuronal⁴⁶.

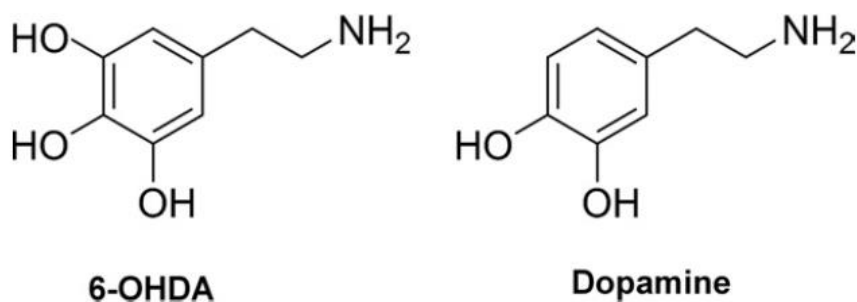


Figura 2. Comparação estrutura 6-OHDA e dopamina⁴⁷.

Como a 6-OHDA atravessa apenas moderadamente a barreira hematoencefálica, ela deve ser injetada diretamente no cérebro para desencadear neurodegeneração, conforme demonstrado por Ungerstedt há mais de 50 anos⁴⁹. Embora a 6-OHDA possa ser injetada em ambos os hemisférios cerebrais, o protocolo preferencial envolve lesão unilateral devido à extensa toxicidade e alta taxa de mortalidade em ratos causada pelas lesões bilaterais⁵⁰.

Dependendo do local da injeção e da dose empregada, diferentes perfis de morte celular podem ser obtidos. Em ratos, as injeções de 6-OHDA na SNpc ou no feixe prosencefálico medial (MFB) resultam em degeneração dopaminérgica já nas primeiras 24 horas e é um dos modelos mais usados para estudar a DP em estágio avançado⁵¹. Já quando injetada no corpo estriado, a 6-OHDA causa degeneração retrógrada dos neurônios nigro-estriatais ao longo de 1 a 3 semanas. Neste modelo, é possível induzir lesões parciais que se assemelham aos estágios mais iniciais da DP⁵².

Animais com lesões unilaterais apresentam déficits motores assimétricos e comportamento rotacional quando sob influência de drogas dopaminomiméticas (Fig. 3)⁴⁸. Injeções de anfetamina ou apomorfina induzem comportamento de rotação marcante que se correlaciona com a extensão da denervação de dopamina, sendo esta uma das maneiras de garantir que o modelo de lesão pela 6-OHDA foi bem sucedido. Enquanto a anfetamina age na liberação de dopamina na fenda sináptica, principalmente do lado intacto (lado oposto à lesão), e induz desbalanço dos níveis de dopamina entre os hemisférios, o animal tem rotações no sentido

ipsilateral à lesão (mesmo lado da lesão)⁵³⁻⁵⁵. Já a apomorfina age nos receptores de dopamina dos neurônios pós-sinápticos, aumentando a sensibilidade desses receptores, principalmente no lado lesionado. Dessa maneira, o desbalanço ocorre favorecendo o lado lesionado e o animal tende a rotar no sentido contralateral à lesão (Fig. 3)⁵³⁻⁵⁵.

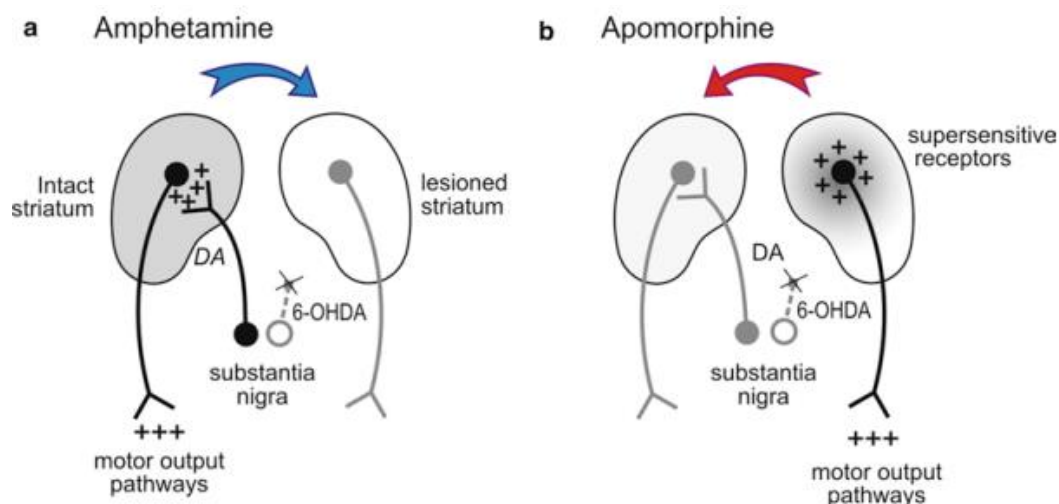


Figura 3. Comportamento rotacional induzido por apomorfina e anfetamina⁵⁶.

Este modelo não mimetiza perfeitamente a DP de humanos, pois não há formação dos corpos de Lewy tradicionais e, na maioria dos protocolos, a morte neuronal acontece de maneira muito rápida e como consequência de lesão unilateral, o que não é comum na história natural da DP^{57, 58}. No entanto, desde que essas limitações sejam compreendidas e levadas em consideração na interpretação dos resultados, o modelo induzido por 6-OHDA permanece na vanguarda dos testes pré-clínicos para DP.

1.3.1.2. 1-metil-4-fenil-1, 2, 3, 6-tetrahidropiridina (MPTP)

No verão de 1976, o estudante de química de 23 anos de idade, Barry Kidston, sintetizou em sua própria casa uma droga recreacional a partir da molécula 1-metil-4-fenil-4-hidroxipiperidina (MPPP). Por vários meses o jovem fez uso da substância produzida, tendo descrito seus efeitos como semelhantes aos da

heroína. Porém, após modificar o processo de síntese, a fim de encurtar seu tempo de preparo, os efeitos da substância não foram mais os mesmos. Em novembro de 1976, após fazer uso deste novo lote por vários dias, Barry passou a desenvolver mudez, rigidez severa, face inexpressiva, tremor e fraqueza, condizentes com estado de parkinsonismo⁵⁹.

Posteriormente, foi demonstrado que a molécula 1-metil-4-fenil-1, 2, 3, 6-tetrahidropiridina (MPTP) (Fig. 4), uma impureza gerada acidentalmente no processo de fabricação da MPPP, seria a responsável⁶⁰.

A MPTP é capaz de ultrapassar a barreira hematoencefálica devido a sua elevada lipofilicidade. É no SNC onde ocorre a conversão ao intermediário 1-metil-4-fenil-2,3, di-hidropiridínio (MPDP⁺) pela ação da enzima monoamino oxidase (MAO-B) e, posteriormente, ao subproduto tóxico 1-metil-4-fenil-2,3-dipiridínio (MPP⁺) (Fig. 4)⁶¹. A MPP⁺ atua como substrato do transportador de dopamina (DAT) e é captada pelos neurônios da SNpc, levando à inibição do complexo mitocondrial I, depleção de ATP, estresse oxidativo, neuroinflamação e morte de neurônios dopaminérgicos⁶². A partir deste incidente, originou-se o modelo de DP animal induzido por MPTP, que ainda é amplamente utilizado.

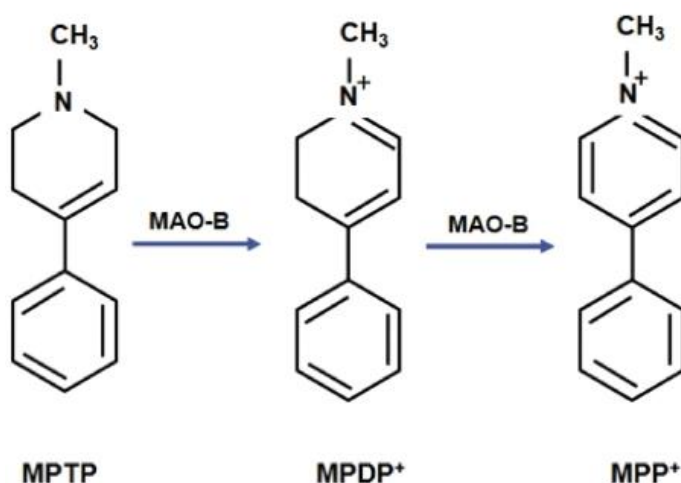


Figura 4. Metabolização da MPTP⁶³.

Inúmeros fatores, incluindo espécie, linhagem, peso e idade do animal, contribuem para a sensibilidade e efeitos tóxicos da MPTP⁴⁸. Por exemplo, primatas não-humanos são bastante sensíveis, em particular, os macacos da família Cercopithecidae (conhecidos como macacos do "Velho Mundo")^{48, 58}. Já gatos, cães, cobaias e ratos apresentam menor sensibilidade⁴⁸. Camundongos, embora menos que primatas não-humanos, apresentam boa resposta à MPTP. No entanto, também é preciso levar em consideração diferenças entre as linhagens dessa espécie: camundongos C57 BL/6 são mais sensíveis, enquanto as linhagens CD-1 e BALB/c são praticamente resistentes⁶⁴. Há evidências de que até mesmo animais de mesma linhagem, embora advindos de diferentes biotérios, possam apresentar diferenças na resposta⁴⁸.

Há uma série de protocolos de administração da MPTP que variam em relação à quantidade total administrada, a via (intraperitoneal ou subcutânea) e o tempo entre as injeções.

Camundongos tratados com regimes agudos (várias injeções - normalmente quatro - em um dia) exibem apenas redução transitória na atividade locomotora, que é perdida ou até mesmo reverte para um estado hiperativo dentro de dois a três dias⁶⁵. Os modelos agudos são, portanto, de pouca utilidade na avaliação da eficácia sintomática dos medicamentos. Em contraste, o regime subagudo (injeções diárias por cinco a 10 dias) produz hipoatividade mais persistente, evidente dentro de três horas pós-tratamento, e dura pelo menos 10 dias. O regime crônico intermitente (normalmente injeções duas vezes por semana por cinco semanas) também produz hipoatividade de longa duração e alteração em alguns testes a partir de duas semanas após o tratamento, com duração de até seis meses⁶⁶⁻⁶⁸. Um modelo promissor, principalmente por sua natureza progressiva, envolve a administração conjunta de MPTP e um adjuvante, a probenecida, que bloqueia a rápida depuração da toxina e seus metabólitos pelos rins e potencializa a neurotoxicidade^{66, 69}. Já a infusão crônica de MPTP (minibomba osmótica com infusão por até aproximadamente 28 dias) produz um fenótipo comportamental variado, podendo até mesmo o animal não apresentar nenhum déficit locomotor e comportamental. Muito provavelmente

essa variação se dá pelos diferentes níveis de neurodegeneração alcançados com este modelo (28 - 90%)^{70, 71}.

As vantagens da MPTP em relação a 6-OHDA são o menor custo e maior facilidade de lesão dos animais, uma vez que não são necessárias cirurgia e estrutura complexas, apesar de necessários cuidados extras na manipulação da toxina⁷². Adicionalmente, as injeções sistêmicas induzem danos bilaterais, que mais se aproximariam do modelo humano. No entanto, os modelos agudos e subagudos sofrem as mesmas críticas da 6-OHDA em relação a não formação dos corpos de Lewy e à rápida degeneração neuronal, que não se assemelha à DP de humanos⁴⁸. Por outro lado, em alguns modelos crônicos é possível observar depósitos de α -sinucleína, embora haja controvérsias e pouca reprodutibilidade, em detrimento de déficits motores pronunciados.

1.3.1.3. Pesticidas

Alguns pesticidas também têm sido utilizados para modelar a DP em animais, mais frequentemente o paraquate e a rotenona.

Há muitas especulações de que, pelo fato de o paraquate ser estruturalmente semelhante à MPTP (mais especificamente à MPP+), ele exerça seus efeitos deletérios pelo mesmo mecanismo^{73, 74}. Porém, foi demonstrado que o paraquate é um fraco inibidor do complexo 1 em roedores⁷⁵. Ainda não é claro se o paraquate é, no entanto, capaz de desencadear disfunção mitocondrial pela produção de EROS devido a seu intenso ciclo redox^{76, 77}. Usualmente não é observada diminuição expressiva da dopamina estriatal nos modelos animais, apesar de ter sido observada degeneração seletiva dos neurônios da SNpc^{78, 79}.

A rotenona, semelhantemente à MPTP, inibe a atividade do complexo mitocondrial I, causando a produção de EROS e disfunção mitocondrial⁸⁰. Além de sua toxicidade central, a rotenona induz toxicidade sistêmica (principalmente cardiovascular), com altas taxas de mortalidade (~ 30% dos animais). Há também variabilidade substancial na resposta individual à toxina, que se traduz em oscilações na porcentagem de animais que desenvolvem lesão nigroestriatal

satisfatória; isto pode tornar o modelo de rotenona inadequado para testar tratamentos potencialmente neuroprotetores⁷⁸.

1.3.2. Modelos genéticos

Estudos genéticos de DP familiar permitiram a identificação de mutações em genes únicos, ou seja, formas monogênicas da DP. Mutações autossômicas dominantes nos genes que codificam as proteínas α -sinucleína (SNCA) e quinase rica em leucina de repetição 2 (LRRK2), bem como as mutações autossômicas recessivas nos genes que codificam parkina (PARK2), quinase 1 induzida por PTEN (PINK1) e DJ-1 (PARK7), são as mais comuns, embora essas formas monogênicas representem apenas cerca de 10% de todos os casos de DP⁴⁶.

O gene da SNCA foi o primeiro conectado à DP familiar¹⁷. Três mutações pontuais autossômicas dominantes totalmente penetrantes foram identificadas: A53T, A30P e E46K^{58, 81, 82}, além de duplicação ou triplicação do gene. Uma das estratégias mais utilizadas para replicar a DP familiar em modelos animais é utilizar camundongos transgênicos que carregam essas mutações mais comumente sob controle do promotor tirosina hidroxilase ou do promotor priônico (permitem a superexpressão do gene em regiões específicas ou mais abrangentes do SNC).

No entanto, apesar de manifestar o acúmulo de α -sinucleína, nem todos os modelos transgênicos induzem neurodegeneração dopaminérgica⁴⁶. Vale ressaltar que as inclusões de α -sinucleína detectadas também não são da mesma composição fibrilar dos corpos de Lewy identificados em humanos.

Embora esses modelos sejam úteis para elucidar a função da α -sinucleína e a relação da DP com outras sinucleinopatias, sua relevância clínica ainda é questionável, uma vez que, a depender do modelo, o transgene pode afetar estágios iniciais da embriogênese em vez de modelar a DP de início tardio⁴⁶. Apenas o modelo de camundongo transgênico carregando a mutação A53T sob controle do promotor priônico apresenta semelhanças com a α -sinucleinopatia e neurodegeneração dependentes da idade observadas em humanos^{57, 83}.

Além dos modelos transgênicos, a superexpressão de α -sinucleína pode ser induzida por vetores virais, comumente vetores adenoassociados e lentivirais⁸⁴. As vantagens deste método incluem o direcionamento da superexpressão de α -sinucleína e lesão ao sistema nigro-estriatal, a indução da doença na idade adulta e a possibilidade de ajuste da dosagem⁸⁴.

A administração de fibrilas pré-formadas de α -sinucleína exógena (*preformed fibrils*, PFF) também é uma estratégia possível para modelar a DP. A α -sinucleína é uma proteína solúvel que forma estruturas mais complexas, como oligômeros, fibrilas e filamentos. Estruturas oligoméricas parecem ser as versões mais tóxicas de α -sinucleína que formam os corpos de Lewy⁸⁵. Em camundongos e ratos, a introdução de inclusões de α -sinucleína no corpo estriado causa disfunção, dano mitocondrial e eventual degeneração retrógrada de neurônios dopaminérgicos nigro-estriatais⁴⁶.

1.4. Tratamento farmacológico da DP

Apesar de a DP ter sido descrita pela primeira vez pelo médico inglês James Parkinson em 1817⁸⁶, seu substrato anatômico permaneceu desconhecido até 1919, quando Konstantin Tretiakoff correlacionou a doença pela primeira vez à perda de neurônios da SNpc⁸⁷. No entanto, a fisiopatologia subjacente da doença só foi revelada várias décadas depois com experimentos animais.

Arvid Carlsson et al. (1957 e 1958) estabeleceram as bases para o uso da levodopa (L-DOPA) como tratamento da DP com a descoberta do envolvimento da dopamina no controle motor^{88, 89}. Eles obtiveram sucesso ao demonstrar reversão do estado acinético induzido por reserpina em ratos e coelhos pela administração de um precursor da dopamina, a L-DOPA⁸⁸ (Fig. 5). Em 1960, Ehringer e Hornykiewicz demonstraram que pacientes com DP apresentam déficit na quantidade de dopamina estriatal, o que pode ser atribuído à perda de neurônios da SNpc⁹⁰.

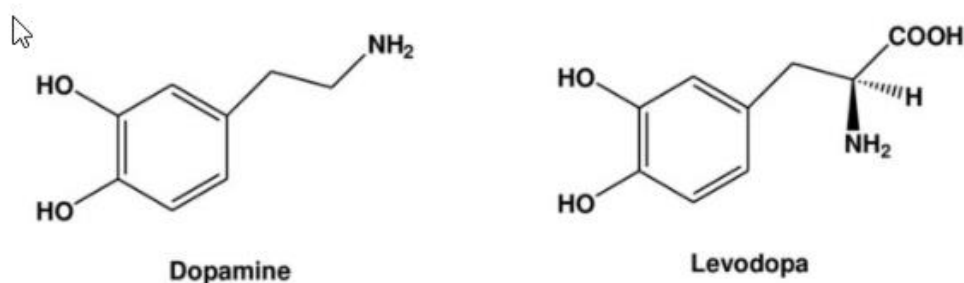


Figura 5. Estruturas dopamina e levodopa⁹¹.

O primeiro ensaio clínico envolvendo L-DOPA em pacientes com DP foi iniciado em menos de cinco anos após o primeiro experimento animal. E cinco anos depois, o desenvolvimento de uma terapia clinicamente útil surgiu com a publicação do artigo de George Cotzias em 1967⁹². Até hoje a L-DOPA continua sendo o tratamento padrão-ouro para a DP.

No entanto, apesar de agir na melhora sintomática dos sinais motores, a L-DOPA não age prevenindo a morte neuronal, de modo que a DP ainda é tida como uma condição progressiva sem cura. Com o tempo de uso da L-DOPA e o agravamento da doença, os pacientes podem manifestar discinesias, caracterizadas pelo excesso de movimentos, e fenômenos “on” e “off”⁹³⁻⁹⁵. Ou seja, tornam-se nítidos os momentos em que o desempenho funcional do paciente é satisfatório devido ao efeito da medicação e os momentos em que seu desempenho é inferior devido à interrupção abrupta do efeito da L-DOPA. Acredita-se que uma das causas para as flutuações motoras observadas como efeito adverso da L-DOPA administrada a longo prazo seja a estimulação intermitente dos receptores dopaminérgicos em função da perda da capacidade de armazenamento de dopamina pela morte da maioria dos neurônios da SNpc em estágios mais avançados da doença^{96, 97}.

Dado o risco mais alto de discinesias induzidas pelo tratamento por L-DOPA em pacientes mais jovens^{98, 99}, outros agonistas dopaminérgicos têm sido considerados o tratamento de primeira escolha, enquanto a L-DOPA pode ser o tratamento inicial em pacientes mais idosos (> 70 anos de idade).

Dentre os agonistas dopaminérgicos não ergolínicos mais utilizados destacam-se o pramipexol, o ropinirol e a rotigotina. Eles podem ser utilizados nos casos mais iniciais da DP, de maneira a adiar a introdução da levodopa, ou ainda, em casos mais avançados da doença, por permitir redução das doses de levodopa, diminuindo as flutuações motoras¹⁰⁰. Com mecanismo diferente, embora com objetivos semelhantes, há os inibidores da MAO-B (*Monoamine oxidase, tipo B*), selegilina e rasagilina, que proporcionam aumento nos níveis de dopamina na fenda sináptica pela inibição de sua enzima degradante¹⁰¹.

1.5. DP e o diabetes mellitus tipo II

Mais recentemente, o Diabetes Mellitus tipo II (DMT2) tem sido associado a sintomas mais graves e progressão acelerada da DP^{101, 102}. A importância da disfunção metabólica na DP está sendo cada vez mais aceita, incluindo a disfunção das vias de sinalização ou de resistência à insulina^{103, 104}.

O DMT2 é caracterizado pela redução na capacidade da insulina de estimular a captação e utilização de glicose pelas células (resistência à insulina) e pela secreção relativamente reduzida de insulina (hipoinsulinemia) pelas células β -pancreáticas em resposta à hiperglicemia¹⁰⁵. No sistema nervoso central (SNC), embora não esteja diretamente envolvida na captação de glicose, a insulina é capaz de modular muitos processos que se encontram desregulados na DP, incluindo apoptose, autofagia, biogênese mitocondrial, estresse oxidativo, neuroinflamação, síntese de proteínas e plasticidade sináptica^{106, 107}.

Wang et al.¹⁰⁸ demonstraram que camundongos mutantes ob/ob e db/db, modelos animais de DMT2, após tratamento com MPTP apresentaram resistência à insulina não apenas no pâncreas e fígado, mas também no mesencéfalo. No SNC desses camundongos com DMT2 houve acúmulo de α -sinucleína intracitoplasmática nos neurônios da SNpc (e adicionalmente no pâncreas), levando a maior perda de neurônios dopaminérgicos na SNpc, além de ativação das células da micróglia (neuroinflamação) quando comparados aos animais sem DMT2 tratados com MPTP. Assim, foi sugerido que os camundongos com resistência à

insulina (ou seja, com perfil diabético) são mais suscetíveis à neurotoxicidade induzida por aquela neurotoxina.

É possível também que exista relação entre o acúmulo α -sinucleína e o desenvolvimento da resistência à insulina¹⁰⁹. Em camundongos transgênicos com superexpressão de α -sinucleína, o acúmulo dessa proteína parece atuar como regulador negativo da via de sinalização da insulina pela desestabilização do substrato de seu receptor (*Insulin receptor substrate-1*, IRS-1), inibindo ativação das outras quinases proteicas dessa via¹¹⁰.

Pesquisas adicionais esclarecerão se a resistência à insulina é causa ou consequência da neurodegeneração na DP, mas, intervenções que visam revertê-la estão sendo cada vez mais reconhecidas como possíveis novas terapias para doenças neurodegenerativas¹⁰⁹. Entre os alvos mais atrativos destacam-se dois receptores capazes de modular a síntese e liberação de insulina no organismo, o GLP-1R e o GIPR.

1.5.1. GLP-1

Em 1932, La Barre et al. apresentaram o conceito de “efeito incretina” para descrever o que pareciam ser fatores humorais liberados pelo intestino capazes de regular a glicose no sangue após as refeições¹¹¹. No entanto, a demonstração quantitativa desse fenômeno só foi possível após três décadas, quando foi observado que, para a mesma quantidade de glicose, a administração oral induz secreção de insulina muito maior do que pela via intravenosa¹¹².

Identificado pela primeira vez no início dos anos 80, o GLP-1 (*glucagon-like peptide- 1*) é um hormônio incretina, derivado do produto da transcrição do gene pró-glucagon, liberado principalmente pelas células enteroendócrinas do intestino delgado em resposta à ingestão alimentar^{109, 113}. A ação incretina do GLP-1 é caracterizada pela indução da secreção de insulina pelo pâncreas e estímulo à proliferação e diferenciação de células β das ilhotas pancreáticas, processos dependentes de glicose. Há também inibição do hormônio glucagon (que tem ação contrária à da insulina), retardamento do esvaziamento gástrico e promoção da

sensação de saciedade^{109, 113}. Portanto, o GLP-1 desempenha papel importante na regulação do metabolismo glicêmico. Análogos sintéticos do GLP-1 têm sido utilizados no tratamento da DMT2. Eles têm a vantagem de superar a curta ação do GLP-1 natural, que é rapidamente degradado pela enzima circulante dipeptidil peptidase (DPP-IV)¹¹⁴.

Além de sua aplicação convencional no tratamento da DMT2, estudos *in vitro* e *in vivo* avaliaram os efeitos neurotróficos dos análogos do GLP-1 em modelos de doenças neurodegenerativas¹⁰⁹. Amplamente expresso nas células das ilhotas pancreáticas, o GLP-1R é também expresso seletivamente no SNC, como no córtex frontal, hipotálamo, tálamo, hipocampo, cerebelo, SNpc, astrócitos e células da glia¹¹⁵⁻¹¹⁷. Por mais que a maior parte do GLP-1 seja produzida periféricamente e adentre ao SNC por difusão pela barreira hematoencefálica, uma parte é localmente produzida por células neuronais e da glia¹¹⁸.

No cérebro, são duas as principais vias de sinalização ativadas pela ligação ao GLP-1R, e consequentemente moduladas pela ação da insulina, que podem ter envolvimento na DP: (i) a via MAPK/ERK, também conhecida como Ras-Raf-MEK-ERK, e a (ii) PI3K/AKT¹¹⁹. Tais vias estão envolvidas em processos como autofagia, apoptose, função mitocondrial e neuroinflamação, bem como no aprendizado, memória e modulação da plasticidade sináptica. Em linhas gerais, a ativação dessas vias promove sobrevivência celular, enquanto inibe vias pró-apoptóticas (Bassil et al., 2014; Hamilton et al., 2011; Spielman et al., 2017; Teramoto et al., 2011).

O primeiro agonista do GLP-1R desenvolvido foi a exenatida, uma versão sintética da exendina-4 - hormônio encontrado na saliva do Monstro-de-Gila, lagarto nativo do sudoeste dos Estados Unidos¹⁰⁹ (Fig. 6). A exendina-4 apresenta aproximadamente 50% de homologia com o GLP-1 humano e é um potente ativador do seu receptor, com a vantagem de ter meia-vida maior que a do GLP-1 endógeno após injeção subcutânea (2,4 horas da exendina-4 *versus* apenas alguns minutos do GLP-1 endógeno)¹²⁰.

Há vários outros agonistas do GLP-1R de desenvolvimento mais recente e já utilizados como segunda linha no tratamento da DMT2: lixisenatida (baseado na estrutura da exendina-4, com alteração de alguns aminoácidos terminais que permitem meia vida ainda maior que da exenatida – cerca de 3 horas), bem como liraglutida e semaglutida (baseados na estrutura do GLP-1 humano, com a adição de uma cadeia lateral de ácidos graxos que permite a ligação reversível à albumina e fuga da ação da DPP-IV) (Fig. 6). As meia-vida da liraglutida e semaglutida são de 11-13 horas a sete dias, respectivamente, e são conhecidos como agonistas do GLP-1R de longa duração¹²⁰. Atividade de ativação do GLP-1R também foi descrita para o geniposídeo, componente ativo da planta ornamental *Gardenia jasminoides*, embora ainda não seja utilizado para fins de tratamento da DMT2¹²¹.

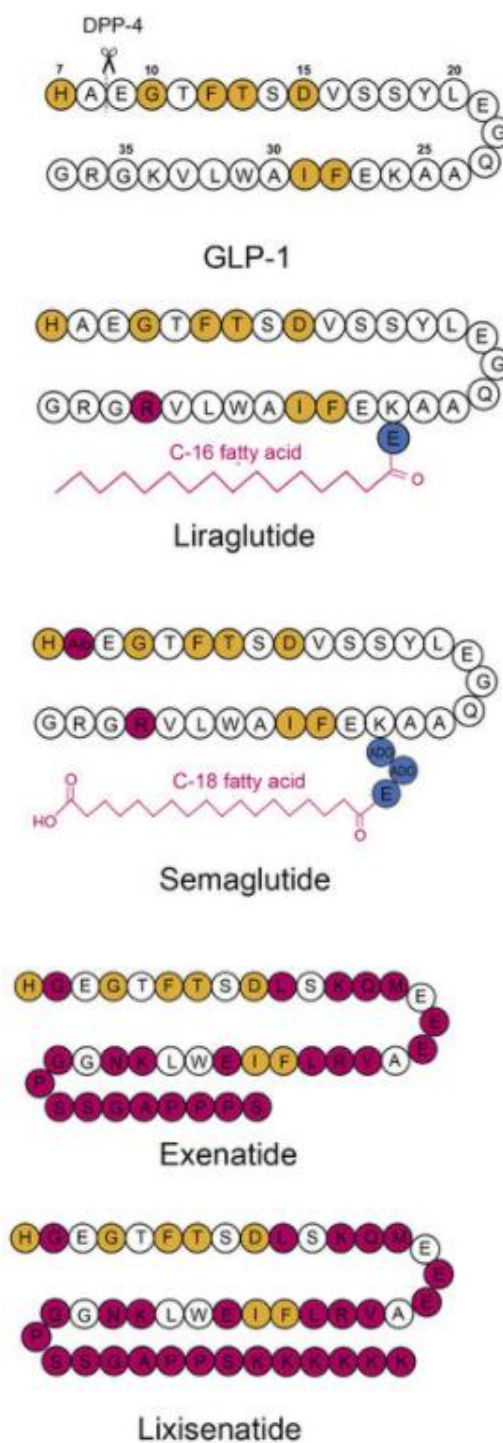


Figura 6. Estrutura do GLP-1 e análogos¹²².

1.5.2. GIP

Descoberto na década de 1970, antes do GLP-1, o GIP (*Gastric inhibitory polypeptide* ou alternativamente *glucose-dependent insulintropic polypeptide*) também é reconhecido como hormônio incretina, com estímulo à proliferação e diferenciação de células β das ilhotas pancreáticas (Fig. 7). No entanto, apesar de potente estímulo para a liberação de insulina em indivíduos saudáveis após as refeições, esse efeito do GIP aparenta ser praticamente inexistente em indivíduos com DMT2¹²³. Em modelos animais foi constatado que o estado de hiperglicemia reduz tanto a expressão do receptor GIP nas células β pancreáticas como a responsividade dessas células para a produção e liberação de insulina^{124, 125}.

Adicionalmente, enquanto o GLP-1 também provoca sensação de saciedade e inibe o apetite, o GIP é desprovido dessas atividades, aparentemente não tendo ação benéfica sobre controle do peso corpóreo. Por esses motivos, o GIP ficou para trás em relação ao GLP-1 como possível tratamento da DMT2¹²⁶.

