UNIVERSIDADE FEDERAL DO PARANÁ

LAIS SOARES RODRIGUES

PREJUÍZO OLFATÓRIO ASSOCIADO À MODULAÇÃO FARMACOLÓGICA DE RECEPTORES D2 DO BULBO OLFATÓRIO EM MODELO ANIMAL DE PARKINSONISMO MODULADO PELA PRIVAÇÃO DE SONO REM

CURITIBA
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Dissertação apresentada ao Programa de Pós Graduação em Farmacologia da Universidade Federal do Paraná, como requisito parcial à obtenção do título de Mestre em Farmacologia.

Orientador: Prof. Dr. Marcelo de Meira Santos Lima

CURITIBA
2014

ATA DO JULGAMENTO DA 97ª DEFESA DE DISSERTAÇÃO DE MESTRADO

Ao trigésimo dia do mês de junho do ano de dois mil e quatorze, às treze horas e trinta minutos, no Auditório do Departamento de Farmacologia, do Setor de Ciências Biológicas da Universidade Federal do Paraná, reuniu-se a Comissão Examinadora da Dissertação de Mestrado de autoria da pós-graduanda **LAÍS SOARES RODRIGUES**, intitulada "PREJUÍZO OLFATÓRIO ASSOCIADO À MODULAÇÃO FARMACOLÓGICA DE RECEPTORES D2 DO BULBO OLFATÓRIO EM MODELO ANIMAL DE PARKINSONISMO MODULADO PELA PRIVAÇÃO DE SONO REM", sob orientação do Prof. Dr. Marcelo de Meira Santos Lima e composta pelos professores Prof. Dr. Marcelo de Meira Santos Lima (Presidente - Fisilogia - UFPR); Prof. Dr. Rui Daniel Schröder Prediger (Farmacologia - UFSC) e Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Farmacologia - UFPR). A Banca Examinadora iniciou os trabalhos. A candidata teve quarenta e cinco minutos para expor oralmente seu trabalho, sendo em seguida arguida durante trinta minutos por cada um dos membros da Banca e tendo trinta minutos para responder a cada uma das arguições. No final da sessão, a Comissão Examinadora emitiu o seguinte parecer: Aprovada. De acordo com o Regimento Interno do Programa de Pós-Graduação em Farmacologia, a pós-graduanda foi aprovada. Para a publicação, o trabalho deverá sofrer as modificações sugeridas, que serão conferidas por seu orientador. Nada mais havendo a tratar, o Presidente deu por encerrada a sessão, da qual foi lavrada a presente ata, que será assinada pelo Presidente e pelos demais Membros da Banca Examinadora em Curitiba, 30 de junho de 2014.

Prof. Dr. Marcelo de Meira Santos Lima (Presidente - Fisiologia - UFPR)

Prof. Dr. Rui Daniel Schröder Prediger (Farmacologia - UFSC)

Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Farmacologia - UFPR)

PARECER

A Comissão Examinadora da Dissertação de Mestrado intitulada "PREJUÍZO OLFATÓRIO ASSOCIADO À MODULAÇÃO FARMACOLÓGICA DE RECEPTORES D2 DO BULBO OLFATÓRIO EM MODELO ANIMAL DE PARKINSONISMO MODULADO PELA PRIVAÇÃO DE SONO REM", de autoria da pós-graduanda LAÍS SOARES RODRIGUES, sob orientação do Prof. Dr. Marcelo de Meira Santos Lima e composta pelos professores Prof. Dr. Marcelo de Meira Santos Lima (Presidente - Fisiologia - UFPR); Prof. Dr. Rui Daniel Schröder Prediger (Farmacologia - UFSC) e Prof.ª Dr.ª Maria Aparecida Barbato Frazão Vital (Farmacologia - UFPR), reuniu-se e, de acordo com o Regimento Interno do Programa de Pós-Graduação em Farmacologia, a pós-graduanda foi Aprovada. Para a devida publicação o trabalho deverá sofrer as modificações sugeridas, que serão conferidas por seu orientador. Em Curitiba, 30 de junho de 2014.

Prof. Dr. Marcelo de Meira Santos Lima (Presidente - Fisiologia - UFPR)

Prof. Dr. Rui Daniel Schröder Prediger (Farmacologia - UFSC)

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RESUMO

Déficits olfatórios são comumente encontrados em indivíduos não tratados com diagnóstico recente da doença de Parkinson (DP). Diferentes estudos observaram quedas nas performances olfatórias durante um curto período de privação do sono. Portanto, levaram à hipótese de que a modulação dos receptores dopaminérgicos D2 no bulbo olfatório pode fornecer uma compreensão mais abrangente dos mecanismos de déficits olfatórios encontrados na DP, depois de um curto período de privação de sono de movimento rápido dos olhos (PSREM). Decidimos investigar alterações na olfação, neuroquímicas e histológicas geradas pela administração de piribedil ou raclopride, dentro da camada glomerular do bulbo olfatório, em ratos submetidos a rotenona intranigral e PSREM. Nossas descobertas mostraram uma evidência notável da ocorrência de uma correlação negativa ($r = - 0,52$, $P = 0,04$) entre o número de neurónios TH -ir periglomerular e os níveis bulbares de dopamina (DA), em rotenona, mas não nos grupos sham. Uma correlação positiva significativa ($r = 0,34$, $P = 0,03$) foi observada entre DA nigral e o índice de discriminação olfatório (IDO), para os grupos sham, indicando que os níveis aumentados de DA na substância negra pars compacta (SNpc) estão associados a um maior desempenho na discriminação olfatória. Além disso, o aumento dos níveis de DA no estriado e bulbo induzido por piribedil no controle rotenona e grupos PSREM rotenona eram consistentes com quantidades reduzidas no IDO. A presente evidência reforça que a DA produzida por neurônios periglomerulares, e em particular os receptores dopaminérgicos D2 bulbares, são participantes essenciais nos processos de discriminação olfatória, bem como SNpc e estriado.

Palavras-chave: bulbo olfatório, discriminação olfatória, dopamina, receptores dopaminérgicos D2 bulbares, privação de sono REM, rotenona intranigral, doença de Parkinson.

ABSTRACT

Olfactory deficits are commonly found in untreated subjects with a recent diagnosis of Parkinson's disease (PD). Different studies observed declines in olfactory performances during a short period of sleep deprivation. Therefore, we have led to the hypothesis that a modulation of the dopaminergic D2 receptors in the olfactory bulb could provide a more comprehensive understanding of the olfactory deficits mechanisms found in PD and after a short period of rapid eye movement sleep deprivation (REMSD). We decided to investigate the olfactory, neurochemical and histological alterations generated by the administration of piribedil or raclopride, within the glomerular layer of the olfactory bulb, in rats submitted to intranigral rotenone and REMSD. Our findings provided a remarkable evidence of the occurrence of a negative correlation ($r = - 0.52$, $P = 0.04$) between the number of periglomerular TH-ir neurons and the bulbar levels of dopamine (DA) in the rotenone, but not sham groups. A significant positive correlation ($r = 0.34$, $P = 0.03$) was observed between nigral DA and olfactory discrimination index (DI), for the sham groups, indicating that increased DA levels in the substantia nigra pars compacta (SNpc) are associated to enhanced olfactory discrimination performance. Also, increased levels in bulbar and striatal DA induced by piribedil in the rotenone control and rotenone REMSD groups were consistent with reduced amounts of DI. The present evidence reinforce that DA produced by periglomerular neurons, and particularly the bulbar dopaminergic D2 receptors, are essential participants in the olfactory discrimination processes, as well as SNpc and striatum.

Key-words: Olfactory bulb, olfactory discrimination, dopamine, bulbar dopaminergic D2 receptors, REM sleep deprivation, intranigral rotenone, Parkinson´s disease.

LISTA DE SIGLAS

5-HT – 5-hidroxitriptamina – Serotonina

6-OHDA – 6-hidroxidopamina

ACh – Acetilcolina

BO – Bulbo olfatório

DA – Dopamina

DAT – transportador de dopamina

DOPAC – Ácido 3,4-dihidroxifenilacético

DP – Doença de Parkinson

EPl – Camada plexiforme externa

Gl – Camada glomerular

HVA – Ácido homovanílico

MAOB – monoamina-oxidase B

MPP+ - 1-metil-4-phenylpyridinium

MPTP - 1-metil-4-fenil-1,2,3,6-tetrahidropiridina

NA – Noradrenalina

PSREM – Privação de sono REM

RBD – Distúrbios comportamentais do sono REM

REM – Movimentos oculares rápidos

ROS – espécies reativas de oxigênio

SN – Substância negra

SNpc – Substância negra pars compacta

TDO – Tarefa de discriminação olfatória

TH – Tirosina hidroxilase

TH-ir – Neurônios imunorreativos à tirosina hidroxilase

SUMÁRIO

1 INTRODUÇÃO

1.1 DOENÇA DE PARKINSON

A doença de Parkinson (DP) é uma doença neurodegenerativa progressiva que atinge cerca de 1 a 3% da população acima de 60 anos (MAYEUX, 2003; BLESA *et al*., 2012; LIMA *et al*., 2014), e foi primeiramente descrita por James Parkinson em 1817, que a denominou de paralisia agitante (“shaking palsy”), relatando alguns sinais como tremor em repouso, fraqueza muscular, anormalidades posturais e de marcha, sem alterações cognitivas, até aquele momento (DAUER e PRZEDBORSKI, 2003).

As características fisiopatológicas da doença são decorrentes, primariamente, da degeneração de neurônios dopaminérgicos da substância negra pars compacta (SNpc), evidenciada macroscopicamente pela despigmentação na porção ventrolateral dessa estrutura (Fig. 1), e que leva à diminuição da aferência dopaminérgica para o estriado dorsal, causando a diminuição da liberação da dopamina (DA) e redução do conteúdo de seus metabólitos, ácido homovanílico (HVA) e ácido 3,4-dihidroxifenilacético (DOPAC). (LANG e LOZANO, 1998; LANE e DUNNETT, 2008; LIMA *et al*., 2012).

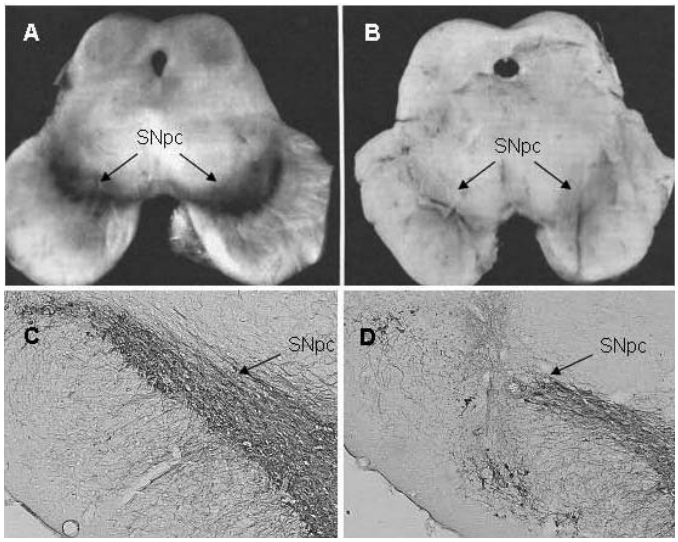

Figura 1. Perda maciça de neurônios dopaminérgicos do mesencéfalo na substância negra pars compacta (SNpc). Os painéis A e B representam o mesencéfalo bilateral humano postmortem de indivíduos normais e parkinsonianos, respectivamente. C e D mostram o equivalente unilateral do mesencéfalo de rato depois de injeção intranigral do veículo (C) ou MPTP (D). As setas indicam a imunoreactividade da tirosina hidroxilase presente na SNpc. Retirado de Lima *et al*., 2012.

Outra característica fisiopatológica importante é a presença de inclusões intracitoplasmáticas neuronais de caráter eosinofílico, constituídas por estruturas de natureza protéica (α-sinucleína e ubiquitina), denominadas corpos de Lewy (Fig. 2), descritos inicialmente como agregados protéicos em encéfalos de pacientes que apresentavam DP, por Fritz Jacob Heinrich Lewy em 1912. Alguns anos depois, em 1919, esses agregados foram nomeados corpos de Lewy, por Konstantin Trétiakoff, que os descreveu na substância negra (SN) (DAUER e PRZEDBORSKI, 2003; RODRIGUES E SILVA *et al*., 2010; DEL TREDICI e DUDA, 2011).

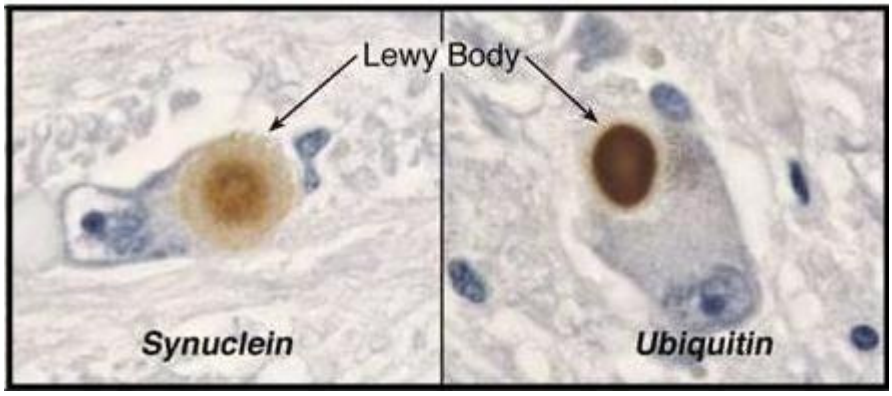

Figura 2. Imunohistoquímica de inclusões intraneuronais, denominadas corpos de Lewy, em um neurônio dopaminérgico da SNpc. Corpo de Lewy (setas pretas). Adaptado de Dauer e Przedborski, 2003.

A redução do conteúdo dopaminérgico é responsável pelos sinais e sintomas motores característicos da DP, que surgem quando ocorre morte de mais de 50% dos neurônios dopaminérgicos do mesencéfalo e perda de 80 a 90% do conteúdo de DA estriatal (LANG e LOZANO, 1998; LANE e DUNNETT, 2008). Essas manifestações motoras caracterizam-se como as mais incapacitantes para o paciente dentro do conjunto sintomatológico apresentado, sendo elas: bradicinesia, rigidez muscular, acinesia, tremor em repouso e anormalidades posturais e de marcha (FAHN, 2003). Todavia, essa doença também apresenta distúrbios não motores, como distúrbios olfatórios, do sono, da fala, autonômicos, do humor, dificuldade de mastigação, fadiga, perda de peso, entre outros (WARRAICH *et al*., 2009) que podem aparecer muito antes dos sintomas motores e, dessa forma, apresentam grande relevância no estudo da DP, uma vez que podem possibilitar um diagnóstico precoce dessa doença.

