UNIVERSIDADE FEDERAL DO PARANÁ

ANA CAROLINA DUARTE NOSEDA

ANÁLISE DO PAPEL DOS RECEPTORES MELATONINÉRGICOS MT2 ESTRIATAIS NA REGULAÇÃO DO HUMOR EM MODELO ANIMAL DE PARKINSONISMO

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Dissertação apresentada como requisito parcial para a obtenção do título de Mestre em Farmacologia, Curso de Pós-Graduação em Farmacologia, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientador: Prof. Dr. Marcelo de Meira Lima Santos

Co-orientadora: Prof[a]. Dr[a]. Maria A. B. F. Vital

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PARECER

A Comissão Examinadora da Dissertação de Mestrado intitulada "ANÁLISE DO PAPEL DOS RECEPTORES MELATONINÉRGICOS MT2 ESTRIATAIS NA REGULAÇÃO DO HUMOR EM MODELO ANIMAL DE PARKINSONISMO", de autoria da pós-graduanda ANA CAROLINA DUARTE NOSEDA, 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. Fernando Mazzilli Louzada (Fisiologia - UFPR) e Prof. Dr. Roberto Andreatini (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, 07 de julho de 2014.

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

Prof. Dr. Fernando Mazzilli Louzada (Fisiologia - UFPR)

Prof. Dr. Roberto Andreatini (Farmacologia - UFPR)

Centro Politécnico – Caixa Postal 19031 – CEP: 81531-990 – Curitiba/PR
Telefones: (41) 3361-1693 – Fax (41) 3266-2042 – pgfarmacologia@ufpr.br
http://farmacologia.bio.ufpr.br/posgraduacao

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RESUMO

Tem sido observado níveis plasmáticos de melatonina alterados em pacientes com Doença de Parkinson (DP) e a administração exógena desse neurohormônio apresenta efeitos benéficos nas alterações não motoras da DP, como distúrbios do humor. Tem sido sugerido que alterações nos receptores MT2 da melatonina estariam envolvidos no aparecimento da depressão. Assim, o presente estudo investigou a modulação de receptores MT2 estriatais no comportamento tipo-depressivo em um modelo de DP induzido por rotenona. Ratos Wistas machos receberam infusão intranigral de rotenona (12 µg/µL) e após sete dias foram submetidos a privação de sono paradoxal (PSREM) durante 24h. Após esse período, receberam infusão no estriado do agonista seletivo MT2, 8-M-PDOT (10 µg/µL), antagonista seletivo MT2, 4-P-DDOT (5 µg/µL), ou do veículo. Após 30 minutos da infusão das drogas, os animais foram avaliados no teste de natação forçada modificado, e então permitidos dormir o sono rebote (REB). Os ratos foram re-testados no dia seguinte, e o estriado, substância negra pars compacta (SNpc) e hipocampo foram coletados para quantificação neuroquímica. O bloqueio de receptores MT2 estriatais demonstrou efeito tipo-antidepressivo, sendo potencializado pela PSREM. A ativação de receptores MT2 aumentou os níveis de DA no estriado e no hipocampo, enquanto que o bloqueio MT2 estriatal aumentou DA e NA na SNpc. O tratamento com 4-P-PDOT no grupo rotenona PSREM reduziu os níveis de 5-HT no estriado, hipocampo e SNpc. Entretanto, foi observado aumento do turnover de 5-HT nessas estruturas. Em conclusão, esses resultados demonstram o envolvimento neuroquímico no efeito tipo-antidepressivo induzido pelo 4-P-PDOT estriatal associado com a PSREM em um modelo de DP induzido pela rotenona.

Palavras-chave: Dopamina, serotonina, 4-P-PDOT, 8-M-PDOT, Doença de Parkinson, rotenona, privação de sono paradoxal, substância negra pars compacta.

ABSTRACT

It has been observed that the secretion pattern of melatonin is modified in Parkinson's disease (PD) patients and consequently the exogenous administration of this neurohormone could demonstrate beneficial effects regarding the non-motor alterations, specially mood disorders. It has been hypothesized that dysregulations of melatonin MT2 receptors may be involved in the installation of depression. Together with recent evidence based on the use of the intranigral rotenone model of PD, have led to the hypothesis that modulating the striatal MT2 receptor could provide a more comprehensive understanding of the antidepressant properties triggered. To further investigate this issue, male Wistar rats were infused with intranigral rotenone (12 µg/µL) and seven days later subjected to a rapid eye movement sleep deprivation (REMSD) for 24 h. After, we injected within the striatum the MT2 selective agonist, 8-M-PDOT (10 µg/µL), the MT2 selective antagonist, 4-P-PDOT (5 µg/µL) or vehicle. Subsequently, they were tested in the forced swimming test and were allowed to perform the sleep rebound (REB). Then, the rats were re-tested and striatum, hippocampus and substantia nigra pars compacta (SNpc) were collected for neurochemical purposes. Results indicated substantial antidepressant effects promoted by the blockade of striatal MT2 receptors that were potentiated by REMSD. MT2 activation increased DA levels in the striatum and hippocampus, while MT2 blockade increase DA in the SNpc. 4-P-PDOT treatment of the rotenone REMSD group generated a decrement in 5-HT levels within the striatum, hippocampus and SNpc. However, increased 5-HT turnover was observed among these structures. Therefore, we demonstrated the neurochemical antidepressant effect induced by striatal 4-P-PDOT associated with REMSD in the rotenone model of PD.

Key-words: Dopamine, serotonin, 4-P-PDOT, 8-M-PDOT, Parkinson´s disease, rotenone, REM sleep deprivation, substantia nigra pars compacta.

LISTA DE ABREVIATURAS

5-HT– 5-hidroxitriptamina (serotonina)

6-OHDA – 6-hidroxidopamina

ATP – Adenosina trifosfato

DA – Dopamina

DAT – Transportador de dopamina

DOPAC – Ácido 3,4-diidroxifenilacético

DP – Doença de Parkinson

EROs – Espécies reativas de oxigênio

GABA – Ácido γ-aminobutírico

GSH – Glutationa

HIAA – Ácido 5-hidroxindolacético

HPLC - *High-performance liquid chromatography (*Cromatografia líquida de alta eficiência)

HVA – Ácido homovanílico

M3C – N-{2-[5-methoxi-1-(4-metoxifenil)-1H-indol-3-il]-etil}-acetamida)

MPP^+-1-metil-4-fenilpiridínio

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

NA – Noradrenalina

NSQ – Núcleo supraquiasmático

REM – Rapid eye movement

SNpc – Substância negra pars compacta

SOD – Superóxido dismutase

SUMÁRIO

1. INTRODUÇÃO

1.1. DOENÇA DE PARKINSON

A Doença de Parkinson (DP) é caracterizada por uma degeneração dos neurônios dopaminérgicos da substância negra pars compacta (SNpc), evidenciada macroscopicamente por uma despigmentação na porção ventrolateral desta estrutura (LANG & LOZANO, 1998). As características fisiopatológicas são referentes à redução da aferência dopaminérgica para o estriado dorsal (caudado/putâmen) levando a uma redução na neurotransmissão dopaminérgica, caracterizada por uma menor capacidade de liberação de dopamina (DA), bem como redução dos níveis estriatais de seus principais metabólitos como o ácido homovanílico (HVA) e ácido 3,4-dihidroxifenilacético (DOPAC) (LANG & LOZANO, 1998; LANE & DUNNET, 2008). Outra característica fisiopatológica importante é a presença de inclusões intracitoplasmáticas neuronais de caráter eosinofílico, organizadas em um núcleo denso de hialina e circundadas por um halo claro, medindo aproximadamente 15 μm de diâmetro denominadas corpúsculos de Lewy, essas inclusões são constituídas por vários agregados protéicos contendo α-sinucleína, ubiquitina, parkina e neurofilamentos (LANG & LOZANO, 1998). O acúmulo de α-sinucleína e a formação dos corpúsculos de Lewy não são achados exclusivos da DP, sendo encontrados em outras doenças neurodegenerativas como a doença de Alzheimer (LANG & LOZANO, 1998; DAUER & PRZEDBORSKI, 2003). O mecanismo de formação dos corpúsculos de Lewy, a importância na patogênese da DP e a função no processo neurodegenerativo ainda não são conhecidos (LANG & LOZANO, 1998). Entretanto a presença de corpúsculos de Lewy na SNpc é utilizado como um marcador fisiopatológico no diagnóstico *post mortem* da DP, juntamente com a neurodegeneração nigroestriatal (DUNNET & BJORKLUND, 1999; SHARMA, *et al*., 2013).

1.2. ETIOLOGIA DA DOENÇA DE PARKINSON

Apesar de se conhecer os sintomas presentes na DP, a etiologia e os mecanismos que levam à neurodegeneração dopaminérgica ainda não são completamente conhecidos. De fato, evidências sugerem que a grande maioria dos casos ocorre pela interação de fatores ambientais e susceptibilidade genética (SUBRAMANIAM & CHESSELET, 2013), entretanto, outros fatores também contribuem para a neurodegeneração dopaminérgica na DP, como a disfunção mitocondrial, estresse oxidativo e neuroinflamação.

1.2.1. FATORES AMBIENTAIS

O envolvimento de toxinas ambientais na patogênese da DP tem sido muito investigado. Especialmente após a descoberta que a administração da substância 1-metil-4-fenil-1,2,3,6-tetraidropiridina (MPTP) leva ao aparecimento de sintomas parkinsonianos pela neurodegeneração nigroestriatal (LANGSTON & BALLARD, 1983). Desde então toxinas tem sido utilizadas sistematicamente para mimetizar a DP em modelos animais. Também tem sido observado que a exposição ocupacional crônica a metais pesados, como chumbo, manganês e cobre, está relacionada com o aparecimento de sintomas da PD (GORELL, *et al*., 2004; ELBAZ & TRANCHANT, 2007). Do mesmo modo, diversos estudos epidemiológicos têm mostrado que a exposição à pesticidas, está relacionada com o aumento no risco para desenvolver PD (RITZ & YU, 2000; PRIYADARSHI, *et al*., 2001; GORELL, *et al*., 2004; HANCOCK, *et al.,* 2008).

Uma meta-análise com 16 estudos analisou fatores ambientais relacionados ao aparecimento da DP. Destes, 14 demonstraram um aumento no risco para essa doença em indivíduos que foram expostos á pesticidas (PRIYADARSHI, et al., 2001). Resultados semelhantes foram obtidos em outro estudo com 319 pacientes sendo observada uma correlação positiva entre a DP e exposições à pesticidas (HANCOCK, *et al*.,2008). Os autores relataram que os organoclorados, organofosforados, ésteres/ácidos de clorafenoxi e a rotenona são os pesticidas mais relacionados com um aumento no risco para o aparecimento do parkinsonismo. A exposição à pesticidas também influencia no aumento da mortalidade de pacientes com DP, relacionando esse efeito com uma maior utilização de pesticidas

comparado a locais onde não havia uso dessas substâncias (RITZ & YU, 2000). A administração crônica do herbicida rotenona, em ratos, gera neurodegeneração dopaminérgica nigroestriatal associado a comportamentos parkinsonianos, como hipocinesia e rigidez (BETARBET, *et al*., 2000), desta forma, demonstrando que a inibição do complexo I mitocondrial, promovida por essa substância assim como por outras neurotoxinas, pode contribuir para a patogênese da DP. Entretanto, as chances da rotenona ser a principal causa da DP são baixas, pois esse herbicida apresenta baixa biodisponibilidade por via oral e rápida degradação no meio ambiente (SHERER, *et al*., 2002).

1.2.2. MODELO ANIMAL DE PARKINSONISMO INDUZIDO POR ROTENONA

A rotenona é um herbicida de origem natural, presente em extratos das raízes de *Lonchocarpusutilise Lonchocarpus urucu* (CABONI, *et al*., 2004). A citotoxicidade da rotenona se deve à sua ação na cadeia transportadora de elétrons, inibindo o complexo I mitocondrial, gerando, assim, o aumento de EROs no organismo, consequentemente intensificando o estresse oxidativo e levando a apoptose (Tada-Oikawaa, *et al*., 2003). A rotenona é altamente lipossolúvel e atravessa com facilidade a barreira hematoencefálica, e diferentemente do MPTP, a rotenona não depende do transportador de dopamina (DAT) para atravessar as membranas celulares (SHERER, *et al*., 2002). O modelo animal utilizando rotenona foi proposto por Betarbet *et al.*(2000), e foi demonstrado que a administração crônica, em ratos, dessa neurotoxina levou a redução seletiva de neurônios dopaminérgicos nigroestriatais, além de apresentar formação de inclusões citoplasmáticas fibrilares contendo ubiquitina e α-sinucleína, como os corpúsculos de Lewy (BETARBET, *et al*., 2000), achado não encontrado nos modelos animais de MPTP e 6-OHDA.

Diversos estudos têm demostrado alterações neuroquímicas e neuropatológicas na via nigroestriatal após a administração de rotenona, além de alterações motoras no teste o campo aberto, como redução na frequência de locomoção e de levantar, e aumento no tempo de imobilidade (LIN, *et al*., 2008; MOREIRA, *et al*., 2012). Prejuízos cognitivos também foram relatados após a administração intranigral dessa neurotoxina (DOS SANTOS, *et al*., 2013). Além de gerar redução nos níveis de DA, 5-HT e da imunoreatividade para a tiroxina hidroxilase nigral (CABONI, *et al*., 2004; SARAVANAN, *et al*., 2007; LIN, *et al*., 2008;

SANTIAGO, *et al*., 2010; MOREIRA, *et al*., 2012), entretanto a noradrenalina (NA) não foi alterada (SANTIAGO, *et al*., 2010). A administração de rotenona diretamente no feixe procencéfalico medial é capaz de promover a redução progressiva e concentração-dependente de neurônios dopaminérgicos, assim como a geração de agregados protéicos (RAVENSTIJN, *et al*., 2008). Estudos *in vitro* também verificaram a citotoxicidade dessa neurotoxina em reduzir o potencial de membrana mitocondrial e aumentando a produção de radicais livres (RADAD, *et al*., 2006). Também foi observado que a rotenona, assim como outras neurotoxinas (MPTP e 6-OHDA), geram comportamento tipo-depressivo em ratos, quando avaliados no teste de natação forçada e no teste de preferência a sacarose (SANTIAGO, *et al*., 2010)**.** Esses dados demostram que a rotenona, uma toxina exógena, pode mimetizar características da DP, como neurodegeneração dopaminérgica seletiva, formação de corpúsculos de Lewy, inibição do complexo I mitocondrial, comportamento tipo-depressivo e apresenta relevância por ser um modelo de exposição ambiental ao herbicida (SANDERS & GREENAMYRE, 2013). Desta forma, a indução do parkinsonismo pela rotenona é um modelo animal apropriado para DP, pois que gera características patológicas e comportamentais presentes na doença (HAUSER & HASTINGS, 2013).