No entanto, moléculas modificadas para terem atividade concomitante em ambos os receptores, GIP e GLP-1, têm teoricamente apresentado resultados mais positivos do que agonistas apenas do receptor GLP-1 no controle glicêmico e do peso corpóreo em alguns ensaios clínicos com portadores de DMT2 obesos¹²⁷, o que nos leva à teoria de esses agonista duplos possam provocar alteração na via de sinalização do GLP-1 e ativar um conjunto diferente de vias em comparação ao GLP-1 ou GIP individualmente¹²³.

Assim como o receptor GLP-1, o receptor GIP também é expresso em diversas regiões do cérebro, como nas camadas do córtex cerebral, hipocampo, giro dentado, amígdala, bulbo olfatório e áreas do tronco cerebral¹²⁸⁻¹³⁰. A ativação desse receptor no cérebro pode ter outras ações além do controle metabólico, como a de restaurar a sensibilidade à insulina neuronal pela ativação de vias específicas moduladas pela insulina. O GIP estimularia a proliferação de células progenitoras neuronais, melhoraria déficits cognitivos de memória e aprendizagem e reduziria a neuroinflamação¹³¹. Moléculas análogas ao GIP, bem como agonistas duplos (GLP-

1/GIP), têm sido investigadas por suas ações neurotrópicas no tratamento da DP *in vitro* e *in vivo*¹³²⁻¹³⁷.

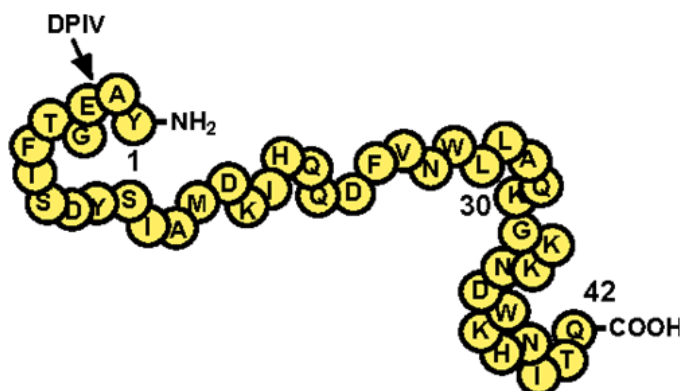


Figura 7. Estrutura do GIP¹³⁸.

1.6. Revisões sistemáticas e metanálises

Revisões sistemáticas (RS) são ferramentas objetivas e transparentes para coletar e sintetizar informações científicas a respeito de um tema específico¹³⁹. Em contraste com as revisões tradicionais da literatura, que baseiam suas conclusões em artigos selecionados aleatoriamente e tendem a ser parcialmente enviesadas pela opinião de seus autores, as revisões sistemáticas oferecem rigor metodológico e possibilidade de otimização dos resultados por meio de metanálises^{139, 140}.

As metanálises são a maneira mais refinada de resumir os dados obtidos por revisões sistemáticas, por meio da compilação estatística dos resultados¹³⁹. Porém, se estivermos trabalhando com estudos diferentes entre si, nem sempre é possível conduzir uma metanálise. Essa heterogeneidade, representada geralmente por I^2 , indica o percentual da variação dos resultados entre os estudos que ultrapassa o efeito do acaso. Assim, quando há alto nível de heterogeneidade, assume-se que existem divergências reais entre os estudos, não sendo recomendável a combinação de seus resultados para realização de uma metanálise¹⁴⁰. Neste caso, os dados são apresentados apenas narrativamente.

As RS e metanálises são mais amplamente utilizadas na área da saúde para comparar a eficácia de tratamentos farmacológicos e procedimentos cirúrgicos,

tipicamente a partir de ensaios clínicos randomizados¹⁴¹. Apesar de menos comuns que as RS de ensaios clínicos, cada vez mais se encontram publicações de RS envolvendo estudos pré-clínicos conduzidos em animais. Ferramentas específicas desenvolvidas por entidades como SYRCLE^{142, 143} e CAMARADES¹⁴⁴ auxiliam na avaliação do risco de viés e qualidade dos artigos focando nas especificidades dos delineamentos de estudos animais.

A proposta do trabalho apresentado no capítulo 2 desta tese foi realizar revisão sistemática e metanálise de estudos tanto clínicos, quanto pré-clínicos, avaliando a eficácia e segurança dos agonistas dos receptores GLP-1 e GIP no tratamento da DP.

Duas revisões sistemáticas já foram publicadas com temática semelhante. A revisão de estudos animais e humanos publicada por Erbil et al. (2019)¹¹³ indicou que os agonistas dos receptores GLP-1 são eficazes na reversão parcial ou total dos efeitos induzidos por neurotoxinas, complicações neurovasculares da diabetes, alterações neuropatológicas relacionadas à doença de Alzheimer, DP ou oclusão vascular. No entanto, nessa revisão sistemática não há clareza na definição do PICO (“participants”, “intervention”, “comparators”, “outcome”) ou da estratégia de busca utilizada e não há metanálise dos resultados, avaliação do risco de viés dos estudos ou da qualidade das evidências (validade interna e externa). Como atualização desta revisão sistemática, além da inclusão dos agonistas dos receptores GLP-1 proposta por Erbil et al. (2019), as análises foram aqui estendidas aos agonistas dos receptores GIP e as avaliações faltantes supracitadas foram realizadas.

Outra revisão sistemática abordando este tópico, publicada por Mulvaney et al. (2020)¹⁴⁵ indicou qualidade baixa ou muito baixa dos poucos ensaios clínicos disponíveis na literatura envolvendo GLP-1 e DP. Apesar do rigor metodológico, focou apenas em ensaios clínicos randomizados. A proposta da atual RS foi avaliar rigorosamente as evidências em estudos também animais para correlação com os achados em humanos e identificar fortalezas e fraquezas dos possíveis medicamentos candidatos a futuros ensaios clínicos envolvendo a DP e agonistas dos receptores GLP-1/GIP.

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Capítulo 2.

Artigo científico

**GLP-1 and GIP receptor agonists in the treatment of Parkinson's
disease: Translational systematic review and meta-analysis of clinical
and preclinical studies**

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Abstract

Background: Parkinson's disease (PD) is a progressive multifactorial neurodegenerative condition. Epidemiological studies have shown that patients with type 2 diabetes mellitus (T2DM2) are at increased risk for developing PD, indicating a possible insulin-modulating role in this latter condition. It is possible that drugs capable of activating the receptors glucagon-like peptide-1 (GLP-1) and gastric inhibitory polypeptide (GIP), potentially used in the treatment of T2DM2, may play a role in PD. **Objectives:** The purpose of this study was to systematically review and meta-analyse data of preclinical and clinical studies evaluating the efficacy/safety of GLP-1 and GIP receptor agonists in the treatment of PD. **Methods:** Two reviewers independently evaluated the studies available in the Ovid Medline, Ovid Embase, Web of Science, Cochrane Central Register of Controlled Trials, and Cinahl. Preclinical rodent or non-human primate studies and randomized controlled human clinical trials were included, without language or publication period restrictions. Outcomes of interest in preclinical studies were primarily locomotor improvements in animal models of PD. For clinical trials, motor and non-motor improvements rated by validated scales were evaluated. The risk of bias of preclinical studies was assessed by an adapted version of the SYRCLE and CAMARADES tools and the clinical studies were assessed by the Cochrane tool (RoB 2); GRADE was used for the certainty of the evidence. **Results:** Of the 1108 studies retrieved, 2 clinical trials and 31 preclinical studies were included. In clinical trials, exenatide (GLP-1) improved the motor impairment in the practically off-medication state exceeding the minimal clinically difference for improvement after the washout period (GRADE low level of certainty). However, there were no beneficial effects for the other primary outcomes, such as motor activities of daily living, non-motor symptoms, or motor complications (all GRADE very-low levels of certainty). One trial was at low risk of bias, whereas the other had some concerns. In preclinical studies, the overall meta-analyses for all GLP-1, GIP and GLP-1/GIP dual receptor agonists showed improvement of the locomotor function of rodents in the open field, rotarod, traction, and footprint tests in studies with different PD lesion models and different treatment regimens with the drugs. Studies were overall at high or unclear risk of bias. **Discussion and conclusion:** The locomotor improvement was present in both preclinical and clinical studies for exendin-4/exenatide, the commonly drug. Since other drugs with registered protocols for clinical trials, such as lixisenatide, semaglutide, liraglutide, and NLY01, showed comparable or superior results in animal preclinical studies, it is possible that at least equal or more promising results are achieved in the future studies compared to exenatide. However, although the present results show a possible anti-parkinsonian activity, biases and/or limitations were identified. Better-designed experimental studies and clinical trials with more participants and longer washout periods are necessary to advance in the understanding of these potential drugs as treatment options for PD.

Systematic review registration: PROSPERO registration number CRD42020223435.

Background

Glucagon-like peptide-1 (GLP-1) and glucose-dependent insulintropic polypeptide (GIP) are incretin hormones secreted by specific intestinal enteroendocrine cells in response to nutrient ingestion and absorption, preferably of carbohydrate and fat¹⁻³.

Both hormone effects are characterized by stimulation of pancreatic islet β cell proliferation and differentiation, as well as induction of glucose-dependent pancreatic insulin secretion^{2, 4}. Together, they play an important role in regulating glycemic homeostasis and are responsible for around 60% of insulin release in healthy subjects in a post-prandial state⁵. However, although the insulintropic activity of GLP-1 is preserved in type II diabetes mellitus (T2DM) patients after administration of GLP-1, the GIP activity is still impaired even with supra-physiological levels of GIP. For this reason, GLP-1 receptor agonists have been approved as possible second line treatments for T2DM, while, so far, GIP receptor agonists have not^{6, 7}.

However, several in vitro and in vivo studies⁸⁻¹³ have evaluated the GLP-1 and GIP actions in extrapancreatic tissues, such as the brain, since the incretin hormone receptors are also expressed in the CNS¹⁴⁻¹⁷. In the CNS, insulin is behind many processes that could be dysregulated in Parkinson's disease (PD), including apoptosis, autophagy, mitochondrial dysfunction, oxidative stress, neuroinflammation, and synaptic plasticity¹⁸⁻²³.

The binding to GLP-1 and GIP receptors by GLP-1 and GIP agonists may restore brain insulin sensitivity by activation of specific insulin-modulated pathways that promote cell survival, while inhibiting pro-apoptotic pathways²⁴⁻²⁷. Therefore, interventions aimed at reversing insulin resistance are being increasingly recognized as possible new therapies for PD.

However, the GLP-1 and GIP receptor agonists may differ in their neuroprotective effects in view of pharmacodynamic and pharmacokinetic differences, and it is not quite clear if they would act as adjuvants in the treatment of PD with other usual antiparkinsonian drugs, or if they really could alter the progression of the disease.

Studies comparing different animal models of PD and different experimental designs with incretin hormones are scarce²⁸. Either, there are a few randomized controlled trials with these analogues available in the literature; therefore, the possibility of

translating the efficacy of this group of drugs from preclinical studies to the clinic has not yet been evaluated.

Translating the knowledge of basic research into new therapeutic approaches following the concept of "bench to bedside" is just one of the stages of translational medical research^{29, 30}. Therefore, we propose to perform a systematic review and meta-analysis of randomized controlled clinical trials and preclinical studies evaluating the anti-parkinsonian efficacy and safety of GLP-1 and GIP receptor agonists. The ultimate goal is to verify if the results from preclinical studies can be translated into the clinical practice.

Methods

Standards

This protocol followed established guidelines for systematic reviews and meta-analyses of clinical trials (PRISMA-P)³¹, and specific guidelines for conducting systematic reviews of preclinical studies³².

Protocol registration and publication number

The protocol of this systematic review is registered in the International Prospective Register of Systematic Reviews (PROSPERO registration number: CRD42020223435), and publicly available at <https://doi.org/10.1371/journal.pone.0255726>.

Eligibility criteria

For clinical studies

Study design - Randomized controlled trials.

Participants – Studies including adults (≥ 18 years old) with a specialist-confirmed PD diagnosis (ICD-10: G20) according to the International Parkinson and Movement Disorder Society (MDS) criteria or the UK Parkinson's Disease Society Brain Bank diagnostic criteria, or on the basis of clinical neurological assessment³³. There were no restrictions regarding sex, ethnicity, or severity of PD.

Intervention – Studies including patients on treatment of PD with drugs capable of activating the following receptors:

- GLP-1
- GIP
- GLP-1/GIP (dual agonists)

There were no restrictions regarding duration and doses. Patients concomitantly receiving usual antiparkinsonian drugs (e.g., levodopa or dopaminergic drugs, such as monoamine oxidase inhibitors, catechol-O-methyltransferase inhibitors, or dopaminergic agonists) were accepted.

Comparators – Studies including specialist-confirmed PD patients who have not received treatment with GLP-1, GIP or dual GLP-1/GIP receptor agonist drugs. Participants on GLP-1/GIP placebos receiving standard of care (e.g., background treatment with antiparkinsonian drugs) were also accepted.

Primary outcomes – Studies that measured:

- PD motor signs rated by the “Movement Disorders Society Unified Parkinson's Disease Rating Scale - part III ³⁴”. A difference of -3.25 points was considered a minimal, but clinically important, improvement and 4.63 points was considered minimal, but clinically important, worsening ³⁵.
- Non-motor symptoms rated by the MDS-UPDRS Scale, part I ³⁴ (a difference of -2.65 points for minimal, but clinically important, improvement and 2.45 points for minimal, but clinically important, worsening)³⁶, Non-motor Symptoms scale (NMSS)³⁷ or by the Non-Motor Symptoms Questionnaire (NMSQ) ³⁸;
- Activities of daily living rated by the MDS-UPDRS Scale, part II ³⁴. A difference of -3.05 and was considered a minimal, but clinically important, improvement and 2.51 points was considered minimal, but clinically important, worsening³⁶.
- Dyskinesia or motor fluctuations rated by the Unified Dyskinesia Rating Scale ³⁹ (a difference of -2.1 points for minimal, but clinically important, improvement and 1.8 points for minimal, but clinically important, worsening)⁴⁰; or by the MDS-UPDRS Scale part IV ³⁴ (a difference of -0.9 points for minimal, but clinically important, improvement and 0.8 points for

minimal, but clinically important, worsening)⁴⁰.

- Adverse effects or serious adverse effects as reported in the included studies.

Secondary outcomes – Studies that measured:

- Quality of life rated by the Parkinson's Disease 39 item Quality of life questionnaire ⁴¹.
- Psychological alterations, such as dementia and depression rated by the Mattis Dementia Rating Scale ⁴² or the Montgomery-Asberg Depression Rating Scale ⁴³.

Exclusion criteria – Considering the potential association between diabetes and PD, we excluded studies that exclusively enrolled patients with diabetes (any type). Furthermore, studies involving participants with advanced dementia were excluded because cognitive impairment could affect the accuracy of patient-reported outcomes.

For preclinical studies

Study design - Any non-human studies using the following most common neurotoxin-induced animal models of PD for the establishment of therapeutic measures:

- 6-hydroxydopamine, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, lipopolysaccharide, rotenone (or other possible pesticide exposure);
- Transgenic animal models that explore the function of PD-linked genes (e.g. α -synuclein, DJ-1, LRRK2, Parkin, UCH-L1, PINK1);
- Other models mentioned in the screened studies.

Animal species – Studies with adult rodents or non-human primates, without restriction as to strain, sex, life stage of exposure and/or moment of outcome evaluation.

Intervention – Studies including PD treatment with drugs capable of activating the following receptors:

- GLP-1
- GIP
- GLP-1/GIP (dual agonists)

Only studies that performed exposure to each drug separately were accepted. There was no restriction on dose level, route of administration or duration of exposure. Animals concomitantly treated with usual antiparkinsonian drugs were accepted.

Comparators – Studies including PD or vehicle animals not exposed to the GLP-1, GIP or GLP-1/GIP receptor agonist drugs. Control animals treated with usual antiparkinsonian drugs were accepted.

Primary outcomes – Studies that measured:

- Motor signs (bradykinesia, coordination and balance, muscle strength of fore paws, and alterations of gait) in tests commonly used to assess neurological parameters in rodents/non-human primates (open field, rotarod, traction, and footprint tests);
- Mortality or adverse effects, such as body weight changes, organ weight changes, macroscopical and histopathological analysis, liver or pancreatic enzyme levels, glucose levels, or others as reported in the included studies.

Secondary outcomes – Studies that measured cognitive functions/neurological behaviors and/or morphological changes that could be indicative of the drugs' mode of action, such as:

- Depression behavior measured by tests commonly used to assess neurological parameters in rodents/non-human primates;
- Number of nigrostriatal dopaminergic neurons by positive immunostaining of the tyrosine hydroxylase enzyme (TH) of neurons in the SNpc and other CNS regions;
- Quantification of dopamine levels and its metabolites in the striated nucleus or other CNS regions;
- Quantification of antiapoptotic signaling molecules;
- Evaluation of neuroinflammation by quantifying inflammatory cytokines;
- Anomalous α -synuclein accumulation in the SNpc and other CNS regions.

Search methods for primary studies

Electronic searches

Peer-reviewed original studies published in the following electronic databases were searched: Ovid Medline, Ovid Embase, Web of Science, Cochrane Central Register of Controlled Trials, and Cinahl, without language and year of publication restrictions. Clinicaltrials.gov was consulted especially concerning possible unpublished trials with

available (or not available) data. All electronic searches were performed from database inception to December 2020.

Search strategy

Key terms, MeSH terms and free terms related to “Parkinson’s Disease”, “Parkinsonian Disorders”, “Glucagon-Like Peptide 1”, “Glucagon-Like Peptide 1 receptor”, “Exenatide”, “Liraglutide”, “Lixisenatide”, “Semaglutide”, “Geniposide”, “Oxytomodulin”, “D-Ala2-GIP-glu-PAL”, “DA-JC1”, “DA-JC4”, “DA-CH5”, “DA3-CH”, and “Glucose-dependent insulintropic polypeptide” were combined for each database (see Appendix 1 for an example of search strategy that was adapted for each database).

Eligibility determination

Two reviewers (CV and DG), after calibration exercises, independently screened all titles and abstracts identified by the literature search, obtained full-text articles of all potentially eligible studies, and evaluated them for eligibility. Disagreements were resolved by consensus and, eventually, through consultation with technical advisors (JLVC, LCL, TVP) to improve accuracy and consistency among screeners.

Data extraction

Reviewers (CV and DG) underwent calibration exercises and worked in pairs to independently extract data from the included studies. A standardized, piloted form was used to extract information from the clinical studies: reference, name of the evaluated drug, study design, severity of PD, mean age, percentage of males, treatment characteristics (dose, duration of administration, and interval between administrations), measured primary and secondary outcomes.

For preclinical studies, the following information was extracted: reference, name of the evaluated drug, description of the PD animal model (including strain, age, sex, and weight of animals and name of the PD-inducing neurotoxin or transgenic model), experimental design (number of animals/group), treatment (doses, number of applications, duration of treatment, route of administration), and primary and secondary measured outcomes.

Whenever results in the preclinical studies were presented in graphs, the measures were estimated by the Digitzeit software (version 2.4) in pairs, independently (CV and DG), and the mean of both measures were extracted in case of minor divergence. Disagreements were resolved by consensus and, eventually, through consultation with technical advisors (JLVC, LCL, TVP) to improve accuracy and consistency among screeners.

Assessment of internal validity of individual studies

Two reviewers (CV and LCL) independently assessed the internal validity of each study. Disagreements were resolved by consensus and, eventually, through consultation with technical advisors.

For clinical studies, the Cochrane risk of bias tool (RoB 2) was used to assess the risk of bias ^{44, 45}. The following domains were considered: a) selection bias (random sequence generation/allocation concealment); b) performance bias (blinding of participants and personnel); c) detection bias (blinding of outcome assessment); d) attrition bias (incomplete outcome data); e) selective outcome reporting (reporting bias); f) other sources of bias.

The possible answers for each domain in the risk of bias assessment of clinical studies were “Yes” (high risk of bias), “Probably yes” (high risk of bias), “No” (low risk of bias), “Probably no” (low risk of bias) or “Some concerns”. Results were presented in tables and graphs.

For preclinical studies, specific assessment tools were applied to evaluate the risk of bias, including an adapted version of the SYRCLE risk of bias tool ⁴⁶ and the CAMARADES checklist ⁴⁷. The complete adapted tool is available in Appendix 2.

The following domains were considered for the risk of bias assessment: a) selection bias (random sequence generation/baseline characteristics/allocation concealment); b) performance bias (random housing/blinding of researchers during intervention); c) detection bias (random outcome assessment/outcome assessment blinding); d) attrition bias (incomplete outcome data); e) other sources of bias.

The possible answers for each domain in the risk of bias assessment of preclinical studies were “Yes” (high risk of bias), “No” (low risk of bias), or “Unclear” (unclear risk of bias). Results were presented in tables and graphs.

Data synthesis and statistical analysis

Meta-analyses were performed separately for preclinical and clinical studies, for each outcome through an inverse-variance random effects model.

The same measure of association was calculated for the studies:

- Continuous outcomes - Mean differences (MD) or standardized mean differences (SMD) were used as the measure of effect size, with 95% confidence intervals.

For preclinical studies, studies that investigated three or more groups had their standard error estimates adjusted for the correlation created when the same comparator group is employed to assess the effect of two or more interventions. This adjustment was necessary to avoid the artificial increase in precision in the summary estimates caused by the repeated use of the same comparator group.

For the present work, the “exact adjustment” described by Rücker et al. (2017) was used⁴⁸. However, for some studies, this approach provided inconsistent results due to the small sample size of some groups. For these cases, the “approximate adjustment” was used – as suggested by Rücker et al. (2017)⁴⁸, where the standard error is inflated by the square root of half the number of groups evaluated in the study. After adjustment, from a statistical point of view, the estimates can be considered “uncorrelated” and traditional methods of meta-analyses can be employed – without the need to incorporate covariance between groups.

All adjustments were conducted in the Stata (version 16, StataCorp, College Station, Texas, USA) and R (version 4.0.2, R Core Team, 2021, Vienna, Austria) statistical packages. All forest plots were created by the Review Manager package (version 5.3, Copenhagen: The Nordic Cochrane Centre).

Whenever feasible, inter-species comparison analyses were performed to contrast the treatment effects observed in animal studies to those detected in human trials (e.g., a drug's effect on motor function evaluated in neurobehavioral tests in animals was compared to the corresponding effects on humans). Inter-species comparisons considered the direction, magnitude, and uncertainty of effects. Morphological outcomes (secondary outcomes) in preclinical studies were used to elucidate the GLP-1/GIP possible mode of action that could be an indication of anti-parkinsonian/neuroprotective activity and were just narratively described.

Heterogeneity assessment

Heterogeneity among individual studies was assessed using the I^2 statistic and Cochran's Q test ⁴⁹. The latter was considered statistically significant when $P < 0.10$.

Subgroup and meta-regression analyses

Meta-regression models with the REML estimator of the between-study variance were fitted whenever 10 or more estimates were available. If less than 10 estimates were available, subgroup analyses were performed.

Meta-regression and subgroup analyses were conducted whenever possible to investigate possible sources of heterogeneity in clinical studies, such as due to the inclusion of participants with different PD severity (severe, moderate and mild scored on the MDS-UPDRS part III), subtypes of PD (tremor dominant, mixed and akinetic/rigid), genomic status of each patient's PD related genes (α -synuclein, DJ-1, LRRK2, Parkin, UCH-L1 or PINK1), use of other antiparkinsonian drugs (levodopa or dopaminergic drugs, such as monoamine oxidase inhibitors, catechol-O-methyltransferase inhibitors or dopaminergic agonists).

We considered whether the patients were in an “off medication” state (with the withdrawal of the medication at least the day before the evaluations). Whenever possible, “on” and “off” state measures were compared separately since it is expected a higher magnitude of effect in an “on medication” state.

For preclinical studies, heterogeneity was investigated considering the use of different GLP-1/GIP receptor agonists and different treatment moments (pre, concomitantly or post-PD lesion).

If there were enough studies (at least 2), the impact of high risk of bias studies on the meta-analyses' results was investigated.

Funnel plot asymmetry

The asymmetry of the funnel plot was evaluated using contour-enhanced plots, highlighting four regions of interest:

- Region without statistical significance with P value > 0.10 ;
- Region without statistical significance with a borderline P value between 0.05

and 0.10;

- Region of statistical significance with a borderline P value between 0.01 and 0.05;

- Region of statistical significance with P value < 0.01.

In addition, the Egger test was used using a linear regression model, where the dependent variable is the standardized mean Hedges difference and the inverse of the standard error of the mean Hedges difference is the independent variable. In this linear model, the weight of each estimate is proportional to the inverse of its variance.

The funnel plot asymmetry was investigated only for meta-analyses with 10 or more estimates. Egger's test P values < 0.10 were considered as evidence of funnel plot asymmetry^{50, 51}.

GRADE

The GRADE system⁵²⁻⁵⁴ was used to rate the certainty of evidence provided by clinical studies. The certainty of evidence was determined for each outcome. Randomized controlled clinical trials, when well designed, can eliminate much of the confounding factors by random allocation of participants to the different study groups. Thus, they start from the initial classification of "high certainty".

However, there are several factors that can downgrade the initial classification of the study groups in relation to the reliability of the evidence that they generated: risk of bias⁵⁵, imprecision⁵⁶, inconsistency⁵⁷, indirectness⁵⁸, and publication bias⁵⁹.

Four descriptors were used to indicate the level of confidence in the evidence generated by clinical studies, separately: high certainty level (++++); moderate certainty level (+++); low certainty level (++); very low certainty level (+). All results will be presented as tables.

Highly reliable evidence implies that further studies will hardly change the available results; on the other hand, very low levels of evidence indicate that future studies may alter not only the confidence in the available results, but the results themselves.

Results

Literature search

Of the 1108 studies retrieved in the different databases, 376 were excluded after duplicates were removed. After analyzing 732 studies based on titles and available abstracts, 678 were excluded since they were literature reviews, systematic reviews, *in vitro* studies, animal studies in species other than rodents or non-human primates, abstracts presented in congresses, commentaries or that did not address the GLP-1/GIP analogues of interest (e.g., dipeptidyl peptidase 4 inhibitors) (figure 1).

Fifty-four studies were assessed in full in the absence of abstracts or if doubts remained after the abstract level assessment, accounting for 17 human studies and 36 animal studies (figure 1).

Of the 17 human studies, 14 were excluded after new assessment: eight were study protocols of randomized clinical trials with no posted results, one was not a randomized control trial, three were post-hoc analyses of one of the included studies, one was a commentary, and one was an abstract presented in a congress (figure 1).

Of the 36 animal studies, five were excluded: one was a review of the literature, one did not investigate the drugs of interest, one used a systemic LPS model of inflammation and not PD, one was an abstract presented in a congress, and one was a retracted article. Indeed, two retracted articles were retrieved in our searches showing apparently considerable overlap and duplication of results. Both were published in different Journals within one day of difference and neither paper cited the other. Since the scientific validity of both papers has not been questioned, we have included one of the papers and excluded the other with the overlap and duplication of results (figure 1).

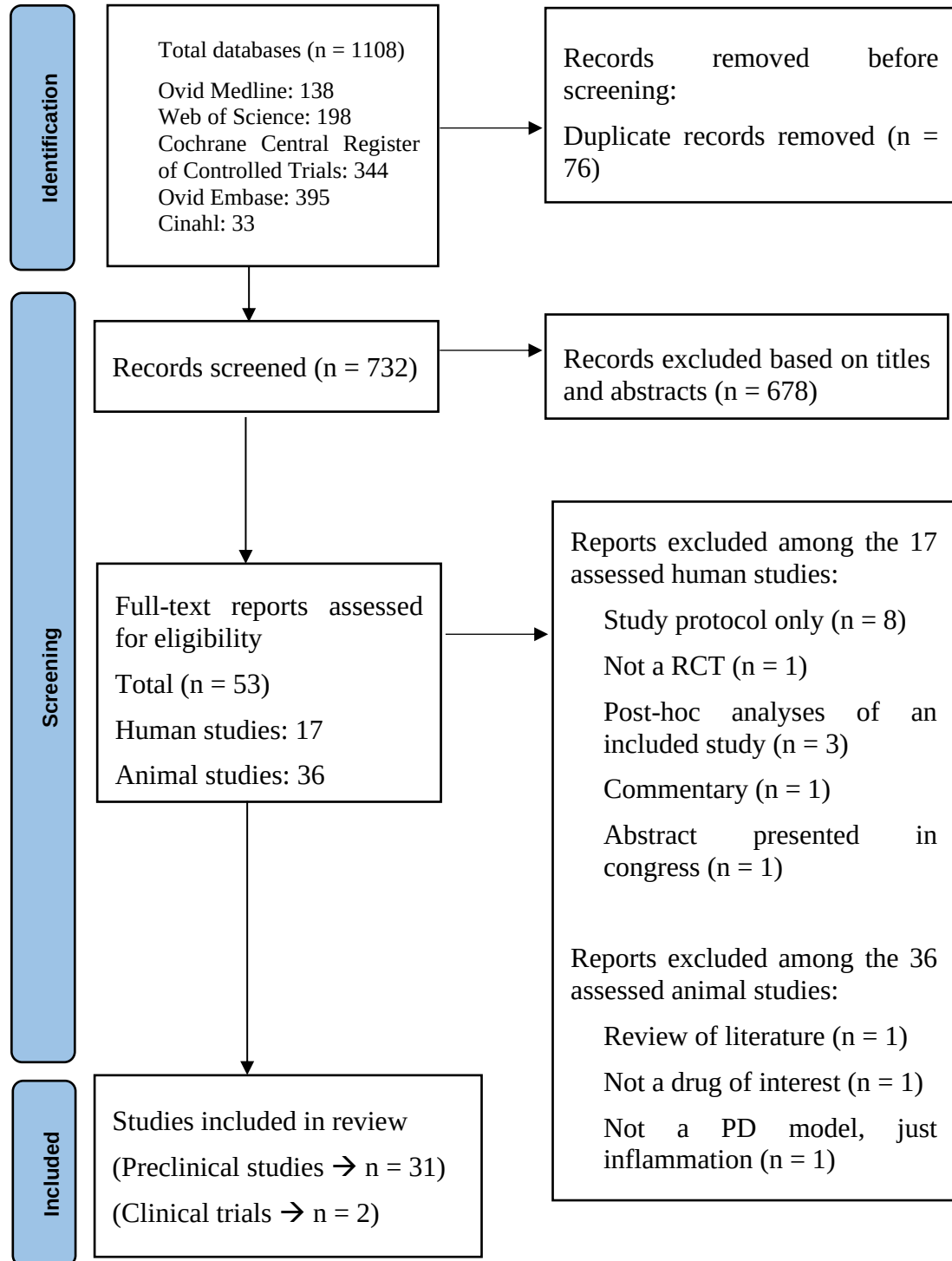


Figure 1. Study flow diagram.