1.2 DISTÚRBIOS NÃO MOTORES DA DOENÇA DE PARKINSON

A abordagem terapêutica atual tem dado uma conotação diferente quanto à importância dos sintomas não motores da DP por apresentam grande relevância para a qualidade de vida dos indivíduos que apresentam a doença. Dentre os distúrbios não motores estão os distúrbios de humor, como a depressão, distúrbios do sono, distúrbios olfatórios, distúrbios autonômicos e dor (POEWE, 2008; GRINBERG et al., 2010). Esses distúrbios são relatados anos ou décadas antes dos pacientes apresentarem alguma alteração motora , como corroborado pela proposta de evolução da doença na teoria de Braak e colaboradores, que identificou uma evolução cronológica e topográfica de lesões em diferentes áreas encefálicas envolvendo diversos núcleos (BRAAK, DEL TREDICI *et al.*, 2003). Nesse sentido, esses autores descreveram seis fases de evolução da doença (Fig. 3) que se iniciaria (fases 1 e 2) em áreas associadas à olfação como o bulbo olfatório (BO) e áreas como o núcleo pedúndulo-pontino (envolvido no controle do ciclo vigília-sono) para então somente atingir áreas como a SNpc (fase 3-4) e córtex (fase 6) (BRAAK, DEL TREDICI *et al.*, 2002; BRAAK, DEL TREDICI *et al.*, 2003; BRAAK, RUB *et al.*, 2003; BRAAK, GHEBREMEDHIN *et al.*, 2004). Essa evolução coincide com o aparecimento dos corpos de Lewy no tronco encefálico e córtex (Fig. 3) (HALLIDAY *et al.*, 2011; DOTY, 2012).

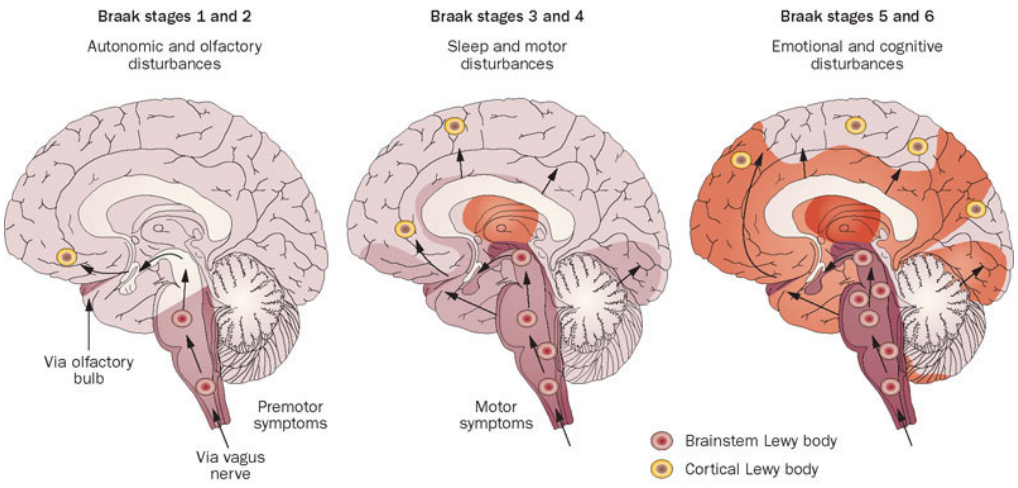

Figura 3. O estagio esquemático da doença de Parkinson proposto por Braak, que mostra os locais de inicio no bulbo olfatório e medula oblonga, através do aparecimento de corpos de Lewy em regiões corticais. A patologia relacionada com α-sinucleína é possivelmente iniciada na periferia através do epitélio olfatório ou do estômago, talvez envolvendo fatores de xenobióticos. O sombreamento vermelho representa o padrão topográfico da patologia. Retirado de Doty, 2012.

1.2.1 DISTÚRBIOS DE SONO E OLFATÓRIOS NA DOENÇA DE PARKINSON

Distúrbios de sono, como os distúrbios comportamentais associados ao sono de movimentos oculares rápidos - REM (RBD) e fragmentação de sono são frequentemente relacionados à sinucleinopatias como a doença dos corpúsculos de Lewy, atrofia sistêmica múltipla e a própria DP (POSTUMA, GAGNON *et al*., 2009; POSTUMA e MONTPLAISIR, 2011). Aproximadamente 50% dos pacientes que possuem RBD a pelo menos uma década irão eventualmente desenvolver uma ou mais dessas doenças (POSTUMA e MONTPLAISIR, 2011). Esse longo intervalo associado ao risco aumentado de desenvolvimento de uma doença neurológica compõe uma importante oportunidade de observação dos estágios pré-clínicos da neurodegeneração que resultará no Parkinsonismo.

Dentro desse contexto, outro fator preditivo importante é a olfação. Os seres humanos são extremamente sensíveis a odores, antes de chegarem à idade avançada que, por si só, é responsável pela diminuição da acuidade olfativa. Essa perda foi observada no estudo de Doty *et al*., (1984), que verificou que 50% das pessoas com idades entre 65 e 80 anos apresentavam prejuízo olfatório em uma tarefa de identificação de odores. Além da idade, observa-se que na DP, há hiposmia e/ou anosmia que também pode ser observada em modelo animal da DP (ANSARI *et al*., 1975; PREDIGER *et al*., 2006).

Perdas da capacidade discriminativa olfatória ocorrem em 90% dos pacientes com DP (DOTY *et al*., 1988), sendo caracteristicamente detectáveis no diagnóstico da doença (POSTUMA e MONTPLAISIR, 2011). Estudos prospectivos sugerem que as perdas olfatórias ocorrem antes mesmo da ocorrência dos prejuízos motores (ROSS *et al*., 2008), ou seja, tais condições se assemelham aos distúrbios de sono que são igualmente pré-motores (HENDERSON, LU *et al*., 2003; PONSEN *et al*., 2004; CHAUDHURI, YATES *et al*., 2005; CHAUDHURI E NAIDU, 2008).

Ainda, outras evidências indicam que a privação de sono REM (PSREM) em humanos e ratos é capaz de produzir um intenso prejuízo numa tarefa de discriminação olfatória (GREINER, MORIGUCHI *et al*., 2001; FANTINI, POSTUMA *et al*., 2006; KILLGORE E MCBRIDE, 2006; KILLGORE, KILLGORE *et al*., 2011), sugerindo uma correlação entre esses dois distúrbios.

1.3 SISTEMA OLFATÓRIO

A evolução do sistema olfatório permitiu aos organismos vivos a detecção e discriminação de moléculas odoríferas, contribuindo para os sistemas de alerta, defesa, reprodução e, sobretudo, alimentação (FIRESTEIN, 2001; HAWKES E DOTY, 2009). O sistema olfatório de roedores apresenta no epitélio olfatório, neurônios receptores de moléculas de odores (odorantes), que têm seus axônios projetados para os glomérulos olfatórios dentro do BO e também o órgão vomeronasal e nervo vomeronasal que inerva o bulbo olfatório acessório (Fig 4) (DEMARIA e NGAI, 2010).

O BO é a principal estrutura do sistema olfatório, pois é nele que ocorrem as sinapses glomerulares, entre os neurônios receptores e células mitrais e tufosas, que são as células responsáveis por encaminhar a resposta olfatória até as áreas corticais, para a percepção do odor específico (Fig.5) (DUDA, 2010).

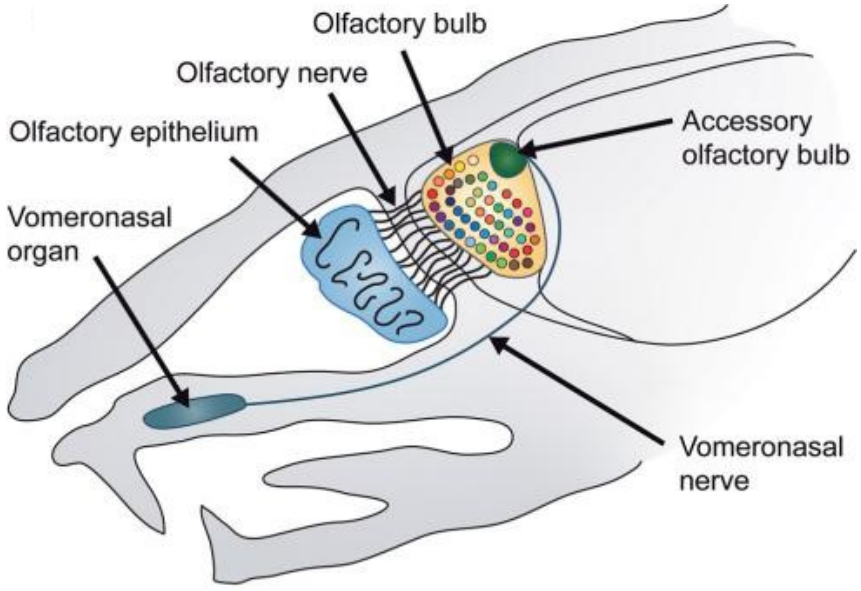

Figura 4. Anatomia do sistema olfatório de roedores. Esquema representivo de uma seção parasagital da cabeça de um rato adulto. Os axônios dos neurônios receptores olfatórios no epitélio olfatório compreendem o nervo olfatório e inervam o bulbo olfatório. Neurônios sensoriais vomeronasais projetam seus axônios através de um aparelho separado, o nervo vomeronasal, que inervam o bulbo olfatório acessório. Vomeronasal organ (órgão vomeronasal); Olfactory epithelium (epitélio olfatório); olfactory nerve (nervo olfatório); olfactory bulb (bulbo olfatório); Accessory olfactory bulb (bulbo olfatório acessório); vomeronasal nerve (nervo vomeronasal). Retirado de DeMaria e Ngai, 2010.

Cada odor é reconhecido por múltiplos receptores, e cada receptor reconhece múltiplos odores. Todos os axônios dos neurônios receptores que contêm os mesmos receptores convergem sempre para os glomérulos específicos em determinadas zonas do BO, de modo que o padrão de ativação desses glomérulos é específico para cada odor. Dentro desses glomérulos ocorrem sinapses dos axônios dos neurônios receptores de odores e células mitrais e tufosas, havendo também a interconexão de glomérulos a partir de interneurônios denominados células periglomerulares. As células mitrais/tufosas são as responsáveis por levar a informação olfatória para áreas corticais onde cada odor específico é, de fato, identificado (MORI *et al*., 1999).

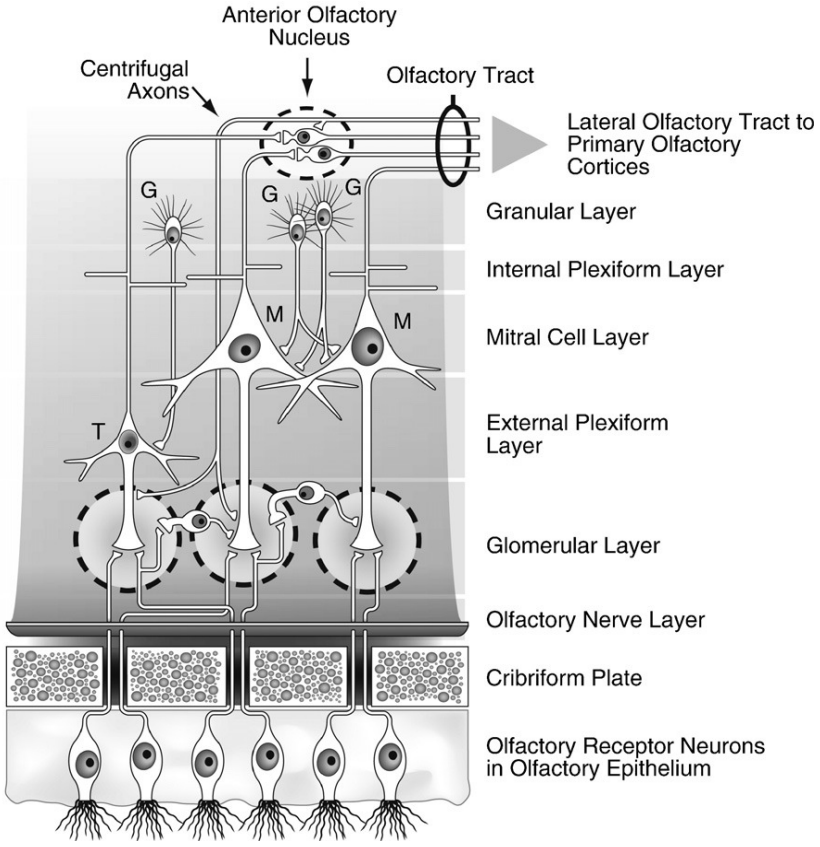

Figura 5. Neuroanatomia esquemática do bulbo olfatório. Neurônios receptores olfatórios projetam seus axônios para o epitélio através da placa crivóide (cribiforme) que divergem ao entrar no bulbo olfatório para formar glomérulos. Nos glomérulos, os neurônios receptores olfativos fazem sinapse com células mitrais (M) e células tufosas (T) e são modulados pelos neurônios e axônios periglomerulares. Células mitrais e tufosas são os principais neurônios do bulbo olfatório, projetando para a informação olfatória para áreas corticais. Olfactory receptor neurons (neurônios receptores olfatórios); cribform plate (placa crivóide); olfactory nerve layer (camada de nervos olfatórios); glomerular layer (camada glomerular); external plexiform layer (camada plexiforme externa); mitral cell layer (camada de células mitrais); internal plexiform layer (camada plexiforme interna); granular layer (camada granular); lateral olfactory tract to priimary olfactory cortices (trato olfatório lateral para o córtex olfatório primário); olfactory tract (trato olfatório); anterior olfactory nucleous (núcleo olfatório anterior); centrifugal axons (axônios centrífugos. Retirado de Duda, 2010.

1.4 PAPEL DA DOPAMINA NA OLFAÇÃO E NO SONO

1.4.1 RECEPTORES DOPAMINÉRGICOS

Existem duas grandes famílias de receptores dopaminérgicos: família D1 e família D2. A família D1 engloba os receptores do tipo D1 e D5, considerados como receptores acoplados a proteínas Gs e que, quando ativados, ativam a enzima adenilato ciclase. São receptores pós-sinápticos excitatórios. A família D2 engloba os receptores do tipo D2, D3 e D4. Esses receptores acoplados a proteína Gi, quando ativados, inibem a adenilato ciclase, podendo agir como autorreceptores ou receptores pós-sinápticos inibitórios (MONTI; MONTI, 2007).

1.4.2 DOPAMINA NA OLFAÇÃO

O BO de mamíferos contém uma abundante população de interneurônios que expressam tirosina hidroxilase (TH), enzima responsável pela síntese de catecolaminas (HÖKFELT *et al*.,1976, 1977; HALÁSZ *et al*., 1981).Sabe-se que, na fisiopatologia da DP, o BO apresenta uma maciça presença de inter-neurônios dopaminérgicos na camada glomerular (Gl), bem como na camada plexiforme externa (EPl) (LIBERIA, BLASCO-IBANEZ *et al*., 2012). Assim, na DP tem sido demonstrado que o número de neurônios periglomerulares tirosina-hidroxilase imunorreactivos (TH-ir) é aumentado em relação aos controles pareados por idade, refletindo possivelmente uma atividade dopaminérgica aumentada (HUISMAN *et al*, 2004;. MUNDIÑANO *et al*., 2011). Consequentemente, essa modulação observada no BO poderia desencadear efeitos de hiposmia ou anosmia, em particular devido ao efeito inibitório da DA na transmissão entre os neurônios receptores e células mitrais/tufosas nos glomérulos olfatórios, mediada, principalmentepor receptores D2, que são abundantes no BO (DOTY e RISSER, 1989; KOSTER *et al*., 1999; GUTIÈRREZ-MECINAS *et al*., 2005).