1.2.3. ESTRESSE OXIDATIVO E DISFUNÇÃO MITOCONDRIAL

A mitocôndria atua na produção de adenosina trifosfato (ATP) através da cadeia respiratória mitocondrial, que contém diversos complexos de enzimas (complexos I, II, III e IV) responsáveis pela fosforilação oxidativa (SUBRAMANIAM & CHESSELET, 2013). E a inibição do complexo I gera o aumento do estresse oxidativo celular, caracterizado pela produção de espécies reativas de oxigênio (EROs) e radicais livres, sendo a principal fonte de formação desses compostos. A inibição do complexo I e o estresse oxidativo, são alguns dos mecanismos propostos para a neurodegeneração dopaminérgica observada na DP (KHANDHAR & MARKS, 2007; SARAVANAN, *et al*., 2007). Estudos utilizando encéfalos *post-mortem* de pacientes com DP (SANDERS & GREENAMYRE, 2013)*, e* modelos animais (MAYO, *et al*., 2005), demonstram a presença de um intenso dano oxidativo por EROs e radicais livres. Foi observada a inibição das enzimas da cadeia respiratória

mitocondrial após administração de 6-OHDA em ratos (DABBENI-SALA, *et al*., 2001), enquanto que um modelo crônico de MPTP em camundongos alterou os níveis da enzima antioxidante endógena, superóxido dismutase (SOD), além de reduzir os níveis de ATP mitocondrial no estriado (PATKI & LAU, 2011). Esses efeitos também demonstrados em um modelo animal de DP induzido pela rotenona (SARAVANAN, *et al*., 2007). Assim, esses dados sugerem que a disfunção na cadeia respiratória mitocondrial, especialmente do complexo I, e a subsequente formação de radicais livres pode contribuir diretamente ou indiretamente na patogênese da DP (SUBRAMANIAM & CHESSELET, 2013).

1.3. DISTÚRBIOS MOTORES DA DOENÇA DE PARKINSON

A DP é caracterizada pela presença de três sinais motores, conhecidos como cardinais da doença, sendo a rigidez muscular, tremor em repouso e bradicinesia (BARTELS & LEENDERS, 2009). A rigidez é o sinal dominante na maioria dos pacientes, e caracteriza-se como o mais incapacitante. O tremor se refere ao aumento da resistência a movimentos passivos dos membros (BARTELS & LEENDERS, 2009). Ocorrendo no repouso e diminui com os movimentos voluntários. E a bradicinesia define-se como a redução ou lentificação dos movimentos, assim o paciente leva mais tempo para realizar tarefas diárias, como se alimentar e se vestir (DAUER & PRZEDBORSKI, 2003). A bradicinesia pode levar ao aparecimento de sinais secundários como a hipofonia (voz fraca e baixa), micrografia (a letra se torna menor), hipersalivação e pouca expressão facial (*masked faces*) (BARTELS & LEENDERS, 2009). O paciente com DP também pode apresentar outras alterações motoras como anormalidades posturais e de marcha, podendo levar a quedas, e o congelamento na marcha, (*freezing of gait)*, que se caracteriza pela inabilidade do paciente em iniciar um movimento voluntário, como caminhar (DAUER & PRZEDBORSKI, 2003), gerando redução na mobilidade e qualidade de vida desse pacientes (GEORGY, 2010). O surgimento dos primeiros achados clínicos motores acontece após a ocorrência de uma redução da ordem de 60-70% dos neurônios dopaminérgicos da SNpc (FAHN, 2003). Apesar do avanço da idade ser um fator de risco para a DP, a perda de neurônios dopaminérgicos na

DP apresenta uma topografia diferente do que é visto em idosos saudáveis (DAUER & PRZEDBORSKI, 2003).

1.4. DISTÚRBIOS NÃO MOTORES DA DOENÇA DE PARKINSON

Pacientes com DP também apresentam distúrbios não motores como alterações neuropsiquiátricas (depressão, ansiedade, apatia), distúrbios do sono (insônia, síndrome das pernas inquietas, distúrbios comportamentais do sono REM, sonolência diurna excessiva), distúrbios olfatórios e gastrointestinais (LIMA, 2013) (CHAUDHURI & SCHAPIRA, 2009). O aparecimento destes distúrbios podem surgir anos antes das manifestações clínicas da PD, podendo ser considerados distúrbios pré-motores, portanto, sendo de grande valor preditivo (BARTELS & LEENDERS, 2009). Pacientes com DP que desenvolvem alterações não motoras apresentam grande redução na qualidade de vida, demonstrando ser de grande importância o diagnóstico preciso e rápido destes distúrbios, além do tratamento adequado (CHEN & MARSH, 2013). Shulman *et al*. (2002) demostraram em um estudo clínico, que neurologistas não identificaram os distúrbios não motores como ansiedade, depressão e distúrbios do sono em 40-75% dos pacientes com DP, sendo estes distúrbios são na maioria das vezes sub-diagnosticados e não tratados (SHULMAN, *et al*., 2002).

1.4.1. DISTÚRBIOS DE SONO

Os distúrbios de sono têm ganhado papel de destaque na DP (LIMA, *et al*., 2012), haja visto que são comumente presentes nesses pacientes. Distúrbios de sono como os distúrbios comportamentais associados ao sono REM, distúrbio comportamental do sono REM, e fragmentação de sono são frequentemente relacionados à sinucleinopatias como a doença dos corpúsculos de Lewy, atrofia sistêmica múltipla e a própria DP (POSTUMA, *et al*., 2009, POSTUMA & MONTPLAISIR, 2011). Aproximadamente 50% dos pacientes que possuem distúrbio comportamental do sono REM há pelo menos uma década irão eventualmente desenvolver uma ou mais dessas doenças (POSTUMA & 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 poderá resultar no parkinsonismo.

1.4.2. ANSIEDADE

A ansiedade é um distúrbio neuropsiquiátrico importante e está presente em 40-55% dos pacientes com DP, entretanto, pouca atenção tem sido dada a esse distúrbio não motor (QUELHAS & Costa, 2009; Walsh & Bennett, 2001). Os pacientes com DP podem apresentar diversos tipos de ansiedade, como transtorno da ansiedade generalizada, transtorno do pânico, fobia social, agorafobia e transtorno obsessivo compulsivo (CHAUDHURI & SCHAPIRA, 2009; LIMA, *et al*., 2012). Entretanto, em um estudo com 127 pacientes com DP foi observado que a fobia social e o transtorno do pânico foram mais prevalentes nesses pacientes (PONTONE, *et al*., 2009). Os autores observaram que o transtorno do pânico está associado ao aparecimento precoce da DP, podendo ser utilizado como um marcador para esse fenótipo da doença (PONTONE, *et al*., 2009). A presença de ansiedade não gera apenas alterações no humor, mas também pode agravar os distúrbios motores preexistentes como o tremor, a discinesia e a instabilidade postural, sendo de grande importância uma terapia ansiolítica adequada (CHEN & MARSH, 2014).

Diversas hipóteses relacionam a grande prevalência da ansiedade na DP, mas, no entanto, pouco se sabe sobre o assunto. Sugere-se que esse distúrbio de humor está relacionado com o grande estresse e a incapacidade que a DP gera nos pacientes (CHEN & MARSH, 2014), sendo observado maior severidade na ansiedade de pacientes com DP quando comparado com pacientes sem DP e com os controles (MENZA, *et al.*, 1993; SHIBA, *et al*., 2000). Também tem sido investigada a presença de transtornos de ansiedade pelo uso de medicamentos anti-parkinsonianos. De fato, ratos lesionados com 6-OHDA e que receberam tratamento crônico com L-DOPA, apresentaram comportamento tipo-ansiogênico (JAUNARAJS, *et al*., 2012). Entretanto, em pacientes com DP foi visto que o uso de L-DOPA não alterou a ansiedade (MENZA, *et al*., 1993). Outra hipótese sugere o envolvimento das alterações neuroquímicas e da neurodegeneração, que ocorrem durante a DP, na patogênese da ansiedade na DP, pois, os pacientes apresentam ansiedade anos

antes do aparecimento dos sinais motores, sendo considerada uma manifestação pré-motora (SHIBA, *et al*., 2000). A associação entre a severidade da DP e ansiedade sugere um possível processo fisiopatológico em comum. Alterações no DAT e perda de neurônios dopaminérgicos estão associadas com a ansiedade na DP (ERRO, *et al*., 2012; PREDIGER, *et al*., 2012). Entretanto, sugere-se que a patogênese da ansiedade não esteja relacionada apenas com a perda de neurônios dopaminérgicos. Na DP também ocorre a neurodegeneração de estruturas não dopaminérgicas, como demonstrado por Braak e colaboradores, sendo observada que a neurodegeneração serotoninérgica dos núcleos da rafe e noradrénergica do locus coeruleus, ocorrem antes da perda de neurônios dopaminérgicos nigroestriatais (BRAAK, *et al*., 2004). Assim, a interação entre a neurodegeneração do sistema dopaminérgico, serotoninérgicos e noradrenérgicos pode deflagrar na ansiedade na DP (MENZA, *et al.*, 1993).

1.4.3. DEPRESSÃO

Outra importante comorbidade na DP é a depressão, estando presente em 57% dos pacientes (ROJO, *et al*., 2003), enquanto que em um estudo realizado no Brasil, com 50 pacientes com DP, a depressão foi diagnosticada em 34% destes (STELLA, *et al.*, 2008). O nível de severidade da depressão varia de acordo com o estudo, sendo relatada depressão leve em 82% dos pacientes, e depressão moderada a severa varia entre 8,7% (PRADO & BARBOSA, 2005) e 60% (QUELHAS & Costa, 2009). Também foi observada a associação entre a severidade da depressão e o aumento da incapacidade de realizar atividades diárias em pacientes com DP pela redução da autonomia (STELLA, *et al.*, 2008). Hipóteses relacionam o aparecimento da depressão na DP como um fator secundário gerado pelo estresse psicossocial que os distúrbios motores geram no paciente (MCDONALD, *et al.*, 2003). Contudo, o aparecimento da depressão antes do início dos sinais motores característicos da DP sugere que esse distúrbio do humor seja devido às alterações neuroquímicas e neuroanatômicas presentes na DP. A degeneração da SNpc, que projeta vias para o estriado e áreas mesocorticais e mesolímbicas, resulta da redução de DA, 5-HT e NA, implicando no aparecimento da depressão e outros distúrbios (MCDONALD, *et al*., 2003). Um estudo demonstra uma maior neurodegeneração no locus coeruleus e na SNpc em pacientes com DP

e depressão, comparado com pacientes com DP sem depressão (FRISINA, *et al.*, 2009). Foi descrita a redução de ligação ao [^{11}C]-RTI-32, um ligante para o DAT e para o transportador de NA, no locus coeruleus e no sistema límbico de pacientes com DP e depressão, comparado a pacientes sem depressão (REMY, *et al*., 2005). Estes estudos sugerem um maior envolvimento da NA e DA com a depressão na DP. Entretanto, também foi visto o envolvimento da 5-HT, sendo descrito concentrações reduzidas de ácido 5-hidroxi-indolacético (5-HIAA), o metabólito da 5-HT, no líquido cefalorraquidiano de pacientes com DP e depressão (MAYEUX, *et al*., 1988).

Apesar de drogas dopaminérgicas apresentarem efeitos antidepressivos e ansiolíticos (CHAUDHURI & SCHAPIRA, 2009), nem todos os pacientes respondem a esse tratamento (MCDONALD, *et al*., 2003). Sendo assim, são necessárias novas intervenções terapêuticas para o tratamento desses sintomas em pacientes com DP (CHAUDHURI, *et al*., 2011). Dentro desse contexto, observa-se que a privação de sono total em pacientes parkinsonianos, melhorou os parâmetros associados à depressão por um período de aproximadamente uma semana (BERTOLUCCI, *et al.*, 1987). Portanto, sugere-se um forte envolvimento do sono, particularmente do sono REM (*rapid eye movement*) (VOGEL, *et al*., 1980), nos eventos associados à regulação do humor, haja vista a hipótese levantada por Vogel (1983) que sugere que um dos mecanismos desencadeados pelos antidepressivos seja em decorrência da redução na porcentagem dessa fase do sono. Observa-se que após a privação de sono REM (PSREM) os pacientes apresentaram melhoras nos sintomas depressivos, que foram potencializadas pelo tratamento com sertralina (GORGULU & CALIYURT, 2009). Além disso, verificou-se um importante efeito tipo-antidepressivo desencadeado pela PSREM (por 48h) num modelo animal de depressão induzido por bulbectomia olfatória em ratos (MATURANA, *et al*., 2014). Tal resposta foi correlacionada com um aumento na expressão nigral do fator neurotrófico derivado do encéfalo (BDNF), assim como por um aumento dos níveis hipocampais de 5-HT. Outras intervenções e alvos terapêuticos têm sido investigados para o tratamento dos distúrbios não motores presentes na DP. A administração de melatonina demonstrou modular o sono de animais (HOLMES & SUGDEN, 1982; Ying, *et al*., 1996; AKANMUA, *et al*., 2004; FISHER & SUGDEN, 2009). Assim como apresenta um importante papel no tratamento de distúrbios de sono em pacientes com DP (DOWLING, *et al.,* 2005; MEDEIROS, *et al.,* 2007).

Deste modo, a relação entre distúrbios de sono, ansiedade e depressão na DP (HAVLIKOVA, *et al*., 2011), bem como o uso de melatonina nesse contexto, tem se apresentado como uma possível estratégia terapêutica.

2. MELATONINA

2.1. CARACTERÍSTICAS GERAIS

A melatonina é um neurohormônio pertencente ao grupo das indolaminas, e está envolvida na sinalização do ciclo claro-escuro e nos biorritmos sazonais (DUBOCOVICH, *et al*., 1996; CARPENTIERI, *et al*., 2012). Outra função importante desse neurohormônio, descrita em 1994, é de ser um potente agente antioxidante (POEGGELER, *et al*., 1994). Consequentemente, a função da melatonina tem sido intensamente investigada (sendo encontrados 15.541 artigos publicados buscando as palavras-chave melatonina + antioxidante no PubMed). Essa aplicação encontra especial interesse no contexto das doenças neurodegenerativas como na doença de Alzheimer (MAYO, *et al*., 2005; PANDI-PERUMAL, *et al*., 2008) e na DP (ACURIA-CASTROVIEJO, *et al*., 1997; DABBENI-SALA, et al., 2001; ANTOLÍN, *et al*., 2002; SHARMA, *et al*., 2006). Foi observado em estudos clínicos e pré-clínicos, que a melatonina reduziu os distúrbios motores da DP (CAPITELLI, *et al*., 2008; LIN, *et al*., 2008; PANDI-PERUMAL, *et al*., 2013). Além disso, a melatonina atua inibindo o crescimento de tumores (ANINIMOV, *et al*., 2006), aumentando a neurogênese (CHERN, *et al*., 2012; RUKSEE, *et al*., 2014), atuando na fototransdução (HUANG, *et al*., 2013) e apresentando efeito anti-apoptótico (Wang, 2009) e anti-nociceptivo (YU, *et al*., 2000).

A melatonina é sintetizada e liberada pela glândula pineal, entretanto sua produção não está limitada a essa glândula, sendo produzida, em menor quantidade, em outros órgãos e tecidos como na retina, ossos, pele, trato gastrintestinal, plaquetas e linfócitos humanos (DUBOCOVICH & MARKOWSKA, 2005). A presença dessa indolamina também foi descrita em invertebrados, como protozoários, plantas, bactérias, fungos e algas (HARDELAND & POEGGELER, 2003; HARDELAND, *et al.,* 2006). Os níveis plasmáticos da melatonina apresentam uma ritmicidade circadiana, sendo que encontramos níveis elevados durante a noite, com pico máximo às 3:00-4:00h, e níveis baixos durante o dia (RAJARATNAM, *et*

al., 2009). A melatonina não é armazenada, sendo assim, após a sua síntese é liberada diretamente na corrente sanguínea (DELAGRANGE, *et al*., 2003). Por apresentar caráter anfifílico, a melatonina é bem absorvida, e através de difusão passiva passa da circulação periférica para diversos tecidos (CARDINALI & PEVET, 1998)**.** Entretanto, apresenta uma farmacocinética desfavorável, com elevado metabolismo de primeira passagem e um tempo de meia vida curto (20-30 min) (Zlotos, *et al*., 2013). Após passar por uma hidroxilação hepática, formando a 6-hidroximelatonina, a melatonina é eliminada, pela urina, de forma conjugada com um grupamento sulfóxido (Von Gall, *et al*., 2002).