Study characteristics

Clinical studies

Specific data on patients' baseline characteristics, drug treatment scheme, and measured outcomes are described in table 1.

Briefly, the two included studies tested exenatide only. One trial is a randomized, double-blind, placebo-controlled, parallel study ⁶⁰, and one is an open label randomized, controlled design, parallel study ⁶¹. Both were single-centric and published in 2017 or 2013.

Participants

A total of 104 participants were included in both studies, being 51 in the treatment groups and 53 in the comparator groups. Participants had moderate Parkinson's disease (97%⁶⁰ and 68.2%⁶² at a Hoehn and Yahr scale stage 1 or 2) and were mostly man (73.3⁶⁰ and 79.5%⁶¹), with no diabetes of any kind or severe dementia. Age ranged from 25 to 75 years old in one study⁶⁰ (mean age 61.5 years old) and from 45 to 70 (mean 58.6 years old) in the other⁶¹.

Intervention

In Athauda et al. (2017) ⁶⁰, the subcutaneous injections of exenatide were of 2 mg/week for 48 weeks. Participants were followed up at 60 weeks, after a 12-week washout period.

In the Aviles-Olmes et al. (2013) study ⁶¹, 5 µg of exenatide (Byetta 5 µg) was self-administered twice-daily in the first month. Then, the dose was increased to 10 µg (Byetta 10 µg) twice-daily for the subsequent 11 months. Participants had a two-month washout period and were followed up until 14 months.

All participants were on L-DOPA treatment and using other possible PD medication.

Comparator

In Athauda et al. (2017), the control group took placebo injections for 48 weeks in the same scheme as the exenatide group, besides their regular PD medication. In the Aviles-Olmes et al. (2013) study ⁶¹, patients in the control group did not take any placebo,

just followed their regular PD medication.

Outcomes

Primary outcomes (as defined by this systematic review) measured by the studies were motor improvements (clinician's perspective) measured as changes from baseline on the MDS-UPDRS, part III, in the "on" and "off-medication" states^{60, 61}; motor and non-motor symptoms from the patient's perspective on each subsection of the MDS-UPDRS, parts I and II - "on medication")^{60, 61} on NMSS⁶¹, and on NMSQuest⁶¹, and motor complications rated by the MDS-UPDRS, part IV, and on the UDyRS^{60, 61}. Adverse effects and serious adverse effects were also considered as primary outcomes. The serious adverse events observed in either treatment or placebo groups in both studies were not judged to be related to exenatide according to the authors.

Secondary outcomes (as defined by this systematic review) measured by the studies were depression, dementia and quality of life, rated as changes from baseline on the Montgomery and Åsberg Depression Rating Scales^{60, 61}, on the Mattis Dementia Rating Scale^{60, 61}, on the Parkinson's Disease 39-item Quality of Life Questionnaire^{60, 61}, and on EQ-5D⁶¹.

Ongoing studies

Seven ongoing studies were identified in Clinicaltrials.gov assessing the effects of NLY01 (NCT04154072), exenatide (NCT04232969 and NCT04305002), PT320 (NCT04232969), liraglutide (NCT02953665), lixisenatide (NCT03439943) and semaglutide (NCT03659682). Results were not posted yet.

Preclinical studies

Specific data related to the PD models, animal's characteristics, drug treatment schemes, and measured outcomes are described in table 2. Methodological details are described in Appendix 4.

Briefly, studies were published from 2008 to 2020. Of the 31 preclinical studies, 30 were neurotoxin-based models of PD: 10 used the 6-OHDA model^{8-10, 26, 63-68}; in one of those 10 studies¹⁰, LPS was also administered in another set of experiment. Eighteen studies used the MPTP model^{12, 69-85}, and two used the rotenone model^{86, 87}. Only one study used the hA53T transgenic mice in the α -synucleinopathy model and the α -synuclein

performed fibril (PFF) model⁸⁸.

Of the 10 6-OHDA studies, eight injected the neurotoxin in the medial forebrain bundle (MFB) (left or right)^{8-10, 26, 63, 65-67}, two injected in the SNpc^{10, 64}, and one injected in the striatum⁶⁸. Of the 18 MPTP studies, five used an acute-lesion protocol^{11, 72, 74, 77, 81}, 11 studies used a sub-acute-lesion protocol^{69-71, 73, 78, 79, 82, 83, 85, 89}, and three studies used chronic-lesion protocols^{75, 80, 84}.

Animals

Considering all 31 studies, a total of 2154 animals were used. Twelve of 31 studies tested in rats: Wistar (five studies)^{10, 63, 64, 66, 68}, Sprague-Dawley rats (six studies)^{8, 26, 65, 67, 86, 90}, or albino not specified rats (one study)⁸⁷. Nineteen studies tested in the C57BL/6 mice^{11, 13, 69-75, 77-85, 88}. Only one study tested the transgenic mice expressing the mutant human A53T alpha-synuclein under the direction of the mouse prion protein promoter⁸⁸. No primate non-human was used.

In 30 studies, only male animals were used; in one study⁸⁸, males and females were included with no specification of how many males and females in each group. Animal's age ranged from 6 to 24 weeks old; five studies just referred the animals as “adults”^{26, 64, 77, 79, 86}, and 11 studies did not report the age of animals^{8, 10, 63, 66, 67, 71, 73, 75, 76, 81, 87}. The weight range of rats was from 175 to 450 g and weight of mice ranged from 15 to 30 g. In four studies^{65, 80, 81, 88}, weight was not reported.

Intervention

Of the 31 preclinical studies, 24 tested the GLP-1 natural hormone or GLP-1 analogues. There are results for GLP-1⁷¹, exenatide⁸⁶, NLY01 (a pegylated form of exenatide)⁸⁸, exendin-4^{8, 10, 63, 66, 74, 77}, PT302 (an encapsulated form of exendin-4)⁶⁵, liraglutide^{9, 12, 64, 72, 82-84, 87, 91}, semaglutide^{83, 84}, lixisenatide⁹¹, oxyntomodulin⁷⁹, geniposide^{70, 80}, and (Val8)GLP-1-Glu-pal⁸⁵ (the same study could have studied more than one drug). One study only tested exendin-4 in association with L-DOPA⁶³.

Four studies tested the GIP natural hormone or GIP analogues: GIP²⁶, D-Ala²-GIP⁸¹, and D-ALA²-GIP-glu-pal^{75, 76}.

Additionally, other six studies tested drugs capable of activating both receptors, GLP-1 and GIP: there are results for DA3-CH⁸², DA-CH5^{12, 72}, DA-JC1^{67, 69, 72, 73}, and DA-JC4⁷² (the same study could have studied more than one drug).

Doses of GLP-1, GIP or dual GLP-1/GIP receptor agonists varied widely, as well as treatment duration, as described in details in table 2 and Appendix 4.

Comparator

Lesioned animals (PD-like) treated with GLP-1, GIP or GLP-1/GIP dual agonists were compared to not lesioned animals treated with vehicle, to not lesioned animals treated with GLP-1, GIP or GLP-1/GIP dual receptor agonists or to lesioned animals only.

Outcomes

The most common primary outcomes were general locomotor activity, motor coordination and balance, abnormal gait, and muscle strength, measured by neurobehavioral tests, such as the open field^{11, 26, 67, 69-71, 75, 77-79, 81, 83, 85, 89}, rotarod^{12, 67, 69, 70, 72, 73, 75, 77-79, 82-85, 88}, traction test^{69, 72, 73, 79, 81, 82, 84}, and footprint^{83, 84, 89} tests. Less frequent tests were for the amplitude and frequency of tremors and grasping reflex (muscle rigidity)⁸¹.

Few studies reported body weight measures^{9, 26, 65, 67, 72, 82, 87, 88}, food consumption⁶⁷ or glucose parameters^{26, 72, 82, 88} as primary outcomes to evaluate possible adverse effects of the drugs. Most studies provided the animals' weight range at the beginning of the study and did not report the variation and body weight gain at the end of the study. For the ones that reported the body weights in the end, only one study indicated weight loss (10%) after 4 weeks of treatment with liraglutide⁹⁰. There were no effects in glucose levels or food consumption.

Only one study assessed secondary effects like anhedonia and depression-like behaviors in the absence of locomotor impairments (pre-motor model), which would avoid biases in the evaluation of these outcomes. Tests such as the sucrose preference⁶⁸, and forced swim⁶⁸ were performed⁶⁸.

The most common secondary outcomes (morphological, biochemical) that could be an indication of the drugs' anti-parkinsonian activity or mode of action were reported as SNpc or striatal tyrosine hydroxylase (TH) immunoexpression or dopamine fibers^{8-12, 65-75, 77-80, 82-88}, measurement of striatal dopamine levels and its metabolites^{10, 63, 66-68, 77, 81, 87}, pro- or anti-apoptotic protein expression (B-cell lymphoma 2 - Bcl-2, and Bcl-2-associated X protein - Bax)^{12, 67, 69, 70, 73, 79, 83-85, 91}, microglia (ionized calcium binding adaptor molecule 1- IBA-1) and astroglia (glial fibrillary acidic protein - GFAP)

activation^{11, 12, 71, 72, 75, 82-84, 88}, expression of neuroinflammation markers (tumor necrosis factor- α - TNF- α , IL-1 α , interleukin-1 β - IL- β , interleukin-6 - IL-6)^{64, 71, 72, 74, 79, 86-88}, glial cell-derived neurotrophic factor (GDNF)^{67, 72, 82, 84, 87}, synaptophysin levels, lipid peroxidation (4-hydroxynonenal - 4-HNE) as a result of oxidative stress, autophagy mediated proteins expression^{12, 67, 83, 84}, and α -synuclein expression^{11, 71, 80, 84, 88}. The activation of the NF- κ B signaling pathway was less frequently assessed^{71, 72}, as well as malondialdehyde (MDA) levels as a marker for oxidative stress^{81, 86}, the number of apoptotic cells (TUNEL test)^{12, 85}, and the brain-derived neurotrophic factor (BDNF) expression^{73, 75}.

Qualitative description of morphological/biochemical secondary outcomes

The binding to neuronal GLP-1 and GIP receptors by GLP-1 and GIP agonists may have similar role as in the peripheral protection, proliferation, and differentiation of β -pancreatic cells. The possible mechanisms of action of GLP-1 and GIP receptor agonists in the brain were their anti-apoptotic and anti-inflammatory activities, which would rescue the dying neurons that are not yet “fatally” damaged.

More often, levels of the anti-apoptotic molecule Bcl-2 were reduced, while the pro-apoptotic signaling molecule Bax levels were increased^{12, 58, 60, 61, 64, 70, 74-76}. One study did not show increase in the Bax/Bcl-2 ratio⁹¹ for liraglutide. Liraglutide, semaglutide and dual agonists rescued the decrease of GDNF levels, a trophic factor that plays an important role in the survival of dopamine neurons, in the neurotoxin models^{67, 72, 82, 84, 87}. Inflammation in the brain was also shown to be reduced by increased levels of GFAP in astrocytes and IBA-1 in microglia. Lower concentrations of pro-inflammatory cytokines such as IL-1 β , IL-6, and TNF- α were identified after treatment with the drugs^{64, 71, 72, 74, 79, 86-88}, which would improve insulin resistance. Lipid peroxidation, as a result of marked oxidative stress, seemed ameliorated by decreased levels of 4-HNE^{12, 83, 84} and MDA^{81, 86} in lesioned animals treated with GLP-1, GIP or GLP-1/GIP dual agonists.

Most drugs seemed to increase the expression of TH-positive neurons in the SNpc, or to increase the density of dopaminergic fibers in the striatum^{8, 10-12, 65-75, 77, 79, 80, 82-88}. Concordantly, striatal/basal ganglia tissue dopamine levels seemed to be restored^{10, 63, 66-68, 77, 81, 88}. Two studies did not show effects for liraglutide⁹⁰ and exendin-4⁹¹ in the expression of TH-positive neurons and another study showed increase in TH-positive neurons in the SNpc for exendin-4 only at the highest dose⁸⁹.

Although there are controversies and little reproducibility in the literature regarding the observation of α -synuclein inclusions in MPTP protocols, in three chronic MPTP studies and in one sub-acute MPTP study^{71, 75, 80, 84}, it was possible to observe increases in the α -synuclein expression in the SNpc and/or striatum by westernblot and/or immunohistochemistry, reversed by GLP-1, semaglutide, liraglutide, geniposide or D-Ala²-GIP-glu-PAL.

Only one study used the human A53T α -synuclein transgenic mouse model under the control of the prion protein promoter and the PFF mice model to evaluate the effects of NLY01, a pegylated form of exenatide⁸⁸. NLY01 also seemed to significantly reduce the phosphorylated version of α -synuclein (p- α -synSer¹²⁹) in the striatum, ventral midbrain and specifically in TH-positive neurons.

Clinical studies

Table 1. Study characteristics – clinical studies.

Author, year	N randomized	Mean age yrs (SD)	% males	Treatment scheme		Duration of disease years (SD)		Severity of PD (N)		Primary outcomes measured	Secondary outcomes measured
				Intervention	Control	Intervention	Control	Intervention	Control		
Athauda et al. 2017 ⁶⁰	62 60 included in the analysis (I = 31, C = 29)	61.5 (7.1)	73.3	s.c. injections 2 mg/week for 48 weeks	s.c. injections (placebo) for 48 weeks	6.4 (3.3)	6.4 (3.3)	Hoen and Yahr stage 1 - 2: 29 Hoen and Yahr stage 2.5: 2	Hoen and Yahr stage 1 - 2: 29 Hoen and Yahr stage 2.5: 0	Motor and non-motor symptoms, motor complications (MDS- UPDRS parts I, II, III, and IV, NMSS, UDyRS) Adverse events	Depression dementia, quality of life (MADRS, MDRS, PDQ-39)
Aviles- Olmes et al. 2013 ⁶¹	45 44 included in the analysis (I = 20; C = 24)	58.6 (8.2)	79.5	5 µg twice- daily for 1 month + 10 µg twice- daily for 11 months	Conventional PD treatment	9.6 (3.4)	11 (5.9)	Hoen and Yahr stage 2: 16 Hoen and Yahr stage 2.5: 8	Hoen and Yahr stage 2: 14 Hoen and Yahr stage 2.5: 6	Motor and non-motor symptoms, motor complications (MDS- UPDRS parts I, II, III, and IV, NMS Quest, UDyRS) Adverse events	Depression dementia, quality of life (MADRS, MDRS, PDQ-39, EQ-5D)

Abbreviations: MDS-UPDRS: Movement Disorders Society Unified Parkinson's Disease Rating Scale; UDYRS: Unified Dyskinesia Rating Scale; NMS Quest: Non-Motor Symptoms Questionnaire; NMSS: Non-Motor Symptoms Scale; MDRS: Mattis Dementia Rating Scale; MADRS: Montgomery-Asberg Depression Rating Scale; PDQ-39: Parkinson's Disease Questionnaire; I: intervention; C: comparator.

Table 2. Study characteristics – preclinical studies.

Author and year	Sex/ Specie/ Strain (weight g)	Total number of animals / animals per group	Age of animals	PD model	GLP-1/GIP Treatment doses Duration of treatment	Primary outcomes measured	Secondary outcomes measured
GLP-1 and dual agonists							
Alabassi et al. 2017 ⁶⁴	Male / Rat / Wistar (200-250 g)	36 / 3 groups of 12 animals	Adults (not specified)	6-OHDA Right SNpc injection (unilateral)	Liraglutide 25 nmol/kg i.p. Once daily for 30 days	Not reported	1. Quantification of IL-1 β levels 2. Glutathione peroxidase expression
Abuirmeileh et al. 2012 ⁶³	Male / Rat / Wistar (210 - 240 g)	72 / 12 groups of 6 animals	Not reported	6-OHDA MFB injection (unilateral)	Ex-4 ^s 0.1 or 0.5 μ g/kg i.p. Twice daily for 7 or 16 days in acute and chronic experiments	1. Rotational test	1. External dopamine and striatal tissue dopamine 2. LIDS after L-DOPA chronic administration
Aksoy et al. 2017 ⁸⁶	Male / Rat / Sprague Dawley (200 - 210 g)	18 / 3 groups of 6 animals	Adults (not specified)	Rotenone SNpc and VTA infusion	Exenatide 20 μ g/kg i.p. Once daily for 28 days	1. Rotational test (pre- and post-treatment)	1. Expression of TH in the striatum 2. MDA and TNF- α levels
Badawi et al. 2017 ⁸⁷	Male / Rat / Albino - not specified (200 - 250 g)	Liraglutide: 60 / 5 groups of 12 animals	Not reported	Rotenone s.c. daily for 10 days	Liraglutide 25, 50 and 100 μ g/kg s.c. Once daily for 16 days	1. Cylindrical test 2. Catalepsy test	1. Histopathological changes in SNpc 2. Expression of TH in the striatum 3. Expression of Bax and Bcl-2 4. Striatal dopamine levels 4. Expression levels GDNF, TGF- β 1, IL-1 β , IL-6
Bertilsson et al. 2008 ⁸	Male / Rat / Sprague	26 / 2 lesion groups of 8 animals and 2	Not reported	6-OHDA	Ex-4 0.1 μ g/kg i.p.	1. Rotational test	1. Expression of TH and VMAT-2 in the SNpc

	Dawley (280 - 350 g)	sham groups of 5 animals		Right MFB injection (unilateral)	Twice daily for 3 weeks		
Cao et al. 2016 ⁶⁹	Male / Mice / C57BL6 (20-25g)	40 / 4 groups of 10 animals	10 ± 2- weeks old	MPTP i.p. daily for 7 days (sub-acute)	DA-JC1 50 nmol/kg i.p. Daily injections for 7 days	1. Rotarod test 2. Traction test 3. Open field test	1. Expression of TH in the SNpc and striatum 2. Expression levels of GFAP and IBA-1 3. Synaptophysin expression in the SNpc and striatum
Chen et al. 2015 ⁷⁰	Male / Mice / C57BL6 (20-25g)	56 / 4 groups of 14 animals	11 ± 2- weeks old	MPTP i.p. daily for 8 days (sub-acute)	Geniposide 100 mg/kg i.p. Daily for 8 days	1. Rotarod test 2. Open field test 3. Swim test	1. Expression of TH in the SNpc 2. Expression of Bcl-2, Bax, Pro-caspase3 and Cleaved- caspase3
Chen et al. 2018 ⁶⁵	Male / Rat / Sprague Dawley (weight not reported)	57 / 3 groups of 10, 9 and 9* (pre-treatment) and 2 groups of 11 and 8 (treatment)	8 weeks-old	6-OHDA MFB injection (unilateral – side not mentioned)	PT302 (Ex-4 sustained release) ^{&} 0.4 and 2 mg/kg s.c. Once every 2 weeks	1. Rotational test	1. Expression of TH in the SNpc and striatum
Fang et al. 2019 ⁷¹	Male / Mice / C57BL6 (25–30 g)	72 / 6 groups of 12 animals Final treated groups had 9, 11, 9, 6 and 10 animals (mortality)	Not reported	MPTP i.p. daily for 7 days (sub-acute)	GLP-1 [#] one group 10 ⁷ CFU MG1363-pMG36e- GLP-1 and 10 ⁹ CFU MG1363- pMG36e-GLP-1 oral 7 or 14 days	1. Pole test 2. Open field test	1. GFAP, IBA-1, and α -syn levels in the SNpc 2. Expression of TH in the SNpc 3. IL-1 β , IL-6 and TNF- α 4. TLR-4 and p-NF-kB expression

Feng et al. 2018 ⁷²	Male / Mice / C57BL6 (15-20 g)	60 / 6 groups of 10 animals	8 weeks-old	MPTP i.p. 4 injections in the same day (acute)	DA-JC1, DA-JC4, DA-CH5 and liraglutide 25 nmol/kg i.p. Once daily for 6 days	1. Rotarod test 2. Grip strength test	1. Expression of TH in the SNpc 2. Levels of IBA-1 expression in the SNpc 3. Levels of pro-inflammatory cytokines (IL-1 β , TNF- α , NF- kB), and synaptophysin 4. Levels of GDNF expression in the SNpc
Hansen et al. 2016 ⁹	Male / Rat / Sprague- Dawley (175-200 g)	Non-diabetic rats 100 / 5 groups of 20 animals	6 weeks-old	6-OHDA Right MFB injection (unilateral)	Liraglutide* from 50 - 500 μ g/kg/day s.c. Daily for 3, 4 or 6 weeks	1. Rotational test	1. Expression of TH in the midbrain
Harkavyi et al. 2008 ¹⁰	Male / Rat / Wistar (210-240 g)	42 / 7 groups of 6 animals	Not reported	6-OHDA Right MFB injection (unilateral) LPS SNpc injection	Ex-4 0.1 or 0.5 μ g/kg i.p. Twice daily for 7 days	1. Rotational test	1. Tissue dopamine assay 2. Ex vivo tyrosine hydroxylase assay 3. Expression of TH in the SNpc
Harkavyi et al. 2013 ⁶⁶	Male / Rat / Wistar (250-300 g)	30 / 5 groups of 6 animals Other 5 groups of 6 animals were separated	Not reported	6-OHDA Left MFB injection (unilateral)	Ex-4 (plus antagonists) 0.5 μ g/kg i.p. Twice daily for 7 days	1. Rotational test	1. Extracellular and tissue dopamine 2. Expression of TH in the SNpc and BrdU immunostaining in the SVZ and SNpc

		for BrDu staininng					
Jalewa et al. 2017 ⁶⁷	Male / Rat / Sprague Dawley (350 - 450 g)	24 / 3 groups of 8 animals	Not reported	6-OHDA Left MFB injection (unilateral)	DA-JC1 25 nmol/kg i.p. Once daily for 6 weeks	1. Rotational test 2. Rotarod test 3. Open field test	1. Expression of TH in the SNpc 2. Dopamine levels in the basal ganglia 3. Expression of GDNF, pAkt/CREB and Beclin1
Ji et al. 2016 ⁷³	Male / Mice / C57BL (20 - 22 g)	50 / 5 groups of 10 animals	Not reported	MPTP i.p. Daily for 7 days (sub-acute)	DA-JC1 50 nmol/kg i.p. Once daily for 7 days	1. Rotarod test 2. Traction test	1. Expression of TH in the SNpc 2. Expression of Bax and Bcl-2 in the SNpc and striatum 3. BDNF expression in the SNpc and striatum 4. Protein levels from isolated midbrain
Kim et al. 2009 ⁷⁴	Male / Mice / C57BL (22 - 24 g)	40 / 8 groups of 5 animals	8 weeks-old	MPTP i.p. 4 injections in the same day (acute)	Ex-4 10 µg/kg i.p. 4 injections in one day	-	1. Expression of TH in the SNpc and density of striatal TH+ fibers 2. Expression of TNF- α and IL- 1 β in the midbrain 3. Expression of Mac-1 in the SNpc and striatum 4. Expression of MMP-3 in the SNpc

Li et al. 2009 ⁷⁷	Male / Mice / C57BL6 (22-24 g)	30 / 3 groups of 10 animals	Adults (not specified)	MPTP i.p. 4 injections in the same day (acute)	Ex-4* 20 nM, 0.25 µL/h (mini pump - right lateral ventricle) Over 7 days	1. Open field test 2. Pole test 3. Rotarod test 3. Beam walk 4. Behaviour assessment (vibrissae placing, reaching reflex, righting reflex, turning and gait)	1. Expression of TH in the SNpc and striatum 2. Levels of dopamine and its metabolites DOPAC and HVA in striatum
Liu et al. 2015a ⁷⁸	Male / Mice / C57BL6 (25-30g)	96 / 8 groups of 12 animals	8 weeks-old	MPTP i.p. Daily for 7 days (sub-acute)	Liraglutide, lixisenatide, Ex-4 25 nmol/kg, 10 nmol/kg and 10 nmol/kg i.p. 14 days	1. Rotarod test 2. Open-field test 3. Catalepsy test	1. Expression of TH in the SNpc and striatum 2. Expression levels of Bax and Bcl-2 in the striatum
Liu et al. 2015b ⁷⁹	Male / Mice / C57BL6 (20-25 g)	48 / 4 groups of 12 animals	Adults (not specified)	MPTP i.p. Daily for 7 days (sub-acute)	Oxyntomodulin Not reported (i.p.) Once daily for 14 days	1. Rotarod 2. Traction test 3. Open-field test 4. Swim test	1. Expression of TH in the SNpc and striatum 2. Bcl-2 levels 3. TNF-α levels 4. Akt activation
Rampersaud et al. 2012 ⁶⁸	Male / Rat / Wistar (180-250 g)	440 / 11 groups of 40 animals (subdivided in 4 groups of 10 animals)	7.4 to 8.4-weeks old (56-63 days)	6-OHDA Striatal injection (bilateral) plus DPS-4 and pCA i.p	Ex-4 0.5 µg/kg i.p. Twice daily for 7 days	1. Open field test	1. Extracellular and tissue levels of DA, NA, and 5-HT in the frontal cortex and striatum 2. Expression of TH in the SNpc and locus coeruleus 3. Depressive-like behavior 4. Anhedonia 5. Memory

Su et al. 2016 ⁸⁰	Male / Mice / C57BL6N (not reported)	64 / 3 groups of 8 animals and 2 groups of 16 animals	~10 weeks- old	MPTP i.p. Every 8 hours for 21 days (chronic)	Geniposide 100 mg/kg oral gavage Once daily for 21 days	-	1. Expression of TH in the SNpc 2. Relative miR-21, LAMP2A and α -synuclein levels
Yuan et al. 2017 ⁸²	Male / Mice / C57BL6 (15-20g)	40 / 4 groups of 10 animals	8 weeks-old	MPTP i.p. Daily for 7 days (sub-acute)	DA3-CH and liraglutide 25 nmol/kg i.p. Once daily for 7 days	1. Rotarod test 2. Grip strength test	1. Expression of TH in the SNpc and striatum 2. Expression of GFAP in the cortex 3. Expression of IBA-1 in the cortex 4. Expression of GDNF in the cortex
Yun et al. 2018 ⁸⁸	PFF model: Male and female / Mice / C57BL6 (weight not reported) Transgenic model: Male and female / Mice / Prnp-SNCA A53T (weight not reported)	120 / 8 groups of 15 animals	PFF model: 12 weeks- old Transgenic model: 24 weeks-old	Unilateral α -syn PFF injection hA53T α -syn transgenic mouse model	NLY01 (pegylated form of exenatide) 3 mg/kg s.c. PFF model: Twice a week for 5 months Transgenic model: treated from 6 to 10 months old or death twice a week	1. Rotational test 2. Pole test 3. Rotarod test 4. Cylinder test	1. Expression of TH in the SNpc 2. Dopamine and its metabolites levels (ng/mg) in striatum 3. % TH fiber density in striatum 4. Expression of GFAP and IBA-1 5. Inflammatory markers 6. α -syn expression and aggregation

Zhang et al. 2015 ⁸⁵	Male / Mice / C57BL6 (23 ±2g)	56 / 4 groups of 14 animals	10±2 weeks- old	MPTP i.p. daily for 8 days (sub-acute)	(Val8)GLP-1-Glu-PAL 25 nmol/kg i.p. Once daily for 8 days	1. Rotarod test 2. Open field test 3. Swim test	1. Expression of TH in the SNpc 2. Apoptotic cells in the SNpc (TUNEL) 3. Levels of Bcl-2 and Bax 4. Levels of the pro-caspase3 protein expression
Zhang et al. 2018 ⁸³	Male/ Mice / C57BL6 (20-25g)	72 / 6 groups of 12 animals	10 weeks- old	MPTP i.p. Daily for 7 days (sub-acute)	Semaglutide and liraglutide 25 nmol/kg i.p. Once daily for 7 days	1. Rotarod test 2. Open field test 3. Footprint test	1. Expression of TH in the SNpc and striatum/dopaminergic fibers in striatum 2. GFAP and IBA-1 expression in SNpc and striatum 3. Lipid peroxidation in the SNpc and striatum 4. Bax, Bcl-2 and Beclin levels in SNpc and striatum 5. ATG7, LC3, P62 expression in SNpc
Zhang et al. 2019 ⁸⁴	Male / Mice / C57BL6 (20-25g)	72 / 6 groups of 12 animals	8 weeks-old	MPTP i.p. Daily for 30 days (chronic)	Semaglutide and liraglutide 25 nmol/kg i.p. Semaglutide: once every 2 days for 30 days Liraglutide: once daily for 30 days	1. Footprint test 2. Grip strength test 3. Rotarod test	1. Expression of TH in the SNpc 2. GFAP, IBA-1 expression in striatum 3. Lipid peroxidation in striatum 4. Bax, Bcl-2 levels in SNpc 5. α-syn expression in SNpc 7. Beclin1, ATG7, LC3, P62, GDNF expression in SNpc

Zhang et al. 2020 ¹²	Male / Mice / C57BL6 (20-22g)	DA-CH5 and ex-4 64 / 8 groups of 8 animals DA-CH5 and liraglutide 72 / 6 groups of 12 animals	8 weeks-old	MPTP i.p. Daily for 7 days (sub-acute)	DA-CH5, Ex-4 and liraglutide DA-CH5 and Ex-4 0.1; 1 and 10 mg/kg i.p. DA-CH5 and liraglutide: 10 and 25 mg/kg i.p. Once daily for 7 days	1. Rotarod test 2. Open field test (DA-CH5 and liraglutide only) 3. Footprint test (DA- CH5 and liraglutide only)	1. Expression of TH in SNpc and dopaminergic fibers in striatum 2. GFAP and IBA-1 expression in SNpc and striatum 3. Lipid peroxidation in the SNpc and striatum 4. Bax, Bcl2 levels in SNpc and striatum 5. Beclin1, LC3, P62 expression in SNpc 6. Apoptosis cell number in striatum (TUNEL)
Li et al. 2016 ⁷⁵	Male / Mice / C57BL6 (20 -25 g)	32 animals/ 4 groups of 8 animals	Not reported	MPTP + probenecid i.p. twice/week for 5 weeks (chronic intermittent)	D-Ala ² -GIP-glu-PAL 25 nmol/kg i.p. Once daily for 6 weeks	1. Open field test 2. Pole test 3. Rotarod test	1. Expression of TH in the SNpc 2. Expression of α -synuclein in the SNpc and striatum 3. Expression of GFAP and IBA-1 4. Expression of 4-HNE 5. Expression of BDNF
Li et al. 2017 ¹¹	Male / Mice / C57BL (25 - 30 g)	48 animals / 4 groups of 12 animals	Not reported	MPTP i.p. 4 injections in the same day (acute)	D-Ala ² -GIP-glu-PAL 25 nmol/kg i.p. Once daily for 7 days	1. Open field test 2. Pole test	1. Expression of TH in the SNpc 2. Expression of GFAP and IBA-1 3. Bcl-2 and Bax levels in the SNpc 4. Synaptophysin levels 5. Levels of total CREB growth factor signaling in the SNpc.