Ambos os receptores D2 e D1 são expressos no bulbo olfatório, porém, os receptores D2 são o sub-tipo predominante e são expressos nos terminais de axônios dos neurônios receptores de odores (ver fig. 8) e nos elementos pré-sinápticas das células mitrais/tufosas e também nos dendritos de células periglomerulares GABAérgicas/dopaminérgicas. Os receptores D1 são pouco expressos no bulbo olfatório de ratos (O'CONNOR E JACOB, 2008; DOTY, 2012b).

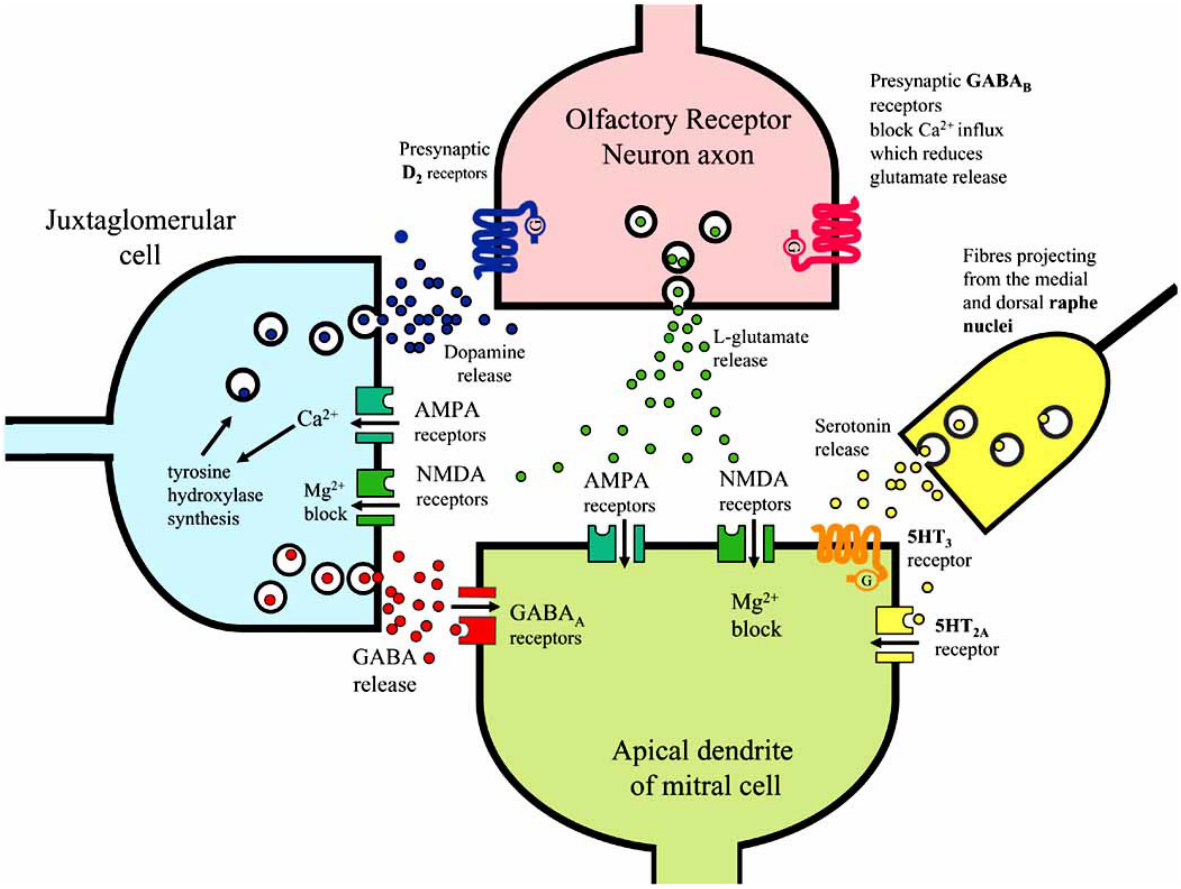

Figura 8. Sinapses glomerulares, mostrando a diversidade de receptores. Os axônios dos neurônios receptores olfatórios fazem sinapses com os dendritos das células mitrais. L-glutamato é o transmissor excitatório principal nesta sinapse, que se liga a receptores NMDA, AMPA na membrana pós-sináptica. Células justaglomerulares são interneurônios inibidores GABAérgicos/dopaminérgicos que medeiam a inibição glomerular. Fibras centrífugas dos núcleos da rafe se projetam para os glomérulos modulando a atividade das células mitrais através de receptores pós-sinápticos de 5HT. Os receptores D2 presentes nas células justaglomerulares e mitrais não estão representados nesta figura. Retirado de O'Connor e Jacob, 2008.

1.4.3 DOPAMINA NA REGULAÇÃO DO SONO

Diversos neurotransmissores como a noradrenalina (NA), acetilcolina (ACh), serotonina (5-HT), DA e neuropeptídeos como a orexina/hipocretina foram, pelo menos parcialmente, caracterizados dentro da circuitaria de regulação do sono (LIMA, ANDERSEN *et al.*, 2007a; LIMA, ANDERSEN *et al.*, 2008; LIMA, REKSIDLER *et al.*, 2008; LIMA, REKSIDLER *et al.*, 2009). Dentre esses neurotransmissores, destaca-se a DA como sendo possivelmente a molécula mais controversa em relação a seu papel neurofisiológico, haja vista que classicamente ela é associada quase que exclusivamente com a regulação da vigília (MONTI, 1982; MONTI e MONTI, 2007). Entretanto, uma série de trabalhos clínicos e em modelos animais têm apresentado a DA como sendo um neurotransmissor de caráter dual, portanto, estando relacionado também com a regulação dos processos

de sono, em particular do sono REM (LIMA, ANDERSEN *et al.*, 2007a; LIMA, ANDERSEN *et al.*, 2007b; LIMA, ANDERSEN *et al.*, 2008; LIMA, REKSIDLER *et al.*, 2008; LIMA, REKSIDLER *et al.*, 2009). A partir de tais estudos, têm-se sugerido que o sistema dopaminérgico nigroestriatal apresenta um papel chave para a regulação de sono, principalmente num contexto de neurodegeneração como no caso da DP (LIMA *et al.*, 2009). Ainda, sabe-se que uma lesão dos neurônios dopaminérgicos na SNpc dificulta geração de sono REM em ratos (LIMA *et al.*, 2007b) e macacos *rhesus* (BARRAUD *et al.*, 2009), e que o bloqueio de receptores dopaminérgicos D2 leva à redução ou supressão de REM após PSREM (LIMA, ANDERSEN *et al.*, 2008).

A observação de que indivíduos Parkinsonianos apresentam distúrbios olfatórios como a hiposmia assim como apresentam distúrbios de sono como RBD, sugere uma convergência desses eventos, sendo modulados pela DA, mediada por receptores D2.

1.5 NEUROTOXINAS UTILIZADAS EM MODELO ANIMAL DA DOENÇA DE PARKINSON

No contexto da DP, é proposta a utilização de neurotoxinas para mimetizar tal condição em modelos animais. Como exemplo dessas neutoxinas estão a 6-hidroxidopamina (6-OHDA), paraquat, 1-metil-4-fenil-1,2,3,6-tetrahidropiridina (MPTP) e a rotenona. O modo de ação de cada uma está representado na Figura 6 (BOVÉ e PERIER, 2012) .

Estudos recentes propõem a administração intranasal de MPTP, no intuito de promover uma degeneração dopaminérgica mais progressiva, mimetizando a DP (PREDIGER, BATISTA *et al.*, 2006; FRANCO, PREDIGER *et al.*, 2007). O modelo intranasal foi o primeiro a sugerir uma relação mais direta entre olfação e a via nigroestriatal num modelo animal de DP, A rotenona, por sua vez, apresenta basicamente os mesmos mecanismos de neurotoxicidade que o MPTP, ou seja, inibe o complexo I da cadeia respiratória mitocondrial, gerando morte neuronal principalmente por apoptose, porém ela está associada a uma importante condição de exposição ambiental, haja vista que é utilizada amplamente como pesticida (BETARBET, CANET-AVILES *et al.*, 2006). Nesse sentido, vários casos de Parkinsonismo decorrentes dessa exposição já foram relatados na literatura, o que

confere uma maior validade ao modelo animal (BETARBET, SHERER *et al*., 2000; DHILLON, TARBUTTON *et al*., 2008). Ainda, sabe-se que o modelo de rotenona intranigral é efetivo para mimetizar estágios iniciais da DP (MOREIRA *et al*., 2012; DOS SANTOS *et al*., 2013), e, nesse sentido, poderia mimetizar distúrbios olfatórios em modelo animal, já que a hiposmia tem sido relacionada a degeneração nigrostriatal nos estágios iniciais da DP (SIDEROWF *et al*., 2005).

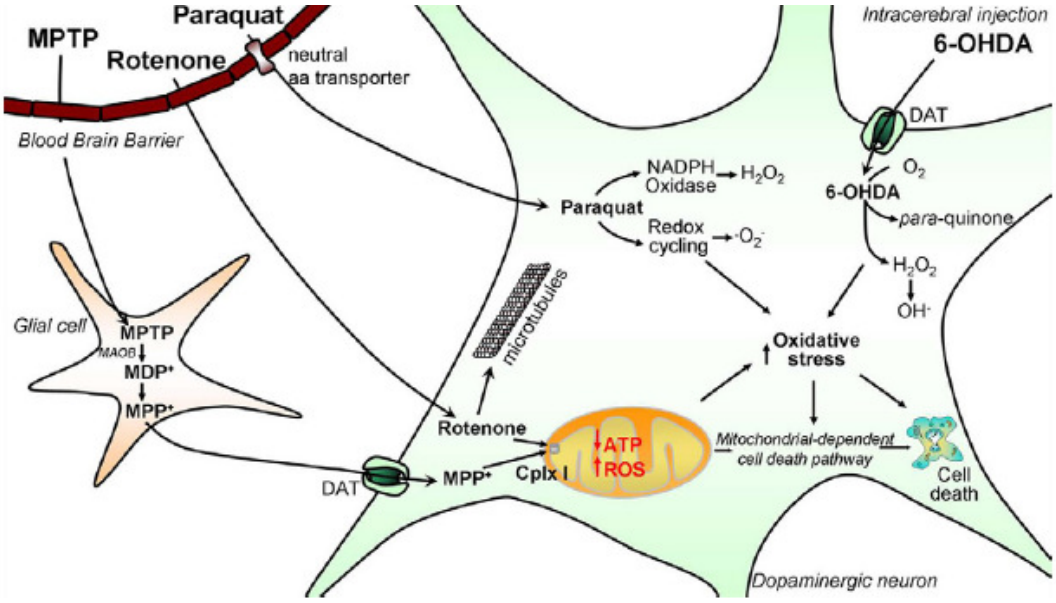

Figura 6. Mecanismo de ação de neurotoxinas utilizadas para mimetizar a doença de Parkinson. A 6-hidroxidopamina (6-OHDA) entra nos neurônios dopaminérgicos através do transportador (DAT). Na sequência, ocorrem várias reações onde aneurotoxina é oxidada, produzindo peróxido de hidrogênio (H2O2) e para-quinona, induzindo assim a morte neuronal através da produção de espécies reativas de oxigênio (ROS). O MPTP e a rotenona, como compostos lipofílicos, pode atravessar a barreira hemato-encefálica. Uma vez no interior do cérebro, MPTP é metabolizado em 1-metil-4-phenylpyridinium (MPP+) Pela enzima monoamina-oxidase B (MAOB) em células gliais. MPP+ entra nas células dopaminérgicas através do DAT. MPP+ e rotenona acumulam-se nas mitocôndrias onde inibem complexo I (Cplx I) da cadeia respiratória mitocondrial. A inibição do Complexo I leva a uma diminuição dos níveis de ATP, um aumento de produção de ROS e, finalmente, para a ativação de uma via de morte celular dependente de mitocôndrias. Rotenona também pode induzir a despolimerização de microtúbulos celulares, que participam no processo de morte celular. Paraquat entra no neurônio, com o auxílio do aminoácido neutro (aa), transportadores e catalisa a formação de ROS através de dois mecanismos: (1) ciclo redox e (2) de ativação de ROS, gerando enzimas como NADPH oxidases. Adaptado de Bovè e Perier, 2012.

2 JUSTIFICATIVA

Diante dos dados da literatura, os distúrbios olfatórios acometem cerca de 90% dos pacientes com DP, e distúrbios de sono acometem cerca de 80%, resultando em dificuldade em tarefas simples de discriminação olfatória, sonolência diurna excessiva, fragmentação de sono, alucinações relacionadas ao REM, ataques de sono e até distúrbios respiratórios. Desta forma, há um agravamento na qualidade de vida desses pacientes. Ainda, sabe-se que esses dois distúrbios podem estar intimamente relacionados, pois um prejuízo olfatório pode ser potencializado ou modulado por privação de sono REM. A literatura aponta as manifestações desses distúrbios em pacientes Parkinsonianos, no entanto, observa-se uma grande carência de evidências visando elucidar, em modelos animais, esses mecanismos que se inter-relacionam. Nesse sentido, o estudo dessas inter-relações torna-se fundamental para a compreensão da evolução fisiopatológica da DP, bem como para a busca de novas formas de tratamento.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Investigar os eventuais prejuízos olfatórios advindos da PSREM e da modulação farmacológica dos receptores D2 do BO, dentro de um paradigma de lesão da via nigroestriatal utilizando o modelo animal de Parkinsonismo induzido por rotenona intranigral.

3.2 OBJETIVOS ESPECÍFICOS

- Analisar os efeitos promovidos pela lesão da via nigroestriatal associada ou não à PSREM sobre o desempenho olfatório.
- Analisar os efeitos de um agonista de receptores D_2 (piribedil) do BO sobre o desempenho olfatório e motor.
- Analisar os efeitos de um antagonista de receptores D_2 (raclopride) do BO sobre o desempenho olfatório e motor.
- Quantificar por cromatografia líquida de alta eficiência (HPLC) os níveis de DA, DOPAC e HVA no BO, SNpc e estriado dorsal, dentro do paradigma da lesão da via nigroestriatal, PSREM e modulação farmacológica dos receptores D_2 do BO.
- Quantificar através de imuno-histoquímica para tirosina hidroxilase (TH) os efeitos sobre o número de neurônios dopaminérgicos no BO dentro do paradigma da lesão da via nigroestriatal, PSREM e modulação farmacológica dos receptores D_2 do BO.