2.2. SÍNTESE DA MELATONINA

A melatonina tem como precursores para a sua síntese o triptofano e a 5-HT. O processo enzimático ocorre a partir da hidroxilação do aminoácido aromático triptofano pela triptofano-hidroxilase com a formação de 5-hidroxitriptofano. A 5-HT é formada pela ação da enzima L-aminoácido descarboxilase. A N-acetiltranferase é responsável pela n-acetilação da 5-HT em N-acetilserotonina. A última etapa da síntese é a metilação catalisada pela hidroxiindole-o-metil-transferase transformando a N-acetilserotonina em melatonina (CARDINALI & PEVET, 1998; CARPENTIERI, *et al*., 2012). A síntese da melatonina é controlada pelo ciclo claro-escuro através do marca-passo biológico presente no NSQ. A informação do fotoperíodo chega ao NSQ através na via retino-hipotalámica (Figura 1), projeções oriundas da retina passam a informação de presença ou ausência de luz para o NSQ, sendo que a presença de luz inibe a síntese de melatonina no organismo (CARDINALI & PEVET, 1998).

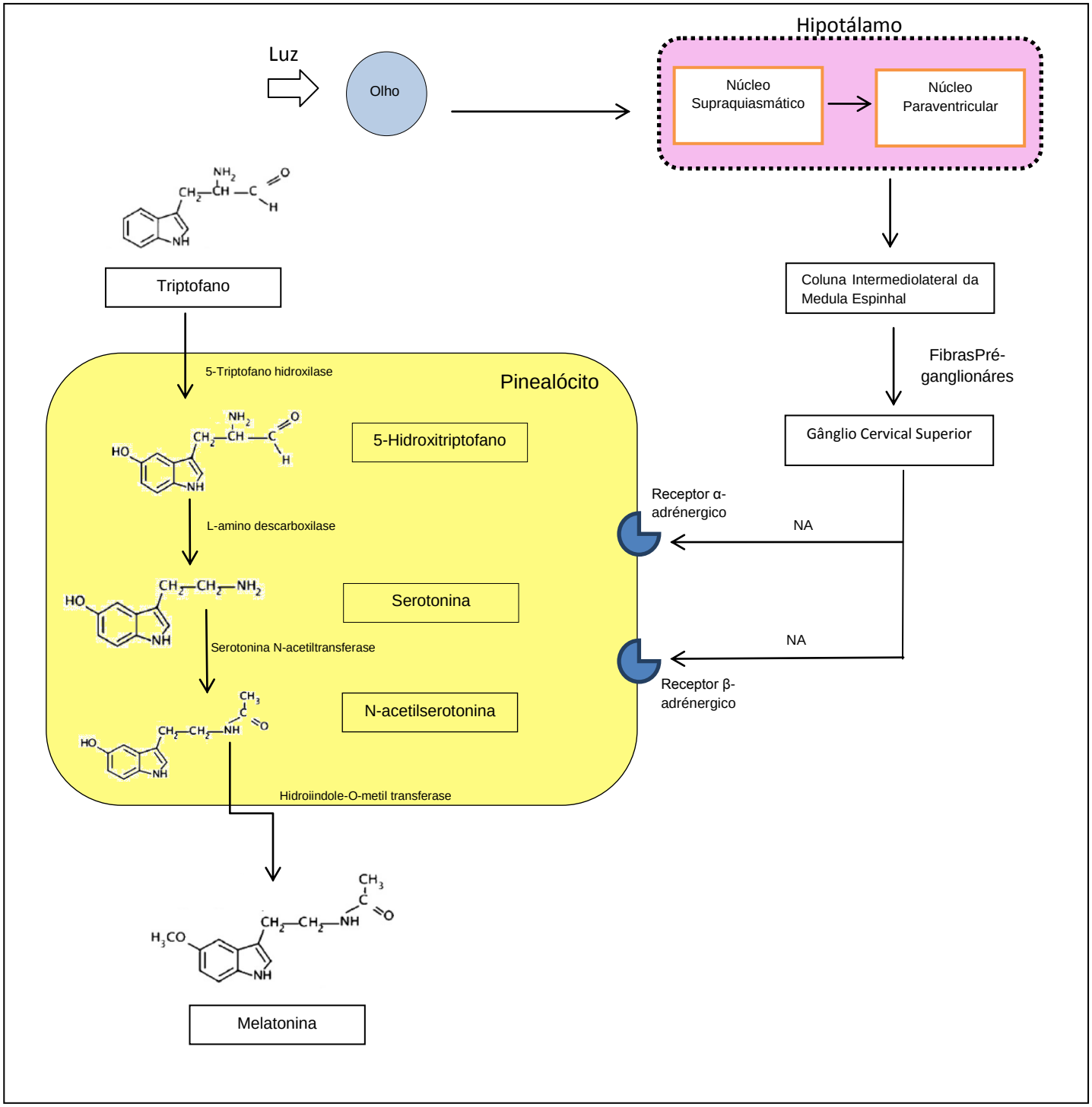

Figura 1. Esquema representativo da síntese de melatonina pela glândula pineal de mamíferos. Esquema com a via retino-hipotalâmica. A luz incide sobre a retina, que envia informação para o núcleo supraquiasmático (NSQ). Este se projeta sobre o núcleo paraventricular (PVN), que projeta-se sobre a coluna intermediolateral da medula espinhal. Fibras pré-ganglionares simpáticas projetam-se sobre o gânglio cervical superior, que, por sua vez projeta-se sobre a pineal. O neurotransmissor simpático, noradrenalina, participa da síntese de melatonina, cuja via biossíntética é representada ao lado.

2.3. RECEPTORES MELATONINÉRGICOS

A melatonina atua na ativação de receptores transmembrana acoplados à proteína G, classificados como MT1 e MT2. Esses receptores atuam na transdução de cascatas de sinais intracelulares desencadeados pela ativação da adenilatociclase, fosfolipase C, fosfolipase A2, canais de potássio e de cálcio (CARPENTIERI, *et al.*, 2012). Os receptores MT1 foram encontrados no encéfalo, sistema cardiovascular, sistema imunológico, testículos, ovários, pele, fígado, rins, córtex da adrenal, placenta e retina. No sistema nervoso central está presente no hipotálamo, área ventral tegmental, cerebelo, hipocampo e na substância negra (PANDI-PERUMAL, *et al*., 2008). Esse receptor apresenta 350 aminoácidos em sua estrutura, e está acoplado à proteína Gi ($Gi_{\alpha 2}$, $Gi_{\alpha 3}$ e $G_{q/11}$) (Slominski, *et al*., 2012). Estudos com animais nocaute para MT1 encontraram alterações no comportamento tipo-depressivo no teste de natação forçada (Weil, *et al*., 2006). Em camundongos da linhagem "*White-footed"* foi observado o envolvimento do receptor MT1 no controle da liberação de melatonina, sugerindo um novo papel regulatório para esse tipo de receptor (BEDROSIAN, *et al*., 2013).

Os receptores MT2 apresentam 363 aminoácidos em sua estrutura, e 60% de homologia com o receptor MT1 (Slominski, *et al*., 2012). Estão presentes no sistema imunológico, vasos sanguíneos, testículos, rins, trato gastrointestinal, glândulas mamárias, tecido adiposo e pele (PANDI-PERUMAL, *et al*., 2008). Também se encontram no NSQ e no estriado (DUBOCOVICH & MARKOWSKA, 2005). Em um modelo animal acidente vascular cerebral isquêmico (CHERN, *et al*., 2012), a administração de melatonina aumentou a neurogênese, reduziu a inflamação e a formação de radicais livres, entretanto, os efeitos da melatonina foram inibidos com a administração de 4-P-PDOT, um antagonista seletivo para receptores MT2. Dessa forma, sugere-se o envolvimento desses receptores na ação neuroprotetora da melatonina, já verificada em outros modelos animais, inclusive da DP (Capitelli *et al*., 2008).

Foi descrito por Nosjean e cols. a existência de um terceiro do receptor, o MT3, por meio de purificação de amostras de rins de hamsters. Diferentemente dos outros dois receptores, o MT3 apresenta baixa afinidade com a melatonina (NOSJEAN, *et al*., 2000; NOSJEAN, *et al*., 2001), e pertence ao grupo de receptores acoplados à enzima quinona redutase II, enzima envolvida no processo de

desintoxicação do organismo e na proteção contra danos oxidativos (PANDI-PERUMAL, *et al*., 2008). Apesar de apresentar 95% de homologia com a quinona-redutase 2 humana, o receptor MT3 ainda não foi isolado em humanos (Slominski, *et al*., 2012).

2.4. DOENÇA DE PARKINSON E MELATONINA

Diversos estudos mostraram efeitos neuroprotetores da melatonina no sistema nigroestriatal em modelos animais de parkinsonismo induzidos pela 6-OHDA (SHARMA, *et al*., 2006; GUTIERREZ-VALDEZ, *et al*., 2012), MPTP (ACURIA-CASTROVIEJO, *et al*., 1997; CAPITELLI, *et al*., 2008; MA, *et al*., 2009) e rotenona (SARAVANAN, *et al.,* 2007). Entretanto, alguns trabalhos relataram a potencialização da neurodegeneração após administração de melatonina em modelos animais (MORGAN & NELSON, 2001; Tapias, *et al*., 2010). Por outro lado, a administração do neurohormônio através de minibombas osmóticas, em um modelo de parkinsonismo induzido por 6-OHDA, reduziu o número de rotações induzidas pela apomorfina (DABBENI-SALA, *et al*., 2001; SHARMA, *et al*., 2006). A administração de melatonina através da água consumida pelos ratos, em um modelo induzido por 6-OHDA levou a melhora da atividade motora dos animais (GUTIERREZ-VALDEZ, *et al*., 2012). Esse efeito também foi visto em um modelo induzido por MPTP intranigral, apresentando melhora na locomoção após o grupo receber uma injeção intraperitoneal de melatonina (CAPITELLI, *et al*., 2008). Em contraste com estes dados, foi demonstrada a piora da atividade locomotora após tratamento com melatonina em presença de 6-OHDA, enquanto que a sua supressão, por pinealectomia ou luz constante, reduziu os distúrbios motores nesses animais (WILLIS & ARMSTRONG, 1999).

Foram observadas diferenças nos níveis plasmáticos de melatonina entre jovens e idosos, estando em menor concentração em idosos, principalmente nos que apresentavam diagnóstico de demência (MAGRI, *et al*., 2004). Corroborando com esse estudo, foi observado um declínio progressivo da melatonina endógena com o aumento da idade, sendo que idosos com média de 80 anos apresentavam menores níveis comparados com pacientes com média de 60 anos de idade (GRAHAM & MCLHLAN, 2004). Já em pacientes com DP foi observado níveis do neurohormônio aumentados no início da manhã (LIN, *et al*., 2014). Também foi

observado a redução na expressão de receptores MT1 e MT2 na substância negra e amígdala de pacientes com DP, sendo assim, os autores sugerem um possível envolvimento da melatonina no mecanismo fisiopatológico da DP (ADI, *et al*., 2010). A presença de receptores MT1 e MT2 no estriado foi descrito primeiro em camundongos (Uz, *et al*., 2005), e mais recentemente em ratos (SHARMA, *et al*., 2006). Níveis de melatonina no estriado de ratos apresentam um padrão circadiano, aumentando durante a noite e diminuindo durante o dia. Ainda, observam-se níveis elevados durante as quatro primeiras semanas pós-lesão nigroestriatal induzida por 6-OHDA. Possivelmente esse aumento seja resultado de um efeito compensatório para proteger os neurônios dopaminérgicos (LIN, *et al*., 2013).

2.5. EFEITOS DA MELATONINA NA REGULAÇÃO DO SONO

Tem sido investigado o envolvimento do sistema melatoninérgico nos mecanismos dos distúrbios de sono da DP, assim como na fisiopatologia dessa doença (Srinivasan, *et al*., 2011). Diversos estudos têm demonstrado a importância da melatonina na iniciação e qualidade do sono, assim como no tratamento dos distúrbios de sono, como sonolência diurna, latência do sono, redução do tempo total de sono, distúrbio comportamental do sono REM e a baixa qualidade do sono presente em pacientes com DP (KUNZ, *et al*., 2004). Como demonstrado em um estudo com 40 pacientes com DP, a administração de melatonina (5mg e 50 mg) por via oral, 30 minutos antes do horário habitual de dormir durante 14 dias, melhorou os distúrbios de sono desses pacientes. Foi observada a melhora na quantidade de sono e a redução da sonolência diurna (DOWLING, *et al*., 2005). Em outro estudo, 22 pacientes com DP receberam 3 mg de melatonina 1h antes do horário de dormir, durante 28 dias (MEDEIROS, *et al*., 2007). A avaliação da qualidade de sono desses pacientes foi realizada pelo índice de qualidade de sono de Pittsburgh (*Pittsburgh Sleep Quality*) e a sonolência diurna pela escala de sonolência de Epworth (*Epworth Sleepiness Scale*). As análises do sono desses pacientes com DP demonstraram: baixa qualidade de sono (70%), sonolência diurna excessiva (40%), aumento da latência do sono (50%), REM sem atonia muscular (66%) e eficiência de sono reduzida (72%). A administração de melatonina melhorou a qualidade do sono, sem melhoras motoras aparentes (MEDEIROS, *et al*., 2007). Em um trabalho que buscou

avaliar os efeitos da melatonina em 14 pacientes com redução de sono REM (redução de 25% ou mais), foi observado que a administração desse neurohormônio aumentou a porcentagem e continuidade do sono REM. Os pacientes relataram no estudo a redução da fadiga diurna e aumento da sonolência durante a noite, melhorando o bem estar desses pacientes (KUNZ, *et al*., 2004)

2.6. EFEITOS DA MELATONINA NA ANSIEDADE

Foi demonstrado, em estudos pré-clínicos, que a melatonina apresenta efeitos ansiolíticos, sendo observado o aumento do comportamento exploratório nos braços abertos do labirinto em cruz elevado (GOLOMBEK, *et al*., 1993; KARAKAS, *et al*., 2011; NAVA & CARTA, 2001). Entretanto, há limitações no uso da melatonina devido ao seu curto tempo de meia vida, sendo assim outras moléculas melatoninérgicas têm sido testadas. Agonistas melatoninérgicos como o Neu-P11 (Tian, *et al*., 2010), MC3 (BUSTAMENTE-GARCÍA, *et al.,* 2014) e a agomelatina (LOISEAU, *et al*., 2006), demonstraram efeitos ansiolíticos em modelos animais, além de apresentarem tempo de meia vida mais longo e maior afinidade pelos receptores melatoninérgicos (Tian, *et al*., 2010). As propriedades ansiolíticas da melatonina podem estar relacionadas com diversos neurotransmissores, sendo observado aumento na concentração encefálica de 5-HT após a administração de melatonina em ratos (COTZIAS, *et al*., 1971). A interação com o sistema GABAérgico também foi sugerida. Foi observado que a melatonina aumentou o *turnover* do ácido γ-aminobutírico (GABA) no hipocampo e na glândula pineal, demonstrando um possível envolvimento da melatonina com a síntese e degradação de GABA nessas regiões (ROSENSTEIN & CARDINALI, 1986). Sua atividade ansiolítica foi, por outro lado, inibida com a co-administração de flumazenil, um antagonista benzodiazepínico, reforçando a ideia de um possível envolvimento com o sistema GABAérgico (GOLOMBEK, *et al.*, 1993). Outro agonista, o M3C (N-{2-[5-methoxi-1-(4-metoxifenil)-1H-indol-3-il]-etil}-acetamida), demonstrou atividade ansiolítica no teste de esconder esferas e no LCE em ratos pinealectomizados, sendo mais efetivo em reduzir a ansiedade que a própria melatonina (BUSTAMENTE-GARCÍA, *et al.*, 2014). O M3C apresenta maior afinidade por receptores MT2, sugerindo um possível envolvimento desses receptores nos

processos associados com a ansiedade. De fato, diversos estudos demonstram o envolvimento de receptores MT2 com a ansiedade e também com a depressão (DUBOCOVICH, *et al*., 1990; SUMAYA, *et al*., 2005; OCHOA-SANCHEZ, *et al*., 2012).