Verma et al. 2017 ⁸¹	Male / Mice / C57BL6 (weight not reported)	72 animals / 9 groups of 8 animals	Not reported	MPTP i.p. 3 injections in the same day (acute)	D-Ala2-GIP* 50–100 nmol/kg i.p. Once daily for 7 days	1. Open-field test 2. Rotarod test 3. Rigidity test 4. Pole test 5. Akinesia test 6. Tremor test	1. Brain MDA levels 2. Brain glutathione levels 3. Striatal concentration of dopamine, HVA and DOPAC levels
Yu et al. 2018 ²⁶	Male / Rats / Sprague Dawley (270-340g)	Total of animals unclear	Adults (not specified)	6-OHDA Left MFB injection (unilateral)	GIP# 15 nmol/kg/day s.c. (mini osmotic pumps) Over 14 days (2 weeks)	1. Rotational test 2. Open field	1. Anxiety behavior (open field test)

Abbreviations: SNpc: *substantia nigra pars compacta*; i.p.: intraperitoneal; s.c.: subcutaneous; α -syn: α -synuclein; MFB: medial forebrain bundle; Ex-4: exendin-4; GP: geniposide; L-DOPA: levodopa; CFU: colony-forming unit; VTA: ventral tegmental area; α -syn PFF: α -synuclein pre-formed fibril; LAMP2A: lysosomal-associated membrane protein 2A; DPS-4: N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; pCA: p-chloroamphetamine; LIDS: levodopa-induced dyskinesias; DA: dopamine; NA: noradrenaline; 5-HT: serotonin; DOPAC: 3,4-dihydroxyphenylacetic acid; HVA: homovanillic acid; TH: tyrosine hydroxylase; GFAP: glial fibrillary acidic protein; IBA-1: ionized calcium binding adaptor molecule 1; IL-6: interleukin-6; IL-8: interleukin-18; TNF- α : tumor necrosis factor alpha; TGF- β : Transforming growth factor beta; GDNF: Glial cell line-derived neurotrophic factor; BDNF: Human Brain-Derived Neurotrophic Factor; MDA: Malondialdehyde levels; MM3: matrix metalloproteinase; Mac-1: Macrophage-1 alpha; NF- κ B: nuclear factor kappa-light-chain-enhancer of activated B cells; SVZ: subventricular zone; BrdU: Bromodeoxyuridine; CREB: cAMP Response Element-Binding Protein; TLR-4: Toll-like receptor 4; VMAT-2: Vesicular monoamine transporter.

* Treatment before lesion.

Treatment before and during lesion.

& Treatment before and after lesion.

^sGLP-1 and L-DOPA were administered together.

Risk of bias – clinical studies

The complete RoB 2 toll is already established and published as a complete guideline. According to the tool, the possible risk-of-bias judgements are: low risk of bias, some concerns, and high risk of bias. Complete results are specifically presented in figures 4 and 5, and table 5 of Appendix 3.

Athauda et al. (2017)⁶⁰ was considered to be at low risk of bias in all domains. Regarding “allocation sequence generation”, “allocation sequence concealment” and “baseline characteristics”, patients and investigators were masked to treatment allocation throughout the study. SealedEnvelope generated the online randomisation list, with a block design of two strata according to disease severity. The trial statistician allowed the allocation of masked study drug kits at randomisation and follow-up visits by assessing clinicians. Despite randomisation, the exenatide group had higher MDS-UPDRS part III scores and lower L-DOPA equivalent dose at baseline than the placebo group. However, since there were adjustments in the primary analysis, study was still considered as “low risk of bias”.

Regarding “deviations from the intended intervention”, participants were at first not aware of the intervention during the drug trial; however, they might have been partly unblinded to their treatment allocation as a result of adverse effects (although injection site reactions were similar across both groups). Still, no changes from the assigned intervention arose because of the trial context. All efficacy analyses were based on an appropriate modified intention-to-treat principle, which included all patients who completed any post-randomisation follow-up assessments. The study was considered as “low risk of bias” for this domain.

Data were missing for two out of 60 randomised participants (that dropped out before first follow-up); for one participant data were missing only in the 60-week time-point (after washout). Since data in all time-points were present for at least 95% of participants, the study was considered as “low risk of bias” for the “missing outcome” domain.

For the “measurement of the outcome” domain, appropriate methods were used to measure primary and secondary outcomes of interest and assessors/clinicians were blinded to the intervention received by each patient.

As for the “selection of the reported result” domain, primary and secondary

outcomes were as specified in the registered original protocol. Also, all study analyses were done according to a predefined statistical analysis plan also mentioned in the registered protocol.

For Aviles-Olmes et al. (2013)⁶¹, a computed list was generated by a statistician using two strata according to patient PD severity. The list was kept by the trial pharmacist securely and completely separately from the trial team; therefore, there were no problems with sequence allocation and concealment. There were no significant differences between the two groups at baseline, although the control group had slightly longer disease duration than that of exenatide-treated patients.

Since this was an open-label from the patient's perspective study given the complex design of the exenatide pen device, patients on the comparator group did not take any placebo injections; instead, they received only their usual PD medication. Therefore, participants knew whether they were on the treatment group or not. Despite of that, no changes from the assigned intervention arose because of the trial context. An appropriate intention to treat analysis was performed for participants completing a single follow-up. The study was considered as "low risk of bias" in the "deviations from the intended interventions" domain.

One participant dropped out before the first follow-up and two participants had missing outcomes in the last time-point of 14 months (93.3% of participants with data in all time-points). For these two participants, a "last observation carried forward" analysis was used, and since reasons to discontinue treatment was due to health outcomes (worsening of PD symptoms), it was not possible to exclude that missingness in the outcome was not influenced by its true value. Therefore, the study was framed as "some concerns" in the "missing outcome" domain.

Assessors were blinded to interventions and analyses on the severity of PD considering the additional effects on rigidity scores (which were only evaluated unblinded) were separated from the blinded assessment. The study was considered as low risk of bias in the "outcome assessment" domain.

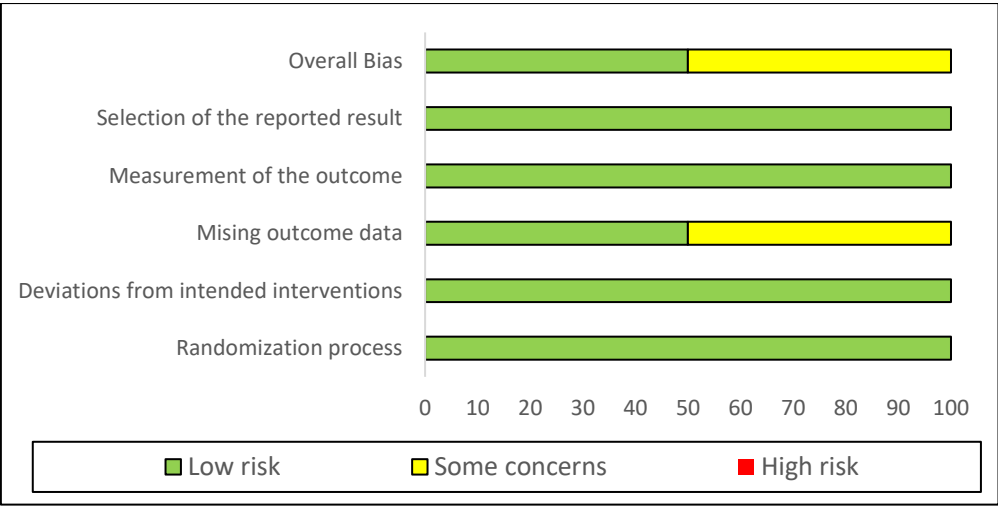


Figure 2. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all clinical included studies (extracted from the RoB 2 tool).

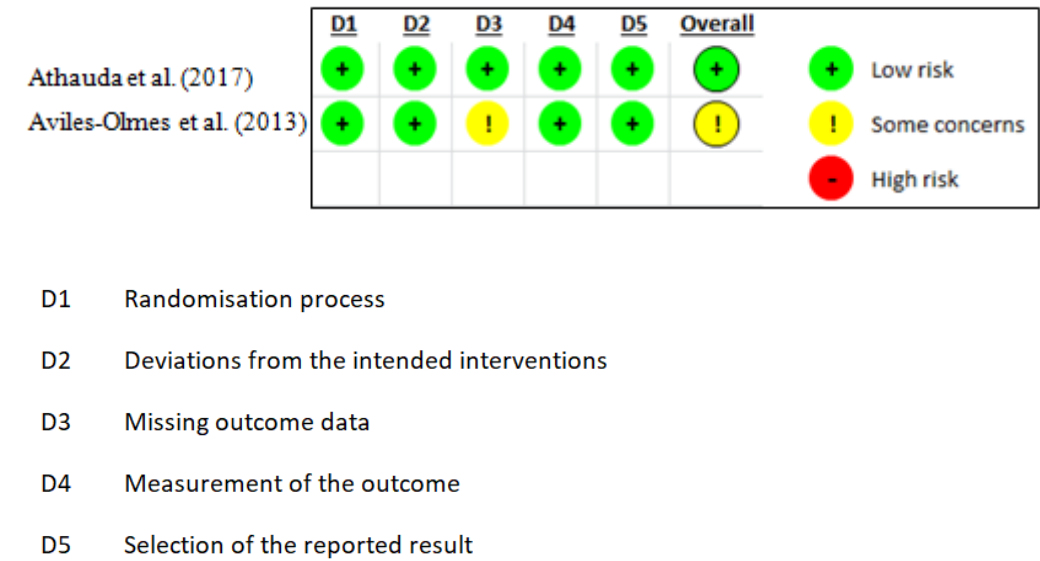


Figure 3. Risk of bias summary: judgements about each risk of bias item for each clinical included study (extracted from the RoB 2 tool).

Risk of bias – preclinical studies

The complete SYRCLE/CAMARADES adapted risk of bias tool for preclinical studies is available in Appendix 2. Complete results are specifically presented in figures 2 and 3, and tables 3 and 4 of Appendix 3, with reasons for “high risk of bias” judgements.

No studies described the method of randomisation into the different study groups and were, therefore, ranked as “unclear” in the “allocation sequence generation” and “allocation sequence concealment” domains^{8, 12, 65, 69, 70, 72, 74, 80-84, 86, 88-91}. The use of appropriate randomisation methods ensures that each experimental group has an equal probability of receiving a particular treatment. Simply selecting an animal “at random” is not statistically random as the process involves human judgement and can introduce bias. The exact randomisation method, including how the allocation sequence was generated and whether this was a blinded process, should be better described in all studies for a better judgment. Studies that did not mention a randomisation process at all were judged as “high risk of bias”^{10, 11, 26, 63, 64, 66-68, 71, 73, 75, 77, 79}.

Animals’ characteristics at baseline were often not reported with as much details as needed. Usually, only the body weight range of all animals at the beginning of the study was reported, without differentiating the mean weight of the animals in each group. Also, age was usually not specified in weeks or months and, if reported, it was mentioned as a range and, as the body weight, it is was not possible to know exactly the mean age of each group, which makes it difficult to assess whether the animals of different groups had similar characteristics at baseline. Additionally, it was often not mentioned if PD was induced before randomisation of the lesioned groups to vehicle or GLP-1/GIP treatment groups and behavioral/locomotor tests were not performed before treatment to assure that PD animals had similar baseline responses to the neurotoxin/insult. Thus, most studies were considered as “unclear”^{10, 65, 68, 71, 81, 86-88} or at “high risk of bias”^{8, 11, 12, 26, 63, 64, 66, 67, 69, 70, 72-75, 77-80, 82, 83, 85} in the “baseline characteristics” domain.

Ideally, investigators should be unaware of the treatment animals have received, from the start of the experiment (allocation concealment) until the data is analysed. Many outcomes used in animal models are dependent upon subjective interpretation. While subjective evaluations are generally an efficient way to score behavioral endpoints, it can create bias if the assessor is aware of the animal's treatment⁹².

Most studies did not mention blinding in any stage of the experiment, therefore, they were also considered as “unclear” in the domains “blinding from knowing which intervention each animal received”^{8-10, 26, 65-69, 72, 77-79, 83, 85-87, 89}. When studies did not have the same design for all groups and it was possible to know which group each animal was in, studies were judged as “high risk of bias”^{11, 26, 63, 64, 70, 71, 73-75, 80-82, 84}. When at least all primary outcomes were not clearly stated as being evaluated in a blinded fashion,

studies were considered as “unclear”^{8-10, 12, 26, 63-70, 72-75, 77-87} in the “blinding during the outcome assessment” domain. When non-blinding was clearly stated, they were judged as “high risk of bias”⁷¹. Only two studies reported blinding at least for behavioral tests^{76, 88}.

Overall, possible conflicts of interests of funders and study executors were disclosed in a clear and transparent manner. Only four studies were totally or partially funded by pharmaceutical companies^{9, 65, 81}. In ten studies, one of the authors was the named inventor on patent applications that cover GLP-1 and dual GLP-1/GIP receptor agonists use as treatment for PD^{12, 67, 72, 75, 76, 78, 82-85}. The absence of a “competing interests” section framed the study as “unclear” regarding a possible conflict of interest interfering results^{8, 80}.

Also, the anesthetic drug used (not for euthanasia purposes) was usually associated at some level with dopamine metabolism interference or neuroprotective proprieties. Thus, it was not possible to exclude a possible dopamine interaction with the GLP-1/GIP receptor agonist drugs, and nine studies were framed as “high risk of bias”^{8-10, 64-67, 72, 88} regarding the anesthetic used.

Most studies did not mention the animals' acclimatization period before the experiment started. An acclimatization period allows animals to get used to the new environment and promotes not only animal welfare, but reproducible experimental results, especially when the outcomes of interest are dopamine levels and behavioral changes that can easily be influenced by stress. Therefore, if acclimatization period was not reported, studies were judged as “unclear”^{8-10, 26, 63-74, 77, 79, 80, 82-86, 88}. Only six studies clearly stated that the acclimatization period was of at least seven days^{11, 13, 75, 78, 81, 87}.

Treatment was frequently initiated either before or concomitantly with the disease induction. This contrasts with the PD clinical situation in which treatment is started after onset of symptoms and a probable/possible PD diagnosis. Thus, a potential pharmacological effect could be overestimated in the animal model simply because the therapeutic intervention occurred earlier in the disease process as compared to the clinical situation. This framed studies as “high risk of bias” regarding the treatment start^{11, 12, 26, 64, 69-71, 73-75, 77, 79-85, 91}. Twelve studies started treatment after establishment of the lesion and were considered as “low risk of bias”^{8-10, 63, 65-68, 72, 86-88}.

If studies presented a “high risk of bias” response in the “conflict of interest”, “anesthesia”, “acclimatization period” and/or “treatment start”, they were judged as “high

risk of bias” 8, 10-12, 26, 64-75, 77-86, 88 in the “other possible problems that could lead to risk of bias” domain.

It is yet not common practice to register study protocols for animal studies before publishing results, therefore, it was not possible to clearly assess whether the studies reported the assessments they proposed previously. The "selective outcome reporting" domain originally included in the SYRCLE toll was, therefore, disregarded.

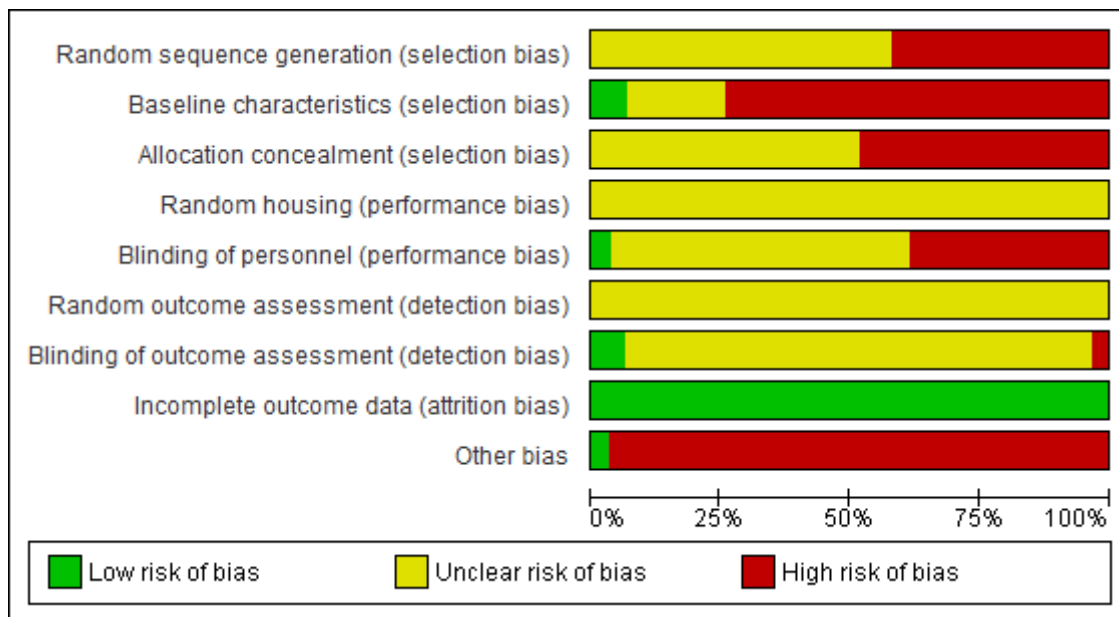


Figure 4. Risk of bias graph: review authors' judgements about each risk of bias item presented as percentages across all preclinical included studies (extracted from RevMan software v.5.3).

	Random sequence generation (selection bias)	Baseline characteristics (selection bias)	Allocation concealment (selection bias)	Random housing (performance bias)	Blinding of personnel (performance bias)	Random outcome assessment (detection bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias)	Other bias
Abdulkareem et al. 2017	●	●	●	?	●	?	?	●	●
Abuirmeileh et al. 2010_exenatide	●	●	●	?	●	?	?	●	●
Aksoy et al. 2017_exenatide	?	●	?	?	?	?	?	●	●
Badawi et al. 2017_liraglutide	●	?	●	?	?	?	?	●	●
Bertilsson et al. 2008_ex-4	?	●	?	?	?	?	?	●	●
Cao et al. 2016_DA-JC1	?	●	?	?	?	?	?	●	●
Chen et al. 2015_geniposide	?	●	?	?	●	?	?	●	●
Chen et al. 2018_ex-4	?	?	?	?	?	?	?	●	●
Fang et al. 2019_GLP-1	?	?	●	?	●	?	●	●	●
Feng et al. 2018_DA-CH5_DA-JC1_DA-JC4_liraglutide	?	●	?	?	?	?	?	●	●
Hansen et al. 2016_liraglutide	?	●	?	?	?	?	?	●	●
Harkavyi et al. 2008_ex-4	●	●	●	?	?	?	?	●	●
Harkavyi et al. 2013_ex-4	●	●	●	?	?	?	?	●	●
Jalewa et al. 2017_DA-JC1	●	●	●	?	?	?	?	●	●
Ji et al. 2016_DA-JC1	●	●	●	?	●	?	?	●	●
Kim et al. 2009_ex-4	?	●	?	?	●	?	?	●	●
Li et al. 2009_ex-4	●	●	●	?	?	?	?	●	●
Li et al. 2016_D-Ala2-GIP-glu-PAL	●	●	●	?	●	?	?	●	●
Li et al. 2017_D-Ala2-GIP-glu-PAL	●	●	●	?	●	?	●	●	●
Liu et al. 2015a_ex-4_lira_lxi	?	●	?	?	?	?	?	●	●
Liu et al. 2015b_oxy	●	●	●	?	?	?	?	●	●
Rampersaud et al. 2012_ex-4	●	?	●	?	?	?	?	●	●
Su et al. 2016_geniposide	?	●	?	?	●	?	?	●	●
Verma et al. 2017_D-Ala2-GIP	?	?	●	?	●	?	?	●	●
Yuan et al. 2017_DA-CH3_lira	?	●	?	?	●	?	?	●	●
Yu et al. 2018_GIP	●	●	●	?	?	?	?	●	●
Yun et al. 2020_NLY01 (exenatide)	?	?	?	?	●	?	●	●	●
Zhang et al. 2015_(Val8)GLP-1-Glu-PAL	?	●	?	?	?	?	?	●	●
Zhang et al. 2018_lira_sema	?	●	?	?	?	?	?	●	●
Zhang et al. 2019_lira_sema	?	●	?	?	●	?	?	●	●
Zhang et al. 2020_DACH5_ex-4_lira	?	●	?	?	?	?	?	●	●

Figure 5. Risk of bias summary: judgements about each risk of bias item for each preclinical study (extracted from RevMan software v.5.3).

Meta-analyses

Clinical studies - Primary outcomes

The MDS-UPDRS is one the most accepted scale to evaluate different aspects of PD including the patient's non-motor and motor experiences of daily living (parts I and II), a complete motor assessment from the clinician's perspective (part III), and also an assessment of motor complications (part IV)³⁴.

Since MDS-UPDRS, part I, was applied in both included studies, it was used in the meta-analyses instead of NMSS and NMSQ for a uniform analysis.

A scale to assess dyskinesia and motor fluctuation that is shown to be superior in detecting treatment effects compared with MDS-UPDRS, part IV, is the Unified Dyskinesia Rating Scale (UDyRS)⁹³. Since both studies applied the UDYRS, this scale was used in the meta-analyses instead of MDS-UPDRS, part IV.

Non-motor symptoms (daily living)

The pooled results for MDS-UPDRS, part I, assessing non-motor experiences of daily living in an “on medication” state from the patient's perspective showed a MD of -1.82 [95% CI: -3.93, 0.30; $I^2=15\%$; $P = 0.09$] after the washout period (figure 6). It was lower than the minimal, but clinically important, difference of -2.65 points for improvement. Therefore, no significant effects were observed for non-motor symptoms.

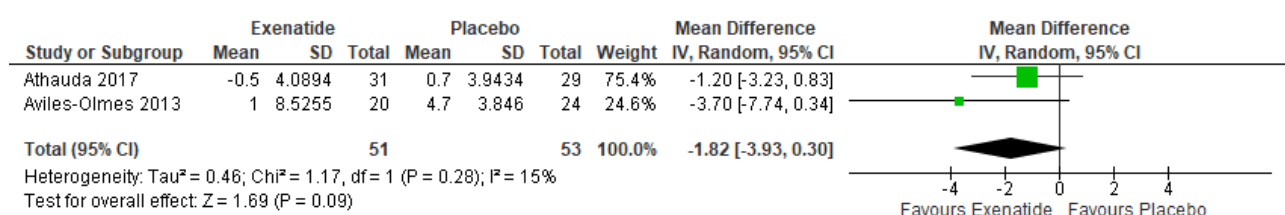


Figure 6. Forest plot. Non-motor symptoms - MDS-UPDRS part I (on medication).

Motor symptoms (daily living)

When assessing motor impairment through activities of the daily living in an “on medication” state by MDS-UPDRS, part II, both studies showed a non-statistically significant MD of -1.48 points [95% CI: -3.24, 0.27; $I^2=0\%$; $P = 0.10$] after the washout period (figure 7). This was lower than the difference of -3.05 considered a minimal, but clinically important, improvement.

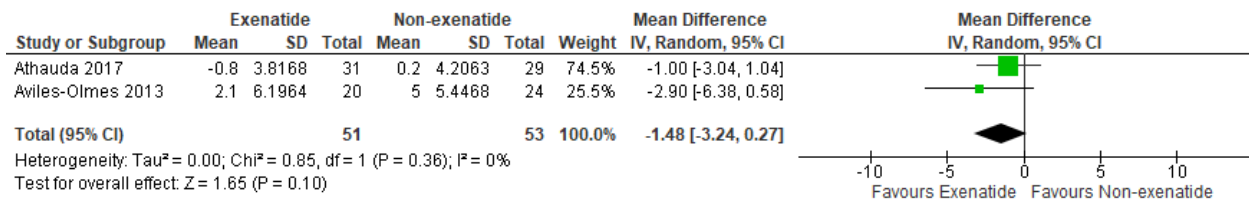


Figure 7. Forest plot. Activities of daily living - MDS-UPDRS part II (on medication) – after washout.

Motor symptoms (full assessment – clinician’s evaluation)

When assessing motor impairment in an “off medication” state by MDS-UPDRS, part III, both studies evaluating exenatide led to a statistically significant MD of -3.60 points [95% CI: -6.01, -1.19; $I^2=0\%$; $P=0.003$] in the end of the washout period compared to baseline. This exceeded the minimal clinically difference for improvement in motor impairment (-3.25) (figure 8).

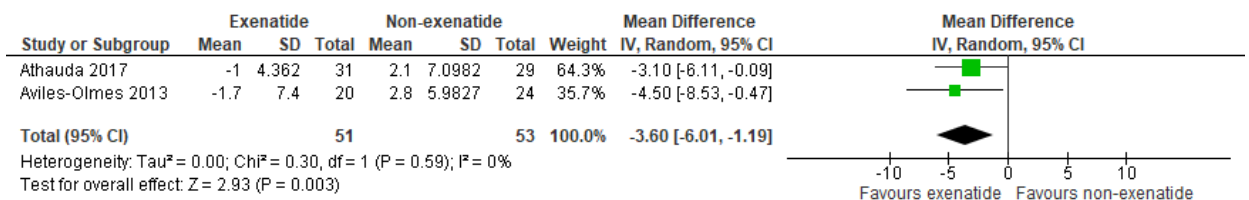


Figure 8. Forest plot. Motor impairment - MDS-UPDRS part III (off medication) – after washout.

When assessing motor impairment in an “on medication” state by MDS-UPDRS, part III, both studies showed a higher MD, although not statistically significant, of -4.48 [95% CI: -14.58, 5.63; $I^2=93\%$; $P=0.39$] after the washout compared to baseline (figure 9).

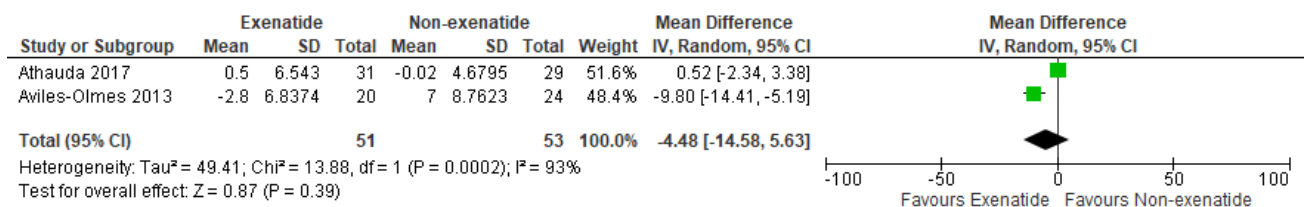


Figure 9. Forest plot. Motor impairment - MDS-UPDRS part III (on medication) – after washout.

Motor complications

The pooled results for UDYRS assessing dyskinesia and motor fluctuations in an “on medication” state showed a non-statistically significant MD of 0.30 [95% CI: -1.65, 2.26; $I^2=0\%$; $P=0.76$] after the washout period compared to baseline. The value did not

exceed a difference of -2.1 points for minimal, but clinically important, improvement. Therefore, no effect favoring exenatide was observed for motor complications (figure 10).

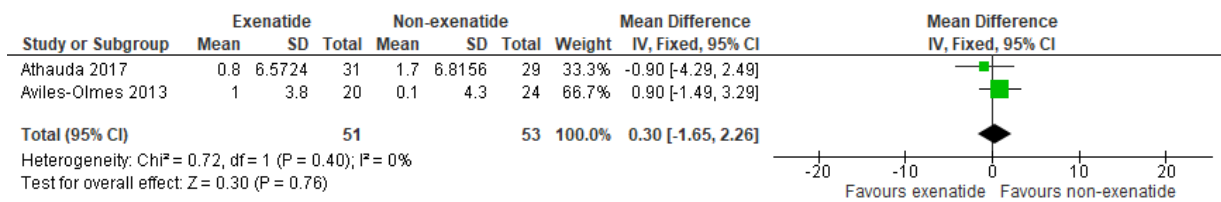


Figure 10. Forest plot. Motor complication – UdyRS (on medication).

Clinical studies - Secondary outcomes

Depression

The pooled results for MADRS assessing depression in an “on medication” state showed a non-statistically MD of -1.03 [95% CI: -2.54, 0.47; $I^2=0\%$; $P=0.18$] after the washout period (figure 11).

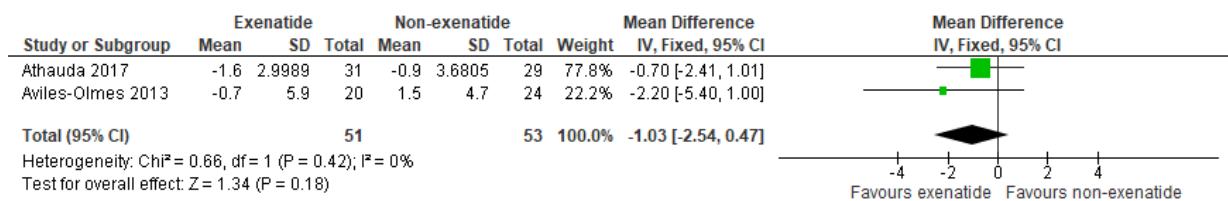


Figure 11. Forest plot. Depression – MADRS (on medication).