4 ARTIGO CIENTÍFICO

Olfactory impairment in the rotenone model of Parkinson's disease is associated to dopaminergic D2 receptor activity in the glomerular layer of the olfactory bulb

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Abstract

Olfactory deficits are commonly found in untreated subjects with a recent diagnosis of Parkinson's disease (PD). Different studies observed declines in olfactory performances during a short period of sleep deprivation. Therefore, we have led to the hypothesis that a modulation of the dopaminergic D2 receptors in the olfactory bulb could provide a more comprehensive understanding of the olfactory deficits mechanisms found in PD and after a short period of rapid eye movement sleep deprivation (REMSD). We decided to investigate the olfactory, neurochemical and histological alterations generated by the administration of piribedil or raclopride, within the glomerular layer of the olfactory bulb, in rats submitted to intranigral rotenone and REMSD. Our findings provided a remarkable evidence of the occurrence of a negative correlation ($r = - 0.52$, $P = 0.04$) between the number of periglomerular TH-ir neurons and the bulbar levels of dopamine (DA) in the rotenone, but not sham groups. A significant positive correlation ($r = 0.34$, $P = 0.03$) was observed between nigral DA and olfactory discrimination index (DI), for the sham groups, indicating that increased DA levels in the substantia nigra pars compacta (SNpc) are associated to enhanced olfactory discrimination performance. Also, increased levels in bulbar and striatal DA induced by piribedil in the rotenone control and rotenone REMSD groups were consistent with reduced amounts of DI. The present evidence reinforce that DA produced by periglomerular neurons, and particularly the bulbar dopaminergic D2 receptors, are essential participants in the olfactory discrimination processes, as well as SNpc and striatum.

Keywords: Olfactory bulb, olfactory discrimination, dopamine, bulbar dopaminergic D2 receptors, REM sleep deprivation, intranigral rotenone, Parkinson´s disease.

Introduction

Olfactory deficits in odor detection (the threshold or the perception of odors at low concentrations), identification (the ability to name an odor), and discrimination (the nonverbal distinction of different smells) are commonly found in untreated subjects with a recent diagnosis of Parkinson's disease (PD) (Tissingh *et al*., 2001). These early dysfunctions are supported by neuropathologic studies, with Lewy pathology present in the olfactory bulb, olfactory tract and anterior olfactory nucleus in preclinical Braak stages prior to significant nigral degeneration (Del Tredici *et al*., 2002). Olfaction is impaired in approximately 90% of early-stage PD cases and can precede the onset of motor symptoms by years (Doty, 2012).

Moreover, it is reported a massive presence of dopaminergic inter-neurons in the glomerular portion of the olfactory bulb and the external plexiform layer (Liberia *et al*., 2012). Thus, in PD it has been demonstrated that the number of tyrosine hydroxylase immunoreactive periglomerular neurons (TH-ir) is increased with respect to age-matched controls, possibly reflecting a higher dopaminergic activity (Huisman *et al*., 2004; Mundinano *et al*., 2011). Accordingly, such effect in the olfactory bulb could lead to a suppression of olfactory information, particularly due to the inhibitory effect of dopamine (DA), mediated by D2 receptors activity, on the transmission between olfactory receptor cells and mitral cells within the olfactory glomeruli, thus, promoting the hyposmia (Doty and Risser, 1989; Koster *et al*., 1999).

Another intriguing prodromal dysfunction found in PD concerns the REM sleep disorders (Lima *et al*., 2012; Lima, 2013). Indeed, several findings demonstrated that normal rapid eye movement (REM) sleep can be suppressed in both normal and DA transporter knockout mice without affecting motor functions by diminishing dopaminergic tone (Dzirasa *et al*., 2006). Besides, it was reported that dopaminergic

D2 blockade may produce the reduction or even suppression of REM sleep after a period of REM sleep deprivation (Lima *et al*., 2008). Furthermore, the lesion of the SNpc dopaminergic neurons provoked a major impairment in REM sleep generation in rats (Lima *et al*., 2007) and rhesus monkeys (Barraud *et al*., 2009). Remarkably, the observation that REM sleep deprivation (REMSD), both in humans and rodents, is capable to produce a significant impairment in an olfactory discrimination task, similar to PD (Fantini *et al*., 2006; Killgore and McBride, 2006; Killgore *et al*., 2010).

These observations, together with recent evidence based on the use of the intranigral rotenone model of PD (Moreira *et al*., 2012; Dos Santos *et al*., 2013), have led to the hypothesis that a modulation of the D2 receptors in the olfactory bulb could provide a more comprehensive understanding of the olfactory deficits mechanisms found in PD and after REMSD. Then, we decided to investigate the olfactory, neurochemical and histological alterations generated by the administration of piribedil or raclopride (selective D2 agonist and antagonist, respectively), within the glomerular layer of the olfactory bulb, in rats submitted to intranigral rotenone infusion. Concurrently, we evaluated the effects of REMSD in the present experimental paradigm.

Material and Methods

Ethics statement

The studies were carried out in accordance with the guidelines of the Committee on the Care and Use of Laboratory Animals, United States National Institutes of Health. In addition, the protocol complies with the recommendations of Federal University of Paraná and was approved by the Institutional Ethics Committee (approval ID #658).

Animals

Male Wistar rats from our breeding colony weighing 280–320 g at the beginning of the experiments were used. The animals were randomly housed in groups of five in polypropylene cages with wood shavings as bedding and maintained in a temperature-controlled room (22±2°C) on a 12-h light-dark cycle (lights on at 7:00 AM). The animals had free access to water and food throughout the experiment.

Experimental design

Before the stereotaxic surgeries the animals were distributed randomly in two groups: Sham (n=102) and Rotenone (n=102). After the surgery procedure, the animals were redistributed in twelve groups (n=17/group): sham control vehicle, sham control piribedil, sham control raclopride, sham REMSD vehicle, sham REMSD piribedil, sham REMSD raclopride, rotenone control vehicle, rotenone control piribedil, rotenone control raclopride, rotenone REMSD vehicle, rotenone REMSD piribedil, rotenone REMSD raclopride. The concentrations of these drugs were determined according to a concentration-effect curve previously defined in an experiment based on locomotor and olfactory parameters (see supplementary material, Fig. 1). In addition, regarding the olfactory discrimination evaluation, we included another control group (n=12) treated with Zicam (Matrixx Initiatives, Inc., Phoenix, AZ) intending to produce loss of olfaction (Lim *et al*., 2009).

Stereotaxic surgeries were performed on day zero and on day 4 all animals were kept individually in their home cages for 48 h to collect sawdust for the olfactory discrimination task (ODT). On day 6, the REMSD procedure started. Twenty four hours after, on day 7, the rats received the intrabulbar administration of piribedil or raclopride, subsequently (2 h after) they were tested (due to pharmacokinetics).

Without delay, after the ODT, 10-12 animals/group were decapitated, their brains removed and dissection of the striatum, substantia nigra and olfactory bulb was taken to neurochemical and molecular purposes. Others 3-5 animals/group had their brains perfused and fixed for subsequent immunohistochemical analysis.

Stereotaxic Surgery

Rats were sedated with intraperitoneal xylazine (10 mg/kg; Syntec do Brasil Ltda, Brazil) and anaesthetized with intraperitoneal ketamine (90 mg/kg; Syntec do Brasil Ltda, Brazil). The following coordinates were used to the bilateral injury, bregma as a reference: substantia nigra pars compacta (SNpc) (AP) = - 5,0 mm, (ML) = ± 2,1 mm e (DV) = - 8,0 mm (Paxinos and Watson 2005). Needles were guided to the region of interest for a bilateral infusion of 1 µL of rotenone (12 µg/µL) or of dimethylsulfoxide - DMSO (Sigma-Aldrich®, United States) using an electronic infusion pump (Insight Instruments, Ribeirão Preto, Brazil) at a rate of 0,33 µL/min for 3 minutes (Saravanan *et al*., 2005; Moreira *et al*., 2012; Dos Santos *et al*., 2013). Sham operations followed the same procedure, but 1 µL of DMSO was injected instead. Complementarily, a guide cannula was implanted in the olfactory bulb of each rat allowing a subsequent 1 µL infusion of piribedil (3 µg/µL) (Tocris Bioscience®, United Kingdom), raclopride (10 µg/µL) (Sigma-Aldrich®, United States) or vehicle (DMSO) at a rate of 0,33 µL/min for 3 minutes, in their respective groups. Coordinates with reference to bregma for implantation of guide cannula were: (AP) = +7.08 mm (ML) = 0.0 mm and (DV) = -3.6 mm (Paxinos and Watson 2005).

Intranasal administration of Zicam (zinc gluconate + zinc acetate solution)

The administration of Zicam® Oral Mist (Matrixx Initiatives, Scottsdale, AZ, USA) was performed as previously reported by (Chioca *et al*., 2013): the animals were sedated with an intraperitoneal administration of 90 mg/kg ketamine and 3 mg/kg xylazine, and approximately 30 µL of Zicam solution was slowly delivered into the nasal cavity using a Hamilton syringe connected to a blunted 30-gauge needle through a polyethylene tube. The needle was inserted 15 mm past the right external nostril to help irrigate the olfactory epithelium. The procedure was repeated in the left nostril. During respiration, part of the solution was expelled through the nostril and dried with absorbent paper to allow the animal to continue breathing.

REMSD procedure

REMSD was attained by means of the single platform method. Rats were individually placed on a circular platform (6.5 cm in diameter) in a cage (23 x 23 x 30 cm) filled with water up to 1 cm below the platform level. At the onset of each REM sleep episode, the animal experiences a loss of muscle tonus and falls into the water, thus being awakened. When platforms of this size are used, REM sleep is completely eliminated (Machado *et al*., 2004). Throughout the study, the experimental room was maintained at controlled conditions (22±2 ◦C, 12 h light/dark cycle, lights on 7:00 a.m.). The control group was kept in the same room as the REMSD rats during the study. Food and water were provided *ad libitum* by placing chow pellets in a dispenser positioned inside the cage and water bottles on a grid located on top of the tank.

Olfactory discrimination task (ODT)

This test was previously described by Lamberty Soffie and subsequently modified by Prediger and colleagues (Soffié and Lamberty, 1988; Prediger *et al*., 2005a; Prediger *et al*., 2005b). The version used has been modified from Prediger *et al*., 2005a. The apparatus consists of a box (60 x 40 x 50cm) equally divided into two compartments, connected by a door that gives free access to the animal. Before the test, it was performed an adaptation period to the apparatus of 5 minutes, in both compartments with fresh sawdust. After that, clean sawdust is added on one side of the box (non-familiar odor). On the other side of the box, is added sawdust which animals remained isolated for 48 hours before testing (familiar odor). The ODT consists of placing the rat in the middle of olfactory discrimination box and record, up to 5 min, the time of investigation of each compartment. The animal that shows olfactory impairment tends to explore both compartments equally, indicating absence of discrimination. As a measure of discrimination, a “discrimination index (DI)” was calculated by dividing the difference in exploration time between the two compartments (compartment non-familiar - compartment familiar) by the total amount of exploration for both compartments (compartment non-familiar + compartment familiar). DI was then multiplied by 100 to express it as a percentage.

Neurochemical analysis of striatal, olfactory bulb and substantia nigra neurotransmitters, metabolites and turnovers

The striatum, olfactory bulb and the substantia nigra of the rats were rapidly dissected, placed on dry ice, and stored at -80°C until the neurochemical quantification. The endogenous concentrations of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) were assayed by reverse-phase high

performance liquid chromatography (HPLC) with electrochemical detection. The system consisted of a Synergi Fusion-RP C-18 reverse-phase column (150 x 4.6 mm i.d., 4 μm particle size) fitted with a 4 x 3.0 mm pre-column (Security Guard Cartridges Fusion-RP); an electrochemical detector (ESA Coulochem III Electrochemical Detector) equipped with a guard cell (ESA 5020) with the electrode set at 350 mV and a dual electrode analytical cell (ESA 5011A); a LC-20AT pump (Shimadzu) equipped with a manual Rheodyne 7725 injector with a 20 μL loop. The column was maintained inside in a temperature-controlled oven (25ºC). The cell contained two chambers in series: each chamber including a porous graphite coulometric electrode, a double counter electrode and a double reference electrode. Oxidizing potentials were set at 100 mV for the first electrode and at 450 mV for the second electrode. The tissue samples were homogenized with an ultrasonic cell disrupter (Sonics) in 0.1 M perchloric acid containing sodium metabisulfite 0.02% and internal standard. After centrifugation at 10,000 x *g* for 30 min at 4ºC, 20 μL of the supernatant was injected into the chromatograph.

The mobile phase, used at a flow rate of 1 mL/min, had the following composition: 20 g citric acid monohydrated (Merck), 200 mg octane-1-sulfonic acid sodium salt (Merck), 40 mg ethylenediaminetetraacetic acid (EDTA) (Sigma), 900 mL HPLC-grade water. The pH of the buffer running solution was adjusted to 4.0 then filtered through a 0.45 μm filter. Methanol (Merck) was added to give a final composition of 10% methanol (v/v). The neurotransmitters and metabolites concentrations were calculated using standard curves that were generated by determining in triplicate the ratios between three different known amounts of the internal standard. The unit was expressed as ng/g of wet weight.

TH- immunohistochemistry within the olfactory bulb

Total number of TH-ir neurons was estimated in the glomerular and plexiform layers of the olfactory bulb. Animals were deeply anesthetized with ketamine immediately after the ODT, and were intracardially perfused with saline first, then with 4% of the fixative solution formaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed from the skulls and were immersed for 48 hours in that fixative solution at 4ºC. Subsequently, the brains were placed in 30% sucrose solution for 3 days and were freeze at -80ºC before sectioning. Twelve 40 µm sections per animal were taken from the olfactory bulb (+7.56 mm and +7.08 mm an interval of 480 µm). Tissue sections were incubated with primary mouse anti-TH antibody, diluted in phosphate-buffered saline containing 0.3% Triton X-100 (1:500; Chemicon, CA, USA) overnight at 4℃. Biotin-conjugated secondary antibody incubation (1:200 anti-mouse #Vector Laboratories, USA), was performed for 2 h at room temperature. After several washes in phosphate-buffered saline, antibody complex was localized using the ABC system (Vectastain ABC Elite kit, Vector Laboratories, USA) followed by 3,3´- diaminobenzidine reaction with nickel enhancement. The sections were then mounted onto gelatin-coated slides and coverslipped after dehydration in ascending concentrations of ethanol-xylene solutions. Cell counts were conducted making use of the software Image-Pro Express 6. The mean number of TH-ir neurons in each hemisphere was considered to be representative of the olfactory bulb neuronal cells in each animal. For each group a mean value was calculated (percentage relative to the sham control vehicle), and compared with those of the other groups. The images were obtained using motorized Axio Imager Z2 microscope (Carl Zeiss, Jena, DE), equipped with a automated scanning VSlide (Metasystems, Altlussheim, DE).

Statistical analysis

Differences between groups in the ODT were analyzed by two-way analysis of variance (ANOVA) followed by the Bonferroni post hoc test. Olfactory discrimination index, neurochemical analysis and TH-immunohistochemistry were analyzed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls multiple comparison test. Pearson's correlation coefficients (r) were calculated to establish relationships between neurochemical and behavioral parameters or molecular and neurochemical parameters. Values were expresses as mean ± standard error of mean (SEM). The level of significance was set at $P \leq 0.05$.