2.7. EFEITOS DA MELATONINA NA DEPRESSÃO

Estudos pré-clínicos utilizando ratos, demonstraram que a melatonina apresenta efeitos tipo-antidepressivos no teste de natação forçada (RAGHAVENDRA, *et al*., 2000; ERGUN, *et al*., 2008; RUKSEE, *et al*., 2014). Esse efeito também foi observado no modelo de estresse crônico moderado em camundongos (DETANICO, *et al*., 2009). Entretanto, a administração apenas de melatonina em pacientes não apresenta ação antidepressiva, apesar de ser observada uma melhora no sono destes pacientes com depressão (DOLBERG, *et al*., 1998; DALTON, *et al*., 2000). Por outro lado, a agomelatina um agonista de receptores MT1/MT2 e antagonista de receptores 5HT2C, além de melhorar o sono também demonstrou atividade antidepressiva em pacientes (KENNEDY, *et al*., 2014). Assim, sugere-se que a combinação de mecanismos de ação que envolve o sistema melatoninérgico e serotoninérgico seja capaz de promover efeitos antidepressivos mais intensos.

A desregulação na liberação de melatonina tem sido relacionada com a depressão (DUBOCOVICH, *et al*., 1990). Pacientes com depressão apresentam concentrações elevadas do metabólito da melatonina, 6-sulfatoximelatonina, durante a noite, e baixas durante o dia. Valores contrários ao observado nos controles, demonstrando uma mudança de fase na secreção de melatonina durante a depressão (CRASSON, *et al*., 2004). Nesse sentido, pacientes com DP também mostraram aumentos nos níveis de melatonina (BOLITHO, *et al.*, 2014). Entretanto, os autores sugerem que essa alteração pode estar relacionada com o tratamento dopaminérgico que esses pacientes recebem, evidenciando uma possível relação regulatória entre DA e o sistema melatoninérgico. Ainda, nesse contexto, observam-se níveis elevados de melatonina, no início da manhã, em pacientes com DP comparado aos controles (LIN, *et al.,* 2013). Assim, a melatonina endógena pode estar aumentando a neurodegeneração dopaminérgica em estágios avançados da

doença, enquanto em estágios iniciais esse neurohormônio atua na neuroproteção (LIN, *et al*., 2013).

Tem sido sugerido que alterações nos receptores da melatonina estão relacionadas com o aparecimento dos distúrbios do humor (JOCKERS, *et al*., 2008). Um estudo demonstrou que polimorfismos no gene para o receptores MT2 estão relacionados com o maior risco de pacientes apresentarem o transtorno de depressão recorrente (GALECKA, *et al*., 2011). E foi observado que pacientes com depressão apresentam redução na expressão dos receptores melatoninérgicos no NSQ (WU, *et al*., 2013). Desta forma, é necessário inverstigar o envolvimento das alterações do humor com os receptores da melatonina.

3. JUSTIFICATIVA

A administração de melatonina demonstrou modular o sono de animais (HOLMES & SUGDEN, 1982; Ying, *et al*., 1996; AKANUMA, *et al*., 2004; FISHER & SUGDEN, 2009). Assim como apresenta um importante papel no tratamento de distúrbios de sono em pacientes com DP (DOWLING, *et al*., 2005; MEDEIROS, *et al*., 2007). Deste modo, a associação entre distúrbios de sono e depressão na DP (HAVLIKOVA, *et al*., 2011), bem como a ocorrência de efeitos antidepressivos gerados pela PSREM convergem para um possível papel da melatonina, e, particularmente de seus receptores MT1 e MT2, nesses mecanismos. Complementarmente, uma maior compreensão desses fenômenos pode permitir novas abordagens terapêuticas para a depressão no contexto da DP.

4. OBJETIVOS

4.1. OBJETIVO GERAL

Investigar o papel dos receptores melatoninérgicos MT2 presentes no estriado dorsal, frente às alterações comportamentais e neuroquímicas no modelo animal da DP induzido por rotenona intranigral em ratos.

4.2. OBJETIVOS ESPECÍFICOS

- Analisar os efeitos promovidos pela lesão da via nigroestriatal, induzida por rotenona intranigral, associada à PSREM e a modulação farmacológica pelo agonista MT2 (8-M-PDOT) e o antagonista MT2 (4-P-PDOT), sobre parâmetros de depressão, utilizando o teste de natação forçada modificado.
- Quantificar por cromatografia líquida de alta eficiência (HPLC) os níveis de dopamina (DA), ácido 3,4-diidroxifenilacético (DOPAC), serotonina (5-HT), ácido 5-hidroxindolacético (5-HIAA) e noradrenalina (NA) no estriado, substância negra e hipocampo, após a lesão com rotenona intranigral, associada ao rebote a modulação farmacológica pelo agonista MT2 (8-M-PDOT) e o antagonista MT2 (4-P-PDOT).
- Quantificar por imuno-histoquímica para tirosina hidroxilase (TH) os efeitos sobre o número de neurônios dopaminérgicos na SNpc promovidos pela lesão com rotenona intranigral, associada ao rebote a modulação farmacológica pelo agonista MT2 (8-M-PDOT) e o antagonista MT2 (4-P-PDOT).

5. ARTIGO CIENTÍFICO

Os materiais e métodos[1], resultados e discussão do trabalho encontram-se no artigo científico a seguir.

[1] Trabalho realizado de acordo com os procedimentos descritos na Lei Arouca (Lei nº 11.794, de 08 de outubro de 2008)

Putative role of monoamines in the antidepressant-like mechanism induced by striatal MT2 blockade

Ana Carolina D. Noseda[1], Lais S. Rodrigues[1], Adriano D. S. Targa[1], Mariana H. Fortes[1], Maria A. B. F. Vital[2],Cláudio Da Cunha[2] and Marcelo M. S. Lima[1] ✉

[1] Laboratório de Neurofisiologia. Departamento de Fisiologia. Universidade Federal do Paraná, Curitiba, PR, Brasil;

[2] Laboratório de Fisiologia e Farmacologia do Sistema Nervoso Central. Departamento de Farmacologia. Universidade Federal do Paraná, Curitiba, PR, Brasil;

✉Corresponding author:

Marcelo M. S. Lima, PhD

Universidade Federal do Paraná

Setor de Ciências Biológicas

Departamento de Fisiologia

Av. Francisco H. dos Santos s/n

ZIP: 81.531 – 990

Caixa Postal: 19031

Curitiba – Paraná – Brasil

Phone number: 0055-041-3361 1722

E-mail: mmslima@ufpr.br or marcelomslima.neuro@gmail.com

Abstract

It has been observed that the secretion pattern of melatonin is modified in Parkinson's disease (PD) patients and consequently the exogenous administration of this neurohormone could demonstrate beneficial effects regarding the non-motor alterations, specially mood disorders. It has been hypothesized that dysregulations of melatonin MT2 receptors may be involved in the installation of depression. Together with recent evidence based on the use of the intranigral rotenone model of PD, have led to the hypothesis that modulating the striatal MT2 receptor could provide a more comprehensive understanding of the antidepressant properties triggered. To further investigate this issue, male Wistar rats were infused with intranigral rotenone (12 µg/µL) and seven days later subjected to a rapid eye movement sleep deprivation (REMSD) for 24 h. After, we injected within the striatum the MT2 selective agonist, 8-M-PDOT (10 µg/µL), the MT2 selective antagonist, 4-P-PDOT (5 µg/µL) or vehicle. Subsequently, they were tested in the forced swimming test and were allowed to perform the sleep rebound (REB). Then, the rats were re-tested and striatum, hippocampus and substantianigra pars compacta (SNpc) were collected for neurochemical purposes. Results indicated substantial antidepressant effects promoted by the blockade of striatal MT2 receptors that were potentiated by REMSD. MT2 activation increased DA levels in the striatum and hippocampus, while MT2 blockade increase DA in the SNpc. 4-P-PDOT treatment of the rotenone REMSD group generated a decrement in 5-HT levels within the striatum, hippocampus and SNpc. However, increased 5-HT turnover was observed among these structures. Therefore, we demonstrated the neurochemical antidepressant effect induced by striatal 4-P-PDOT associated with REMSD in the rotenone model of PD.

Keywords: Dopamine, serotonin, 4-P-PDOT, 8-M-PDOT, Parkinson´s disease, rotenone, REM sleep deprivation, substantianigra pars compacta

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 old [1, 2]. It is characterized by major cardinal motor disturbances, namely rigidity, rest tremor and bradykinesia[3].These alterations are the result of the progressive dopaminergic neuronal loss in the substantianigra pars compacta (SNpc) and consequently reductions in the striatal levels of dopamine (DA) [4].PD patients also experience prodromal, non-motor features of the disease, such as neuropsychiatric symptoms (depression, anxiety and apathy), sleep disorders (insomnia, restless legs and periodic limb movements, rapid eye movement (REM) sleep behavior disorder and excessive daytime somnolence) and autonomic dysfunctions [3, 5].These early dysfunctions are supported by neuropathologic studies with Lewy pathology present in non-dopaminergic nuclei in preclinical Braak stages prior to significant nigral degeneration [6].

A number of animal models have been development to research DP and to permit the investigation of new treatments. Rotenone, a specific inhibitor of mitochondrial complex I, that produce behavioral, pathological and biochemical features of PD in rats [7].

There is a growing interest in exploring novel pharmacotherapeutic targets for the treatment of depression in the context of PD [8-10]. Among these emerging targets, rapid eye movement sleep deprivation (REMSD) and melatoninergic drugs have gained considerable attention.

REMSD has been shown to improve depression in humans [11] and other animals models [12]. There is compelling evidence that antidepressant drugs improve depression by REM sleep deprivation [11, 13, 14].In addition, REMSD

increases the levels of brain-derived neurotrophic factor (BDNF) in the SNpc of rats, even after sleep rebound [12].

Melatonin is a neurohormone synthesized and secreted during the dark phase, from the mammalian pineal gland [15, 16].The main roles of melatonin are related to the control of the circadian sleep-wake states, regulation of sleep and seasonal biorhythm [17-19]. Furthermore, it has been observed that the secretion pattern of melatonin is modified in PD patients [20-23] and consequently the exogenous administration of this hormone could demonstrate beneficial effects regarding the motor and non-motor alterations [24-28]. Melatonin levels also was been demonstrated to be an alternative index of PD severity [23] Melatonin acts mainly through MT1 and MT2 receptors, two G-protein-coupled membrane receptors, and subsequently, MT3 was identified as a quinone reductase enzyme [29]. It has been hypothesized that dysregulations of these receptors may be involved in the installation of mood disorders [30]. In fact, MT1 and MT2 receptors are altered in the suprachiasmatic nucleus of depressed patients [31] and are down-regulated in the amygdala and the substantianigra of PD patients [32]. Moreover, polymorphisms of the MT2 receptor gene may be related with increased risk for recurrent depressive disorder [33]. Besides, the interaction of melatonin with the dopaminergic system may play a significant role in the nonphotic and photic entrainment of the biological clock as well as in the fine-tuning of motor coordination in the striatum [34]. In view of that, MT2 has been suggested to be the pivotal melatonin receptor involved in depression [15, 35, 36].

These observations, together with recent evidence based on the use of the intranigral rotenone model of PD [37, 38], have led to the hypothesis that modulating pharmacologically the striatal MT2 receptor could provide a more comprehensive

understanding of the antidepressant properties triggered by this receptor. Therefore, in the present study we investigated the alterations generated by REMSD and the modulation of striatal MT2 receptors on depressive-like behavior and neurochemical alterations in a rotenone animal model of DP.

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 # 695).

Animals

Male Wistar rats from our breeding colony were used, weighing 280-320g at the beginning of the experiments. The animals were housed in groups of five, in polypropylene cages and maintained under standard conditions of temperature (22 ± 2^0C) and illumination (12/12 h light-dark cycle). The animals had free access to water and food throughout the experiment.

Drugs

Rotenone was purchased from Sigma-Aldrich (United States) and dissolved in dimethylsulfoxide (DMSO) (Sigma-Aldrich, United States) at a final concentration of 12 μg/μL. This solution was administered by bilateral intranigral injections through stereotaxic surgery. The selective MT2 agonist 8-Methoxy-2-propionamidotetralin (8-

M-PDOT) and the selective MT2 antagonist 4-Phenyl-2-propionamidotetralin (4-P-PDOT) were purchased from TOCRIS (San Diego, CA, USA) and were dissolved with DMSO. The final concentrations were 10 µg/µl and 5 µg/µl, respectively, which were injected into the striatum, through bilateral cannulas. For the vehicle group was injected DMSO.

Experimental design

Seven days after the stereotaxic surgeries, the animals underwent the training session of the forced swimming test. Afterwards, the animals were subjected to 24 h of REM sleep deprivation (REMSD), than were injected into the striatum the 8-M-PDOT, 4-P-PDOT or vehicle, allowing the pharmacological modulation of the striatal MT2 receptors. Therefore it was obtained, randomly 12 groups: sham control vehicle (n=15), sham control 8-M-PDOT (n=15), sham control 4-P-PDOT(n=15), sham REMSD vehicle (n=15), sham REMSD 8-M-PDOT (n=15), sham REMSD 4-P-PDOT (n=15), rotenone control vehicle (n=15), rotenone control 8-M-PDOT (n=15), rotenone control 4-P-PDOT (n=15), rotenone REMSD vehicle (n=15), rotenone REMSD 8-M-PDOT (n=15) and rotenone REMSD 4-P-PDOT (n=15). Thirty minutes after, the animals were evaluated in the forced swimming tested. At the end of this test, the rats were allowed to sleep for 24 h, known as rebound period (REB) from 12:00 p.m. to 12:00 p.m. Afterwards, the groups were re-tested for the same behaviors and immediately decapitated for tissue dissection of striatum, SNpc and hippocampus for neurochemical purposes or intracardially perfused and the brains were processed for immunohistochemistry to assess tyrosine hydroxylase immunoreactivity (TH-ir) neurons density within the SNpc.

Stereotaxic surgery

Rats were sedated with intraperitoneal xylazine (10 mg/kg; Syntec do BrasilLtda, Brazil) and anaesthetized with intraperitoneal ketamine (90 mg/kg; Syntec do BrasilLtda, Brazil). The following coordinates were used to the bilateral injury, bregma as a reference: substantianigra pars compacta (SNpc) (AP) = - 5,0 mm, (ML) = ± 2,1 mm e (DV) = - 8,0 mm [30]. Needles were guided to the region of interest for a bilateral infusion of 1 µL of rotenone (12 µg/µL) using an electronic infusion pump (Insight Instruments, Ribeirão Preto, Brazil) at a rate of 0,33 µL/min for 3 minutes [37, 40, 41]. Sham operations followed the same procedure, but 1 µL of DMSO was injected instead. Complementarily, bilateral guide cannulas were implanted in the dorsal striatum of each rat allowing a subsequent infusion 1 µL of 8-M-PDOT (10 µg/µL) (Tocris Bioscience®, United Kingdom), 4-P-PDOT (5 µg/µL) (Sigma-Aldrich®, United States) or vehicle (DMSO) at a rate of 0,33µL/min for 3 minutes, in their respective groups. Coordinates with reference to bregma for implantation of guide cannulas were: (AP = - 1.0 mm, ML = ± 3.0 mm e DV = - 6.0 mm) [30]. This administration protocol was performed during the light-cycle between 7:00 a.m. to 9:00 a.m.