Dementia

The pooled results for MDRS assessing dementia in an “on medication” state showed a statistically significant MD of 2.59 [95% CI: 0.92, 4.26 $I^2=0\%$; $P=0.002$] after the washout period, not favoring exenatide treatment (figure 12). Therefore, there was no effect in ameliorating dementia symptoms.

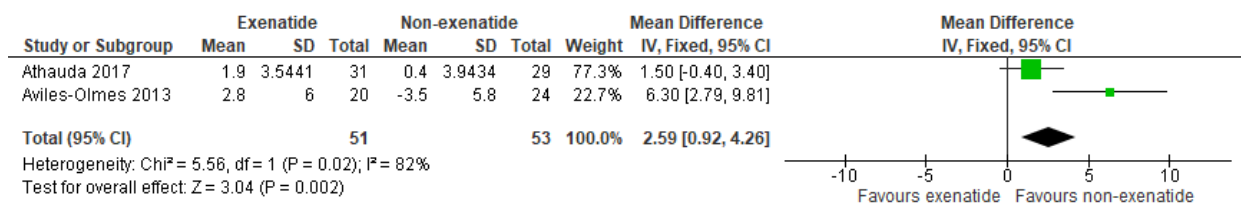


Figure 12. Forest plot. Dementia – MDRS (on medication).

Quality of life

The pooled results for PQ-39 assessing quality of life in an “on medication” state showed a non-statistically significant MD of 0.56 [95% CI: -4.61, 5.72; $I^2=40\%$; $P=0.83$] after the washout period (figure 13). Therefore, there was no effect in increasing quality of life of patients.

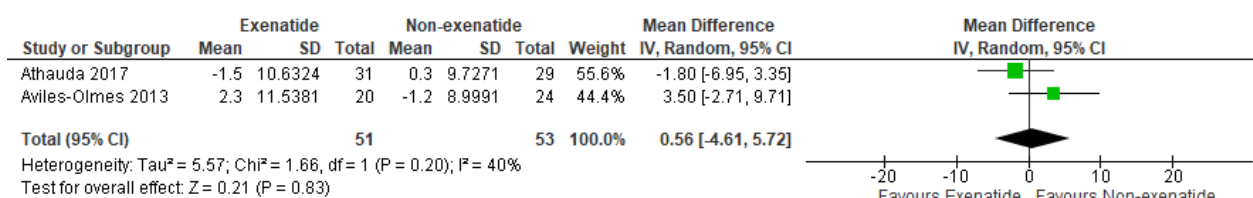


Figure 13. Forest plot. Quality of life - PQ-39 (on medication).

Preclinical studies - Primary outcomes

Studies were separately investigated as GLP-1, GIP or GLP-1/GIP dual receptor agonists. Only studies that performed the same test for the specific outcome and with similar units of measure were included in the meta-analyses. The result of the last evaluated timepoint was considered for meta-analyses in case more than one measure was taken, or in the absence of this data, the average result of all timepoints was plotted.

General locomotor activity

The open field test was commonly performed to evaluate the general locomotor activity of “lesioned animals only” vs. “lesioned animals” treated with the drugs of interest. Animals placed in an arena had their free movements recorded for a period of time (5 or 10 min). The total distance traveled by the animals was reported in cm; studies that reported the number of line crossing and the distance in % were not included. Results were imputed in the meta-analyses as the distance moved proportionally per minute (e.g., cm/minute).

GLP-1 receptor agonists

Meta-analyses assessed GLP-1, Ex-4, liraglutide, lixisenatide, semaglutide, oxyntomodulin, and (Val8)GLP-1-Glu-PAL^{71, 78, 79, 83-85}.

Overall meta-analysis, considering all GLP-1 receptor agonists, has led to a statistically significant SMD of 1.65 [95% CI: 1.05, 2.25; $I^2=59\%$; $P<0.00001$] (figure

14).

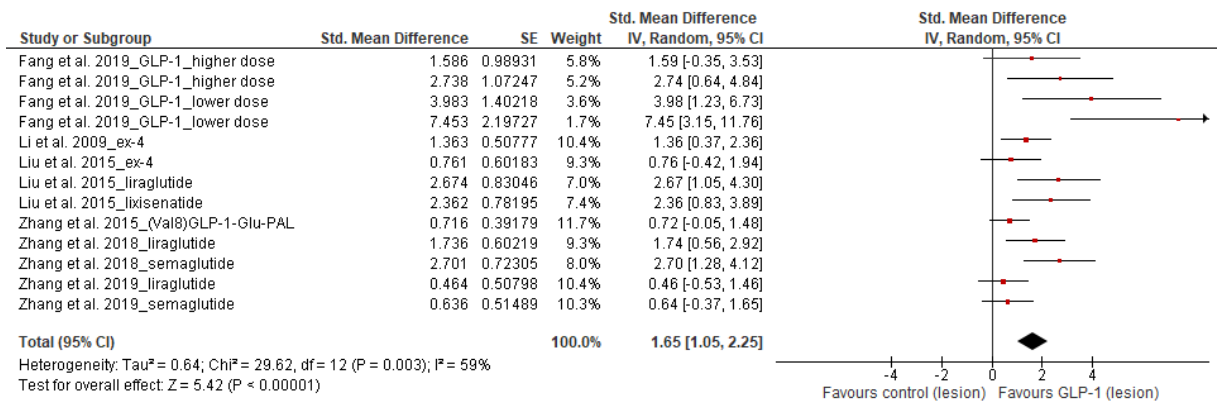


Figure 14. Forest plot. General locomotor activity – open field test (GLP-1 receptor agonists).

GIP receptor agonists

Only one study²⁶ performed the open field test with the distance traveled in cm, which limits the interpretation of results. A statistically significant SMD of 1.36 [95% CI: 0.08, 2.64; $P=0.04$] was obtained for this study (figure 15).

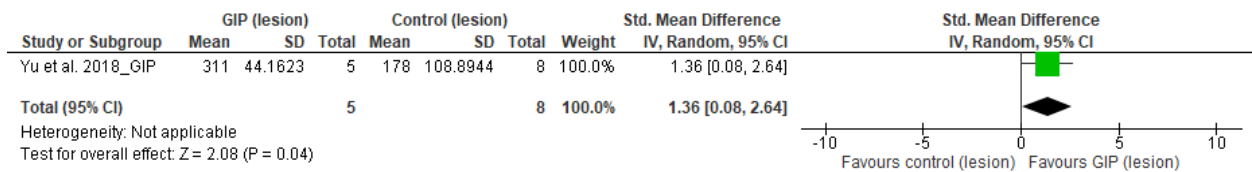


Figure 15. Forest plot. General locomotor activity – open field test (GIP receptor agonists).

GLP-1/GIP receptor agonists

For the dual agonists, two DA-JC1 studies had open field results^{67, 69}. Considering both studies together, the overall result was a statistically significant SMD of 0.72 [95% 0.04, 1.41; $I^2=0\%$; $P=0.04$] (figure 16).

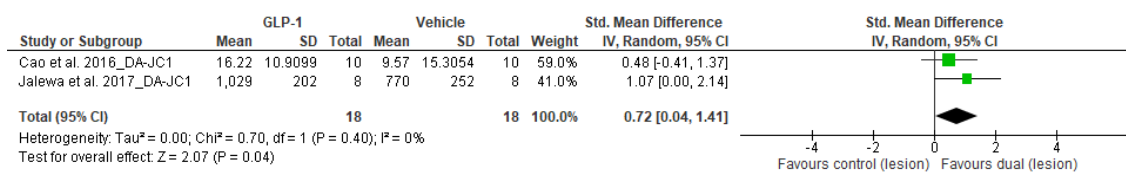


Figure 16. Forest plot. General locomotor activity – open field test (GLP-1/GIP dual agonists).

Coordination and balance

To assess coordination and balance of “lesioned animals only” vs. “lesioned animals” treated with the drugs of interest, the most common test performed was the

rotarod test. Animals were placed on a rotating rod at an either constant rotation or a steady acceleration; the latency to fall in seconds was recorded.

GLP-1 receptor agonists

Results for liraglutide, semaglutide, exendin-4, geniposide, (Val8)-GLP-1-glu-pal, oxyntomodulin, lixisenatide, and NLY01 were plotted^{70, 72, 78, 79, 82-85, 88}. The overall result led to a statistically significant SMD of 1.11 [95% CI: 0.66, 1.57; $I^2=61\%$; $P<0.00001$] (figure 17).

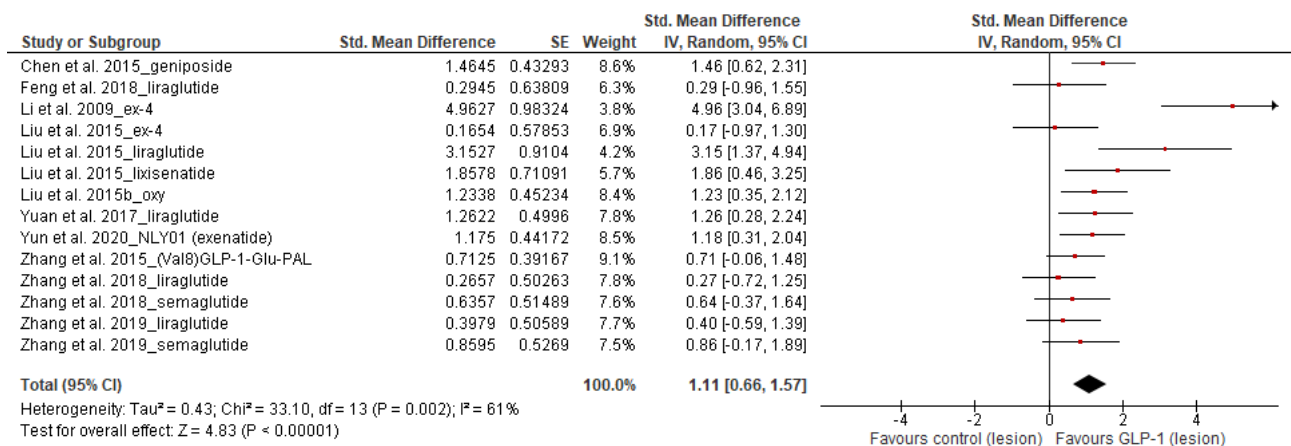


Figure 17. Forest plot. Motor coordination and balance – rotarod test (GLP-1 receptor agonists)

GIP receptor agonists

Two studies had results for D-ALA²-GIP and D-ALA²-GIP-glu-pal^{75, 81}. The overall result was a SMD of 1.88 [95% CI: 0.36, 3.40; $I^2=79\%$; $P=0.02$] (figure 18).

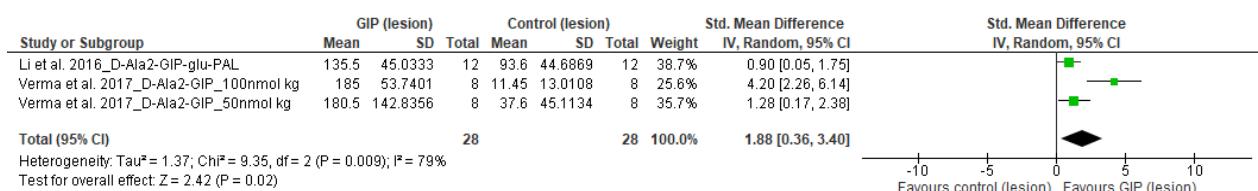


Figure 18. Forest plot. Motor coordination and balance – rotarod test (GIP receptor agonists)

GLP-1/GIP dual receptor agonists

The dual agonists DA-JC4, DA-JC1, DA-CH3 and DA-CH5^{67, 72, 73, 82} resulted in a statistically significant SMD of 1.10 [95% CI: 0.14, 2.06; $I^2=65\%$; $P=0.03$] (figure 19).

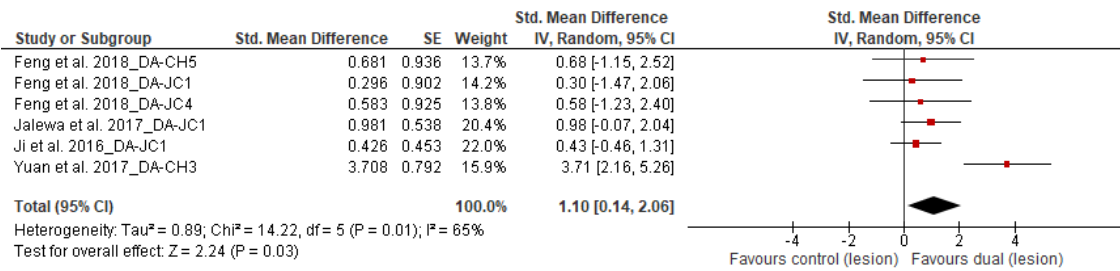


Figure 19. Forest plot. Motor coordination and balance – rotarod test (GLP-1/GIP dual receptor agonists).

Muscle strength

The traction test was used to assess the animal's muscle strength. In this test, the animals were suspended by the forepaws to a metallic bar. The animal was scored as 3 for gripping the wire with both hind paws, 2 for gripping the wire with one hind paw, and 1 for not gripping the wire with either hind paw.

GLP-1 receptor agonists

Liraglutide and oxyntomodulin^{72, 79, 82} were plotted together and led to a statistically significant SMD of 0.47 [95% CI: 0.23, 0.72; $I^2=0\%$; $P=0.0001$] (figure 20).

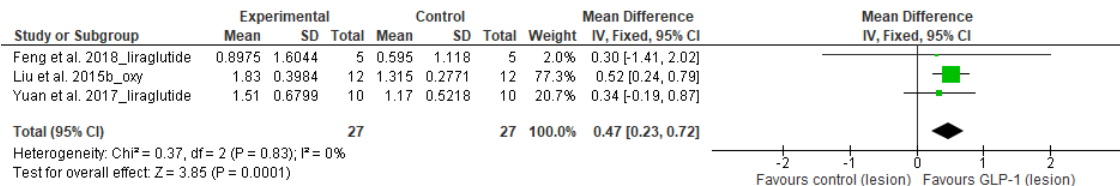


Figure 20. Forest plot. Motor strength - Traction test (GLP-1 receptor agonists).

GIP

No GIP receptor agonist was evaluated in the traction test.

GLP-1/GIP dual receptor agonists

The dual agonists DA-JC4, DA-JC1, DA-CH3, and DA-CH5^{72, 73, 82} resulted in a statistically significant SMD of 1.00 [95% CI: 0.24, 1.77; $I^2=32\%$; $P=0.01$] (figure 21).

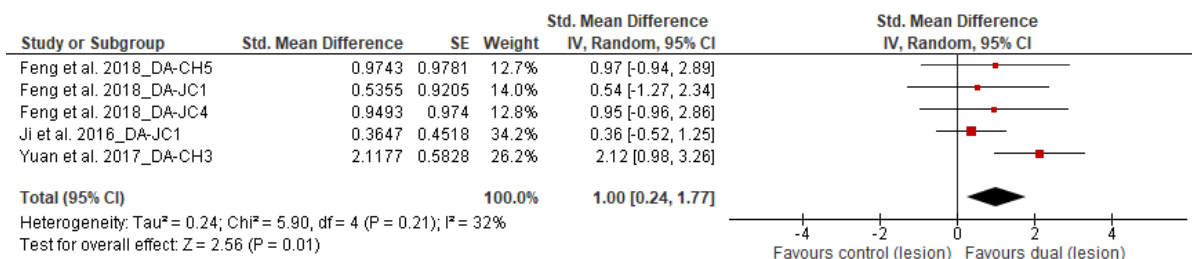
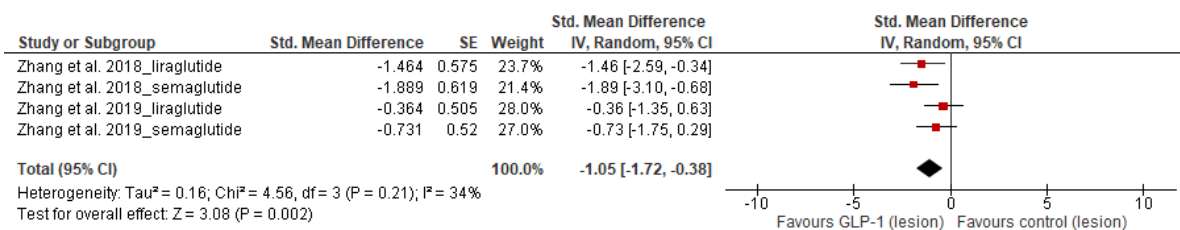


Figure 21. Florest plot. Motor strength - Traction test (GLP-1/GIP dual receptor agonists).**Gait abnormalities**

The footprint test was performed to evaluate gait abnormalities. Animals' forepaws and hind paws were painted with different colors to record footprints as they walked through a white paper in a dark tunnel. Stride lengths were determined by measuring the distance (cm) between each step on the same side of the body. The length of the shortest stride was subtracted from the length of the longest stride to determine the variation rate (cm).

GLP-1 receptor agonists

Liraglutide and semaglutide together^{83, 84} led to a statistically significant SMD of -1.05 [-1.72, -0.38, $I^2=34\%$; $P=0.002$], favouring treatment in ameliorating gait disturbances (figure 22).

**Figure 22.** Gait abnormalities – Footprint test (GLP-1 receptor agonists).**GIP receptor agonists**

No GIP receptor agonist was evaluated in the footprint test.

GLP-1/GIP dual receptor agonists

No GLP-1/GIP dual receptor agonist was evaluated in the footprint test.

Preclinical studies - Secondary outcomes

Only one study⁶⁸ assessed depression and anhedonia in the absence of locomotor effects (pre-motor model), which is important to account for possible biases in assessment of these behaviors.

Exendin-4 improved depression [SMD -5.92; 95% CI: -8.15, -3.69; $P<0.00001$] (figure 23) and anhedonia [SMD 3.36; 95% CI: 1.90, 4.81; $P<0.00001$] (figure 24) behaviors in the forced swim test (% immobility time) and sucrose preference test (%), respectively.

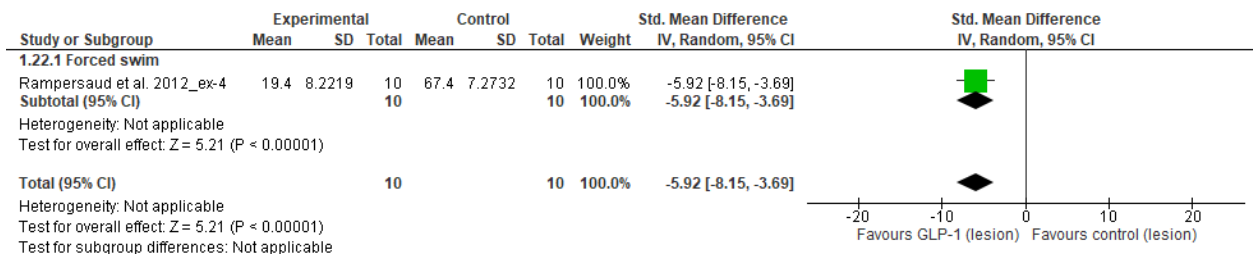


Figure 23. Depression – Forced swim test (GLP-1 receptor agonists).

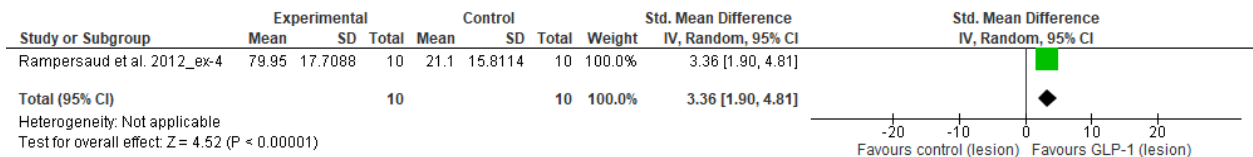


Figure 24. Anhedonia – Sucrose preference test (GLP-1 receptor agonists).

Subgroup analyses

Clinical studies

It was not possible to perform subgroup analyses for the two included clinical trials. There was no specific result for subtypes of PD, genomic status of each patient's PD related genes, or use of other antiparkinsonian drugs.

Preclinical studies

General locomotor activity

GLP-1 receptor agonists

Subgroups were performed for each GLP-1 receptor agonist, when appropriate, as shown in figure 18.

Lixisenatide and (Val8)-GLP-1-Glu-Pal^{78, 85} were individually tested in two studies. Their individual results were: a statistically significant SMD of 2.36 [95% CI: 1.28, 3.45; P<0.0001] for lixisenatide⁷⁸, and a non-statistically significant SMD of 0.72 [95% CI: -0.05, 1.48; P=0.07] for (Val8)-GLP-1-Glu-Pal.

GLP-1 itself was evaluated in one study⁷¹ with multiple arms (different doses and types of treatment). GLP-1 led to an overall statistically significant SMD of 3.33 [95% CI: 1.41, 5.25; I²=55%; P=0.0007]. Liraglutide was tested in three studies^{78, 83, 84}. All three led to a statistically significant SMD of 1.57 [95% CI: 0.31, 2.84; I² 80%; P=0.01]. Exendin-4 had only two studies^{77, 78}, with an overall SMD of 1.01 [95% CI: 0.37, 1.65; I²

=0%; P=0.002]. Semaglutide also had only two studies^{83, 84} included with an overall SMD of 1.63 [95% CI: -0.40, 3.65; I²=88%; P=0.11].

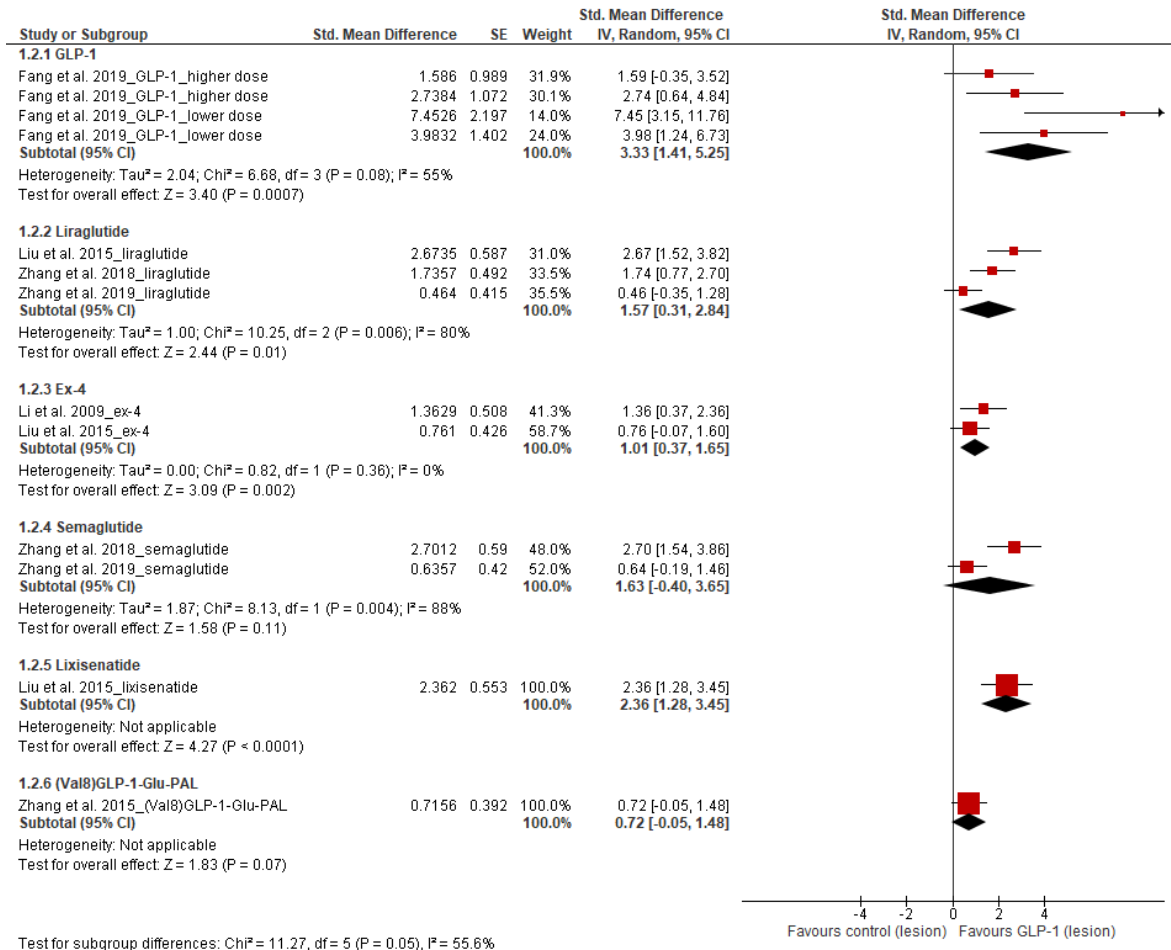


Figure 18. Forest plot. General locomotor activity – open field test (per GLP-1 receptor agonist)

Motor coordination and balance

GLP-1 receptor agonists

Subgroups for each GLP-1 were also performed for the rotarod test, when appropriate, as shown in figure 19.

Lixisenatide, oxyntomodulin, geniposide, (Val8)-GLP-1-Glu-Pal, and NLY01 had only one study each^{78, 79, 85, 88}. Lixisenatide⁷⁸ alone led to a statistically significant SMD of 1.86 [95% CI: 0.87, 2.84; P=0.0002]; oxyntomodulin⁷⁹, a statistically significant SMD of 1.23 [95% CI: 0.35, 2.12; P=0.06]; geniposide⁷⁰, a statistically significant SMD

of 1.46 [95% CI: 0.62, 2.31; $P=0.0007$]; (Val8)-GLP-1-Glu-Pal⁸⁵, a non-statistically significant SMD of 0.71 [95% CI: -0.06, 1.48; $P=0.07$]; and NLY01⁸⁸, a statistically significant SMD of 1.18 [95% CI: 0.31, 2.04; $P=0.08$].

Exendin-4 with two studies^{77, 78} showed a statistically significant SMD of 0.87 [95% CI: 0.13, 1.61; I^2 95%; $P=0.02$]. Liraglutide studies^{72, 78, 82-84} combined showed a statistically significant result of 0.84 [95% CI: 0.41, 1.27; I^2 =77%; $P=0.0001$]^{72, 78, 82-84}. Semaglutide with two studies^{83, 84} led to a SMD of 0.74 [95% CI: 0.16, 1.33; $I^2=0\%$; $P=0.01$].

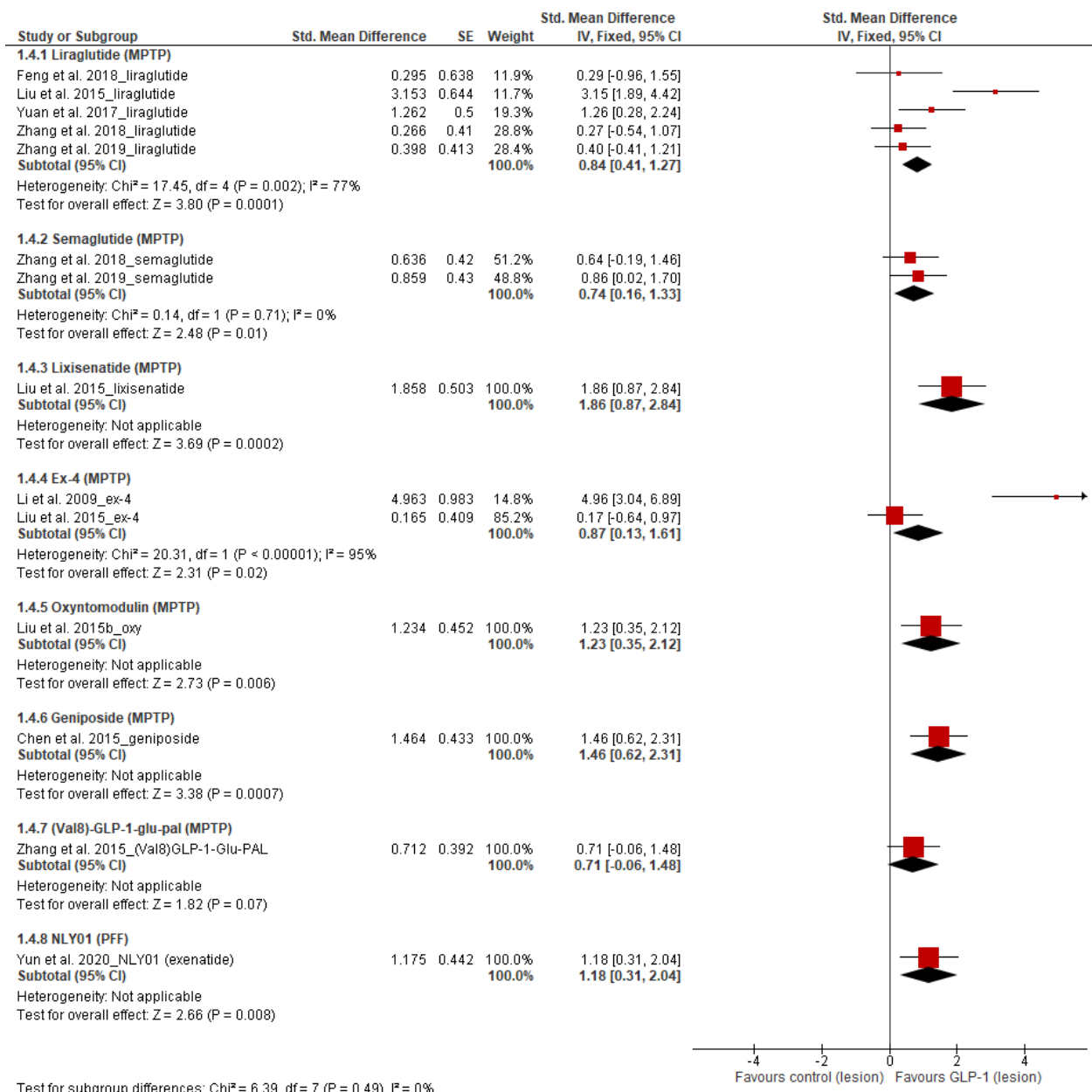


Figure 19. Forest plot. Motor coordination and balance – rotarod test (per GLP-1 receptor agonists).