Results

Olfactory discrimination task (ODT)

Regarding the time spent in each compartment in the ODT, we found that the sham control vehicle group showed an increment in time spent in the non-familiar odor compartment in relation to the familiar ($P \leq 0.001$), as well as the sham control piribedil ($P \leq 0.05$) and sham REMSD vehicle ($P \leq 0.001$) groups (Fig. 1A). In opposite, the sham control raclopride group remained more time exploring the familiar odor compartment in relation to the non-familiar ($P \leq 0.05$) and that time spent in the familiar compartment was significantly higher when compared to the sham control piribedil group ($P \leq 0.05$). In fact, the time spent in the non-falimiar compartment for the sham control raclopride group was significantly lower in comparison to the sham control vehicle ($P \leq 0.001$) and sham control piribedil ($P \leq 0.05$) groups as indicated by the odor [$F(12,292)=8.46$, $P<0.0001$], group [$F(12,292)=0.00$, $P=1.00$] and interaction [$F(12,292)=8.46$, $P<0.0001$] factors (Fig. 1A). Besides, this analysis revealed that sham REMSD piribedil and sham REMSD raclopride did not

discriminated the comparments. A similar result was obtained for all the rotenone groups, as well as for the Zicam group (positive control of olfactory loss) (Fig. 1A).

Fig. 1B shows the DI for each group obtained during the ODT. Accordingly, the sham control raclopride group exhibited a significant impairment in the DI compared to the sham control piribedil ($P<0.001$) and sham control vehicle ($P<0.05$) groups. Likewise, the sham control raclopride group demonstrated a significant reduction ($P<0.05$) in this parameter in comparison to the rotenone control raclopride group [$F(12,146)=4.236$, $P<0.0001$]. In addition, the sham REMSD piribedil presented a reduction ($P<0.05$) in the DI compared to the sham REMSD vehicle.

Quantification of bulbar, nigral and striatal dopamine and metabolites

Fig. 2 shows the alterations in the neurotransmission within the olfactory bulb. Accordingly, DA levels (Fig. 2A) were reduced in the rotenone control vehicle group compared to the sham control vehicle ($P\leq0.01$) and rotenone control piribedil ($P\leq0.01$) groups [$F(11,78)=3.678$, $P=0.0003$]. Considering the DOPAC levels (Fig. 2B) it was observed that the rotenone REMSD vehicle group presented an increase of this metabolite compared to the sham REMSD vehicle ($P\leq0.05$), rotenone control vehicle ($P\leq0.01$) and rotenone REMSD raclopride ($P\leq0.01$) groups [$F(11,86)=3.569$, $P=0.0004$]. Fig. 2C shows the HVA levels in the olfactory bulb, that is increased in the rotenone control vehicle group compared to the rotenone REMSD vehicle group ($P\leq0.05$) [$F(11,80)=2.146$, $P=0.0259$]. The calculation of the bulbar DA turnover (Fig. 2D) indicated no differences between groups [$F(11,41)=0.5327$, $P=0.8696$].

The neurochemical alterations in the substantia nigra are shown in Fig. 3. DA levels (Fig. 3A) were found to be reduced in the sham control raclopride group compared to the sham control vehicle group ($P\leq0.05$). A rather similar result is found for the sham REMSD raclopride group compared to sham REMSD vehicle group

(P≤0.05), as well as for the rotenone control raclopride group compared to rotenone control piribedil group (P≤0.05) [F(11,79)=4.987, P<0.0001]. Regarding the DOPAC levels (Fig. 3B) the rotenone control raclopride group presented a decrease in this metabolite in comparison to the rotenone control vehicle group (P≤0.01) [F(11,80)=2.978, P=0.0023]. Fig.3C shows the HVA levels, that is increased in the sham control raclopride group compared to the rotenone control raclopride group (P≤0.001). Furthermore, the sham REMSD vehicle group exhibited a significant increase in HVA compared to the rotenone REMSD vehicle group (P≤0.05). Besides, the sham REMSD raclopride group presented an increment in HVA levels compared to the rotenone REMSD raclopride group (P≤0.05). Likewise, the rotenone control vehicle group showed an increase in HVA compared to the rotenone REMSD vehicle (P≤0.05) and rotenone control raclopride groups (P≤0.05) [F(11,87)=6.768, P<0,0001]. The calculation of the nigral DA turnover (Fig. 3D) indicated that the rotenone control vehicle group presented an increased turnover (P≤0.05) compared to the rotenone control piribedil and rotenone REMSD vehicle groups [F(11,42)=2.907, P=0,0062].

In the striatum, the neurochemical alterations are shown in Fig. 4. DA levels (Fig. 4A) were found to be reduced in the sham control raclopride, sham REMSD vehicle and rotenone control vehicle groups compared to the sham control vehicle group (P≤0.001). Similar results were found for the rotenone control raclopride group compared to rotenone control piribedil group (P≤0.05) [F(11,75)=5.654, P<0.0001]. Considering the DOPAC levels (Fig. 4B) no differences among the groups [F(11,75)=1.872, P=0.0567] were identified. Absence of statistical significances were also found regarding the HVA levels [F(11,76)=1.572, P=0.1243] (Fig. 4C). The calculation of the striatal DA turnover (Fig. 4D) indicated that the rotenone control

vehicle group presented an increase in this parameter compared to the sham control vehicle (P≤0.05), rotenone control piribedil (P≤0.05), rotenone control raclopride (P≤0.05) and rotenone REMSD vehicle (P≤0.01) groups [F(11,37)=2.593, P=0,0149].

TH-immunohistochemistry

As can be seen in Fig. 5A, the percentage of TH-ir neurons within the glomerular layer of the olfactory bulb indicated that a significant increase in TH-ir neurons was observed in the sham control piribedil (P<0.01) and sham control raclopride (P<0.01) groups in comparison to the sham control vehicle group. Besides, the sham REMSD piribedil group exhibited a significant increase of this parameter compared to the sham REMSD vehicle (P<0.001) and sham REMSD raclopride (P<0.001) groups. Interestingly, the sham REMSD raclopride group also presented a decrease of TH-ir neurons compared to the sham control raclopride group (P<0.001). The intranigral rotenone administration inflicted a rather opposite effect regarding the number of TH-ir neurons, i.e., the rotenone control piribedil group showed a decrease of this labeling compared to the rotenone control vehicle (P<0.001) and rotenone control raclopride (P<0.05). Moreover, the rotenone REMSD piribedil and rotenone REMSD raclopride groups showed increased percentage in TH-ir neurons in comparison to the rotenone REMSD vehicle group. In addition, the rotenone REMSD piribedil group demonstrated an increase in TH-ir neurons compared to the rotenone control piribedil (P<0.001). Remarkably the rotenone control piribedil group exhibited a significant decrease in the percentage of TH-ir neurons in comparison to the sham control piribedil group (P<0.001). In opposite, the rotenone control vehicle group showed an increment in this labeling compared to the sham control vehicle (P<0.001). Moreover, a significant increase (P<0.001) was identified in the rotenone

REMSD raclopride group compared to the sham REMSD raclopride group [F(11,28)=17.80, P<0.0001].

In relation to the plexiform layer of the olfactory bulb (Fig. 5B) a significant increase in TH-ir neurons was observed in the rotenone control raclopride (P<0.05) and rotenone REMSD vehicle (P<0.001) compared to their respective sham groups. Additionally, an important increment in the number of this neurons was perceived in the rotenone REMSD vehicle group compared to the rotenone control vehicle (P<0.05) and rotenone REMSD piribedil (P<0.05) groups [F(11,30)=7.504, P<0.0001].

Statistical correlations between behavioral and neurochemical parameters or molecular and neurochemical parameters

Pearson's correlation coefficients (Table 1) revealed a moderate negative correlation (r = -052; P = 0.04) between periglomerular TH-ir neurons and bulbar DA levels for the rotenone groups. It has been noted a weak, however, significant positive correlation (r = 0.34; P = 0.03) between nigral DA levels and DI for the sham groups. Also, it has been noted a significant positive correlation (r = 0.30; P = 0.04) between striatal DA levels and DI for the rotenone groups.

Discussion

In the present study we observed that both, intranigral rotenone and REMSD, were able to produce olfactory dysfunction, apparently modulated by D2 receptors within the glomerular layer of the olfactory bulb. The extent of the olfactory deficit was moderate, considering the REMSD, and particularly associated to D2 activation. However, rotenone evoked a sustained impairment (possibly a ceiling effect), since no longer the pharmacological manipulation produced any difference. Of note, in this

study we demonstrated that the olfactory effects generated by rotenone were particularly alike than the olfactory deficit inflicted by intranasal Zicam, which was used as a positive control of olfactory impairment. This is an agent that has been described to promote a significant cytotoxicity to both mouse and human nasal tissue given the potential development of long-lasting, and perhaps irreversible smell dysfunction (Lim *et al*., 2009; Chioca *et al*., 2013). The neurotoxic effects of rotenone are typically related to nigrostriatal dopaminergic neurotransmission (Betarbet *et al*., 2000; Moreira *et al*., 2012; Dos Santos *et al*., 2013), however, this is the first study, according to our knowledge, that compares the variations of the monoaminergic neurotransmission within the olfactory bulb, striatum and SNpc after a rotenone exposure. In fact, rotenone alone appears to reduce the DA content in the olfactory bulb and striatum, whilst REMSD produced a similar effect in the striatum. Interestingly, the blockade of bulbar D2 receptors triggered considerable reductions in DA - that also were observed in the REMSD - within the SNpc and striatum. However, a remarkable increment in DA levels in the rotenone control piribedil group is detected in the olfactory bulb, SNpc and striatum. Such increase was completely abolished in the rotenone REMSD piribedil group. Moreover, the number of olfactory TH-ir periglomerular neurons was increased in both, activated or blockade, D2 receptors in the sham control groups. Conversely, only the activation of D2 was able to increment this labeling in a REMSD situation. Also, rotenone associated to REMSD imposed a drastic reduction in the TH-ir neurons that was rescued, most likely, by the D2 agonist piribedil. These data are in accordance to previous reports that also described the involvement of the dopaminergic system in the olfactory function (Mundinano *et al*., 2011; Hutter and Chapman, 2013; Borghammer *et al*.,

2014), strengthening the notion of a particular participation of the periglomerular TH-ir neurons and its D2 receptors.

Rotenone exposure in rodents provides an interesting model for studying mechanisms of toxin-induced dopaminergic neuronal injury (Betarbet *et al*., 2000; Greenamyre *et al*., 2003; Segura Aguilar and Kostrzewa, 2004; Drolet *et al*., 2009). In this model, a massive inhibition of mitochondrial complex I produces selective degeneration of the dopaminergic nigrostriatal system and reproduces key pathological features of clinical PD (Sherer *et al*., 2003; Alam and Schmidt, 2004). The pathophysiology of olfactory loss in PD is unclear. Neuropathologic changes in (1) the olfactory bulb and the anterior olfactory nucleus in the early disease and (2) the olfactory cortex and limbic structures in advances stages may both be involved (Benarroch, 2010). Hyposmia has been related to nigrostriatal denervation in the early stages of PD (Siderowf *et al*., 2005), whereas cholinergic deficits in the limbic cortex may occur in later stages (Bohnen *et al*., 2010). Thus, the use of intranigral rotenone, as a model of PD, becomes attractive because it is strongly associated to the presymptomatic state of PD (Moreira *et al*., 2012; Dos Santos *et al*., 2013), without motor influence (supplementary material Fig. 3 – Open field test).

Accordingly, different studies observed declines in olfactory performances during a short period of sleep deprivation (Killgore and McBride, 2006; Killgore *et al*., 2008; Killgore *et al*., 2010). Furthermore, the relationship between dopaminergic neurotransmission and REM sleep is a recent theme in the literature, and growing evidence suggests a significant impact of PD in REM sleep disturbances (Lima, 2013). Besides, electrophysiological data indicated that the absence of half of the SNpc TH-ir neurons, in rats, provoked a major impairment in the sleep-wake parameters, predominantly in REM sleep (Lima *et al*., 2007). In addition, REM sleep

could be recovered in the dopaminergic transporter knockout (DAT-KO) mice by selective activation of the D2, but not the D1, suggesting a particular role of this receptor in the regulation of REM sleep (Dzirasa, *et al*., 2006). Such involvement of DA has been previously reported subsequent to sleep deprivation protocols, as being directly involved in the generation of burly dopaminergic D2 supersensitivity (Tufik *et al*., 1978; Tufik, 1981; Nunes *et al*., 1994). In that respect, our findings provided a remarkable evidence of the occurrence of a negative correlation ($r = - 0.52$, $P = 0.04$) between the number of periglomerular TH-ir neurons and the bulbar levels of DA in increased decreased TH-ir neurons, in the olfactory bulb, tends to promote deficits in DA that is produced exclusively in periglomerular cells (Halasz et al. 1977). This is also associated to the olfactory deficit promoted by rotenone and counteracted by piribedil, but not by raclopride. In this context, REMSD purportedly generates D2 supersensitivity promoting a restorative effect of the DA storages in the different areas investigated. Besides, piribedil associated to REMSD generated a synergic induction of c-Fos content within the SNpc, thus indicating that a strong positive correlation between striatal DA levels and nigral c-Fos activation occurs (Proenca *et al*., 2014). However, the impact of that neurochemical mechanism was identified as an olfactory impairment in the presence of D2 modulation.

Complementarily, a significant positive correlation ($r = 0.34$, $P = 0.03$) was observed between nigral DA and DI, for the sham groups, indicating that increased DA levels in the SNpc are associated to enhanced olfactory discrimination performance. Nevertheless, our study fail in detect possible significant negative correlations between the groups ($r = -0.12$; $P = 0.40$ for sham groups and $r = -0.10$; $P = 0.49$ for rotenone groups) for these parameters regarding the levels of DA in the olfactory bulb. Also, a similar correlation ($r = 0.30$, $P = 0.04$) was identified

concerning the striatal DA and DI for the rotenone groups. D2 receptors are the most abundant subtype of DA receptors in the olfactory bulb (Coronas *et al*., 1997). It has been reported that one functional effect of D2 receptor activation in the bulb is a significant depression of synaptic transmission between olfactory receptor neurons and mitral cells (Hsia et al. 1999). Interestingly, we used concentrations of piribedil and raclopride, obtained from a preliminary study (supplementary material Fig. 1 - concentration/effect curve), that generated increased and decreased, respectively, of the olfactory discrimination performances without locomotor bias. Apparently, this result contradicts the previous statement, however, a potential limitation of our results relies on the activity of piribedil also in D3 receptors (Cagnotto *et al*., 1996). In fact, there is no other anatomical evidence demonstrating that D2 receptors are presynaptically located in the glutamatergic synapses from the olfactory nerve to mitral/tufted cells and periglomerular cells (Gutierrez-Mecinas *et al*., 2005). Moreover, the presence of D2 receptors in the other elements that integrate the glomerular circuitry (i.e., intraglomerular dendrites of mitral/tufted cells and periglomerular cells) has not been analyzed yet. An alternative explanation is the possible occurrence of a piribedil-induced blockade of D2 in the periglomerular neurons due to the its relatively high concentration injected.