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 [42]. Throughout the study, the experimental room was

maintained at controlled conditions (22±2 ◦C, 12/12 h light/dark cycle, lights on 7:00 a.m. and off on 7:00 p.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.

Modified forced swimming test

This test is a modified version of Detke and colleagues [44] and Cryan *et al*. [45]. Rats were placed in an opaque plastic cylinder (diameter 20 cm; height 50 cm) containing water up to 30 cm (24 ± 1ºC); on day 1 the rats remained in the cylinder for 15 min (training session) and 24 h later they were placed back and tested for 5-min (test session). The test session was video recorded via a camera positioned above the cylinder for subsequent analysis. The behaviors registered during the test session were: immobility (when the rat stopped all active behaviors and remained floating in the water with minimal movements, with its head just above the water), swimming (movements throughout the swim cylinder, including crossing into another quadrant) and climbing (upward movements of the forepaws along the cylinder walls). The time spend in each one of the behavior was analyzed. The water was changed and the cylinder rinsed with clean water after each rat. Following the training and the test sessions, the animals were dried and placed in their home cages.

Quantification of striatal, hippocampal and nigral neurotransmitters and metabolites

The SNpc, striatum and hippocampus of the rats were rapidly dissected and stored at -80°C until the neurochemical quantification. The endogenous

concentrations of DA, 3,4-dihydroxyphenylacetic acid (DOPAC), 5-HT, 5-hydroxyindoleacetic acid (5-HIAA) and noradrenaline (NA) were assayed by reverse-phase high performance liquid chromatography (HPLC) with electrochemical detection. The method used for neurotransmitters and metabolites quantification is the accondance with previosly reported by Maturana *et al*. [12].

TH Immunohistochemistry

Independent groups of animals were analyzed for the immunohistochemical study of neuronal dopaminergic cells within the SNpc. Rats were deeply anesthetized with ketamine immediately after the behaviors tests and were intracardially perfused with saline, followed by 4% of fixative solution (formaldehyde in 0.1M phosphate buffer, pH 7.4). The brain was removed from the skull, and kept in fixative solution for 48h at 4ºC. After this period, the brain was placed in 30% sucrose solution for 3 days, and freeze at -80ºC. Afterwards, three series of 40 µm sections were cut with a cryostat between bregma -4.92 mm and -5.28 mm (with an interval of 360 µm). The sections were incubated with primarily mouse anti-TH antibody diluted phosphate-buffered saline containing 0.3% Triton X-100 (1:500; Chemicon, CA, USA) overnight at 4°C. 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 SNpc neuronal cells in each animal. For each group of rats, a mean value was calculated (percentage relative to 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 an automated scanning VSlide (Metasystems, Altlussheim, DE).

Statistical analysis

Differences between groups were analyzed by two-way analysis of variance (ANOVA), - with treatment and REMSD as the factors - followed by the Bonferroni post hoc test.Pearson's correlation coefficients (r) were calculated to establish relationships between neurochemical and behavioral parameters or molecular and neurochemical or behavioral and molecular parameters. Values are expresses as mean ± standard error of mean (SEM). The level of significance was set at $P \leq 0.05$.

Results

Modified forced swimming test

The swimming parameter evidenced that the sham control 4-P-PDOT group exhibited a significant ($P \leq 0.01$) increase compared to the sham control 8-M-PDOT (Fig. 1A). In addition, the sham REMSD 8-M-PDOT group presented an increase ($P \leq 0.01$) in this parameter compared to the sham control 8-M-PDOT group. Similarly, the rotenone REMSD 4-P-PDOT group demonstrated an increment in the swimming time in comparison to the rotenone REMSD 8-M-PDOT ($P \leq 0.01$) and

rotenone REMSD vehicle (P≤0.001) groups, according to the treatment F[(2,106)=32.83, P≤0.001], REMSD F[(3,106)=1.85, P=0.14] and interaction F[(6,106)=1.13, P=0.34] factors.

The examination of the same parameter after the REB period (Fig. 1B) revealed that, interestingly, the sham control 4-P-PDOT group presented a reduction (P<0.05) in the swimming time compared to the sham control vehicle. However, the REB seems to restore this condition since the sham REB 4-P-PDOT group showed an increment (P<0.05) of this parameter compared to the sham control 4-P-PDOT. Thus, the rotenone control 4-P-PDOT group also presented an increased (P<0.01) swimming time when compared to the sham control 4-P-PDOT as indicated by the treatment F[(2,118)=2.17, P=0.11], REB F[(3,118)=3.43, P≤0.05] and interaction F[(6,118)=3.14, P≤0.01] factors.

As depicted in Fig. 1C, the immobility behavior of the sham control 8-M-PDOT appears to be increased in comparison to the sham control vehicle (P<0.01) and sham REMSD 8-M-PDOT group (P<0.001). On the contrary, the sham REMSD 4-P-PDOT group demonstrated a significant decrease in this parameter compared to the sham REMSD vehicle (P<0.05) and sham control 4-P-PDOT (P<0.05). The rotenone REMSD 4-P-PDOT group significantly decreased their immobility compared to the rotenone REMSD 8-M-PDOT (P<0.001), rotenone REMSD vehicle (P<0.001), rotenone control 4-P-PDOT (P<0.05) and sham REMSD 4-P-PDOT (P<0.05) as indicated by the treatment F[(2,124)=7.20, P≤0.001], REMSD F[(3,124)=3.42, P≤0.05] and interaction F[(6,124)=4.80, P≤0.001] factors. In Fig. 1D it is represented the immobility time obtained after the REB period. It is noticeable that the rotenone control 8-M-PDOT presented a significant increase in this parameter in comparison to the rotenone control vehicle and rotenone control 4-P-PDOT groups (P<0.01 for

both). Also, the reduced immobility time observed in the rotenone control 4-P-PDOT was significant compared to the sham control 4-P-PDOT (P≤0.05) and rotenone REB 4-P-PDOT (P≤0.05) groups as demonstrated by the treatment F[(2,93)=5.59, P≤0.05], REB F[(3,93)=4.31, P≤0.001] and interaction F[(6,93)=2.26, P≤0.05] factors. Besides, the rotenone REB 8-M-PDOT group exhibited an increased immobility (P≤0.05) compared to the rotenone REB vehicle group.

Complementarily, the climbing time of the sham control 4-P-PDOT group was decreased (P≤0.05) when compared to the sham control vehicle group (Fig. 1E). In opposite, the rotenone REMSD 4-P-PDOT group showed an increment (P≤0.01) in this parameter in comparison to the rotenone REMSD vehicle group, that was significantly lower (P≤0.05) than the sham control vehicle group. Interestingly, we did not observe significant differences regarding the treatment F[(2,117)=0.67, P=0.51] and REMSD F[(3,117)=1.12, P=0.34] factor, except for the interaction F[(6,117)=4.00, P≤0.001]. Indeed, the rotenone REMSD 4-P-PDOT group presented a significant increase (P≤0.05) in the climbing time compared to the sham control 4-P-PDOT group. Finally, the analysis of this parameter after the REB period (Fig. 1F) showed absence of statistical differences for the treatment F[(2,87)=1.34, P=0.26] and interaction F[(6,87)=1.81, P=0.10] factors but not for the REB F[(3,87)=4.82, P≤0.01] factor. In view of that, only the rotenone control vehicle group presented a significant increase (P≤0.01) in this parameter compared to the sham control vehicle group.

Quantification of striatal, hippocampal and nigral neurotransmitters and metabolites

Fig. 2 shows the alterations in the neurotransmission within the striatum. Accordingly, DA levels (Fig. 2A) were intensely decreased in the rotenone control

vehicle (P≤0.05) and rotenone control 8-M-PDOT (P≤0.05) groups compared to their respective sham control groups, as indicated by the treatment [F(2,29)=0.88, P=0.42], REB [F(3,29)=8.95, P≤0.001] and interaction factors [F(6,29)=1.49, P=0.21]. Considering the DOPAC levels (Fig. 2B), it was observed an increment in the sham REB 8-M-PDOT compared to the sham control 8-M-PDOT (P≤0.05), sham REB 4-P-PDOT (P≤0.05) and rotenone REB 8-M-PDOT (P≤0.05) groups. Moreover, a significant increase in this metabolite was identified in the rotenone control 4-P-PDOT group in comparison to the rotenone control vehicle (P≤0.01) and rotenone control 8-M-PDOT (P≤0.001) groups. Thus, the rotenone REB 4-P-PDOT group exhibited an increase (P≤0.01) in this parameter when compared to the rotenone REB vehicle, as demonstrated by the treatment [F(2,22)=5.40, P≤0.05], REB [F(3,22)=3.09, P≤0.05] and interaction [F(6,22)=9.66, P≤0.001] factors. Moreover, an increase (P≤0.01) was also observed in the rotenone REB 4-P-PDOT group compared to its respective sham REB group. The analysis of the DA turnover (Fig. 2C) did not show statistical differences among the groups regarding the treatment [F(2,29)=0.42, P=0.66], REB [F(3,29)=1.53, P=0.22], however, significant in the light of the interaction [F(6,29)=4.53, P≤0.01] factor.

Concerning the 5-HT levels detected in the striatum (Fig. 2D), the sham REB 4-P-PDOT group presented a significant increase (P<0.01) compared to the sham control 4-P-PDOT group. In addition, the rotenone control 8-M-PDOT group showed an increment (P≤0.01) in 5-HT in comparison to the sham control 8-M-PDOT group. Furthermore, the rotenone REB vehicle group presented an increment in this parameter when compared to the sham REB vehicle (P≤0.01), rotenone control vehicle (P≤0.001), rotenone REB 8-M-PDOT (P≤0.001) and rotenone REB 4-P-PDOT (P≤0.001) groups. Also, the rotenone REB 8-M-PDOT was significantly higher

(P≤0.001) compared to the rotenone REB 4-P-PDOT group, as indicated by the treatment [F(2,21)=10.48, P≤0.001], REB [F(3,21)=19.91, P≤0.001] and interaction [F(6,21)=14.17, P≤0.001] factors. The 5-HT metabolite quantification (Fig. 2E) demonstrated that the sham REB 4-P-PDOT group presented an increase (P≤0.01) in this parameter in comparison to the sham REB 8-M-PDOT group. Besides, a significant decrease (P≤0.05) of 5-HIAA was observed in the rotenone REB 8-M-PDOT compared to the rotenone REB vehicle group, according to the treatment [F(2,26)=14.59, P≤0.001], REB F[(3,26)=2.01, P=0.13] and interaction [F(6,26)=3.77, P<0.0078] factors. As a result, the evaluation of the striatal 5-HT turnover (Fig. 2F) showed that the sham control 4-P-PDOT presented an increment compared to the sham control 8-M-PDOT (P≤0.01), sham control vehicle (P≤0.01) and rotenone control 4-P-PDOT (P≤0.001) groups. Interestingly, this turnover was remarkably increased (P≤0.001) in the rotenone REB 4-P-PDOT group compared to all other groups tested, according to the treatment [F(2,25)=37.89, P≤0.001], REB [F(3,25)=18.48, P≤0.001] and interaction [F(6,25)=20.19, P≤0.001] factors.

Fig. 3 shows the alterations in the neurotransmission within the hippocampus DA levels (Fig. 3A) have been found to be increased in the sham control 8-M-PDOT group in comparison to the sham control vehicle (P≤0.05), sham control 4-P-PDOT (P≤0.05) and sham REB 8-M-PDOT (P≤0.05) groups. In addition, the sham REB 8-M-PDOT exhibited an increase (P≤0.05) in this parameter compared to the sham REB 4-P-PDOT group, as exhibited by the treatment [F(2, 25)=2.84, P=0.077], REB [F(3, 25)=2.42, P=0.08] and interaction [F(6,25)=1.17, P=0.35] factors. Fig. 3B demonstrates the levels of DOPAC within the hippocampus. In view of that, it is observed that the sham REB 8-M-PDOT group showed a significant increment compared to the sham control 8-M-PDOT (P≤0.05), sham REB 4-P-PDOT (P≤0.05),

rotenone control 8-M-PDOT (P≤0.01) and rotenone REB 4-P-PDOT (P≤0.05) groups, according to the treatment [F(2,32)=2.35, P=0.11], REB [F(3,32)=3.22, P≤0.05] and interaction [F(6,32)=2.24, P=0.06] factors. However, the examination of the hippocampal DA turnover (Fig. 3C) did not indicate significant differences among the groups tested, as indicated by the treatment [F(3,21)=1.51, P=0.24], REB [F(2,21)=0.73, P=0.49] and interactions [F(6,21)=1.84, P=0.14] factors.

Considering the hippocampal 5-HT levels (Fig. 3D) it was observed a significant decrease (P≤0.001) in the sham control 4-P-PDOT group compared to the sham control vehicle. Similarly, a decrement in 5-HT (P≤0.05) was detected in the rotenone control vehicle group in comparison to the sham control vehicle group. Nevertheless, the rotenone REB 8-M-PDOT group showed an increase in this parameter when compared to the rotenone control 8-M-PDOT (P≤0.01) and rotenone REB 4-P-PDOT (P≤0.05) groups, as pointed out by the treatment [F(2,24)=20.11, P≤0.001], REB [F(3,24)=13.23, P≤0.001] and interaction [F(6,24)=2.83, P≤0.05] factors. Intriguingly, according to the treatment [F(2, 35)=2.06, P=0.14], REB [F(3, 35)=0.73, P=0.54] and interaction [F(6,35)=1.30, P=0.28] factors, the 5-HIAA levels did not differed among the groups (Fig. 3E), although, the evaluation of the 5-HT turnover (Fig. 3F) indicated that the sham control 4-P-PDOT presented a significant increase (P≤0.05) compared to the sham control vehicle, as indicated by the treatment [F(2, 27)=8.18, P≤0.01], REB [F(3, 27)=2.77, P=0.06] and interaction [F(6,27)=3.00, P≤0.05] factors. Moreover, the rotenone control 8-M-PDOT showed an increase in comparison to the sham control 8-M-PDOT (P≤0.05), sham REB 8-M-PDOT (P≤0.01), rotenone control vehicle (P≤0.01), rotenone control 4-P-PDOT (P≤0.05) and rotenone REB 8-M-PDOT (P≤0.01) groups. Finally, the rotenone REB 4-P-PDOT presented an increase in

comparison to the rotenone REB vehicle (P≤0.05) and rotenone REB 8-M-PDOT (P≤0.05) groups.