GIP receptor agonists

Subgroups for each GIP were also performed, when appropriate, as shown in figure 20.

Only one study reported results for D-Ala²-GIP-glu-pal⁷⁵. Its individual result was a statistically significant SMD of 0.90 [95% CI: 0.05, 1.75; P=0.04].

Other study with two tested doses was included for D-Ala²-GIP⁸¹, with a non-statistically significant SMD of 2.62 [95% CI: -0.23, 5.48; I²=85%; P=0.07].

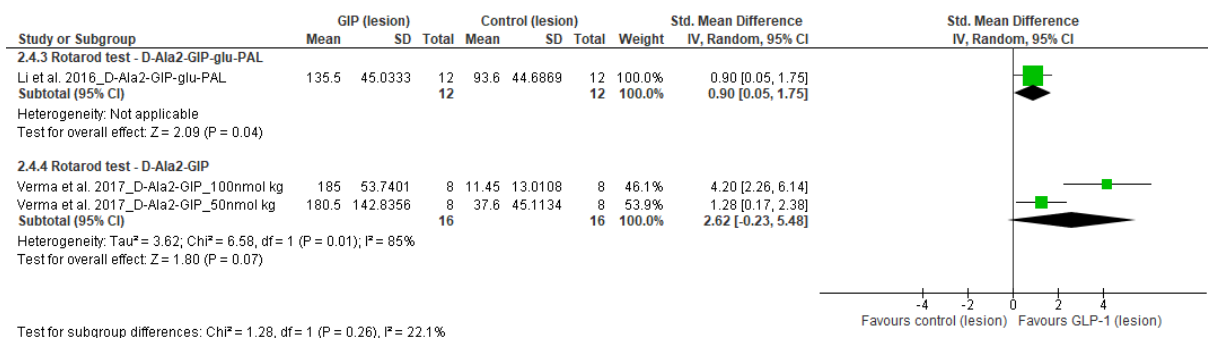


Figure 20. Forest plot. Motor coordination and balance – rotarod test (per GIP receptor agonists).

GLP-1/GIP receptor agonists

Subgroups for each GLP-1/GIP dual agonists were performed, when appropriate (figure 21).

DA-CH5, DA-JC4, DA-CH3 results were reported in only two studies^{72, 88}. Their individual results were: a non-statistically significant SMD of 0.68 [95% CI: -0.62, 1.98; P=0.3] for DA-CH5⁷², a non-statistically significant SMD of 0.58 [95% CI: -0.70, 1.86; P=0.37] for DA-JC4⁷², and a statistically significant SMD of 3.71 [95% CI: 2.16, 5.26; P<0.00001] for DA3-CH⁸².

All three DA-JC1 studies^{67, 72, 73} combined led to a non-statistically significant SMD of 0.57 [95% CI: -0.02, 1.17; I²=0%; P=0.06].

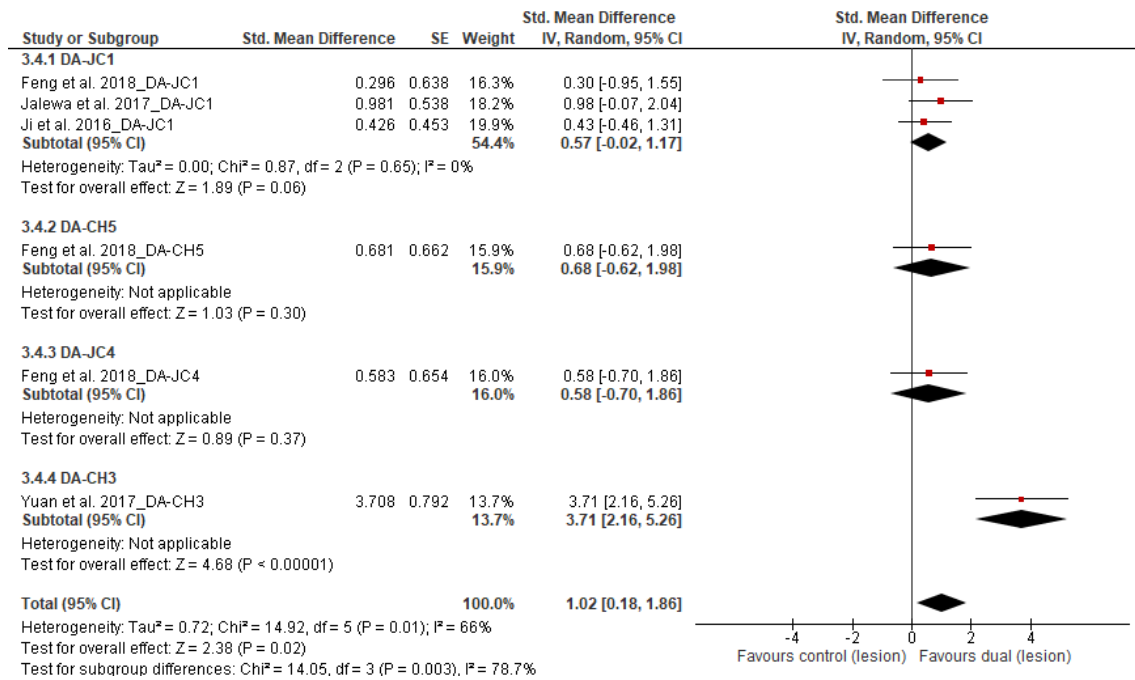


Figure 21. Forest plot. Motor coordination and balance – rotarod test (per GLP-1/GIP receptor agonists).

Muscle strength

GLP-1 receptor agonists

Separating the GLP-1 receptor agonists, liraglutide in two studies^{72, 82} led to a non-statistically significant SMD of 0.42 [95% CI: -0.31, 1.15; $I^2=0\%$; $P=0.26$] and oxyntomodulin in only one study⁷⁹ had a statistically significant SMD of 1.45 [95%CI: 0.53, 2.37; $P=0.002$].

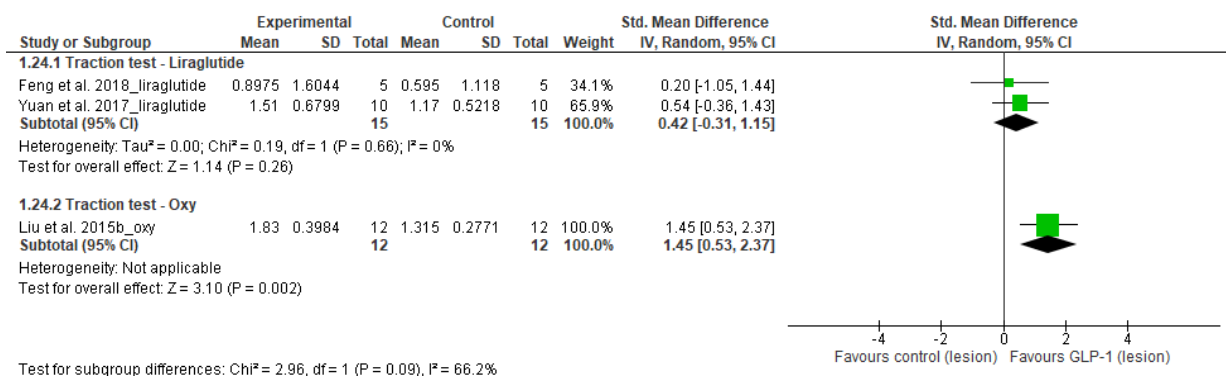


Figure 22. Muscle strength – traction test (per GLP-1).

GIP receptor agonist

No GIP receptor agonist was evaluated in the traction test.

GLP-1/GIP dual receptor agonists

Overall result for DA-JC1 in two studies^{72, 73} was a non-statistically significant SMD of 0.42 [95% CI: -0.31, 1.15; I^2 0%]. Only one study showed non-statistically significant results for DA-CH5⁷² [SMD 0.97; 95% CI -0.38, 2.33], and DA-JC4⁷² [SMD 0.95; 95% CI: -0.40, 2.30]. DA3-CH⁸² was the only one showing significant results [SMD 2.12; 95% CI: 0.98, 3.26] (figure 23).

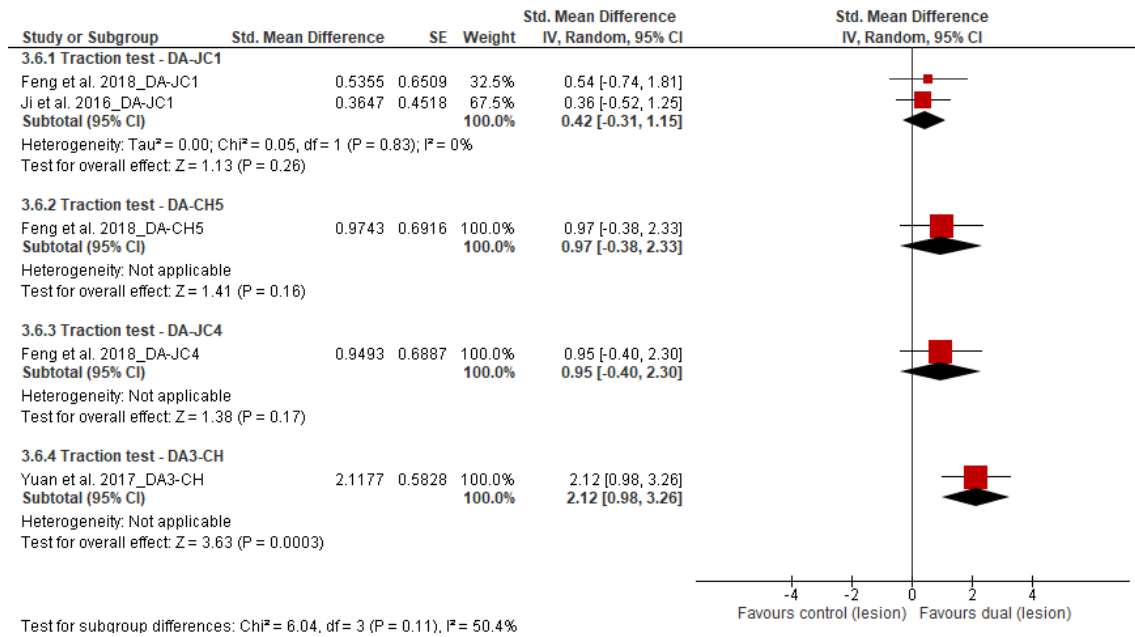


Figure 23. Muscle strength – traction test (per GLP-1/GIP dual receptor agonists).

Gait abnormalities

GLP-1 receptor agonists

Two studies with liraglutide^{83, 84} led to a non-statistically significant SMD of -0.89 [95% CI: -1.97, -0.19], and semaglutide^{83, 84} to a statistically significant SMD of -1.28 [95% CI: -2.41, -0.14], both with 68% heterogeneity, in the footprint test (figure 24).

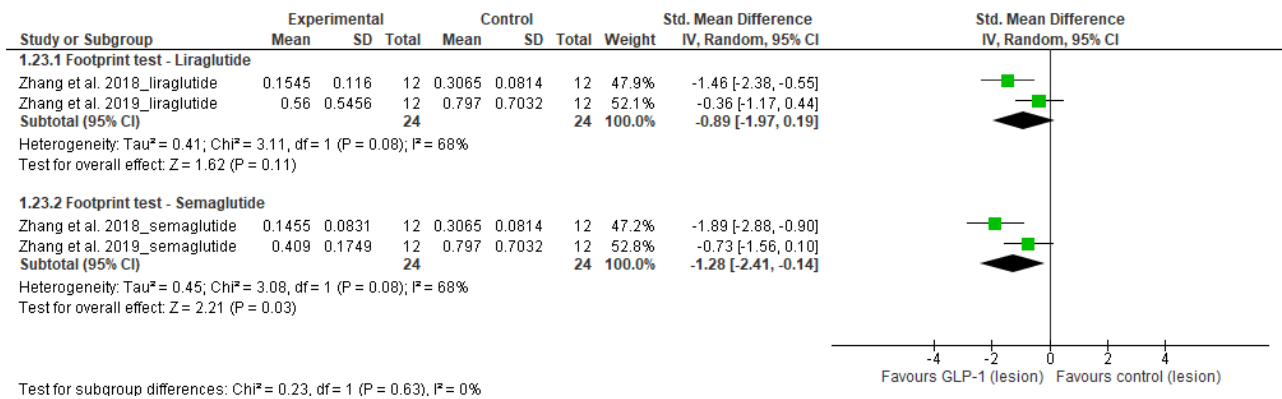


Figure 24. Gait abnormalities – footprint test (per GLP-1/GIP dual receptor agonists).

Meta-regression

Linear meta-regression models were employed to explore possible predictors of statistical heterogeneity in meta-analyses with 10 or more estimates.

Specifically, we tested the hypothesis that the moment the intervention started could explain some of the heterogeneity between studies. For this, we created a dummy variable that assumed a value of 0, if the estimate was obtained from an intervention that started before the PD lesion (pre-lesion model), 1 if the estimate was obtained from an intervention that started after the PD lesion (post-lesion model), or 2 if the estimate was obtained from an intervention that started concomitantly with the PD lesion.

Meta-regression models employed the Knapp-Hartung correction⁹⁴, which keeps the type I error close to the desired alpha level (e.g., 5%).

For the locomotor activity outcome (open field test), based on the evidence of 13 estimates (from five studies with 144 animals in the GLP-1 receptor agonists' group and 144 animals in the control group), there was no evidence that the moment of lesion had a statistically significant association with the summary effect. The model indicates that studies with post-lesion interventions will have, on average, a summary effect size 0.46 units of standard deviation smaller compared to studies with pre-lesion intervention, but this difference is not statistically significant ($\beta = -0.46$, 95% CI: -2.55, 1.64; $P = 0.64$). This variable did not explain any variability in the observed estimates ($R^2 = 0\%$), and residual heterogeneity ($I^2 = 62.1\%$, P for Cochran's residual test = 0.002) remained statistically significant.

The Hedges summary effect size (random effects) predicted for “post-lesion” studies was 2.04 (95% CI: 0.35, 3.74; $P=0.02$), while the corresponding estimate for “pre-

lesion” studies was 1.59 (95% CI: 0.80, 2.38; $P < 0.001$), corroborating the hypothesis of a significant and similar effect of the interventions regardless of the moment of lesion.

However, data from 14 estimates from nine studies (163 animals in the GLP-1 receptor agonist’s group, 163 animals in the control group) revealed a statistically significant association between the moment of lesion and the Hedges summary effect size for the motor coordination and balance outcome (rotarod test).

Taking interventions in pre-lesion studies as a reference, interventions with concomitant and post-lesions were associated with statistically less expressive summary estimates ($\beta_{conco\ vs\ pre} : -4.02$, 95% CI = -6.61, -1.42; $P = 0.006$; $\beta_{post\ vs\ pre} : -4.09$, 95% CI = -6.84, -1.34; $P = 0.007$). The model explained 88.6% of the variability in the estimates, where residual heterogeneity was not statistically significant ($I^2 = 33.8\%$, P for Cochran's residual test = 0.12).

The Hedges summary effect size (random effects) predicted for “post-lesion” studies was 0.87 (95% CI: -0.03, 1.77; $P = 0.06$). The corresponding estimate for the “concomitant with lesion” studies was 0.95 (95% CI: 0.56, 1.33; $P < 0.001$), and the corresponding estimate for the “pre-lesion” studies was 4.96 (95% CI: 2.68, 7.24; $P < 0.001$), supporting the hypothesis that the effect of the intervention for this test may depend on the moment of lesion.

Funnel Plot asymmetry

There is strong evidence of funnel plot asymmetry in the visual inspection (figures 27 and 28), as well as in the Egger’s statistical test (Appendix 5) that could be an indication of publication bias for the locomotor activity and motor coordination and balance outcomes for all GLP-1 receptor agonists (13 and 14 estimates, respectively).

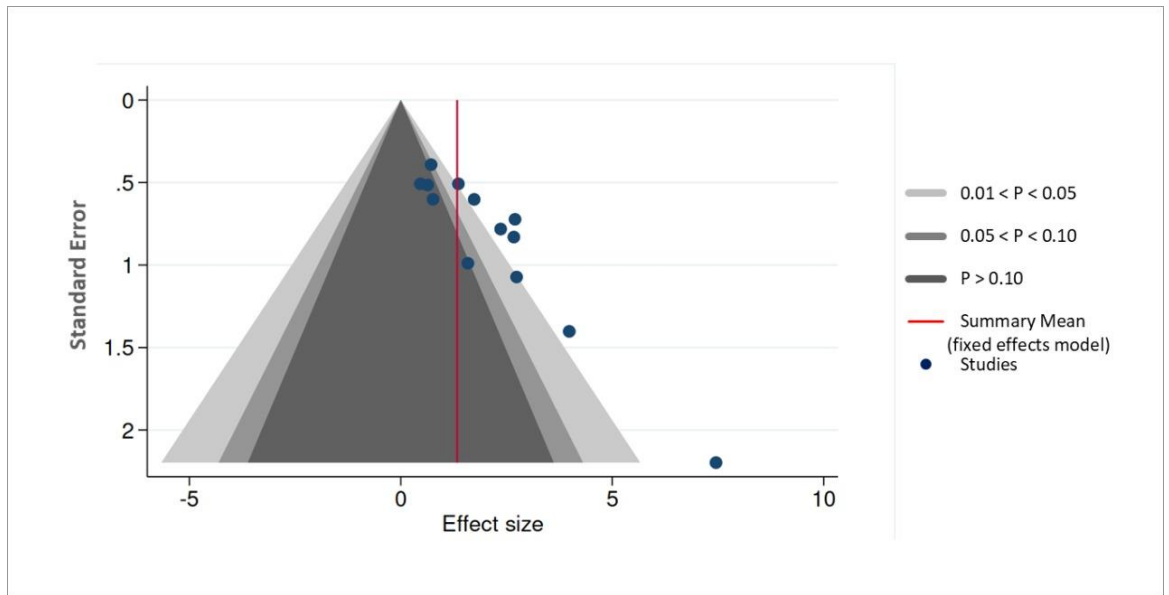


Figure 25. Funnel plot. Open field test.

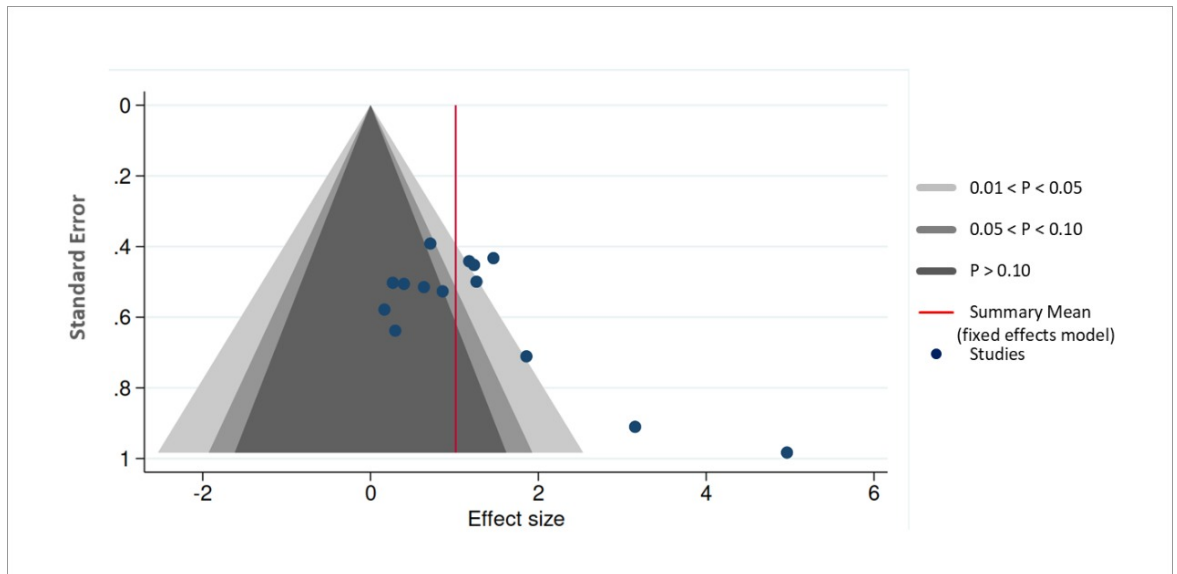


Figure 26. Funnel plot. Rotarod test.

GRADE

The certainty of the evidence for the defined primary outcomes was summarized in the “Summary of findings” table below. Risk of bias ⁵⁵, imprecision ⁵⁶, inconsistency ⁵⁷, indirectness ⁵⁸, and publication bias ⁵⁹ were evaluated to downgrade the level of certainty by one or two points.

Question: Exenatide compared to control for PD

Setting: clinics, research institutes

Certainty assessment							№ of patients		Effect		Certainty	Importance
№ of studies	Study design	Risk of bias	Inconsistency	Indirectness	Imprecision	Other considerations	GLP-1 receptor agonist	no GLP-1	Relative (95% CI)	Absolute (95% CI)		
Non-motor activities of daily living (on medication) (follow-up: mean 60.4 weeks; assessed with: MDS-UPDRS part I)												
2	randomised trials	serious ^a	not serious	not serious	very serious ^b	none	51	53	-	mean 1.91 lower (4.12 lower to 0.3 higher)	⊕○○○ Very low	IMPORTANT
Motor activities of daily living (on medication) (follow-up: mean 60.4 weeks; assessed with: MDS-UPDRS part II)												
2	randomised trials	serious ^a	not serious	not serious	very serious ^b	none	51	53	-	mean 1.48 lower (3.24 lower to 0.27 higher)	⊕○○○ Very low	IMPORTANT
Locomotor activity (off medication) (follow-up: mean 60.4 weeks; assessed with: MDS-UPDRS, part III)												
2	randomised trials	serious ^a	not serious	not serious	serious ^c	none	51	53	-	mean 3.58 fewer (6.02 fewer to 1.15 fewer)	⊕⊕○○ Low	IMPORTANT
Motor complications (follow-up: mean 60.4 weeks; assessed with: UDyRS)												
2	randomised trials	serious ^a	not serious	not serious	very serious ^b	none	51	53	-	0.3 higher (1.65 lower to 2.26 higher)	⊕○○○ Very low	IMPORTANT

CI: confidence interval

Explanations

^a. Participants in the Aviles Olmes et al. (2013) study were aware of their assigned intervention and it is not possible to exclude placebo effects being responsible for the observed differences between patients treated with exenatide and controls

^b. Downgraded by two points: 95% CI touches the nullity line; low number of participants; effect size not higher than the minimal clinically difference for improvement (MCDI)

^c. Downgraded by one point: low number of participants, although 95% CI does not touch the nullity line and effect size is higher than minimal clinically difference for improvement (MCDI)

Discussion

Only few clinical trials assessing the GLP-1 receptor agonists' neuroprotective role in PD are available so far: one proof of concept randomized single-blind trial⁶¹ and a randomized double-blind trial⁶⁰ for exenatide. Other randomized clinical trial protocols are already registered for exenatide, including a phase 3 trial, as well as for PT302, NLY01, liraglutide, semaglutide and lixisenatide. However, no results were posted yet. There is no registered protocol for GIP receptor agonists or GLP-1/GIP dual receptor agonists.

Participants of the exenatide groups had locomotor improvements (off medication) (MDS-UPDRS, part III) in the end of treatment persisting after the washout period exceeding the minimal clinically difference for improvement [MD -3.60, 95% CI: -6.01,-1.19; $I^2=0\%$; $P=0.003$]. However, for activities of daily living, non-motor symptoms and motor complications (on medication) there was no indication of improvements favouring exenatide (MDS-UPDRS, part I: MD -1.82, 95% CI: -3.93, 0.30; $I^2=15\%$; $P=0.09$; MDS-UPDRS, part II: MD -1.48, 95% CI: -3.24, 0.27; $I^2=0\%$; $P=0.10$; UDyRS: MD 0.30, 95% CI: -1.65, 2.26; $I^2=0\%$; $P=0.76$).

Athauda et al. (2017)⁶⁰ was considered as “low” risk of bias. Aviles-Olmes et al. (2013)⁶² only had “some concerns” regarding a “last observation carried forward” analysis performed for patients with missing outcomes. Overall certainty of the evidence was low for MDS-UPDRS, part III, and very low for MDS-UPDRS, parts I and II, and UDyRS.

Since it is not possible to differentiate between symptomatic motor improvements from a disease modifying effect in clinical trials, it is important that studies include a long washout period after treatment to demonstrate a possible disease modification⁹⁵. Both clinical trials for exenatide included a short 2 or 3-month washout period that might have been insufficient to eliminate unexpected long-lasting symptomatic effects of the medication. Sample sizes were small, and some baseline characteristics were different among groups, although no problems were identified in the randomisation processes. Even though patients were under L-DOPA treatment and other possible PD medication, studies aimed at assessing locomotor activities in a practically “off medication” state. Since a medication will hardly entirely dismiss the use of symptomatic therapy, the concomitant use of other medications did not impose a severe limitation, although the variability in results could have been due to differences in the timing from the last dose of the PD medication to the locomotor assessments.

Additionally, since PD is a heterogeneous disorder with varying prognosis, it would be interesting to understand the subtypes of PD of included patients (tremor dominant, mixed and akinetic/rigid) and their genomic status regarding PD related genes (SNCA, DJ-1, LRRK2, Parkin, UCH-L1 or PINK1) since this could be further correlated with results from genetic animal PD models.

As for preclinical studies, the motor outcomes (general locomotor activity, motor coordination and balance, muscle strength of forepaws, and alteration of gait) were evaluated in the open field, rotarod, traction and footprint tests. The overall meta-analyses showed improvement in locomotor function for GLP-1, GIP and GLP-1/GIP dual receptor agonists, regardless of the drug, dose, duration of treatment and PD models, although some heterogeneity was detected.

Meta-analyses for all GLP-1 receptor agonists has led to a SMD of 1.65 [95% CI: 1.05, 2.25; I^2 59%] in the open field test^{71, 78, 79, 83-85}, which means the drugs were effective at improving the general spontaneous locomotion of treated lesioned animals in the open field test when compared to lesioned animals only. Only one study²⁶ tested GIP with a SMD of 1.36 [95% CI: 0.08, 2.64], and as for the dual agonists^{67, 69}, the overall result was a SMD of 0.72 [95% 0.04, 1.41; I^2 0%]. For the rotarod test, the overall result for all GLP-1 together^{70, 72, 78, 79, 82-85, 88} was a SMD of 1.10 [95% CI: 0.66, 1.53; I^2 58%]. GIP receptor agonists^{75, 81} had an overall result of 1.88 [95% CI: 0.36, 3.40; I^2 79%], and, the dual agonists^{67, 72, 73, 82}, a SMD of 0.95 [95% CI: 0.46, 1.45; I^2 0%]. In the traction test, all GLP-1 receptor agonists^{72, 79, 82} showed a MD of 0.47 [95% CI: 0.23, 0.72; I^2 0%]; no GIP receptor agonist was tested. For dual agonists^{72, 73}, a SMD of 1.00 [95% CI: 0.24, 1.77; I^2 32%] was obtained. In the footprint test, all GLP-1 receptor agonists^{83, 84} showed an overall MD of -1.05 [95% CI: -1.72, -0.38; I^2 34%]. There were no results for GIP or dual receptor agonists.

Among GLP-1 receptor agonists, exendin-4^{77, 78}, the natural version of exenatide used in clinical trials, had a combined SMD of 1.01 [95% CI: 0.37, 1.65; I^2 =0%; P =0.002] in the open field test. Exendin-4 was, however, less effective than GLP-1 itself⁷¹, when tested as a probiotic engineered to be continuously expressed in the gut [SMD 3.33; 95% CI: 1.41, 5.25; I^2 =55%; P =0.0007], and liraglutide^{78, 83, 84} [SMD 1.57; 95% CI: 0.31, 2.84; I^2 80%]; semaglutide^{83, 84} had non-statistically significant results [SMD 1.63; 95% CI: -0.40, 3.65; I^2 =88%; P =0.11]. Exendin-4 was less effective than its long-lasting analogue lixisenatide⁷⁸ individually [SMD of 2.36; 95% CI: 1.28, 3.45; P <0.0001]. Compared to

GIP or dual agonists, exendin-4 was also less effective in the open field test than GIP itself²⁶ given through osmotic mini pumps for 14 days [SMD 1.36; 95% CI: 0.08, 2.64].

In the rotarod test, exendin-4^{77, 78} had similar results compared to other GLP-1 or GIP receptor agonists with statistically significant results [exendin-4: SMD 0.87; 95% CI: 0.13, 1.61; $P=0.02$; $I^2=95\%$; semaglutide: SMD 0.74; 95% CI 0.16, 1.33; $I^2=0\%$; $P=0.01$; liraglutide^{72, 78, 82-84}: SMD 0.84; 95% CI 0.41, 1.27; $I^2=77\%$; $P=0.0001$] and to D-Ala2-GIP-glu-pal⁷⁵ with a SMD of 0.90 [95% CI: 0.05, 1.75; $P=0.04$]. Lixisenatide⁷⁸ [SMD 1.86; 95% CI: 0.87, 2.84], geniposide⁷⁰ [SMD 1.46; 95% CI: 0.62, 2.31 $P=0.0007$], oxyntomodulin⁷⁹ (dose not informed) [SMD 1.23; 95% CI: 0.35, 2.12], and NLY01⁸⁸ (long-lasting exendin-4) [SMD 1.18; 95% CI: 0.31, 2.04] had better results in this test individually. DA3-CH (dual receptor agonist) individually^{72, 88} was also better than exendin-4 [DA3-CH: SMD 3.71, 95% CI: 2.16, 5.26; $P<0.00001$].

Exendin-4 was not evaluated in the traction and footprint tests and it did not allow a more straightforward comparison with other GLP-1, GIP or dual receptor agonists in these tests.

Most studies were considered as “unclear” in the risk of bias assessment, because information was not clearly reported, which jeopardizes the assessments and interpretation of results. Rarely PD was reported as induced before randomisation of the lesioned groups to vehicle or treatment groups and the baseline responses to the neurotoxin/insult before treatment were rarely comparable between groups. Randomisation of animals and blinding of investigators/caregivers to the experimental and control groups during the whole experiment were also not common practices. Based on funnel plot visual asymmetry and Egger’s test for the open field and rotarod tests, it is also possible to assume the presence of publication bias.