Still, it is demonstrated that DA and the D2 receptor agonist bromocriptine could modulate GABAA receptors, facilitating GABA responses in cultured mitral and tufted cells from rat olfactory bulb (Brunig *et al*., 1999). A similar effect of DA and D2 receptors cannot be discarded in the intraglomerular dendrites of the mitral and tufted cells in vivo, taking into account that GABAA receptors are present in the mitral/tufted cells of the rat olfactory bulb (Giustetto *et al*., 1998; Panzanelli *et al*., 2004). If the activation of D2 receptors modulates GABAA receptors of the mitral/tufted cells

facilitating the GABAergic neurotransmission from TH-ir periglomerular neurons, this fact should lead to an increase in the inhibition of the mitral/tufted cells (Tillerson *et al*., 2006). It is noteworthy that we observed increased levels in bulbar, nigral and striatal DA induced by piribedil in the rotenone control and rotenone REMSD groups that were consistent with reduced amounts of DI (close to 3%, similar to Zicam 4.5%). Hence, it is conceivable that the activation of D2 by piribedil, associated to the well known dopaminergic D2 supersensitivity induced by rotenone and also by REMSD could enhance this inhibitory effect triggered by D2 receptors promoting a massive impairment in the olfactory function.

The present findings provide new information regarding the role of DA and D2 receptors within the olfactory bulb, SNpc and striatum in olfactory discrimination. Rotenone promoted a remarkable olfactory impairment that was interestingly modulated by REMSD and consequently by D2 receptors. Indeed, D2 receptor activation can enhance or impair the discrimination of odors, presumably by altering the perceived intensity of a given odorant through changes in the effective sensitivity of bulbar neurons to olfactory sensory neuron activity (Escanilla *et al*., 2009). DA levels have also been found to increase during odor learning (Coopersmith *et al*., 1991), suggesting that DA modulation may have important roles in synaptic plasticity in the bulb and also impacting in other areas such as the SNpc and striatum. Therefore, the present evidence reinforce that DA produced by periglomerular TH-ir neurons, and particularly the bulbar dopaminergic D2 receptors, are essential participants in the olfactory discrimination processes, as well as SNpc and striatum. As a consequence, these changes may have direct impact in the prodromal abnormalities found in patients with PD.

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Figure Legends

Figure 1. A. Time (s) spent in familiar and non-familiar compartments in the olfactory discrimination task 7 days after surgery. The bars represent the mean ± standard error of the mean. n=15 per group, *P≤0.05, ***P≤0.001 comparing the time mean spent in familiar and non-familiar compartments. Two-way ANOVA followed by the Bonferroni test. **B**. Olfactory discrimination index (DI) calculated by (NF-F/NF+F)*100, NF is the time spent in the compartment with non-familiar odor and F is the time spent in the compartment with familiar odor. The bars represent the mean ± standard error of the mean, n=15 per group, *P≤0.05, **P≤0.01, ***P≤0.001. One-way ANOVA followed by the Newman-Keuls test.

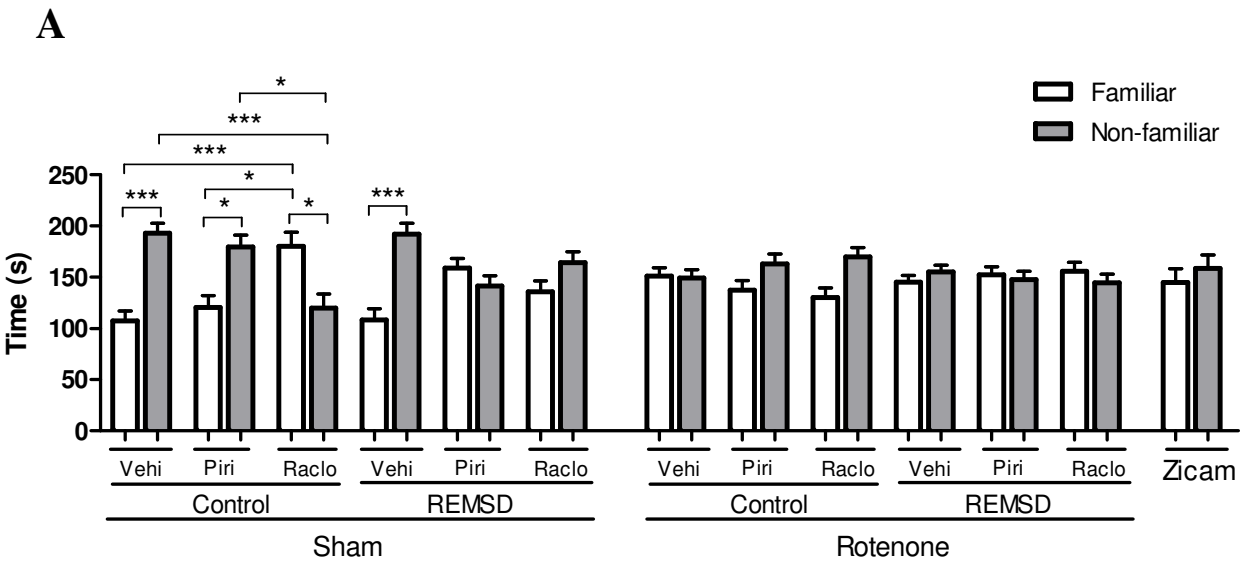
Figure 2. Neurochemical examination of the olfactory bulb content of DA and metabolites. (**A**) DA, (**B**) DOPAC, (**C**) HVA, (**D**) DA turnover. Values are expressed as mean ± SEM. *P≤0.05, **P≤0.01. One-way ANOVA followed by the Newman Keuls test.

Figure 3. Neurochemical examination of the substantia nigra content of DA, and metabolites. (**A**) DA, (**B**) DOPAC, (**C**) HVA, (**D**) DA turnover. Values are expressed as mean ± SEM. *P≤0.05, **P≤0.01, ***P≤0.001. One-way ANOVA followed by the Newman Keuls test.

Figure 4. Neurochemical examination of the striatal content of DA and metabolites. (**A**) DA, (**B**) DOPAC, (**C**) HVA, (**D**) DA turnover. Values are expressed as mean ± SEM. *P≤0.05, **P≤0.01, ***P≤0.001. One-way ANOVA followed by the Newman Keuls test.

Figure 5. A. Number (%) of neurons TH-ir in the glomerular layer of the olfactory bulb in relation to sham control vehicle group. The bars represent the mean ± standard error of the mean, n=5 per group, *P≤0.05, **P≤0.01, ***P≤0.001. One-way ANOVA

followed by the Newman-Keuls test. **B**. Number (%) of neurons TH-ir in the external plexiform layer of the olfactory bulb in relation to sham control vehicle group. The bars represent the mean ± standard error of the mean, n=5 per group, *P≤0.05, ***P≤0.001. One-way ANOVA followed by the Newman-Keuls test. **C**. Schematic diagram representing the coordinates (mm) and sections used in immunohistochemistry for TH in the glomerular layer (triangle) and external plexiform layer (ball) of the olfactory bulb.


A
Familiar
Non-familiar
Time (s)
250
200
150
100
50
0
Vehi
Piri
Raclo
Vehi
Piri
Raclo
Vehi
Piri
Raclo
Vehi
Piri
Raclo
Zicam
Control
REMSD
Control
REMSD
Sham
Rotenone

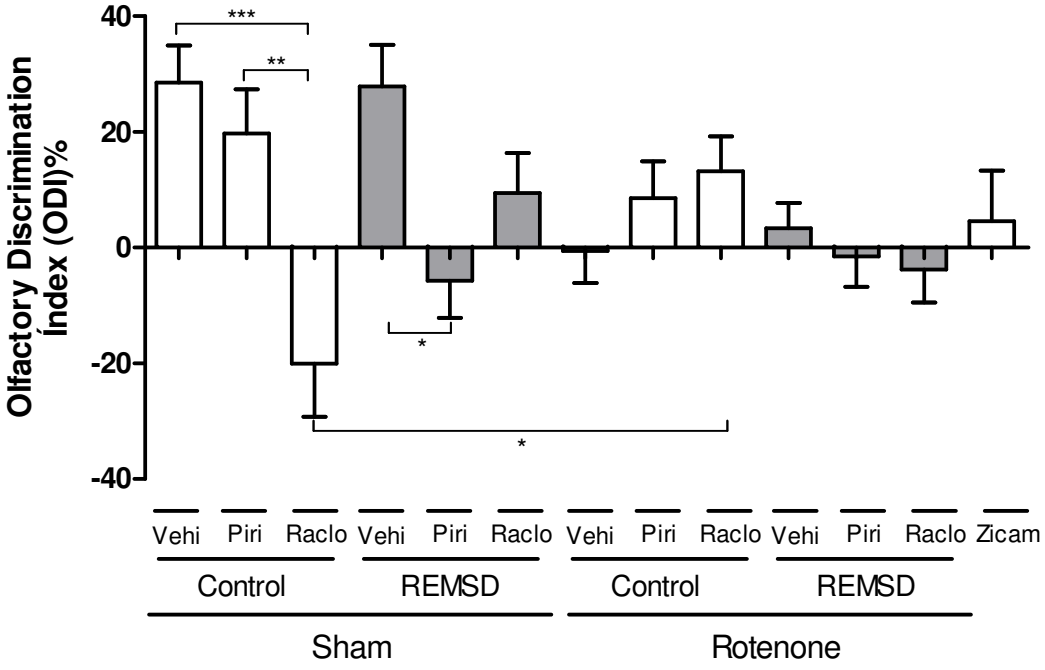

B
Olfactory Discrimination Índex (ODI)%
40
20
0
-20
-40
Vehi
Piri
Raclo
Vehi
Piri
Raclo
Vehi
Piri
Raclo
Vehi
Piri
Raclo
Zicam
Control
REMSD
Control
REMSD
Sham
Rotenone

Figure 1

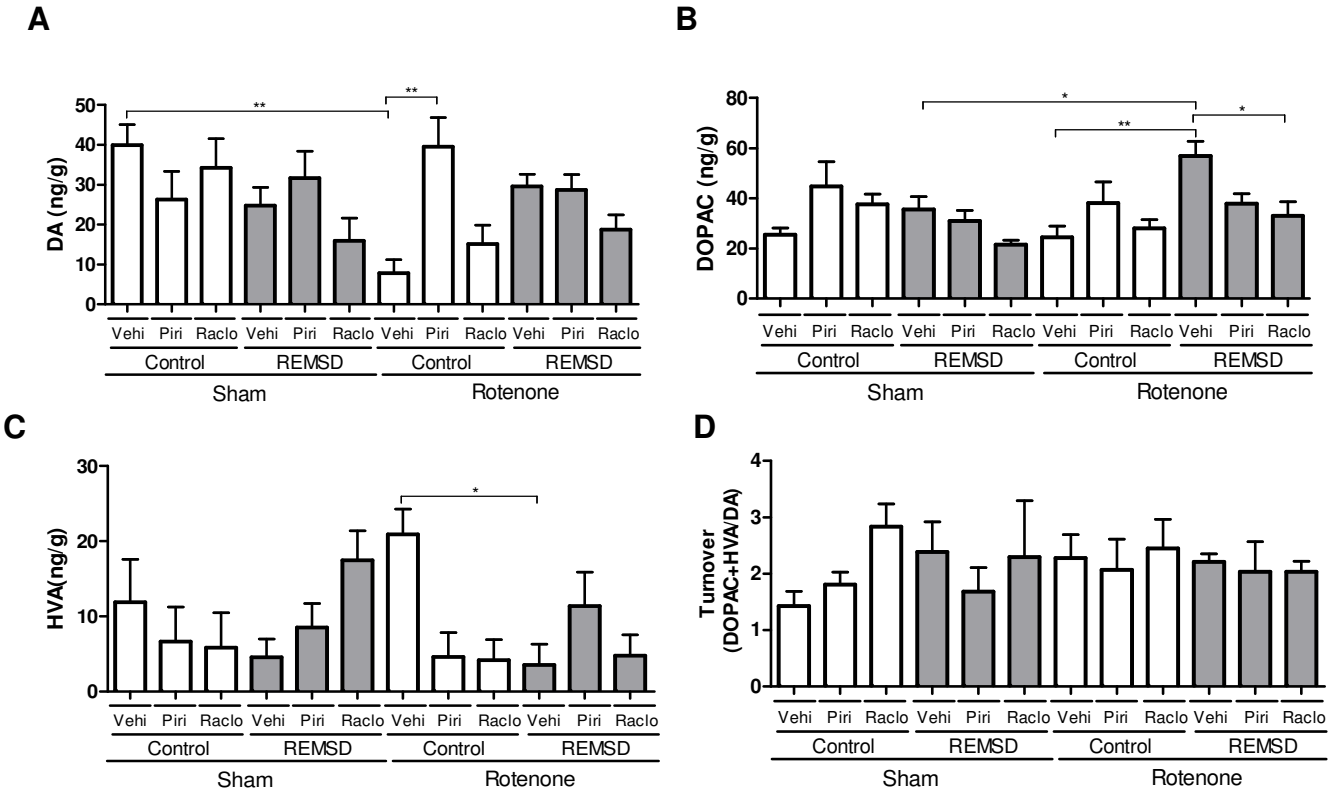

A
B
C
D
DA (ng/g)
DOPAC (ng/g)
HVA(ng/g)
Turnover (DOPAC+HVA/DA)
Vehi
Piri
Raclo
Control
REMSD
Sham
Rotenone