Regarding the nigral levels of DA (Fig. 4 A) it was detected that the sham control 4-P-PDOT group presented a significant increase compared to the sham control 8-M-PDOT (P≤0.05) and rotenone control 4-P-PDOT (P≤0.05) groups. In addition, the rotenone REB 4-P-PDOT is significantly increased (P≤0.05) in comparison to the rotenone control 4-P-PDOT group. Also, the rotenone REB 8-M-PDOT presented an increase (P≤0.05) in this parameter compared to the rotenone REB vehicle group, as demonstrated by the treatment [F(2, 25)=3.99, P≤0.05], REB [F(3, 25)=0.83, P=0.49] and interaction [F(6,25)=2.74, P≤0.05] factors. Nigral DOPAC (Fig. 4B) presented a significant increase in the sham REB vehicle group in comparison to the sham REB 8-M-PDOT (P≤0.05) and rotenone control vehicle (P≤0.05) groups. As well, the sham REB 4-P-PDOT group presented an increase (P≤0.05) in comparison to the sham REB 8-M-PDOT group. In fact, the rotenone control vehicle groups showed a decrease in this metabolite compared to the sham control vehicle (P≤0.05) and rotenone control 8-M-PDOT (P≤0.05) groups. Furthermore, rotenone REB 4-P-PDOT group exhibited a significant increase of DOPAC compared to the rotenone REB 8-M-PDOT (P≤0.05), rotenone REB vehicle (P≤0.01) and rotenone control 4-P-PDOT (P≤0.05) groups. Besides, the rotenone control 4-P-PDOT group showed a decrement (P≤0.01) in this parameter compared to the sham control 4-P-PDOT group, as indicated by the treatment [F(2, 25)=5.67, P≤0.01], REB [F(3, 25)=7.32, P≤0.01] and interaction [F(6,25)=8.93, P≤0.001] factors. As a result, the calculation of the nigral DA turnover (Fig. 4C) indicated the rotenone REB 4-P-PDOT group presented a significant increase in this parameter in comparison to the rotenone REB vehicle (P≤0.01), rotenone control 4-P-PDOT

(P≤0.05) and sham control 4-P-PDOT (P≤0.05) groups, according to the treatment [F(2, 32)=9.83, P≤0.001], REB [F(3, 32)=8.90, P≤0.001] and interaction [F(6,32)=3.19, P≤0.01] factors.

The quantification of the nigral concentrations of 5-HT (Fig. 4D) indicated that the sham control 4-P-PDOT group generated an increase (P≤0.05) in this parameter compared to the sham control vehicle group. Moreover, a significant increase was also detected in the rotenone control vehicle group in comparison to the sham control vehicle (P≤0.05) and rotenone control 8-M-PDOT (P≤0.01) groups. Besides, the rotenone REB vehicle group showed a significant increase in the 5-HT compared to the sham REB vehicle (P≤0.05) and rotenone REB 4-P-PDOT (P≤0.05) groups, as pointed out by the treatment [F(2, 23)=5.83, P≤0.01], REB [F(3, 23)=3.47, P≤0.05] and interaction [F(6,23)=5.50, P≤0.01] factors. In addition, the quantification of the 5-HT metabolite, 5-HIAA (Fig. 4E), indicated that the sham control 4-P-PDOT group showed an increase of the parameter (P0.05), in comparison to the rotenone control 4-P-PDOT group. Also, the rotenone REB 8-M-PDOT (P≤0.01) and the rotenone REB 4-P-PDOT (P≤0.05) groups presented significant increases compared to their respective rotenone control groups, according to the treatment [F(2, 28)=2.31, P=0.1175], REB [F(3, 28)=15.34, P≤0.001] and interaction [F(6,28)=2.07, P=0.08] factors. Finally, the examination of the nigral 5-HT turnover (Fig. 4F) revealed that the rotenone REB 4-P-PDOT group presented a significant increase in this parameter, when compared to the rotenone REB vehicle (P≤0.01) and rotenone control 4-P-PDOT (P<0.05) groups, as indicated by the treatment [F(2, 38)=6.03, P≤0.01], REB [F(3, 38)=3.82, P≤0.05] and interaction [F(6,38)=3.07, P≤0.05] factors.

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As depicted in Fig. 5, TH-ir neurons within the SNpc demonstrated to be significantly increased by the 4-P-PDOT treatment as indicated by the higher labeling in the sham control 4-P-PDOT group compared to the sham control 8-M-PDOT ($P \leq 0.05$) and sham control vehicle ($P \leq 0.01$) groups. Interestingly, a similar, however slightly, increment ($P \leq 0.05$) was also detected in the rotenone control 4-P-PDOT group compared to the rotenone control vehicle group. Besides, the rotenone control 8-M-PDOT showed a significant increase ($P \leq 0.05$) in this parameter, compared to the sham control 8-M-PDOT group. Indeed, such effect elicited by the 4-P-PDOT was unnoticeable in the sham REB groups. However, it is worth noting that after the REB period a significant decrease in the percentage of TH-ir neurons was detected in the rotenone REB 4-P-PDOT compared to the rotenone REB vehicle ($P \leq 0.05$), rotenone control 4-P-PDOT ($P \leq 0.001$) and sham REB 4-P-PDOT ($P \leq 0.001$), as indicated by the treatment $F[(2,46)=11.76, P \leq 0.001]$, REB $F[(3,46)=19.48, P \leq <0.001]$ and interaction factors $F[(6,46)=7.69, P \leq 0.001]$.

Statistical correlations between behavioral and neurochemical parameters

Pearson's correlation coefficients, depicted in Table 1, revealed a moderate positive correlation ($r= 0.49$; $P=0.007$) between striatal DA and immobility time in the sham but not in the rotenone ($r = 0.13$; $P = 0.47$) groups. Interestingly, it was observed a significant correlation between DA x immobility time ($r = 0.34$; $P = 0.05$), 5-HT x swimming time ($r = 0.41$; $P = 0.05$) and NA x climbing time ($r = 0.34$; $P = 0.05$) in the sham groups that were not replicated in the rotenone correlated groups. Nevertheless, within the SNpc, DA significantly correlated with immobility time ($r = 0.44$; $P = 0.01$) only in the rotenone groups. Considering the correlation between

striatal DA x the % of TH-ir neurons within the SNpc it is observed a significant association only in the rotenone groups (r = -0.60; P = 0.0004). However, correlations between striatal NA x % of SNpc TH-ir neurons indicated a significant effects in both sham (r = 0.42; P = 0.01) and rotenone (r = -0.53; P = 0.001) groups. The analysis of the hippocampus revealed the occurrence of a significant, moderate correlation (r = -0.43 ; P = 0.01) between NA x % of SNpc TH-ir neurons, only for the rotenone groups. Lastly, the associations between the SNpc levels of NA x % of SNpc TH-ir neurons showed weak and moderate correlations, respectively, for the sham (r = 0.36 ; P = 0.04) and rotenone (r = -0.48 ; P = 0.008) groups.

Discussion

Concerning the mood disorders, different reports have shown that melatonin promotes antidepressant effects in the forced swimming test [46, 47], potentially due to increases in the 5-HT levels [48]. However, in our study, we observed substantial antidepressant effects promoted by the blockade of striatal MT2 receptors (induced by 4-P-PDOT) that were potentiated by REMSD (as seen in swimming and immobility), particularly in the context of rotenone lesion (according to swimming, immobility and climbing parameters). The observation of the REB period revealed a remaining antidepressant effect inflicted by MT2 blockade (swimming parameter), although, associated to an increment in the immobility time as a result of MT2 activation (8-M-PDOT). In parallel, the striatal deficit in DA levels, induced by the intranigral rotenone administration, is in accordance to this early phase animal model of PD [9, 37, 38, 40]. In view of that, striatal DA significantly correlated to the immobility time (recorded in the REB period) in the sham (r = 0.46; P = 0.007) but not in the rotenone (r = 0.13; P = 0.47) groups. In this context, significant correlations

were observed between the depressive-like behaviors and the related neurotransmitters in the hippocampus of the sham, but not in the rotenone groups (see Table 1). Moreover, there was a significant negative correlation between the striatal DA levels and the percentage of SNpc TH-ir neurons only in the rotenone ($r = -0.60$; $P = 0.0004$) groups, supporting the notion of a compensatory dopaminergic mechanism involved in the antidepressant activity mediated by the striatal MT2 blockade.

Different reports have been described antidepressant properties of melatonin [46, 47, 49], there is some evidence that melatonin antidepressant activity may be related to an antagonism of MT2 receptors [15, 36]. However, to our knowledge, the present study is the first to show antidepressant effects induced by striatal MT2 blockade in the intranigral rotenone model of PD. The antidepressant effects of sleep deprivation has been widely investigated in depressed patients. This procedure has proven its efficacy for alleviating depression in approximately 60% of the cases after a single session, and in almost 90% after three sessions performed at one-week intervals [50, 51]. Furthermore, in a randomized study it was found that lower amounts of REM sleep were correlated with a greater reduction in depressive symptom ratings [52]. However, this effect usually is only transient, and in most cases relapse occurs after the first episode of sleep rebound [53]. Thus, endogenous and rotenone-induced depression is associated with an increase in cholinergic and decrease in serotonergic neurotransmission [9, 10, 54, 55], it has been proposed that this imbalance would be responsible for the disinhibition of REM sleep in depressed patients. Furthermore, a recent report characterized these mechanisms promoted by REMSD, indicating the occurrence of a sustained antidepressant effect

lasting until the REB period, could be explained by the maintenance of the increased nigral BDNF expression [12].

In addition, positive correlations were observed for DA and immobility, in the striatum ($r = 0.46$; $P = 0.007$) and hippocampus ($r = 0.34$; $P = 0.05$), and 5-HT and swimming ($r = 0.41$; $P = 0.05$), respectively, in the sham, but not in the rotenone (DA: $r = 0.13$; $P = 0.47$; $r = -0.23$; $P = 0.2$; 5-HT: $r = 0.15$; $P = 0.44$) groups suggesting a remarkable impairment in both neurotransmitters levels after the nigrostriatal lesion. This is also reinforced by the striatal deficit in DA observed in the rotenone control vehicle group compared to its correlate sham group. Interestingly, DA appears to be increased as a result of MT2 activation in the hippocampus and blockade in the SNpc. Moreover, 5-HT content seems to be particularly affected by REMSD, since it was drastically increased in the rotenone REB vehicle group compared to its correlated groups in the striatum. Interestingly, the 4-P-PDOT treatment of the rotenone REMSD group generated a drastic decrement in the 5-HT levels within the striatum, hippocampus and SNpc, which contrasts to the antidepressant effect observed by the MT2 blockade. However, the significant increase in the 5-HT turnover, among these areas, could explain such apparent discrepancy. In addition, an increased DA turnover, depicted in the SNpc, could also enlighten this antidepressant mechanism. In fact, there is described a mutual association between DA and 5-HT in mood disorders, particularly in PD [56]. This hypothesis considers a 5-HT-induced DA release in the nucleus accumbens which is down-regulated by 5-HT2C receptors [57]. As a result, reductions in the 5-HT content or increases in the 5-HT2C inhibitory activity could be associated to a decline on dopaminergic neurotransmission.

Of note, a complementary explanation relies on the participation of NA as a potential player in that mechanism. We observed a substantial increase in this neurotransmitter content within the striatum and SNpc, but not in the hippocampus (data not shown) induced by the 4-P-PDOT administration. Besides, hippocampal NA showed a positive correlation to climbing in the sham ($r = 0.34$; $P = 0.05$), but not rotenone ($r = -0.13$; $P = 0.47$) groups, suggesting a participation of this neurotransmitter in the antidepressant effect that was counteracted by rotenone. This involvement is also supported by the significant correlations between NA levels and the percentage of TH-ir neurons in the striatum (sham and rotenone groups), hippocampus (rotenone groups) and SNpc (sham and rotenone groups) (see Table 1). In the light of our findings, these explanations seem plausible because DA, 5-HT and complementarily NA presented similar fluctuations in the striatum, hippocampus and SNpc, structures which are closely related to the nucleus accumbens, that collectively define the so called neural circuitry of depression [58, 59].

In mice, the nonselective MT1/MT2 receptor antagonist luzindole displayed antidepressant-like activity by antagonizing the MT2 receptors since luzindole was not able to decrease the duration of immobility during the forced swimming test in MT2 $^{-/-}$ mice [6]. Moreover, a subcutaneous injection of UCM765 (a partial MT2 agonist) at a dose of 40 mg/kg significantly reduced the latency to slow wave sleep while increasing the amount of slow wave sleep during the 24-hour period [60]. This study also demonstrated that the amount and latency of REM sleep were not altered by UCM765. The amount of wakefulness was instead significantly decreased due to the increase in slow wave sleep paralleled by no changes in REM sleep. Like UCM765, no effects on REM sleep amount and latency were reported with IIK7, which is a full MT2 agonist [53]. Hence, this MT2-induced increase of slow wave

sleep in relation to REM sleep, also is in accordance to Vogel, that inferred that REMSD fits the criteria for being the mechanism of action of the antidepressant drugs [13]. In addition, it was observed that the acute administration of fluoxetine in rats generated a quite similar effect to other antidepressants, i.e., suppression of REM sleep [62].

Concerning the modulation of TH-ir neurons within the SNpc, we observed that the pharmacological manipulation of the MT2 receptors generated significant negative correlations between this parameter and the DA and NA levels among the areas analyzed of the rotenone groups. That is, the association of MT2 blockade and REMSD manipulations that produced more predominantly TH-ir neurons decrease tends to elicit increases in the striatal, hippocampal and nigral DA and NA levels, perhaps as a compensatory mechanism.

The evidence of the role of MT2 receptors in depression and depressive-like behaviors, particularly in PD, is still limited and need further investigation. Therefore, the present findings provide new information regarding the antidepressant effect triggered by MT2 receptors within the striatum. Indeed, we demonstrated that this behavior is only successful when such receptors were blocked by 4-P-PDOT and not when activated by 8-M-PDOT. Thus, we demonstrated, a putative role of DA and NA in the antidepressant-like effect induced by striatal 4-P-PDOT, as well as the increment in the 5-HT turnover in the striatum, hippocampus and SNpc.

Conflict of interests

The authors have declared that no conflict of interests exists.

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Figure Legends

Figure 1. Schematic diagram of the experimental design performed in these experiments

Figure 2. Depressive-like parameters during modified forced swimming test. **A**. swimming after REMSD, **B**. swimming after REB, **C**. immobility after REMSD, **D**. immobility after REB, **E**. climbing after REMSD, **F**. climbing after REB. The bars represent the mean ± standard error of the mean. n=15 per group, *P≤0.05, **P≤0.01, ***P≤0.001. Two-way ANOVA followed by the Bonferronipost hoc test.

Figure 3. Neurochemical examination of the striatal content of DA, 5-HT and metabolites, after the REB period.**A**. DA, **B**. DOPAC, **C**. DA turnover, **D**. 5-HT, **E**.5-HIAA, **F**. 5-HT turnover. Values are expressed as mean ± SEM. n = 3-5 per group, *P≤0.05, **P≤0.01, ***P≤0.001. Two-way ANOVA followed by the Bonferronipost hoc test.

Figure 4. Neurochemical examination of the hippocampal content of DA, 5-HT and metabolites, after the REB period.**A**. DA, **B**. DOPAC, **C**. DA turnover, **D**.5-HT, **E**.5-HIAA, **F**. 5-HT turnover. Values are expressed as mean ± SEM. n = 3-5 per group, *P≤0.05, **P≤0.01, ***P≤0.001. Two-way ANOVA followed by the Bonferronipost hoc test.

Figure 5. Neurochemical examination of the substantianigra content of DA, 5-HT and metabolites, after the REB period. **A**. DA, **B**. DOPAC, **C**. DA turnover, **D**.5-HT, **E**.5-HIAA, **F**. 5-HT turnover. Values are expressed as mean ± SEM. n = 3-5 per group, *P≤0.05, **P≤0.01. Two-way ANOVA followed by the Bonferroni post hoc test.