The heterogeneity found was further explored in meta-regression models for the open field and rotarod tests, since they were the only outcomes with more than 10 estimates. The hypothesis was that pre-lesion treatment or treatment starting concomitantly with the lesion could overestimate the pharmacological effect of the drugs, since treatment would start before the establishment of the disease, contrasting with the actual PD clinical situation. This was statistically corroborated for the rotarod test, but not for the open field test. There could be many other explanations for the presence of heterogeneity in the included studies: different PD models and protocols, extension of lesion, duration of treatment, different timepoints for outcome evaluation, differences in

the way the same tests were performed, or biases during the conduction of tests.

Reproducible science requires transparent reporting. The high level of methodological rigor expected for human randomized clinical trials is fundamental for generating reliable and unbiased data⁹⁶. However, although this thought is consolidated for clinical trials, it still does not fully extend to animal preclinical studies.

Besides the biases animal experiments are subjected to, PD animal models face an additional challenge: Parkinson's is not a naturally occurring disease in animals⁹⁷. Therefore, it must be artificially induced somehow. The most used models in the included studies were the neurotoxin-based ones, such as the 6-OHDA, MPTP, rotenone and LPS models. These models mainly aim at inducing mitochondrial dysfunction, oxidative stress, neuroinflammation, insufficient proteasomal protein degradation, loss of dopaminergic neurons in the SNpc, and reduced dopamine levels in the striatum, that we assume are the same mechanisms also taking place in PD patients^{98, 99}.

The shift away from the classical view of PD as a dopamine deficiency syndrome to the idea of a more widespread and complex disorder with the involvement of other neurotransmitters, and where an interaction between α -synuclein and the immune system seems to be in the center^{100, 101}, calls into question the validity of neurotoxin-based models since they fail at reproducing α -synuclein aggregation and Lewy body formation, especially in acute protocols¹⁰².

The other problem of acute/sub-acute toxin-based models is that a single or a few injections of the toxin given over a short period of time is followed by a rapid or immediate onset of symptoms, which would disagree with the slow and progressive nature of PD in humans^{98, 102}. If the goal is to investigate a disease modifying effect, the acute protocols may not be the best alternative, since, with the fast neurodegeneration observed, the characterization of mechanistic changes in the brain that could progressively lead to the neuronal degeneration and symptoms' onset would be restricted.

In an attempt to get closer to the human condition, only one study used the chronic intermittent MPTP plus probenecid protocol with injections once every 3.5 days for 5 weeks¹¹. This model is known to potentiate neurotoxicity by reducing the clearance of MPTP and its metabolites from the kidneys and brain and to induce chronic striatal dopamine depletion¹⁰³. Other two studies administered daily MPTP intraperitoneal injections for longer periods: 21⁸⁰ or 30 days⁸⁴. Only one study tested in the transgenic mice carrying the A53T mutation under the prion promoter control. This is one of few

transgenic models with similarities to the age-dependent α -synucleinopathy and neurodegeneration observed in humans⁹⁸. The same study also used the PFF model, with accumulation of Lewy body-like inclusions in the context of normal endogenous α -synuclein levels, more faithfully modeling the sequence of events in idiopathic PD¹⁰⁴.

Another caveat of animal studies, regardless of the model, is the way PD signs are measured. PD patients may present many motor alterations including bradykinesia, muscular rigidity, resting tremors, gait abnormalities, and postural instability. These signs, except bradykinesia, would probably be easier to characterize in animals if rodents were less dependent on all four limbs for balance⁹⁷. Although there is no single animal test that can be comparable to the most used motor scale applied in humans (MDS-UPDRS, part III), the combination of behavioral tests covering most symptoms could lead to some clues about the anti-parkinsonian activity of a drug.

Bradykinesia and rigidity, the main symptoms, could be measured at some extent by the open field and rotarod tests in rodents. The alteration of gait, resembling postural imbalance, could be represented not just by the footprint test, but also by the rotarod test. Grip strength tasks were evaluated in the traction test to detect forepaw weaknesses. The anti-parkinsonian activity of GLP-1 and GIP receptor agonists observed in behavioral tests is supported mainly by anti-apoptotic^{12, 58, 60, 61, 64, 70, 74-76} and anti-inflammatory proprieties^{64, 71, 72, 74, 79, 86-88} in the CNS detected in the morphological and biochemical assessments; however, the mode of action could be further better investigated in other models, such as *in vitro*, *Drosophila*^{105, 106}, or *C.elegans*^{107, 108}, not included in this systematic review. The restrictions of the current models do not allow us to confirm the potential of the drugs as disease-modifying agents.

There are several limitations in translating results from preclinical PD models to the clinic as described. But the fact that the overall meta-analyses for GLP-1, GIP and GLP-1/GIP dual receptor agonists showed improvement in the locomotor function of animals in the abovementioned tests (even though studies were conducted in the most different ways) shows some consistency in the beneficial effect of the drugs. The locomotor improvement was corroborated in clinical trials for exenatide in the MDS-UPDRS, part III, off medication, after the washout period. Since other GLP-1 analogues or dual agonists showed comparable or higher effects in the evaluated tests in preclinical studies, it could be expected equal or better results in future clinical trials compared to exenatide. A psychological aspect, such as depression, was improved in a pre-model

study with exendin-4; however, this was not corroborated by the clinical trials.

It is necessary to keep in mind the low number of studies (and animals) included in the meta-analyses of preclinical studies, besides the limitations and biases of the PD models. Additionally, GRADE indicated a low level of confidence in the human clinical trials for the motor outcome and a very low level of confidence in other motor-related (activities of daily living and dyskinesias) and non-motor outcomes. Therefore, results should be interpreted with caution.

The classic George Box sentence: “All models are wrong”, and its complementation years later, “but some are useful”, means in biology that hardly an animal model will entirely reproduce the human disease with all its complexities. There is now a consensus that PD is not a single disease entity, but it is represented by many subtypes with differences in the spectrum of symptoms and the nature and distribution of Lewy body pathology^{102, 109}.

However, a combination of different models that represent parts of the same puzzle could eventually come closer to the human PD. When adequately designed and conducted, animal models can contribute to our knowledge of the disease; however, better design and clear reporting are warranted to minimize bias.

Conclusion

Although results of the evaluated tests indicate a possible anti-parkinsonian activity in preclinical studies, limitations and biases were identified (or unclear information for a clear judgment). More studies using chronic protocols (neurotoxin or a combination of genetic and neurotoxin) with the GLP-1, GIP or dual receptor agonists' treatment starting after the establishment of the PD lesion are needed to better reflect the human condition. Additionally, new double-blind randomized clinical trials with a large number of participants and longer washout periods could improve our confidence in the evidence.

Appendix

Appendix 1. Search Strategy OVID MEDLINE. Search strategy example to be adapted for each database.

1.	Parkinson Disease.mp. or exp Parkinson Disease/
2.	Idiopathic Parkinson's Disease.mp.
3.	Lewy Body Parkinson Disease.mp.
4.	Lewy Body Parkinson's Disease.mp.
5.	Primary Parkinsonism.mp.
6.	Parkinsonism, Primary.mp.
7.	Parkinson's disease.mp.
8.	Parkinson's Disease, Idiopathic.mp.
9.	Parkinson's Disease, Lewy Body.mp.
10.	Idiopathic Parkinson Disease.mp.
11.	Paralysis Agitans.mp.
12.	Parkinsonian Disorders.mp. or exp Parkinsonian Disorders/
13.	Parkinsonian Syndrome.mp.
14.	Parkinsonism.mp.
15.	Parkinsonian Syndromes.mp.
16.	Parkinsonian Diseases.mp.
17.	Autosomal Dominant Parkinsonism.mp.
18.	Autosomal Recessive Juvenile Parkinson Disease.mp.
19.	Chromosome 6-Linked Autosomal Recessive Parkinsonism.mp.
20.	Chromosome 6 Linked Autosomal Recessive Parkinsonism.mp.
21.	Parkinsonism, Juvenile, Autosomal Recessive.mp.
22.	Parkinson Disease Autosomal Recessive, Early Onset.mp.
23.	Familial Juvenile Parkinsonism.mp.
24.	Parkinsonism, Experimental.mp.
25.	Experimental Parkinsonisms.mp.
26.	Parkinson Disease, Experimental.mp.
27.	MPTP-Induced Experimental Parkinsonism.mp.
28.	MPTP Induced Experimental Parkinsonism.mp.
29.	Experimental Parkinson Disease.mp.
30.	Experimental Parkinsonism.mp.
31.	Parkinsonism, Juvenile.mp.
32.	Juvenile Parkinsonism.mp.
33.	Juvenile Parkinson Disease.mp.
34.	Autosomal Recessive Parkinsonism.mp.

35.	Parkinsonism, Autosomal Recessive.mp.
36.	exp Glucagon-Like Peptide 1/ or Glucagon-Like Peptide 1.mp.
37.	Glucagon Like Peptide 1.mp.
38.	GLP-1.mp.
39.	GLP 1.mp.
40.	Glucagon-Like Peptide-1.mp.
41.	Exenatide.mp. or exp Exenatide/
42.	Bydureon.mp.
43.	ITCA 650.mp.
44.	AC 2993 LAR.mp.
45.	Ex-4.mp.
46.	Ex4 Peptide.mp.
47.	Exendin 4.mp.
48.	Byetta.mp.
49.	AC 2993.mp.
50.	liraglutide.mp. or exp Liraglutide/
51.	Victoza.mp.
52.	Saxenda.mp.
53.	NN 2211.mp.
54.	2211, NN.mp.
55.	NN2211.mp.
56.	NN-2211.mp.
57.	lixisenatide.mp.
58.	Adlyxin.mp.
59.	ZP10A peptide.mp.
60.	ZP 10.mp.
61.	ZP-10.mp.
62.	Lyxumia.mp.
63.	"AVE 0010".mp.
64.	AVE0010.mp.
65.	Gastric Inhibitory Polypeptide.mp. or exp Gastric Inhibitory Polypeptide/
66.	Inhibitory Polypeptide, Gastric.mp.
67.	Polypeptide, Gastric Inhibitory.mp.
68.	Glucose-Dependent Insulin-Releasing Peptide.mp.

69.	Glucose Dependent Insulin Releasing Peptide.mp.
70.	Glucose Dependent Insulinotropic Peptide.mp.
71.	Glucose-Dependent Insulinotropic Peptide.mp.
72.	Insulinotropic Peptide, Glucose-Dependent.mp.
73.	Peptide, Glucose-Dependent Insulinotropic.mp.
74.	Gastric-Inhibitory Polypeptide.mp.
75.	Polypeptide, Gastric-Inhibitory.mp.
76.	GIP.mp.
77.	Semaglutide.mp.
78.	Glucagon-Like Peptide-1 Receptor.mp. or exp Glucagon-Like Peptide-1 Receptor/
79.	Glucagon Like Peptide 1 Receptor.mp.
80.	Receptor, Glucagon-Like Peptide-1.mp.
81.	GLP-1R Receptor.mp.
82.	GLP 1R Receptor.mp.
83.	Receptor, GLP-1R.mp.
84.	GLP1R Protein.mp.
85.	GLP-1 Receptor.mp.
86.	GLP 1 Receptor.mp.
87.	Receptor, GLP-1.mp.
88.	Receptor, GLP1R.mp.
89.	Geniposide.mp.
90.	Jasminoidin.mp.
91.	D-Ala2-GIP-glu-PAL.mp.
92.	Ala2-GIP-Glu-PAL.mp.
93.	DA-JC1.mp.
94.	DA-JC4.mp.
95.	DA-CH5.mp.
96.	DA3-CH.mp.
97.	Oxytomodulin.mp.
98.	1 or 2 or 3 or 4 or 5 or 6 or 7 or 8 or 9 or 10 or 11 or 12 or 13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24 or 25 or 26 or 27 or 28 or 29 or 30 or 31 or 32 or 33 or 34 or 35

99.	36 or 37 or 38 or 39 or 40 or 41 or 42 or 43 or 44 or 45 or 46 or 47 or 48 or 49 or 50 or 51 or 52 or 53 or 54 or 55 or 56 or 57 or 58 or 59 or 60 or 61 or 62 or 63 or 64 or 65 or 66 or 67 or 68 or 69 or 70 or 71 or 72 or 73 or 74 or 75 or 76 or 77 or 78 or 79 or 80 or 81 or 82 or 83 or 84 or 85 or 86 or 87 or 88 or 89 or 90 or 91 or 92 or 93 or 94 or 95 or 96 or 97
100.	98 and 99

Appendix 2. SYRCLE adapted risk of bias tool for preclinical studies.

1. Was the allocation sequence adequately generated and applied?	
Yes (low risk of bias)	Investigators used and described in detail a valid type of randomisation (simple, stratification, within blocks, minimization). AND Investigators described the method used to generate the randomisation sequence (e.g., flipping a coin, rolling a dice, using a computer-generated randomisation sequence, with details of the algorithm or programme used).
Unclear	Not clear if there was a valid random sequence generation process. Investigators just described that “<i>animals were randomly assigned into the groups</i>” without further specification.
No (high risk of bias)	There was no mention at all about a randomisation process, just that “<i>animals were assigned into the groups</i>”. OR Investigators described in detail that there was no random component in the sequence generation process, such as by: Allocation by judgment or by investigator’s preference. OR Allocation based upon animals’ arrival.

	OR Allocation based on the results of a laboratory test or a series of tests. OR Allocation by availability of the intervention. OR Sequence generated by odd or even date of birth. OR Sequence generated by some rule based on animal number or cage number.
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2. Did the study authors report relevant baseline characteristics, or can we be confident that baseline characteristics were similar among groups?	
Yes (low risk of bias)	Relevant baseline characteristics (species, strain, sex, age, and body weight) were reported. Mean body weight and mean age (defined in weeks or days) of each group were reported in tables or graphs and these important prognostic variables were not statistically different among groups at baseline. OR Tables/graphs with the information from each group were not provided, but at least body weight range and age range (defined in weeks or days) were reported for the animals included in the study and variation between upper and lower weight values was $\leq 10\%$ and upper and lower age values differed 2 weeks or less). OR Tables with the information from each group were not provided, but at least body weight range and age range

	(defined in weeks or days) were reported for the animals included in the study and it was clearly stated that the randomisation process was stratified according to body weight if variation between upper and lower values was > 10%, and to age, if upper and lower values in the range differed more than 2 weeks. AND PD was induced before randomisation of the lesioned groups (vehicle or treatment groups) and behavioral/locomotor tests were performed before treatment to assure that PD animals had similar baseline responses to the neurotoxin/insult.
Unclear	No clear information on strain, sex, body weight or age at baseline (e.g., regarding age: animals were just described as “adults” or age was not reported). OR Not mentioned if PD was induced before randomisation of the lesioned groups (vehicle or treatment groups).
No (high risk of bias)	Relevant baseline characteristics (species, strain, sex, age, and animal weight) were reported in tables or graphs for each group and they were statistically different among all groups. OR PD was induced after randomisation of the groups (vehicle or GLP-1/GIP) and behavioral/locomotor tests were not performed before treatment to assure that PD animals had similar baseline responses to the neurotoxin/insult.

3) Was the allocation to the different groups adequately concealed?

Yes (low risk of bias)	The investigator allocating the animals to the different groups did not foresee assignment due to one of the following or equivalent methods: Central randomisation by a third party. OR Use of sequentially numbered opaque, sealed envelopes.
Unclear	Not clear if there was allocation concealment. Concealment was not described.
No (high risk of bias)	Investigators allocating the animals were clearly able to foresee assignments by using the following methods: Open randomisation schedule. OR Envelopes without appropriate safeguard. OR Alternation or rotation. OR Allocation based on date of birth. OR Allocation based on animal number. OR Any other explicitly unconcealed procedure of a non-random approach.

4) Were the animals randomly housed and were the environmental conditions in the room adequately monitored during the whole experiment?	
Yes (low risk of bias)	Investigators randomly placed the animal cages within the room to ensure groups were similarly affected by a source of

	variability (direct light, temperature, ventilation in the top of the rack may differ from the bottom of the rack). AND Temperature, humidity, and 12-hour light-dark cycles were controlled in the room where animals were housed.
Unclear	Not specified if there was random housing. OR Temperature, humidity and/or 12-hour light-dark cycles were not reported.
No (high risk of bias)	Animal cages were placed on various locations [e.g., vehicle group in room A or on rack A (e.g., top of the rack); treatment group in room B or on rack B (e.g., bottom of the rack)]. OR Temperature, humidity, and light cycles were clearly stated as not controlled in the room/rooms where the animals were housed.

5) Were the caregivers and/or investigators blinded from knowing which intervention each animal received during the experiment?	
Yes (low risk of bias)	Animal care staff and those who administered treatments were unaware of allocation groups to ensure that all animals in the experiment were handled, monitored, and treated in the same way. This may include use of: Central randomisation by a third party. OR Sequentially numbered drug containers identical in appearance. OR

	ID cards of individual animals, or cage/animal labels were coded and identical in appearance. OR Equivalent methods.
Unclear	Not specified if investigators were blinded from knowing which intervention each animal received during the experiment.
No (high risk of bias)	Inappropriate blinding was specified, such as by using colored cage labels (e.g., one color for one group and another color for the other group). OR The individual who prepared the experiment was the same as the one who applied each treatment. OR Route of administration of vehicle and treatment differed. OR There was clear evidence of any other type of inappropriate blinding.

6) Were animals selected at random for outcome assessment?	
Yes (low risk of bias)	Investigators randomly picked an animal/histological slide for outcome assessment (random number table or a computer random number generator).
Unclear	Not specified if animals/histological slides were selected at random for outcome assessment.
No (high risk of bias)	Investigators did not randomly pick an animal/histological slide for outcome assessment.

7) Was the outcome assessor blinded?	
Yes (low risk of bias)	All locomotor/behavior outcomes, histopathological slides and biochemical analyses were assessed by a blinded investigator. OR If the person assessing the outcome could not be blinded to the group allocation, the sources of bias were mitigated by sending data (e.g., automatic video recording, histopathological slides, etc) for analysis to a third party who was not aware of the treatment schemes.
Unclear	Not specified if outcome assessor was blinded to group allocation.
No (high risk of bias)	Locomotor/behavior outcomes and histopathological slides/biochemical analyses were assessed by an investigator that clearly knew which intervention each animal received and yet the data was not sent to a third party for analysis.

8) Were missing outcome data adequately addressed?	
Yes (low risk of bias)	Not all animals were included in the analysis, but the reasons for missing outcome data were unlikely to be related to true outcome (e.g., technical failure). OR Missing outcome data was clearly and properly explained.
Unclear	Not specified how missing data occurred and was handled.
No (high risk of bias)	Not all animals were included in the analysis, but reasons for missing outcome data is likely to be related to true outcome.

9) Was the study apparently free of other problems that could result in high risk of bias?	
Yes (low risk of bias)	The anesthetic drug used (not for euthanasia purposes) was free of dopamine metabolism interference, AKT signaling pathway interference or potential neuroprotective proprieties. AND No conflicts of interests of funders were disclosed. AND Treatment started after lesion was established. AND Acclimatization period was of at least 7 days
Unclear	There was no specification of the anesthetic used. OR Possible conflicts of interests of funders were not disclosed. There was no conflict-of-interest section. OR It was not clear the precise day the treatment started OR Acclimatization period was not reported.
No (high risk of bias)	The anesthetic drug used (not for euthanasia purposes) was not free of dopamine metabolism interference, AKT signaling pathway interference or potential neuroprotective proprieties (e.g., ketamine, xylazine, propofol, haloperidol, chloral hydrate, isoflurane, halothane). AND Clear conflicts of interests of funders were disclosed. AND

	Treatment started before lesion was established. AND Acclimatization period was clearly less than 7 days
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10) Are reports of the study free of selective outcome reporting?
--

Since protocols for preclinical studies are rarely registered in a publicly accessible database, this domain will not be included the Risk of Bias assessment.
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Appendix 3. Complete risk of bias judgment for each included study (preclinical and clinical studies).

Table 3. Complete risk of bias judgment of preclinical studies.

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Alabassi et al. 2017 ⁶⁴	No No mention of randomisation	No Age not specified PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	No Since there was no sham group submitted to surgery, it was possible to know at least who the animals from the control group were. Not clear if there was any blinding for the lesioned groups	Unclear	Unclear No behavioral tests performed. No blinding mentioned for other analyses	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Abuirmeileh et al. 2012 ⁶³	No No mention of randomisation	No Age not reported, weight variation over 10%, PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Unclear	Yes
Aksoy et al. 2017 ⁸⁶	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Badawi et al. 2017 ⁸⁷	No No mention of randomisation	Unclear Age not reported, strain not reported	No No mention of randomisation	Unclear	Unclear	Unclear	Unclear Blinding mentioned for immuno, not for behavioral tests	Yes
Bertilsson et al. 2008 ⁸	Unclear	No Age not reported, (animals just referred as "adults"), weight variation > 10%, PD lesion not induced before randomisation of groups	Unclear	Unclear	Unclear	Unclear	Unclear	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Cao et al. 2016 ⁶⁹	Unclear	No Weight variation > 10%, age variation > 2 weeks, PD lesion not induced before randomisation of groups	Unclear	Unclear	Unclear	Unclear	Unclear Blinding mentioned for Traction test; not mentioned for rotarod, open field and histological readings	Yes
Chen et al. 2015 ⁷⁰	Unclear	No Weight variation > 10%, age variation > 2 weeks, PD lesion not induced before randomisation of groups	Unclear	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Unclear	Yes
Chen et al. 2018 ⁶⁵	Unclear	Unclear PD lesion induced before randomisation of groups (post-lesion	Unclear	Unclear	Unclear	Unclear	Unclear Blinding for immuno;	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
		groups), but body weight not specified <i>* mentioned only in the end of the study</i>					rotational test not mentioned	
Fang et al. 2019 ⁷¹	Unclear	Unclear Weight variation > 10%, age not reported	No No blinding was performed	Unclear	No No blinding was performed	Unclear	No No blinding was performed	Yes
Feng et al. 2018 ⁷²	Unclear	No PD lesion not induced before randomisation of groups	Unclear	Unclear	Unclear	Unclear	Unclear	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Hansen et al. 2016 ⁹	Unclear	Yes	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Harkavyi et al. 2008 ¹⁰	No No mention of randomisation	No Age not reported, PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	Unclear	Unclear	Unclear	Yes
Harkavyi et al. 2013 ⁶⁶	No No mention of randomisation	No Age not reported, PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	Unclear	Unclear	Unclear	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Jalewa et al. 2017 ⁶⁷	No No mention of randomisation	No PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	Unclear	Unclear	Unclear Blinding mentioned for rotational test, but not for rotarod or open field	Yes
Ji et al. 2016 ⁷³	No No mention of randomisation	No PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Unclear	Yes
Kim et al. 2009 ⁷⁴	Unclear	No PD lesion not induced before randomisation of groups	Unclear	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Unclear No behavioral tests were performed	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Li et al. 2017 ⁷⁶	No No mention of randomisation	No Age not reported, PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Yes	Yes
Li et al. 2016 ⁷⁵	No No mention of randomisation	No Age not reported, PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Unclear	Yes
Li et al. 2009 ⁷⁷	No No mention of randomisation	No PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	Unclear	Unclear	Unclear Blinding for immuno; behavioral tests not mentioned	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Liu et al. 2015 ⁹¹	Unclear	No PD lesion not induced before randomisation of groups	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Liu et al. 2015 ⁷⁹	No No mention of randomisation	No PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	Unclear	Unclear	Unclear Blinding for swim forced test, for others not mentioned	Yes
Rampersaud et al. 2012 ⁶⁸	No No mention of randomisation	Unclear	No No mention of randomisation	Unclear	Unclear	Unclear	Unclear	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Su et al. 2016 ⁸⁰	Unclear	No Weight not clear, PD lesion not induced before randomisation of groups	Unclear	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Unclear No behavioral tests performed. For immuno and western blot blinding was not mentioned	Yes
Verma et al. 2017 ⁸¹	Unclear	Unclear	No Weight and age not reported, PD lesion not induced before randomisation of groups	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Unclear	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Yu et al. 2018 ²⁶	No No mention of randomisation	No PD lesion not induced before randomisation of groups	No No mention of randomisation	Unclear	Unclear	Unclear	Unclear	Yes
Yuan et al. 2017 ⁸²	Unclear	No PD lesion not induced before randomisation of groups	Unclear	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Unclear Only immuno analyses are mentioned as blinded, not mentioned for behavioral tests	Yes
Yun et al. 2018 ⁸⁸	Unclear	Unclear No body weight, not mentioned how many males and females in each group	Unclear	Unclear	Yes	Unclear	Yes	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Zhang et al. 2015 ⁸⁵	Unclear	No PD lesion not induced before randomisation of groups	Unclear	Unclear	Unclear	Unclear	Unclear Most behavior tests blinded, rotarod not mentioned	Yes
Zhang et al. 2018 ⁸³	Unclear	No PD lesion not induced before randomisation of groups	Unclear	Unclear	Unclear	Unclear	Unclear	Yes
Zhang et al. 2019 ⁸⁴	Unclear	No PD lesion not induced before randomisation of groups	Unclear	Unclear	No MPTP only group did not receive further i.p. injections of vehicle. It was possible to know who the control and treatment groups among lesioned animals were	Unclear	Unclear	Yes

Author, year	Was the allocation sequence adequately generated and applied?	Can we be confident that baseline characteristics were similar among groups?	Was the allocation adequately concealed?	Were the animals randomly housed during the experiment?	Were the investigators blinded from knowing which intervention each animal received?	Were animals selected at random for outcome assessment?	Were assessors blinded for behavioral evaluation?	Was missing outcome data adequately addressed?
Zhang et al. 2020 ⁸⁹	Unclear	No PD lesion not induced before randomisation of groups	Unclear	Unclear	Unclear	Unclear	Unclear Blinding for open field, for others not mentioned	Yes

Table 4. Complete risk of bias judgment of preclinical studies (continuation).

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Alabassi et al. 2017 ⁶⁴	No	Yes Ketamine and xylazine	Unclear	No Treatment started concomitantly with the lesion	No	Not reported
Abuirmeileh et al. 2012 ⁶³	No	Yes Isoflurane	Unclear	Yes	No	Parkinson's UK
Aksoy et al. 2017 ⁸⁶	No	Yes ketamine and xylazine	Unclear	Yes	No	Not reported

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Badawi et al. 2017 ⁸⁷	Yes	No	Yes	Yes	No	Not reported
Bertilsson et al. 2008 ⁸	No	Yes Halothane	Unclear	Yes	Unclear No conflict-of-interest section reported	Not reported
Cao et al. 2016 ⁶⁹	No	No	Unclear	No Treatment started concomitantly with the lesion	No	Cure Parkinson's Trust UK and a grant to C.H. under the '100 Foreign Expert' of the Shanxi province government

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Chen et al. 2015 ⁷⁰	No	No	Unclear	No Treatment started concomitantly with the lesion	No	Shanxi province government grant to C.H. under the '100 Foreign talent' grant scheme.
Chen et al. 2018 ⁶⁵	No	Yes Chloral hydrate	Unclear	Yes	Yes H.-K. Kim, D.-S. Kim and H.-I. Choi are employees of Peptron Inc. The Intramural Research Program of the National Institute on Aging, NIH, and Peptron Inc. have a Cooperative Research and Development Agreement to develop exendin-4 (exenatide) as a treatment strategy for neurodegenerative disorders for which NIA and Peptron Inc. hold patent rights. N.H. Greig has assigned all patent rights to the NIH and US Government.	Technological Innovation R&D Program funded by the Small and Medium Business Administration (Republic of Korea); Peptron Inc., Daejeon, Republic of Korea; Bio and Medical Technology Development Program of the National Research Foundation (NRF) funded by the Ministry of Science, ICT and Future Planning (MSIP) of Korea; NINDS, NIH grant to BJH; Intramural Research Program of the National Institute on Aging, National Institutes of Health, USA; National Health Research Institutes, Taiwan; Ministry of Science and Technology

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Fang et al. 2019 ⁷¹	No	No	Unclear	No Treatment started concomitantly with the lesion or before	No	National Natural Science Foundation of China, Excellent Youth Foundation of JiangXi Science and Technology Agency, Key R & D plan of JiangXi Science and Technology Agency, JiangXi Provincial Science and technology innovation team, and Science and technology plan of Jianxi Health Planning Committee
Feng et al. 2018 ⁷²	No	Yes Chloral hydrate	Unclear	Yes	Yes C.H. is a named inventor on a patent submitted by Lancaster University on the use of dual GLP- 1/GIP analogues in neurodegenerative. The other authors do not declare a conflict of interest.	Fellowship to C.H. under the '100 Foreign Expert' scheme of the Shanxi province government

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Hansen et al. 2016 ⁹	No	Yes	Unclear	Yes (in post-lesion treatment groups)	Yes Novo Nordisk funding	Novo Nordisk A/S
Harkavyi et al. 2008 ¹⁰	No	Yes Isoflurane	Unclear	Yes	No	Parkinson's Disease Society of Great Britain
Harkavyi et al. 2013 ⁶⁶	No	Yes Isoflurane	Unclear	Yes	No	Parkinson's UK and the Cure Parkinson's Trust

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Jalewa et al. 2017 ⁶⁷	No	Yes Ketamine and zylazine	Unclear	Yes	Yes C.H. is a named inventor on a patent application that list dual GLP-1/GIP analogues as novel treatments for PD. The patents application is owned by Lancaster University. The other authors do not declare a conflict of interest.	Cure Parkinson's Trust UK
Ji et al. 2016 ⁷³	No	No	Unclear	No Treatment started concomitantly with the lesion	No	Cure Parkinson's Trust UK, and a grant to C.H. under the '100 Foreign Expert' of the Shanxi province government
Kim et al. 2009 ⁷⁴	No	No	Unclear	No Treatment started concomitantly with the lesion	No	Korea Research Foundation Grant funded by the Korean Government (MOEHRD, Basic Research Promotion Fund) and by the Korea Science and Engineering Foundation

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Li et al. 2017 ⁷⁶	No	No	Yes	No Treatment started concomitantly with the lesion	Yes C.H. is a named inventor on a patent held by Ulster University on the use of GIP analogues in diseases of loss of synaptic transmission. The other authors do not declare a conflict of interest	Cure Parkinson's Trust UK and a Shanxi province government grant to C.H. under the '100 Foreign talent' grant scheme
Li et al. 2016 ⁷⁵	No	No	Yes	No Treatment started concomitantly with the lesion	Yes C.H. is a named inventor on a patent held by Ulster University on the use of GIP analogues in diseases of loss of synaptic transmission. The other authors do not declare a conflict of interest	Cure Parkinson's Trust UK and a Shanxi province government grant to C.H. under the '100 Foreign talent' grant scheme

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Li et al. 2009 ⁷⁷	No	No	Unclear	No Treatment started concomitantly with the lesion	No	The Intramural Research Programs of the National Institute on Aging and the National Institute on Drug Abuse
Liu et al. 2015 ⁹¹	No	No	Yes	No Treatment started concomitantly with the lesion	Yes C.H. is a named inventor on patent submissions covering the use of GLP-1 analogues in neurodegenerative diseases	Cure Parkinson's Trust UK and Shanxi province government
Liu et al. 2015 ⁷⁹	No	No	Unclear	No Treatment started concomitantly with the lesion	No	Cure Parkinson's Trust UK and Shanxi province government

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Rampersaud et al. 2012 ⁶⁸	No	Yes Isoflurane	Unclear	Yes	No	Michael J. Fox Foundation
Su et al. 2016 ⁸⁰	No	No	Unclear	No Treatment started concomitantly with the lesion	Unclear	Not reported
Verma et al. 2017 ⁸¹	No	No	Yes	No Treatment started concomitantly with the lesion	Yes Lupin limited funding	Lupin Limited

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Yu et al. 2018 ²⁶	No	No	Unclear	No Treatment started concomitantly with the lesion	No	Ministry of Science and Technology; Taipei Medical University; the Intramural Research Program of the National Institute on Aging, NIH; Department of Neurosurgery, Case Western Reserve University School of Medicine; the National Institute of Neurological Disorders and Stroke, NIH
Yuan et al. 2017 ⁸²	No	No	Unclear	No Treatment started concomitantly with the lesion	Yes C.H. is the named patent inventor on the use of dual agonists in PD	Grant of the Cure Parkinson's Trust UK, and a fellowship to C.H. under the '100 Foreign Expert' scheme of the Shanxi province government.