Figure 2

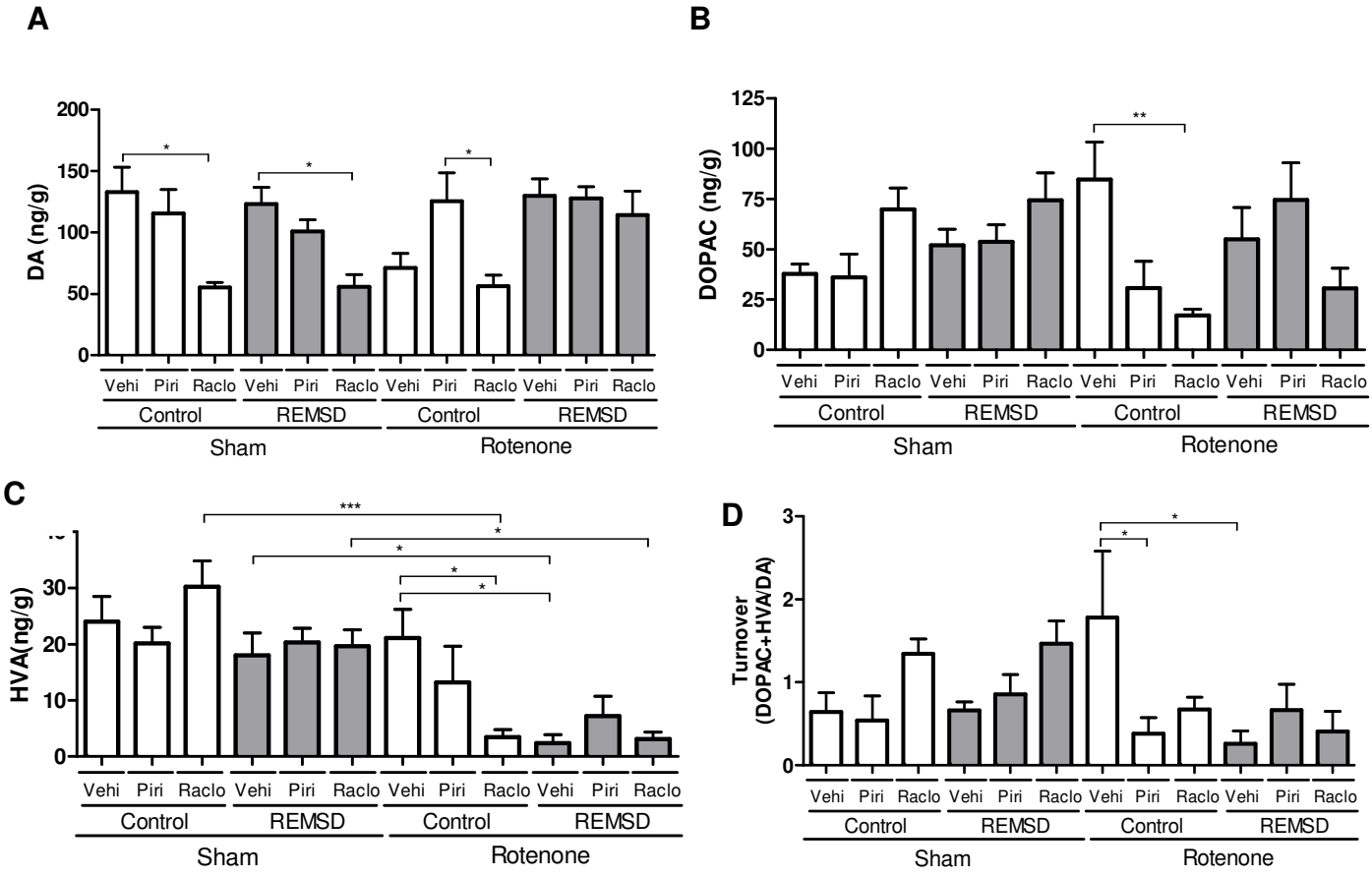

A
DA (ng/g)
200
150
100
50
0
Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo
Control REMSD Control REMSD
Sham Rotenone
B
DOPAC (ng/g)
125
100
75
50
25
0
Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo
Control REMSD Control REMSD
Sham Rotenone
C
HVA(ng/g)
30
20
10
0
Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo
Control REMSD Control REMSD
Sham Rotenone
D
Turnover (DOPAC+HVA/DA)
3
2
1
0
Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo
Control REMSD Control REMSD
Sham Rotenone

Figure 3

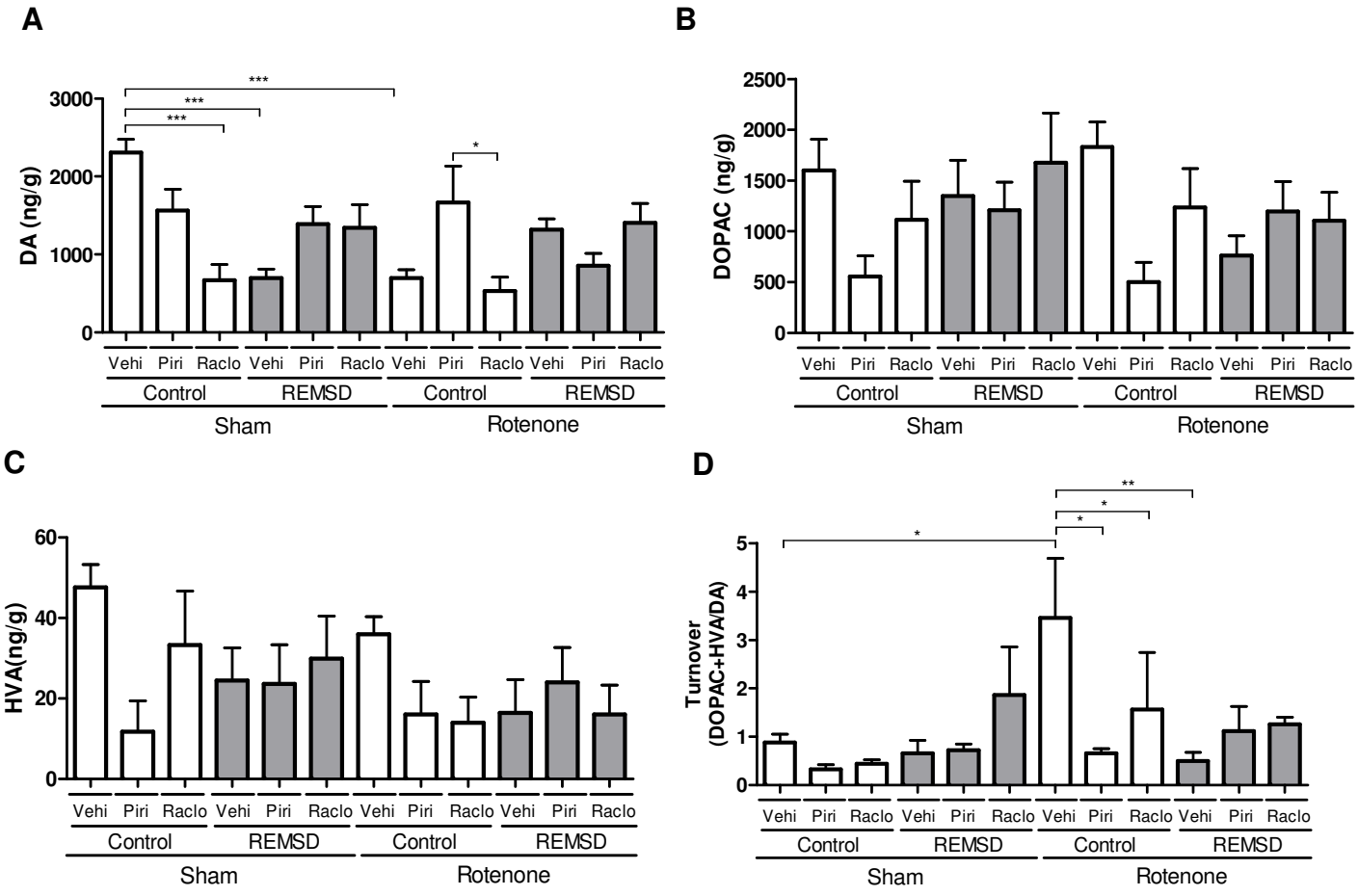
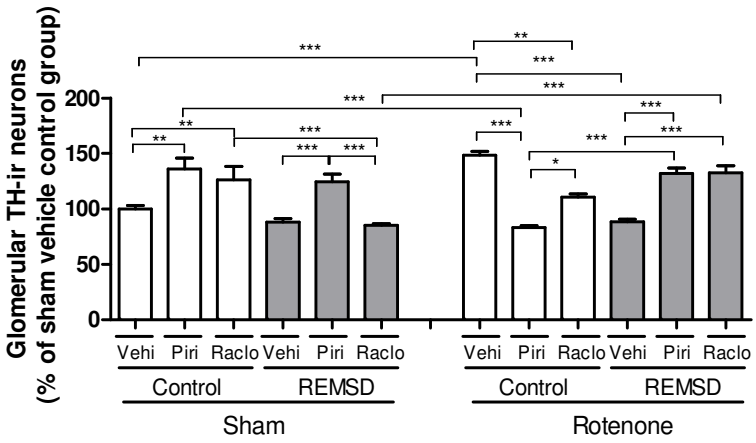
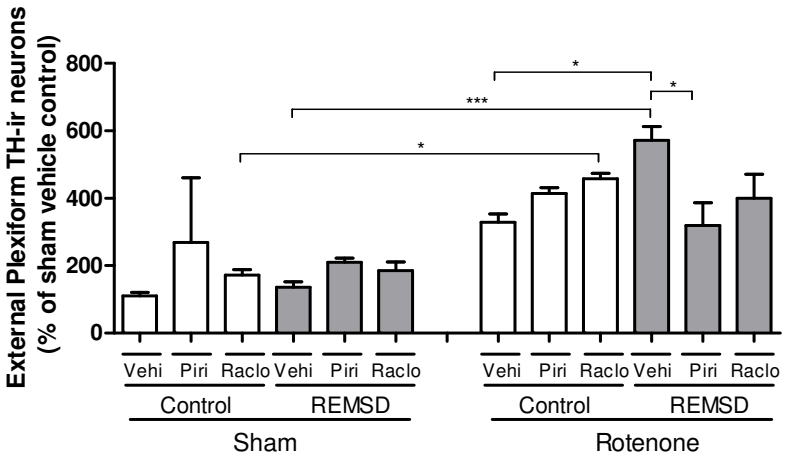

A
B
C
D
DA (ng/g)
DOPAC (ng/g)
HVA(ng/g)
Turnover (DOPAC+HVA/DA)
Vehi
Piri
Raclo
Control
REMSD
Sham
Rotenone

Figure 4

A


Glomerular TH-ir neurons
(% of sham vehicle control group)
200
150
100
50
0
Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo
Control REMSD Control REMSD
Sham Rotenone

B


External Plexiform TH-ir neurons
(% of sham vehicle control)
800
600
400
200
0
Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo Vehi Piri Raclo
Control REMSD Control REMSD
Sham Rotenone

C

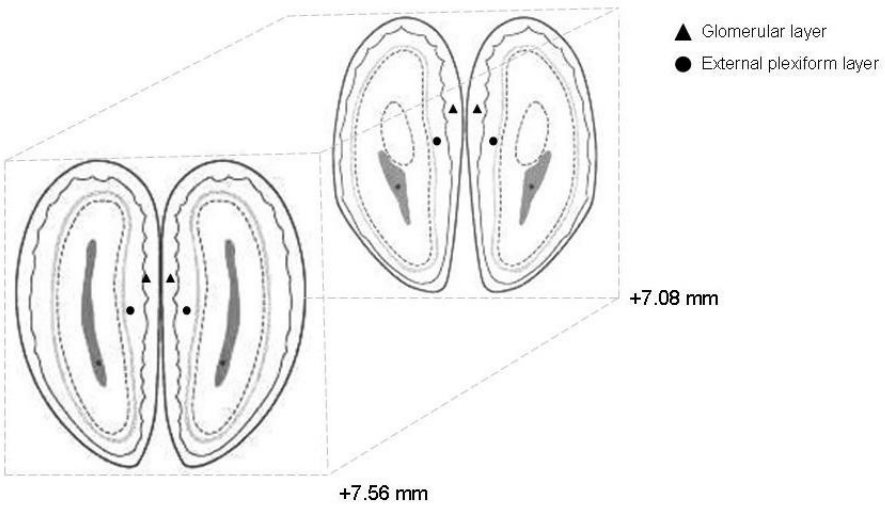

Glomerular layer
External plexiform layer
+7.08 mm
+7.56 mm

Figure 5

Table 1. Pearson's correlations between different behavioral, neurochemical and histological parameters.

Correlations	Groups	
	Sham	Rotenone
Periglomerular TH-ir neurons x bulbar DA	r = 0.24; P = 0.25	r = -052; P = 0.04*
Periglomerular TH-ir neurons x nigral DA	r = -0.21; P = 0.31	r = -032; P = 0.21
Periglomerular TH-ir neurons x striatal DA	r = -0.06; P = 0.76	r = -0.38; P = 0.14
Bulbar DA x DI	r = -0.12; P = 0.40	r = -0.10; P = 0.49
Nigral DA x DI	r = 0.34 ; P = 0.03*	r = -0.21; P = 0.15
Striatal DA x DI	r = 0.13 ; P = 0.37	r = 0.30; P = 0.04*

*Significant correlations are indicated. Discrimination índex (DI); Dopamine (DA)

Supplementary material

Figure 1. ***Concentration/effect curve:***

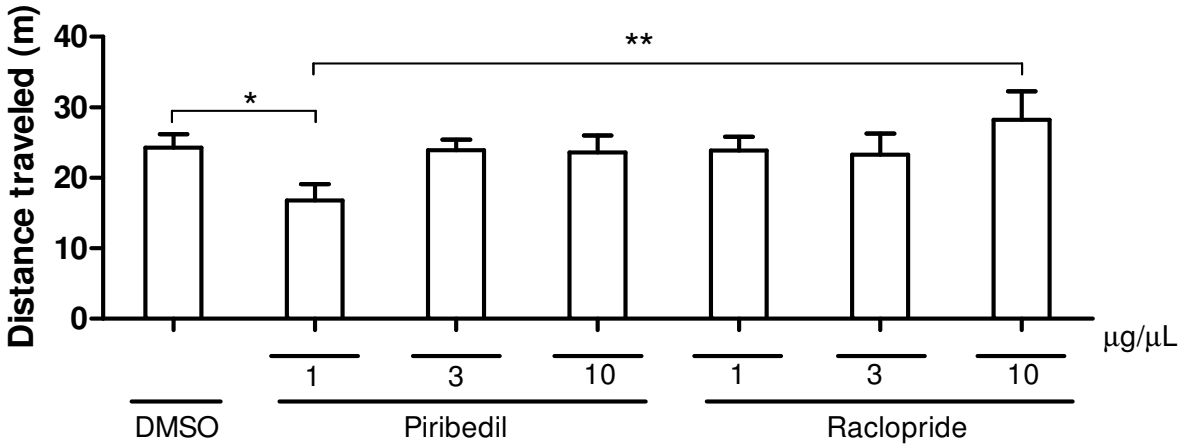


Fig. 1. A. Open field test. Distance traveled (m) of animals in the open field test 7 days after surgery, 120 minutes after drugs (piribedil or raclopride) or vehicle (DMSO) micro infusion, concentration 1, 3 or 10 µg/µL. The bars represent the mean ± standard error of the mean. DMSO n=10, P1 n=10, P3 n=10, P10 n=10, R1 n=10, R3 n=10, R10 n=10, *P≤0.05, **P≤0.01, comparing the means of the distance traveled between groups. One-way ANOVA followed by the Newman Keuls test.