Figure 6. Percentage of neurons expressing TH protein in the substantia nigra pars compacta in relation to sham control vehicle group, after the REB period. The bars represent the mean ± standard error of the mean, n=3-5 per group, *P≤0.05, ***P≤0.001. Two-way ANOVA followed by the Bonferroni post hoc test.

Table Legends

Table 1. Different alterations induced by the rotenone model as indicated by correlations of different parameters in the sham and rotenone groups after the REB period. Pearson's correlation coefficients were calculated considering the following: striatal DA x immobility time, striatal 5-HT x swimming time, striatal NA x climbing time, striatal DA x % SNpc TH-ir neurons, striatal NA x % SNpc TH-ir neurons, hippocampal DA x immobility time, hippocampal 5-HT x swimming time, hippocampal NA x climbing time, hippocampal DA x % SNpc TH-ir neurons, hippocampal NA x % SNpc TH-ir neurons, nigral DA x immobility time, nigral 5-HT x swimming time, nigral NA x climbing time, nigral DA x % SNpc TH-ir neurons, nigral NA x % SNpc TH-ir neurons. Significant correlations are indicated by *P≤0.05.

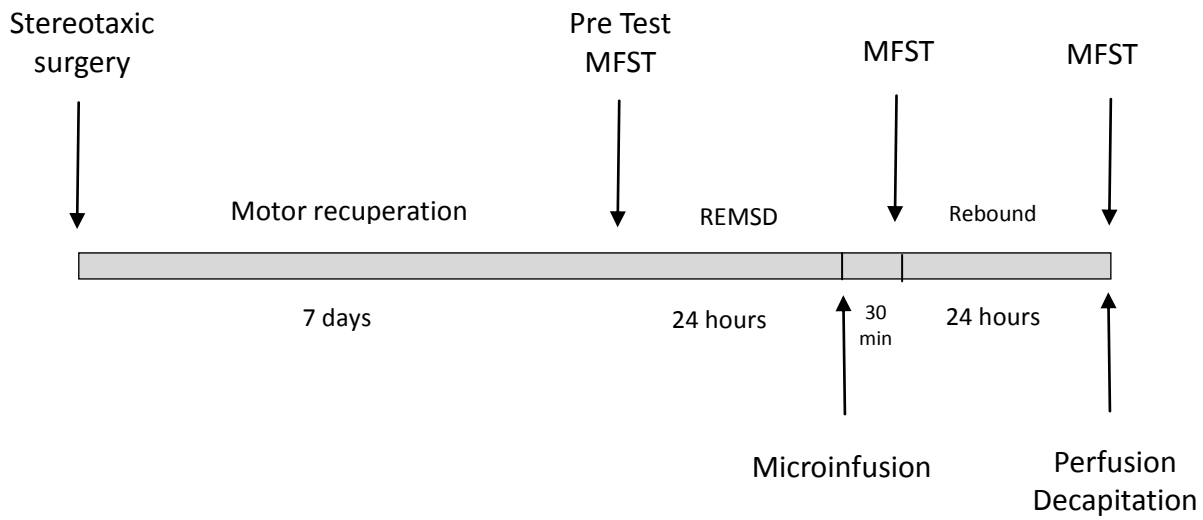
Stereotaxic surgery
Pre Test MFST
MFST
MFST
Motor recuperation
REMSD
Rebound
7 days
24 hours
30 min
24 hours
Microinfusion
Perfusion Decapitation

Figure 1

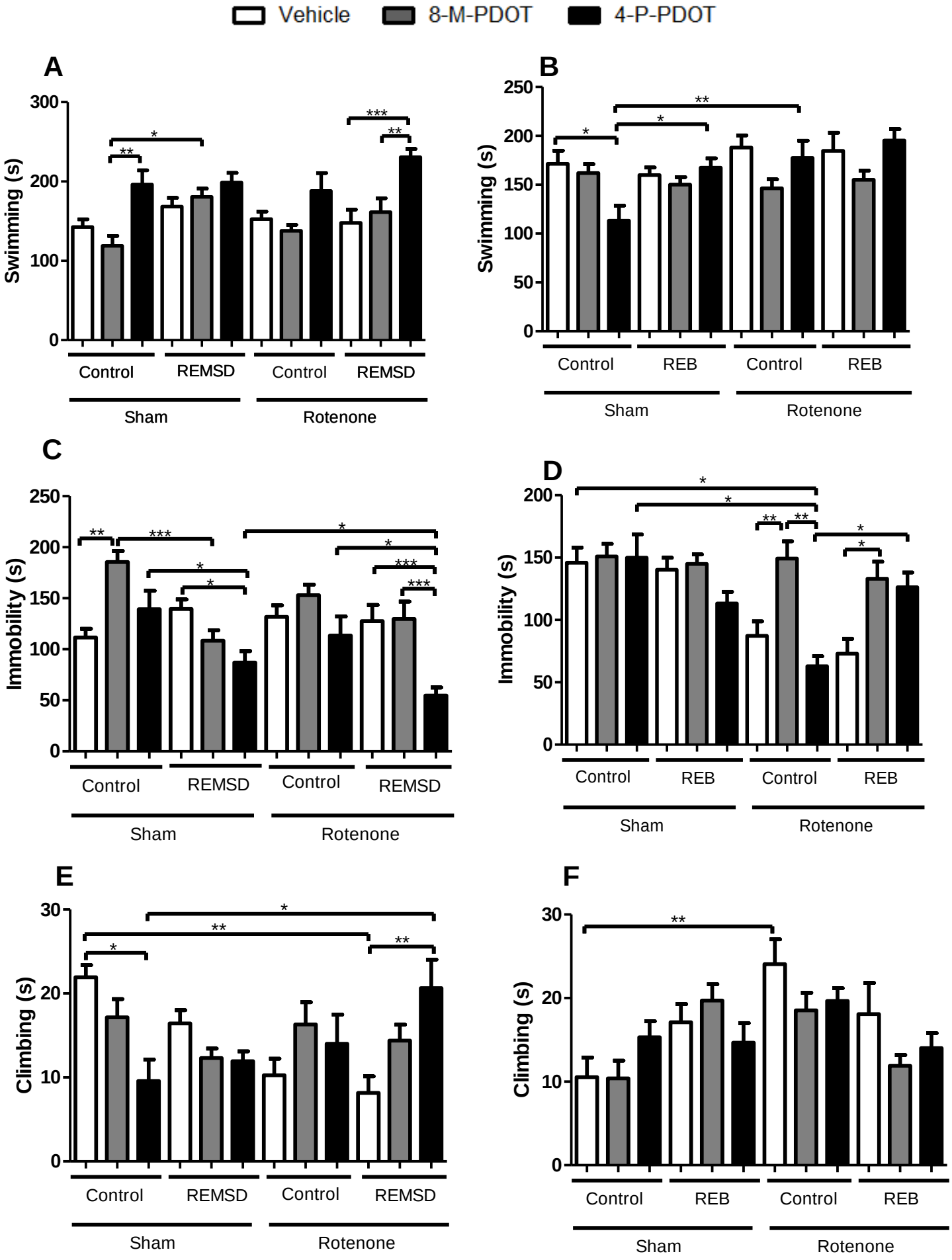
Vehicle
8-M-PDOT
4-P-PDOT
A
Swimming (s)
300
200
100
0
Control
REMSD
Control
REMSD
Sham
Rotenone
B
Swimming (s)
250
200
150
100
50
0
Control
REB
Control
REB
Sham
Rotenone
C
Immobility (s)
250
200
150
100
50
0
Control
REMSD
Control
REMSD
Sham
Rotenone
D
Immobility (s)
200
150
100
50
0
Control
REB
Control
REB
Sham
Rotenone
E
Climbing (s)
30
20
10
0
Control
REMSD
Control
REMSD
Sham
Rotenone
F
Climbing (s)
30
20
10
0
Control
REB
Control
REB
Sham
Rotenone

Figure 2

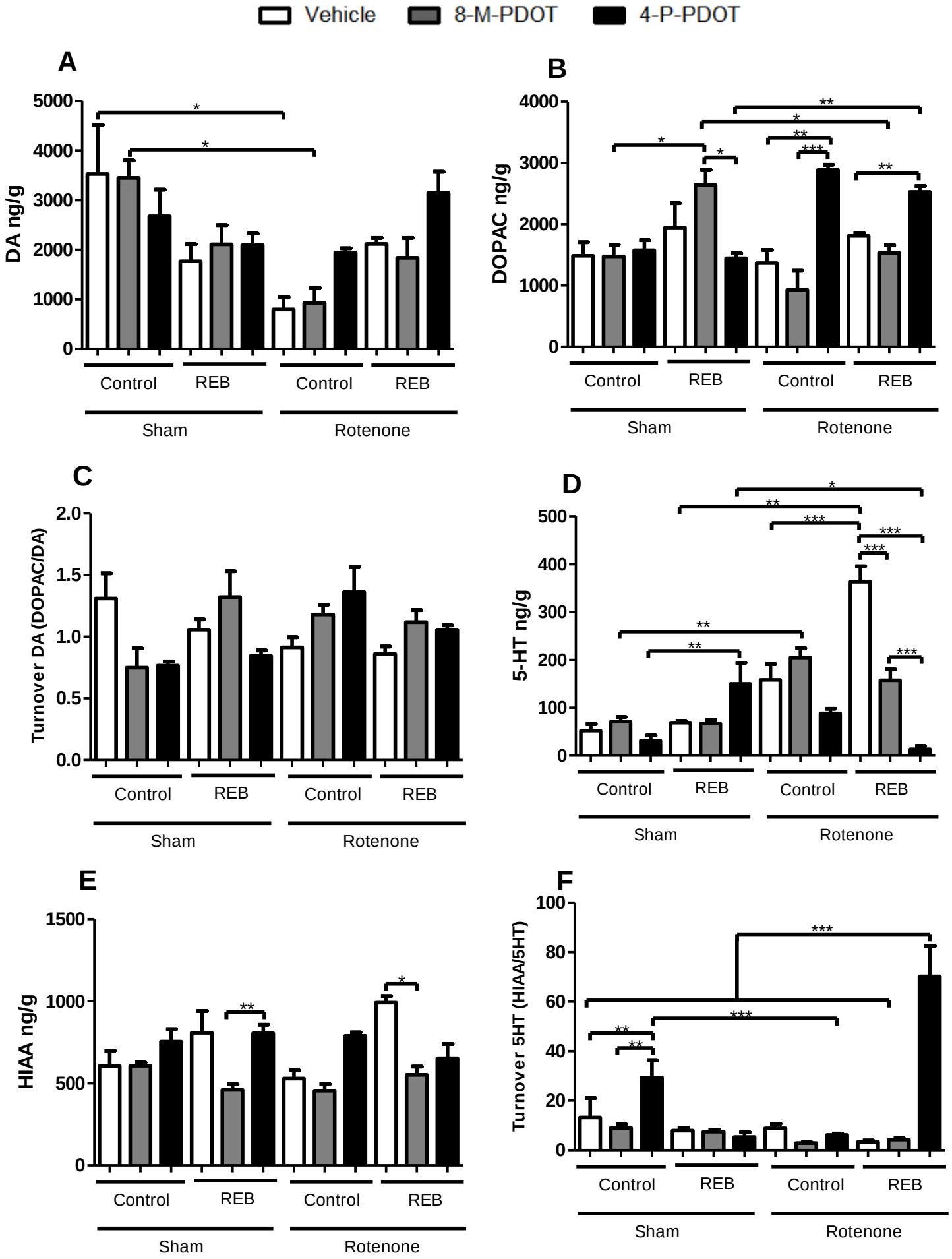
Vehicle
8-M-PDOT
4-P-PDOT
A
DA ng/g
5000
4000
3000
2000
1000
0
Control
REB
Control
REB
Sham
Rotenone
B
DOPAC ng/g
4000
3000
2000
1000
0
Control
REB
Control
REB
Sham
Rotenone
C
Turnover DA (DOPAC/DA)
2.0
1.5
1.0
0.5
0.0
Control
REB
Control
REB
Sham
Rotenone
D
5-HT ng/g
500
400
300
200
100
0
Control
REB
Control
REB
Sham
Rotenone
E
HIAA ng/g
1500
1000
500
0
Control
REB
Control
REB
Sham
Rotenone
F
Turnover 5HT (HIAA/5HT)
100
80
60
40
20
0
Control
REB
Control
REB
Sham
Rotenone

Figure 3

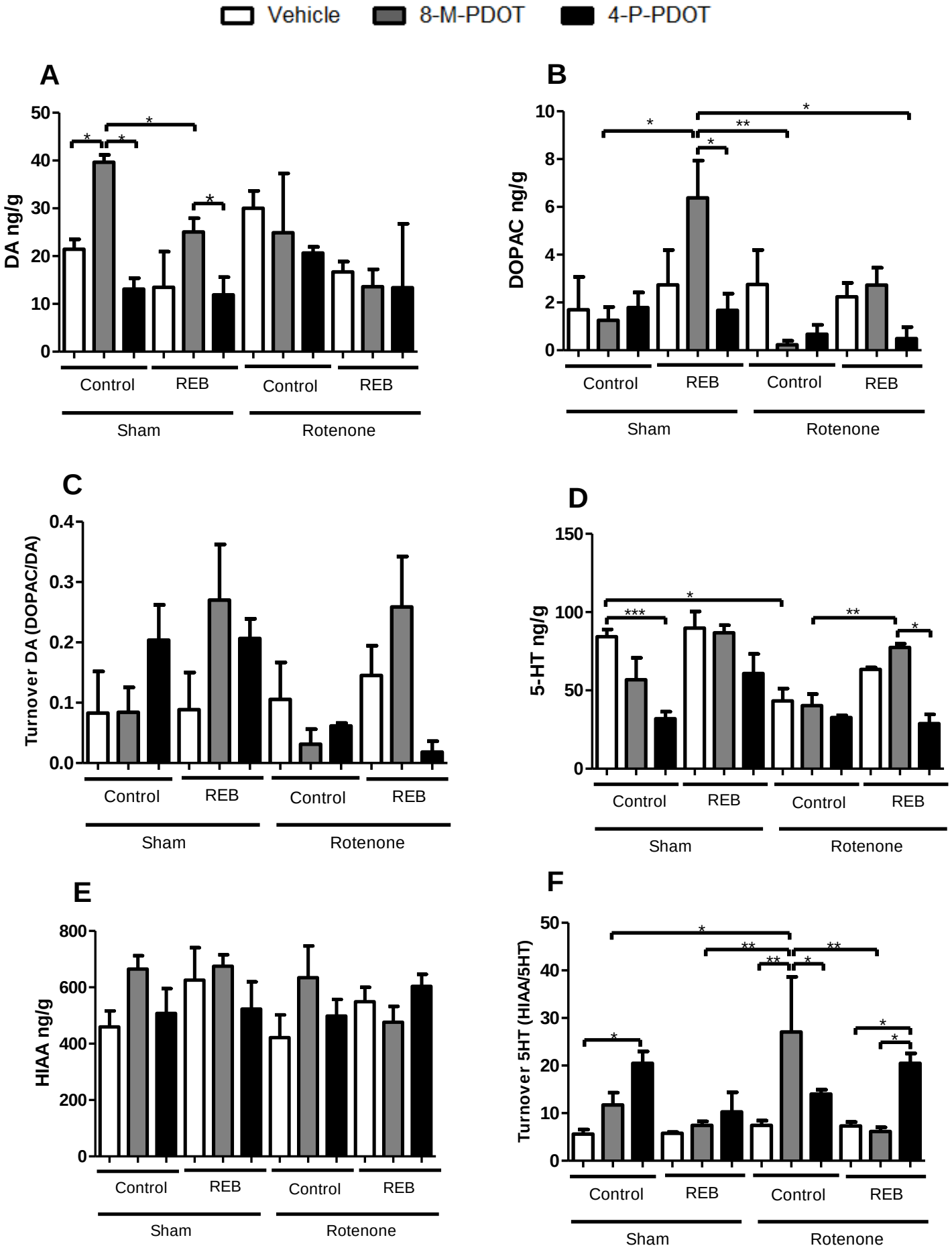
Vehicle
8-M-PDOT
4-P-PDOT
A
DA ng/g
B
DOPAC ng/g
C
Turnover DA (DOPAC/DA)
D
5-HT ng/g
E
HIAA ng/g
F
Turnover 5HT (HIAA/5HT)
Control
REB
Sham
Rotenone