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Yun et al. 2018 ⁸⁸	No	Yes Xetamine and xylazine in the PFF model	Unclear	Yes	Yes Z.M., V.L.D., Se.L., T.M.D. and H.S.K are co-founders of Neuraly Inc. and hold ownership equity in the company. This arrangement has been reviewed and approved by the Johns Hopkins University in accordance with its conflict-of- interest policies. V.V.R. is the CSO of Neuraly Inc.	This work was supported by grants from the NIH/NINDS Maryland Stem Cell Research Foundation, the JPB Foundation, NIH/National Institute on Aging grant, the American Parkinson Disease Association (APDA) Research Grant Awards and the National Research Foundation of Korea
Zhang et al. 2015 ⁸⁵	No	No	Unclear	No Treatment started concomitantly with the lesion	Yes C.H. named inventor on patent is the named inventor on a patent held by Ulster University, UK	Cure Parkinson's trust and Shanxi province government grant to C.H. under the 100 Foreign talent grant scheme

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Zhang et al. 2018 ⁸³	No	No	Unclear	No Treatment started concomitantly with the lesion	Yes CH is a named inventor on a patent application by Lancaster University which covers the use of dual GLP-1/GIP receptor agonists as a treatment for PD	The work was supported by a fellowship to C.H. of the funding programme of the State Administration of Foreign Experts Affairs in China, and Shanxi scholarship council of China and the Shanxi '1331 Project' Key Subjects Construction fund
Zhang et al. 2019 ⁸⁴	No	No	Unclear	No Treatment started concomitantly with the lesion	Yes CH is a named inventor on patent applications that cover GLP-1 use as treatment for PD. Patent is owned by Ulster university, UK	Shanxi Scholarship Council of China and by the Fund for Shanxi '1331 project' Key Subjects Construction

Author, year	Was the study apparently free of other problems that could result in high risk of bias? If any problems are identified in the 4 following boxes, indicate "No" here	Did the anesthetic used somehow interfere with dopamine metabolism or have potential neuroprotective properties?	Was the acclimatization period at least 7 days?	Did the treatment start after lesion was established?	Was there a conflict of interest by funders?	Funding
Zhang et al. 2020 ⁸⁹	No	No	Yes	No Treatment started concomitantly with the lesion	Yes CH is a named inventor on a patent application by Lancaster University which covers the use of dual GLP-1/GIP receptor agonists as a treatment for PD. The other authors declare no conflict of interest.	The work was supported by a fellowship to CH of the funding programme of the State Administration of Foreign Experts Affairs in China, the Shanxi '1331 Project' Key Subjects Construction fund.

Table 5. Complete risk of bias judgment of clinical studies (extracted from the RoB 2 tool).

Domain	Signalling question	Response	
		Athauda et al. 2017 ⁶⁰	Aviles-Olmes et al. 2013 ⁶
Bias arising from the randomisation process	1.1 Was the allocation sequence random?	Yes	Yes
	1.2 Was the allocation sequence concealed until participants were enrolled and assigned to interventions?	Yes	Yes
	1.3 Did baseline differences between intervention groups suggest a problem with the randomisation process?	No	No
	Risk of bias judgement	Low	Low
Bias due to deviations from	2.1. Were participants aware of their assigned intervention during the trial?	No	Yes
	2.2. Were carers and people delivering the interventions aware of participants' assigned intervention during the trial?	No	No

intended interventions	2.3. If Y/PY/NI to 2.1 or 2.2: Were there deviations from the intended intervention that arose because of the experimental context?	Not applicable	Probably no
	2.4 If Y/PY to 2.3: Were these deviations likely to have affected the outcome?	Not applicable	Not applicable
	2.5. If Y/PY/NI to 2.4: Were these deviations from intended intervention balanced between groups?	Not applicable	Not applicable
	2.6 Was an appropriate analysis used to estimate the effect of assignment to intervention?	Yes	Yes
	2.7 If N/PN/NI to 2.6: Was there potential for a substantial impact (on the result) of the failure to analyse participants in the group to which they were randomized?	Not applicable	Not applicable
	Risk of bias judgement	Low	Low
Bias due to missing outcome data	3.1 Were data for this outcome available for all, or nearly all, participants randomized?	Yes	No
	3.2 If N/PN/NI to 3.1: Is there evidence that result was not biased by missing outcome data?	Not applicable	Probably no
	3.3 If N/PN to 3.2: Could missingness in the outcome depend on its true value?	Not applicable	Probably yes
	3.4 If Y/PY/NI to 3.3: Is it likely that missingness in the outcome depended on its true value?	Not applicable	Probably no
	Risk of bias judgement	Low	Some concerns

Bias in measurement of the outcome	4.1 Was the method of measuring the outcome inappropriate?	No	No
	4.2 Could measurement or ascertainment of the outcome have differed between intervention groups?	PN	No
	4.3 Were outcome assessors aware of the intervention received by study participants?	No	No
	4.4 If Y/PY/NI to 4.3: Could assessment of the outcome have been influenced by knowledge of intervention received?	Not applicable	Not applicable
	4.5 If Y/PY/NI to 4.4: Is it likely that assessment of the outcome was influenced by knowledge of intervention received?	Not applicable	Not applicable
	Risk of bias judgement	Low	Low
Bias in selection of the reported result	5.1 Were the data that produced this result analysed in accordance with a pre-specified analysis plan that was finalized before unblinded outcome data were available for analysis?	Yes	Yes
	5.2 ... multiple eligible outcome measurements (e.g. scales, definitions, time points) within the outcome domain?	No	No
	5.3 ... multiple eligible analyses of the data?	No	No

	Risk of bias judgement	Low	Low
Overall bias	Risk of bias judgement	Low	Some concerns

Appendix 4. Methodological characteristics – preclinical studies.

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
GLP-1 and GLP-1/GIP dual agonists							
Alabassi et al. 2017 ⁶⁴	3 animals/cage Plastic cages of 20x25x35 cm	14 days	22 ± 10°C and humidity not reported. Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Lesioned animals (6-OHDA) + saline for 30 days Group 3: Lesioned animals (6-OHDA) + liraglutide for 30 days	Xylazine and ketamine Decapitation	Not reported. Just that food and water were given <i>ad libitum</i>	No
Abuirmeileh et al. 2012 ⁶³	Group housed (number of animals/cage not specified)	Not reported	Not reported	<u>Acute experiments</u> Group 1: Sham animals Group 2: Lesioned animals (6-OHDA) Groups 3 and 4: Lesioned animals (6-OHDA) + one injection of L-DOPA after 7 days (10 or 30 mg/kg) Group 5 and 6: Lesioned animals (6-OHDA) + Ex-4 (0.1 or 0.5 µg/kg) after 7 days for a period of 7 days (twice daily) + L-DOPA (10 mg/kg) Groups 7 and 8: Lesioned animals (6-OHDA) + Ex-4 (0.1 or 0.5 µg/kg) after 7 days for a period of 7 days (twice daily) + L-DOPA (30 mg/kg) <u>Chronic experiments</u> Group 1: Sham not lesioned animals Group 2: Lesioned animals (6-OHDA) Group 3: Lesioned animals (6-OHDA) + L-DOPA after 7 days (10 mg/kg/day) for a period of 16 days Group 4: Lesioned animals (6-OHDA) + Ex-4 (0.5 µg/kg - twice	Pre- and during surgery: isoflurane Not reported	Not reported. Just that food and water were given <i>ad libitum</i>	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
				daily) and L-DOPA (10 mg/kg/day) after 7 days for a period of 16 days			
Aksoy et al. 2017 ⁸⁶	Not reported	Not reported	22 ± 2°C and humidity not reported Standard 12-h light/dark cycle	Group 1: Sham not lesioned animals Groups 2 and 3: Lesioned animals (rotenone). After the confirmation of the model in 10 days, group 2 received saline and group 3 received exenatide for 28 days	Pre-surgery: ketamine and xylazine Intracardial perfusion with PFA	Standard pellet diet and tap water <i>ad libitum</i>	No
Badawi et al. 2017 ⁸⁷	Stainless steel cages (number of animals/cage not specified)	7 days	25 ± 3 °C and 55% humidity Standard 12-h light/dark cycle	Group 1: Sham not lesioned animals Group 2: Lesioned animals (rotenone) for 10 days + saline for 16 days Groups 3, 4 and 5: Lesioned animals (rotenone) daily for 10 days + liraglutide (25, 50 or 100 µg/kg) daily for 16 days	Not reported Light anesthesia (not specified)	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Bertilsson et al. 2008 ⁸	Not reported	Not reported	Temperature-controlled room (not specified) and humidity not reported Standard 12-h light/dark cycle	Group 1: Sham not lesioned animals Group 2: Sham not lesioned animals + Ex-4 after 5 weeks twice daily for 3 weeks Group 3: Lesioned animals (6-OHDA) + PBS twice daily for 3 weeks after 5 weeks Group 4: Lesioned animals (6-OHDA) + Ex-4 twice daily for 3 weeks after 5 weeks	Pres-surgery: halothane Not reported	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No
Cao et al. 2016 ⁶⁹	Not reported	Not reported	22 ± 3°C and 50–55% humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Not lesioned animals + DA-JC1 daily for 7 days Group 3: Lesioned animals (MPTP for 7 days) + saline administered 30 minutes after each MPTP administration Group 4: Lesioned animals (MPTP for 7 days) + DA-JC1 administered 30 minutes after each MPTP administration	Urethane Intracardial perfusion with saline and fixation with PFA (histological analyses)	Not reported. Just that food and water were given <i>ad libitum</i>	No
Chen et al. 2015 ⁷⁰	Not reported	Not reported	22 ± 3°C and 50–55% humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Not lesioned animals + geniposide for 8 days Group 3: Lesioned animals (MPTP for 8 days) Group 4: Lesioned animals (MPTP for 8 days) + geniposide 60 minutes after each MPTP administration	Chloral hydrate or urethane	Not reported. Just that food and water were given <i>ad libitum</i>	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Chen et al. 2018 ⁶⁵	1 or 2 animals/cage	Not reported	Not reported	<u>Prevention + treatment groups:</u> Group 1: Animals were pretreated with diluent on days 16 and 2 prior to the 6-OHDA lesion. Following surgery, animals were again treated on days 12, 26 and 40 with diluent Groups 2 and 3: Animals were pretreated with 0.4 or 2 mg/kg PT302 on days 16 and 2 prior to prior to the 6-OHDA lesion. Following surgery, animals were treated on days 12, 26 and 40 with 2 mg/kg PT302 <u>Treatment groups</u> Group 4: Lesioned animals (6-OHDA) + diluent on days 6, 20 and 34 Group 5: Lesioned animals (6-OHDA) + 2 mg/kg PT302 on days 6, 20 and 34	Pre-surgery: chloral hydrate Not reported	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No
Fang et al. 2019 ⁷¹	Not reported	7 days	23 ± 3°C and 51 ± 13% humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Lesioned animals (MPTP for 7 days) Group 3: Pre-treatment with 10⁷ CFU MG1363-pMG36e-GLP-1 7 days before treatment with MPTP + daily for other 7 days Group 4: MPTP + 10⁷ CFU MG1363-pMG36e-GLP-1 for 7 days Group 5: Pre-treatment with 10⁹ CFU MG1363-pMG36e-GLP-1 7 days before treatment with MPTP + daily for another 7 days Group 6: MPTP in combination with 10⁹ CFU MG1363-pMG36e-GLP-1 for 7 days	Intraperitoneal injection of chloral hydrate. Subsequently, mice were perfused with saline intracardially, and fixed with PFA	Not reported	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Feng et al. 2018 ⁷²	Not reported	Not reported	22 ± 3°C and 50-55% humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Lesioned animals (MPTP) + saline for 6 days starting on day 2 Group 3: Lesioned animals (MPTP) + liraglutide for 6 days starting on day 2 Groups 4, 5 and 6: Lesioned animals (MPTP) + DA-JC1, DA-JC4 or DA-CH5 for 6 days starting on day 2	On the 8th day, mice were injected with chloral hydrate, then perfused with saline and PFA	Not reported. Just that food and water were given <i>ad libitum</i>	No
Hansen et al. 2016 ⁹	2 animals/cage 5 days after surgery Polypropylene cages (480 x 265 x 210 mm, floor area of 940 cm ²). After surgery, animals were single-housed the first week post-surgery, then pair-housed	Not reported	20–22°C and 50-60% humidity Standard 12-h light/dark cycle	Group 1: Partially lesioned animals (6-OHDA) + PBS 6 weeks after the lesion for 4 weeks Group 2: Partially lesioned animals (6-OHDA) + liraglutide (daily from 50 to 500 mg/kg on day 5) 6 weeks after lesion for 4 weeks Group 3: PBS for 21 days prior to 6-OHDA full lesion + PBS for 3 weeks Group 4: PBS for 21 days prior to 6-OHDA full lesion + liraglutide (daily from 50 to 500 mg/kg on day 5) for 3 weeks after lesion Group 5: Liraglutide (daily from 50 to 500 mg/kg on day 5) for 3 weeks + 6-OHDA full lesion + liraglutide for other 3 weeks	Pre- and during surgery: carprofen, midazolam, fluanisone and fentanyl After surgery: carprofen CO ₂ anaesthesia followed by decapitation	Regular chow (Altromin 125, Brogaarden, Hoersholm, Denmark) and water <i>ad libitum</i>	No
Harkavyi et al. 2008 ¹⁰	Group housed (number of animals/cage not specified)	Not reported	Not reported	Group 1: Sham not lesioned animals Group 2: 6-OHDA lesion + saline twice daily after 7 days for 7 days Group 3: LPS injection + after 7 days, saline was given twice daily for 7 days Group 4: 6-OHDA lesion + after 7 days, Ex-4 (0.1 µg/kg) was given twice daily for 7 days	Pre- and during surgery: isoflurane Not reported	Not reported. Just that food and water were given <i>ad libitum</i>	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
				Group 5: 6-OHDA lesion + after seven days, Ex-4 (0.5 µg/kg) was given twice daily for 7 days Group 6: LPS lesion + after 7 days, Ex-4 (0.1 µg/kg) was given twice daily for 7 days. Group 7: LPS lesion + after 7 days, Ex-4 (0.5 µg/kg) was given twice daily for 7 days.			
Harkavyi et al. 2013 ⁶⁶	Group housed (number of animals/cage not specified)	Not reported	Controlled temperature and humidity (not specified) Fixed light/dark cycles (not specified)	Group 1: Sham not lesioned animals Group 2: 6-OHDA lesion + saline twice daily for 7 days after 7 days Group 3: 6-OHDA lesion + after 7 days, Ex-4 was administered twice daily for 7 days Groups 4 and 5: 6-OHDA lesion + Ex-4 twice daily for 7 days after 7 days + Ex-(9-39) or NAF 5 min after each Ex-4 dose *Other 5 groups with the same design of the above mentioned groups were treated with BrdU	Pre- and during surgery: isoflurane in O ₂ Decapitation after anesthesia with isoflurane in O ₂	Not reported. Just that food and water were given <i>ad libitum</i>	No
Jalewa et al. 2017 ⁶⁷	1 animal/cage Ventilated cages (IVC's)	Not reported	21 ± 2°C and humidity not reported Standard 12-h light/dark cycle	Group 1: Sham not lesioned animals Group 2: Lesioned animals (6-OHDA) Group 3: Lesioned animals (6-OHDA) + DA-JC1 once daily for 6 weeks 6 days later	Pre-surgery: ketamine and xylazine, lidocaine and metacam Ketamine and xylazine, followed by cervical dislocation	Food and water were given <i>ad libitum</i> to the animals. During post-surgery recovery, the animals were fed with apples and soft food for 3 days	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Ji et al. 2016 ⁷³	Group housed (number of animals/cage not specified)	Not reported	22 ± 1°C and humidity not reported Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Not lesioned animals + DA-JC1 for 7 days Group 3: Lesioned animals (MPTP for 7 days) Group 4: Lesioned animals (MPTP) + DA-JC1 for 7 days Group 5: Lesioned animals (MPTP) + DA-JC1 + LY294002 for 7 days	Anesthesia not mentioned Intracardial perfusion with saline followed by PFA.	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No
Kim et al. 2009 ⁷⁴	Not reported	7 days	Temperature and humidity not reported Standard 12-h light/dark cycle	<u>Sacrifice 1 day after lesion + treatment</u> Group 1: Not lesioned animals Group 2: Not lesioned animals + Ex-4 Group 3: Lesioned animals (MPTP) + saline 30 min prior to each MPTP injection Group 4: Lesioned animals (MPTP) + Ex-4 30 min prior to each MPTP injection <u>Sacrifice 7 days after lesion + treatment</u> Group 5: Not lesioned animals Group 6: Not lesioned animals + Ex-4 Group 7: Lesioned animals (MPTP) + saline 30 min prior to each MPTP injection Group 8: Lesioned animals (MPTP) + Ex-4 30 min prior to each MPTP injection	Xylazine and ketamine and then transcardial perfusion with PFA	Tap water and food not specified <i>ad libitum</i>	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Li et al. 2009 ⁷⁷	Not reported	Not reported	20–23°C and humidity not reported Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Animals treated with PBS (mini pump) for 7 days + MPTP after 2 hours of the last PBS administration Group 3: Animals treated with Ex-4 (mini pump) for 7 days + MPTP after 2 hours of the last Ex-4 administration	Not reported	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No
Liu et al. 2015a ⁷⁸	Not reported	7 days	Not reported	Group 1: Not lesioned animals Group 2: Lesioned animals (MPTP for 7 days) + 7 other daily injections of saline Group 3: Not lesioned animals + Ex-4 for 14 days Group 4: Lesioned animals (MPTP) + Ex-4 for 7 days + 7 other daily injections of Ex-4 Group 5: Not lesioned animals + liraglutide for 14 days Group 6: Lesioned animals (MPTP) + liraglutide for 7 days + 7 other daily injections of liraglutide Group 7: Not lesioned animals + lixisenatide for 14 days Group 8: Lesioned animals (MPTP) + lixisenatide for 7 days + 7 other daily injections of lixisenatide	Not reported Animals were transcardially perfused PFA	Not reported	No
Liu et al. 2015b ⁷⁹	Not reported	Not reported	22 ± 1°C and 30% relative humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Not lesioned animals + Oxy for 7 days Group 3: Lesioned animals (MPTP for 7 days) + 7 daily injections of saline after 30 min of each MPTP injection Group 4: Lesioned animals (MPTP for 7 days) + 7 daily injections of Oxy after 30 min of each MPTP injection	Urethane and intracardial perfusion with saline (histological analyses). For biochemical analyses, animals were decapitated.	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Rampersaud et al. 2012 ⁶⁸	Not reported	Not reported	18–22° C and 40–60% humidity Standard 12-h light/dark cycle	Group 1: Sham not lesioned animals treated with saline before and after stereotaxic infusion of saline Group 2: Saline 4 days before 6-OHDA bilateral lesion Group 3: pCA 4 days before stereotaxic infusion of saline. After 7 days, saline for 7 days Group 4: DSP-4 4 days before stereotaxic infusion of saline. After 7 days, saline for 7 days Group 5: Saline 4 days after stereotaxic infusion of saline. After 7 days, Ex-4 for 7 days Group 6: pCA 4 days before 6-OHDA bilateral lesion. After 7 days, saline for 7 days Group 7: DSP-4 4 days before 6-OHDA bilateral lesion. After 7 days, saline for 7 days Group 8: DSP-4 and pCA on the same day 4 days before 6-OHDA bilateral lesion. After 7 days, saline for 7 days Group 9: pCA 4 days before 6-OHDA bilateral lesion. After 7 days, Ex-4 for 7 days Group 10: DSP-4 4 days before 6-OHDA bilateral lesion. After 7 days, Ex-4 was administered for 7 days Group 11: DSP-4 and pCA on the same day 4 days before 6-OHDA bilateral lesion. After 7 days, Ex-4 for 7 days	Pre-surgery and in vivo microdialysis: isoflurane through a fitted anesthetic nose mask Pentobarbital	Standard rodent diet and water <i>ad libitum</i>	No
Su et al. 2016 ⁸⁰	Not reported	Not reported	Not reported	Group 1: Not lesioned animals Groups 2: Lesioned animals (MPTP for 21 days) Group 3: Not lesioned animals + geniposide for 21 days Group 4: Lesioned animals (MPTP) + geniposide for 21 days Group 5: Lesioned animals (MPTP) + geniposide for 21 days + miR-21 agomir 1 hour after each geniposide administration also for 21 days	No anesthesia mentioned Cervical dislocation	Not reported	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Yuan et al. 2017 ⁸²	Not reported	Not reported	22 ± 3°C and 50–55% humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Lesioned animals (MPTP) for 7 days Groups 3 and 4: Lesioned animals (MPTP) for 7 days + liraglutide or DA3-CH once daily for 7 days	Pentobarbital before perfusion	Not reported	No
Yun et al. 2018 ⁸⁸	Not reported	Not reported	Not reported	<u>PFF model</u> Group 1: PBS (stereotaxic injection) + PBS twice a week for 5 months after 1 month of surgery Group 2: PBS (stereotaxic injection) + NLY0 twice a week for 5 months after 1 month of surgery Group 3: α-syn PFF (stereotaxic injection) + PBS twice a week for 5 months after 1 month of lesion Group 4: α-syn PFF (stereotaxic injection) + NLY01 twice a week for 5 months after 1 month of lesion <u>Transgenic model</u> Group 5: Wild type mice – PBS twice a week from the 6th to 10th month or death Group 6: Wild type mice – NLY01 twice a week from the 6th to 10th month or death Group 7: transgenic PD mice received PBS twice a week from the 6th to 10th month or death Group 8: transgenic PD mice received NLY01 twice a week from the 6th to 10th month or death	Xylazine and ketamine Not reported	Not reported	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Zhang et al. 2015 ⁸⁵	Not reported	Not reported	25 ± 2°C and 60-70% relative humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Lesioned animals (MPTP for 8 days) Group 3: Not lesioned animals + (Val8)GLP-1-glu-PAL daily for 8 days Group 4: Lesioned animals (MPTP) + (Val8)GLP-1-glu-PAL once daily for 8 days	Urethane before perfusion	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No
Zhang et al. 2018 ⁸³	Not reported	Not reported	24±1°C and 55 ± 5% humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 3: Not lesioned animals + semaglutide daily for 7 days Group 4: Lesioned animals (MPTP for 7 days) Groups 5 and 6: Lesioned animals (MPTP) + liraglutide or semaglutide daily for 7 days	Ethyl carbamate anesthetization before perfusion or dissection	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No
Zhang et al. 2019 ⁸⁴	Not reported	Not reported	Temperature and humidity not reported Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Lesioned animals (MPTP for 30 days) Group 3: Not lesioned animals + liraglutide once daily for 30 days Group 4: Not lesioned animals + semaglutide once every 2 days for 30 days Group 5: Lesioned animals (MPTP) + liraglutide once daily for 30 days Group 6: Lesioned animals (MPTP) + semaglutide once every two days for 30 days	Ethyl carbamate anesthesia before perfusion or dissection	Not reported Just that food and water were given <i>ad libitum</i> to the animals	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Zhang et al. 2020 ¹²	Not reported	Not reported	Controlled temperature and humidity (not specified) Standard 12-h light/dark cycle	<u>DA-CH5 and Ex-4</u> Group 1: Not lesioned animals Group 2: Lesioned animals (MPTP for 7 days) Groups 3, 4 and 5: Lesioned animals (MPTP) + 10, 1 or 0.1 mg/kg DACH5 for 7 days Groups 6, 7 and 8: Lesioned animals treated with MPTP + 10, 1 or 0.1 mg/kg Ex-4 for 7 days <u>DA-CH5 and Liraglutide</u> Group 9: Not lesioned animals Group 10: Lesioned animals (MPTP for 7 days) Group 11: Not lesioned animals + DACH5 for 7 days Group 12: Not lesioned animals + liraglutide for 7 days Groups 13 and 14: Lesioned animals (MPTP) + DA-CH5 or liraglutide for 7 days	Not specified "mice were anesthetized and sacrificed"	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No
GIP							
Li et al. 2016 ⁷⁵	Not reported	at least 7 days	24 ± 1° C and 55 ± 5% humidity Standard 12-h light/dark cycle	Group1: Not lesioned animals Group 2: Not lesioned animals + D-Ala2-GIP-GLU-PAL Group 3: Lesioned animals (MPTP + probenecid for 5 weeks with an interval of 3.5 days between doses) Group 4: Lesioned animals (MPTP + probenecid for 5 weeks with an interval of 3.5 days between doses) + D-Ala2-GIP-glu-PAL daily during lesion treatment plus 1 week after	Ethyl carbamate so brains could be immediately removed (half group). Another group was perfused with PFA	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Li et al. 2017 ¹¹	Not reported	at least 7 days	24 ± 1°C and 55 ± 5% humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Not lesioned animals + D-Ala2-GIP-GLU-PAL for 7 days Group 3: Lesioned animals (MPTP for 7 days) Group 4: Lesioned animals (MPTP) + other 7 daily injections of D-Ala2-GIP-GLU-PAL after MPTP injections	Ethyl carbamate so brains could be immediately removed. Another group was perfused with PFA and then brains were removed	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No
Verma et al. 2017 ⁸¹	Not reported	7 days	21 ± 2 °C and 50-70% relative humidity Standard 12-h light/dark cycle	Group 1: Not lesioned animals Group 2: Lesioned animals (MPTP on day 8) Group 3: 100 nmol/kg D-Ala2GIP daily for 7 days + MPTP on day 8 Group 4: L-DOPA daily for 7 days + MPTP on day 8 <u>Additional groups:</u> Group 5: Not lesioned animals Group 6: Lesioned animals (MPTP on day 8) Group 7: 50 nmol/kg D-Ala2GIP for 7 days + MPTP on day 8 Group 8: 50 nmol/kg (Pro3)GIP (Inhibitor) + 50 nmol/kg D-Ala2GIP for 7 days + MPTP on day 8 Group 9: 100 nmol/kg (Pro3)GIP (Inhibitor) + 50 nmol/kg D-Ala2GIP ip for 7 days + MPTP on day 8	Not reported	Standard rodent diet and water <i>ad libitum</i>	No

Author and year	Cages	Acclimatization period	Control of temperature and humidity light schedule	Description of Treatment/control groups	Anesthetic used Protocol for euthanasia	Source of food and water	Sample size calculation?
Yu et al. 2018 ²⁶	Not reported	Not reported	Controlled temperature and humidity (not specified) Standard 12-h light/dark cycle	Group 1: Lesioned animals treated with saline for 2 days prior to the 6-OHDA surgery for 2 weeks Group 2: Lesioned animals treated with GIP for 2 days prior to the 6-OHDA surgery for 2 weeks Group 3: Not lesioned animals treated with saline for 5 days for GIP biochemical analyses Group 4: Not lesioned animals treated with GIP for 5 days for GIP biochemical analyses (7.8 or 15 nmol/kg/day)	Pre-surgery: Zoletil-Rumpun; anesthesia for implantation of osmotic pumps not reported Not reported	Not reported. Just that food and water were given <i>ad libitum</i> to the animals	No

Abbreviations: Ex-4: exendin-4; L-DOPA: levodopa; CFU: colony-forming unit; DSP-4: N-(2-chloroethyl)-N-ethyl-2-bromobenzylamine; pCA: parachloroamphetamine; α -syn PFF: α -synuclein pre-formed fibril.

Appendix 5. Egger's test.

General locomotor activity – Open field test

Number of estimatives = 13 Root MSE = .82

Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]
slope	-.8164241	.4014573	-2.03	0.067	-1.700026 .0671775
bias	3.574127	.6215771	5.75	0.000	2.206045 4.942209

Test of H0: no small-study effects p = 0.000

Motor coordination and balance – Rotarod test

Number of estimatives = 14 Root MSE = 1.354

Std_Eff	Coef.	Std. Err.	t	P> t	[95% Conf. Interval]	
slope	-.988018	.8353573	-1.18	0.260	-2.808105	.8320693
bias	3.91573	1.590883	2.46	0.030	.4494935	7.381966

Test of H0: no small-study effects $p = 0.030$

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