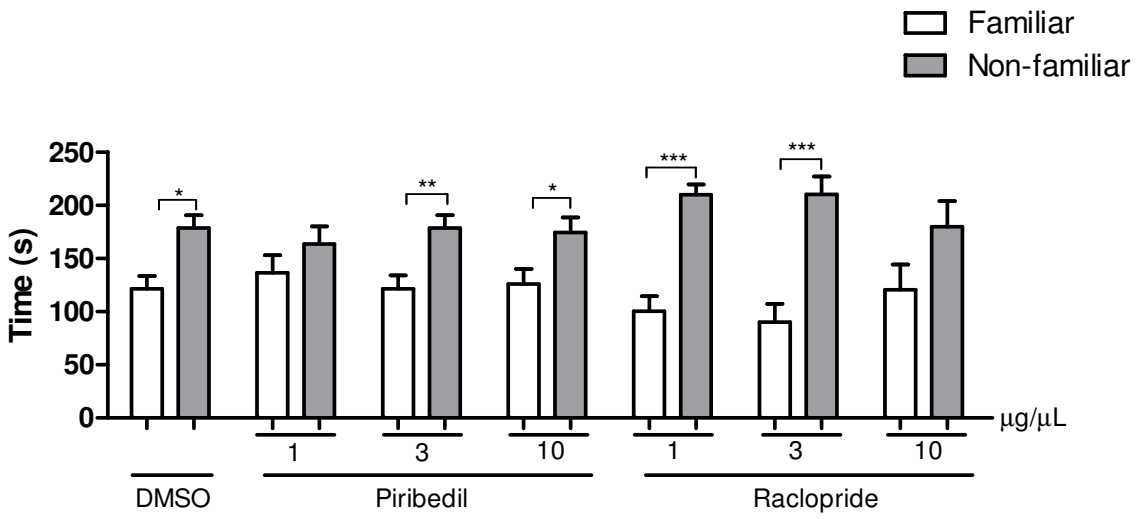


Fig. 1. B. Olfactory discrimination task. Time (s) spent in familiar and non-familiar compartments in the olfactory discrimination task 7 days after surgery, 120 minutes after drugs (piribedil or raclopride) or vehicle (DMSO) micro infusion, concentration 1, 3 or 10 µg/µL. The bars represent the mean ± standard error of the mean. DMSO n=10, P1 n=10, P3 n=10, P10 n=10, R1 n=10, R3 n=10, R10 n=10, *P≤0.05, **P≤0.01, ***P≤0.001 comparing the mean time spent in the familiar and non-familiar compartments. Two-way ANOVA followed by the Bonferroni test.

Figure 2. ***NA levels:***

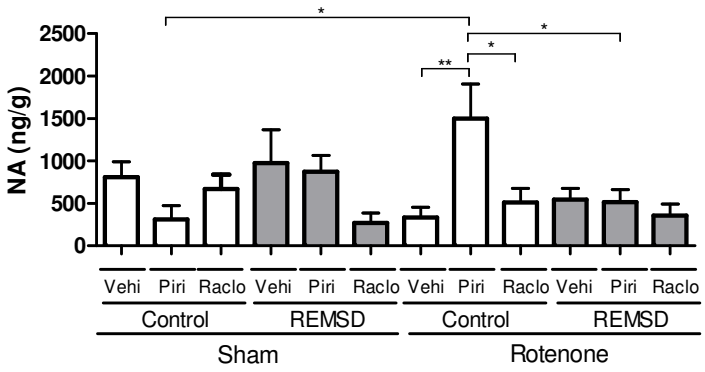


Fig 2. A. Olfactory bulb. Neurochemical examination of the olfactory bulb content of NA. Values are expressed as mean ± SEM. *P≤0.05, **P≤0.01. One-way ANOVA followed by the Newman Keuls test.

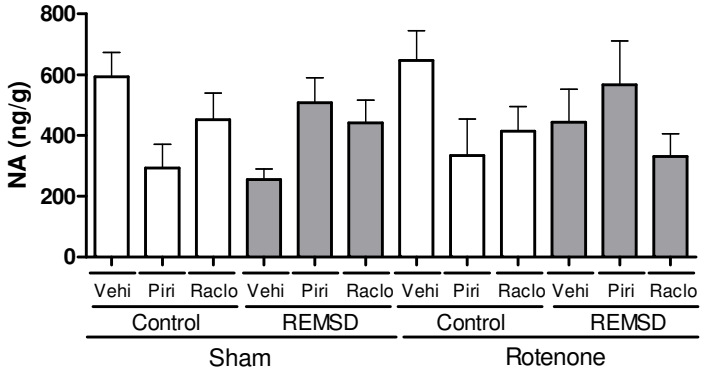


Fig 2. B. Substantia nigra pars compacta. Neurochemical examination of the substantia nigra content of NA. Values are expressed as mean ± SEM. *P≤0.05. One-way ANOVA followed by the Newman Keuls test.

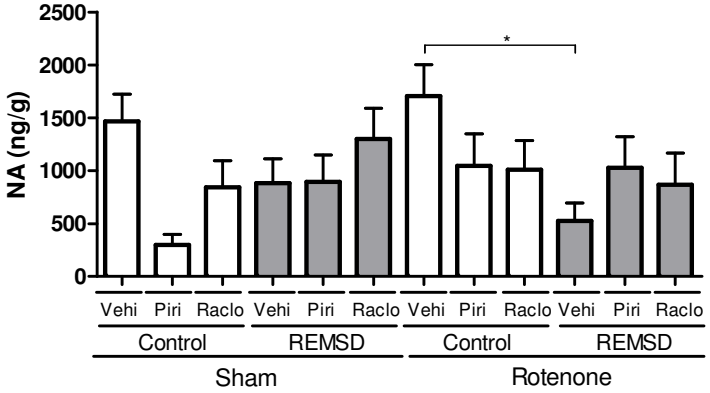


Fig 2. C. Striatum. Neurochemical examination of the striatum content of NA. Values are expressed as mean ± SEM. *P≤0.05. One-way ANOVA followed by the Newman Keuls test.

Figure 3. ***Open field test:***

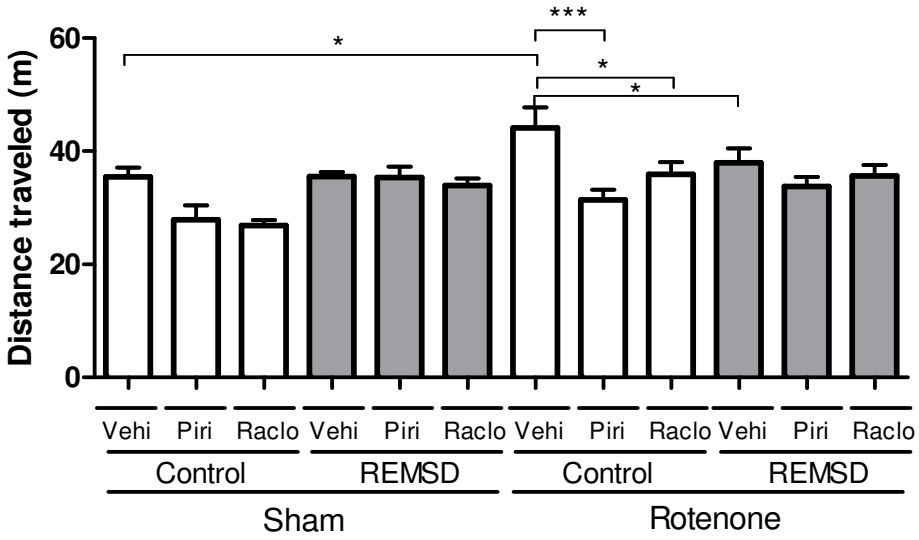


Fig.3. Distance traveled (m) of animals in the open field test 7 days after surgery. The bars represent the mean ± standard error of the mean, n=15 per group, *P≤0.05, ***P≤0.001. One-way ANOVA followed by the Newman-Keuls test.

5 CONCLUSÕES

- Até onde nos consta, este é o primeiro estudo que compara as variações da neurotransmissão dopaminérgica dentro do BO, estriado e SNpc após uma exposição intranigral de rotenona, o que mimetiza um estado pré-sintomático da DP e, como consequência, pode haver impacto direto nos distúrbios prodrômicos encontrados nesses indivíduos.
- A rotenona foi capaz de promover um notável prejuízo olfatório, assim como o medicamento utilizado como controle positivo do prejuízo olfatório, Zicam.
- A PSREM também foi capaz de produzir um prejuízo na TDO quando associada à modulação de receptores D2, no BO, indicando a associação dos distúrbios olfatórios com a PSREM.
- A quantificação de DA e de seus metabólitos indicaram que a modulação dopaminérgica pode ter um papel importante na plasticidade sináptica no bulbo, impactando também em outras áreas, como a SNpc e estriado.
- A DA produzida por neurônios periglomerulares TH-ir, que se mostraram mais reativos nos grupos com a lesão, e os receptores dopaminérgicos D2 do BO, são participantes essenciais nos processos de discriminação olfatória, bem como SNpc e estriado.

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ANEXOS

ANEXO 1 Does Parkinson´s disease and type-2 diabetes mellitus present common pathophysiological mechanisms and treatments?

ANEXO 2 REM Sleep Deprivation Reverses Neurochemical and Other Depressive-Like Alterations Induced by Olfactory Bulbectomy

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Abstract: Parkinson's disease (PD) is the second most common neurodegenerative disease afflicting about 1% of people over 65 years old and 4-5% of people over 85 years. It is proposed that a cascade of deleterious factors is set in motion within that neuron made not of one, but rather of multiple factors such as free radicals, excitotoxicity, neuroinflammation, and apoptosis to cite only some of the most salient. In this scenario, chronic systemic inflammation, as well as impaired mitochondrial metabolism, have also been suspected of playing a role in the development of type-2 diabetes, and the possibility of a shared pathophysiology of PD and type-2 diabetes has been proposed. The discussion about the interactions between PD and type-2 diabetes mellitus began in the 1960's and there is still controversy. Insulin and dopamine may exert reciprocal regulation hence; hypoinsulinaemia induced by streptozotocin decreased the amounts of dopamine transporter and tyrosine hydroxylase transcripts in the substantia nigra pars compacta. Accordingly, dopamine depletion in the striatum is able to decreases insulin signaling in basal ganglia, indicating that, perhaps, PD may be considered as a risk factor for the development of type-2 diabetes mellitus. In this sense, it is described that peroxisome proliferator-activated receptor-γ, ATP-sensitive K^+ channels, AMP-activated protein kinase, glucagon-like peptide-1 and dipeptidyl peptidase-4 are important therapeutic targets for PD and reinforces the association with diabetes. Therefore, the objective of the present review is to contextualize the mutual pathophysiological interactions between PD and type-2 diabetes mellitus, as well as the potential common treatments.

Keywords: Dopamine, Treatment, Peroxisome proliferator-activated receptor-γ, Type-2 diabetes mellitus, Parkinson's disease.

INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disease afflicting about 1% of people over 65 years old and 4-5% of people over 85 years. Typically, PD is the result of the degeneration of neurons in the substantia nigra pars compacta (SNpc), which leads to the subsequent reduction of dopaminergic input to the striatum. Moreover, there is a degeneration of neurons of selected brain stem nuclei (locus coeruleus, raphe nuclei, dorsal motor nucleus of the vagus), cortical neurons (particularly within the cingulated gyrus and the entorhinal cortex), the nucleus basalis of Meynert and of preganglionic sympathetic and parasympathetic neurons. In the soma of these neurons, the existence of intracellular proteinaceous inclusions, called Lewy bodies and Lewy neurites, mainly composed of α-synuclein, have been observed [1]. The characteristic distribution of these aggregations is considered to be the most classical neuropathological hallmark of PD.

Several reports discuss that the mechanism of neuronal death in PD starts with an otherwise healthy dopaminergic neuron being hit by an etiological factor, such as mutant α-synuclein. Besides, type-2 diabetes mellitus, chronic renal failure, past brain insults, or genetically determined differences in drug metabolism were also suggested as a risk factor for PD [2, 3]. Also, the coexistence of dopaminergic neurons and insulin receptors in the SNpc reinforce the occurrence of a direct association between the two diseases [4, 5]. There are various ways in which a shared pathogenesis of diabetes, dementia, and PD may occur. One is that there might be an underlying disorder of mitochondrial bioenergetics, manifest in pancreatic beta-cells and adipose tissue; this might be attributable to limited activation of peroxisome proliferator-activated receptor-γ (PPAR-γ), PPAR coactivator-1α (PGC1α) and its link to AMP kinase in the SNpc and dopaminergic neurons [6]. Another overlapping cytotoxic disorder is that of abnormal protein folding [7, 8] which is associated with amylin-derivative effects on pancreatic beta-cells in diabetes, the neurodegenerative tauopathies (hyperphosphorylation of tau, low levels of soluble tau) [9], the formation of amyloid precursor protein (characteristic of Alzheimer's disease) and with synucleinopathies in neurodegenerative disorders characterized by neurofibrillary aggregates of α-synuclein protein in neurons and glial cells in PD [10].

Studies with animal models have reinforced this proposition indicating that dopaminergic drugs influence insulin production, insulin resistance, and glycaemic control. For instance, intracerebroventricular delivery of bromocriptine, a potent D2 receptor agonist, improved insulin sensitivity in hamsters [11]. These findings suggest that dopamine (DA) activity in the brain contributes to

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REM Sleep Deprivation Reverses Neurochemical and Other Depressive-Like Alterations Induced by Olfactory Bulbectomy

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Abstract There is compelling evidence that sleep deprivation (SD) is an effective strategy in promoting antidepressant effects in humans, whereas few studies were performed in relevant animal models of depression. Acute administration of antidepressants in humans and rats generates a quite similar effect, i.e., suppression of rapid eye movement (REM) sleep. Then, we decided to investigate the neurochemical alterations generated by a protocol of rapid eye movement sleep deprivation (REMSD) in the notably known animal model of depression induced by the bilateral olfactory bulbectomy (OBX). REMSD triggered antidepressant mechanisms such as the increment of brain-derived neurotrophic factor (BDNF) levels, within the substantia nigra pars compacta (SNpc), which were strongly correlated to the swimming time ($r=0.83$; $P<0.0001$) and hippocampal serotonin (5-HT) content ($r=0.66$; $P=0.004$). Moreover, there was a strong correlation between swimming time and hippocampal 5-HT levels ($r=0.70$; $P=0.003$), strengthen the notion of an antidepressant effect associated to REMSD in the OBX rats. In addition, REMSD robustly attenuated the hippocampal 5-HT deficiency produced by the OBX procedure. Regarding the rebound (REB) period, we observed the occurrence of a sustained antidepressant effect, indicated mainly by the swimming and climbing times which could be explained by the maintenance of the increased nigral BDNF expression. Hence, hippocampal 5-HT levels remained enhanced in the OBX group after this period. We suggested that the neurochemical complexity inflicted by the OBX model, counteracted by REMSD, is directly correlated to the nigral BDNF expression and hippocampal 5-HT levels. The present findings provide new information regarding the antidepressant mechanisms triggered by REMSD.

Keywords REM sleep deprivation · Bulbectomy · Intranigral · Dopamine · Substantia nigra pars compacta · Parkinson's disease

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Introduction

The German psychiatrist Walter Schulte (1910–1972) introduced today's practice of treating patients with depressive disorders with sleep deprivation (SD) [1]. Thus, there is compelling evidence that SD is an effective strategy in promoting antidepressant effects in humans [2–6]. Whereas only few studies were performed in relevant animal models of depression [7–10], therefore many questions regarding the antidepressant mechanism triggered by SD still remain.

According to Vogel, rapid eye movement (REM) sleep deprivation (REMSD) fits the criteria for being the mechanism of action of the antidepressant drugs. Hence, REMSD by itself improves endogenous depression; that