Figure 4

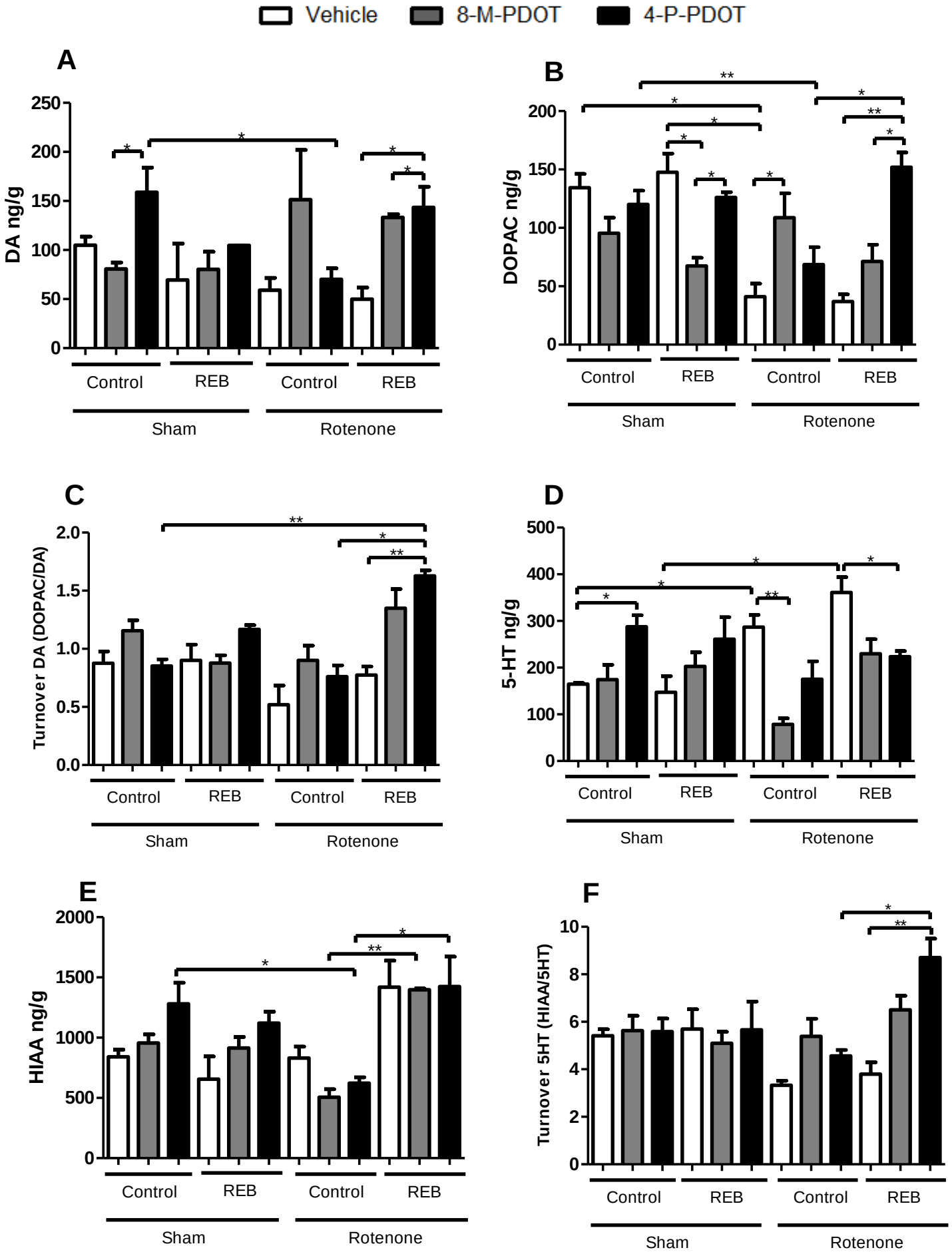
Vehicle
8-M-PDOT
4-P-PDOT
A
DA ng/g
250
200
150
100
50
0
Control
REB
Control
REB
Sham
Rotenone
B
DOPAC ng/g
200
150
100
50
0
Control
REB
Control
REB
Sham
Rotenone
C
Turnover DA (DOPAC/DA)
2.0
1.5
1.0
0.5
0.0
Control
REB
Control
REB
Sham
Rotenone
D
5-HT ng/g
500
400
300
200
100
0
Control
REB
Control
REB
Sham
Rotenone
E
HIAA ng/g
2000
1500
1000
500
0
Control
REB
Control
REB
Sham
Rotenone
F
Turnover 5HT (HIAA/5HT)
10
8
6
4
2
0
Control
REB
Control
REB
Sham
Rotenone

Figure 5

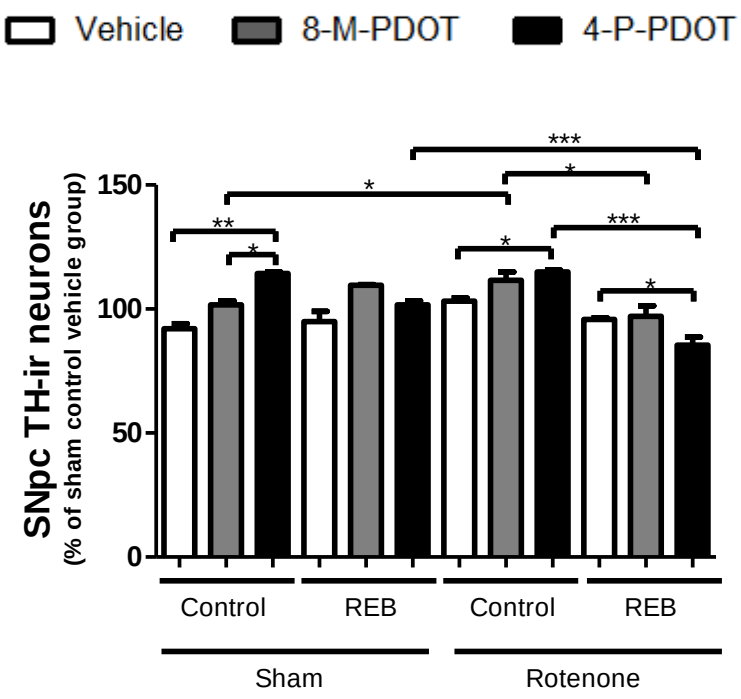
Vehicle
8-M-PDOT
4-P-PDOT
SNpc TH-ir neurons
(% of sham control vehicle group)
150
100
50
0
***
*
*
**
*
*
***
*
Control
REB
Control
REB
Sham
Rotenone

Figure 6

Table 1.Pearson's correlations between different behavioral, neurochemical and molecular parameters after the rebound period.

Correlations	Groups	
	Sham	Rotenone
Striatum		
DA x immobility time	r = 0.46; P = 0.007*	r = 0.13; P = 0.47
5-HT x swimming time	r = 0.31; P = 0.08	r = 0.1; P = 0.6
NA x climbing time	r =0.13; P = 0.46	r = -0.05; P = 0.8
DA x % of SNpc TH-ir neurons	r = -0.33; P = 0.88	r = -0.60; P = 0.0004*
NA x % of SNpc TH-ir neurons	r = 0.42; P = 0.01*	r = -0.53; P = 0.001*
Hippocampus		
DA x immobility time	r = 0.34 ; P = 0.05*	r = -0.23; P = 0.2
5-HT x swimming time	r = 0.41; P = 0.05*	r = 0.15; P = 0.44
NA x climbing time	r = 0.34 ; P = 0.05*	r = -0.13; P = 0.47
DA x % of SNpc TH-ir neurons	r = -0.28 ; P = 0.12	r = -0.26; P = 0.15
NA x % of SNpc TH-ir neurons	r = 0.28 ; P = 0.11	r = -0.43 ; P = 0.01*
Substantianigra pars compacta		
DA x immobility time	r = 0.07; P = 0.7	r = 0.44; P = 0.01*
5-HT x swimming time	r = 0.01 ; P = 0.94	r = - 0.2 ; P = 0.31
NA x climbing time	r = 0.09 ; P = 0.6	r = -0.3; P = 0.09
DA x % of SNpc TH-ir neurons	r = 0.21 ; P = 0.2	r = -0.17 ; P = 0.34
NA x % of SNpc TH-ir neurons	r = 0.36 ; P = 0.04*	r = -0.48 ; P = 0.008*

DA – dopamine, NA – noradrenaline, 5-HT- serotonin, % of SNpc TH-ir neurons - % of SubstantiaNigra pars compacta Tyrosine Hydroxylase immunoreactive neurons.
*Significant correlations are indicated.

6. CONCLUSÕES

- A administração do antagonista de receptores MT2, 4-P-PDOT, no estriado, gerou um efeito tipo-antidepressivo em ratos, verificado pelo teste de natação forçada modificado;
- O efeito tipo-antidepressivo promovido pelo 4-P-PDOT foi potencializado pela PSREM;
- A administração do agonista de receptores MT2, 8-M-PDOT, no estriado, gerou um efeito tipo-depressivo em ratos;
- O bloqueio de receptores MT2 estriatais aumentou os níveis de DA na SNpc, enquanto que a ativação de receptores MT2 estriatais aumentou os níveis de DA no hipocampo;
- O sono rebote promoveu aumento dos níveis de 5-HT estriatais;
- A administração de 4-P-PDOT no estriado reduziu os níveis de 5-HT no estriado, hipocampo e SNpc. Entretanto foi observado aumento de seu *turnover* (hipocampo, estriado e SNpc) bem como de DA (SNpc);
- A administração de 4-P-PDOT aumentou a imunoreatividade a TH nos grupos controle. Entretanto esse efeito foi revertido na presença da rotenona.

Tomados em conjunto, os nossos resultados demonstraram que o 4-P-PDOT apresentou efeitos tipo-antidepressivos, que foram potencializados pela REMSD. Havendo um forte envolvimento do sistema dopaminérgico, noradrenérgico e serotonérgico no efeito tipo-antidepressivo gerado pelo bloqueio de receptores MT2 estriatais no modelo de DP induzido pela rotenona.

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

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

Maira J. Maturana · Cláudia Pudell · Adriano D. S. Targa · Laís S. Rodrigues · Ana Carolina D. Noseda · Mariana H. Fortes · Patrícia dos Santos · Cláudio Da Cunha · Sílvio M. Zanata · Anete C. Ferraz · Marcelo M. S. Lima

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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

M. J. Maturana · C. Pudell · A. D. S. Targa · L. S. Rodrigues · A. C. D. Noseda · M. H. Fortes · P. dos Santos · A. C. Ferraz · M. M. S. Lima
Laboratório de Neurofisiologia, Departamento de Fisiologia, Universidade Federal do Paraná, Curitiba, PR, Brazil

C. Da Cunha
Laboratório de Fisiologia e Farmacologia do Sistema Nervoso Central, Departamento de Farmacologia, Universidade Federal do Paraná, Curitiba, PR, Brazil

S. M. Zanata
Laboratório de Neurobiologia, Departamento de Patologia Básica, Universidade Federal do Paraná, Curitiba, PR, Brazil

M. M. S. Lima (✉)
Setor de Ciências Biológicas, Departamento de Fisiologia, Universidade Federal do Paraná, Av. Francisco H. dos Santos s/n, ZIP: 81.531-990Caixa Postal: 19031, Curitiba, Paraná, Brazil
e-mail: mmslima@ufpr.br

M. M. S. Lima
e-mail: mmeirasantoslima@yahoo.com.br

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

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ANEXO II – Artigo - Does Parkinson's Disease and Type-2 Diabetes Mellitus Present Common Pathophysiological Mechanisms and Treatments?

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Does Parkinson's Disease and Type-2 Diabetes Mellitus Present Common Pathophysiological Mechanisms and Treatments?

Marcelo M.S. Lima*, Adriano D.S. Targa, Ana C.D. Noseda, Laís S. Rodrigues, Ana Márcia Delattre, Fabíola V. dos Santos, Mariana H. Fortes, Maira J. Maturana and Anete C. Ferraz

Laboratório de Neurofisiologia. Departamento de Fisiologia. Universidade Federal do Paraná, Curitiba, PR, Brasil

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; hypoinsulinemia 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

*Address correspondence to this author at the Universidade Federal do Paraná, Setor de Ciências Biológicas, Departamento de Fisiologia, Av. Francisco H. dos Santos s/n, ZIP: 81.531 – 990, Caixa Postal: 19031, Curitiba, Paraná, Brasil; Tel: 0055-041-3361 1722; E-mails: mmslima@ufpr.br, mmeirasantoslima@yahoo.com.br

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ANEXO III- Material complementar – resultados do Teste Labirinto em Cruz Elevado (LCE)

O LCE foi realizado como demonstrado na figura 1, e os resultados estão representados na figura 2 (A, B, C e D).

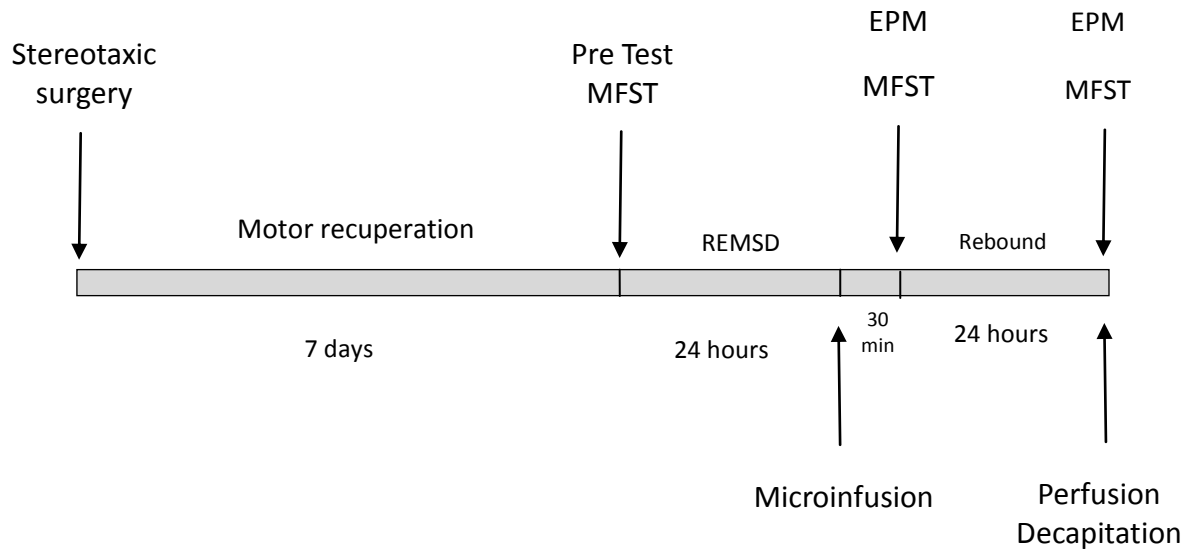

Figure 1

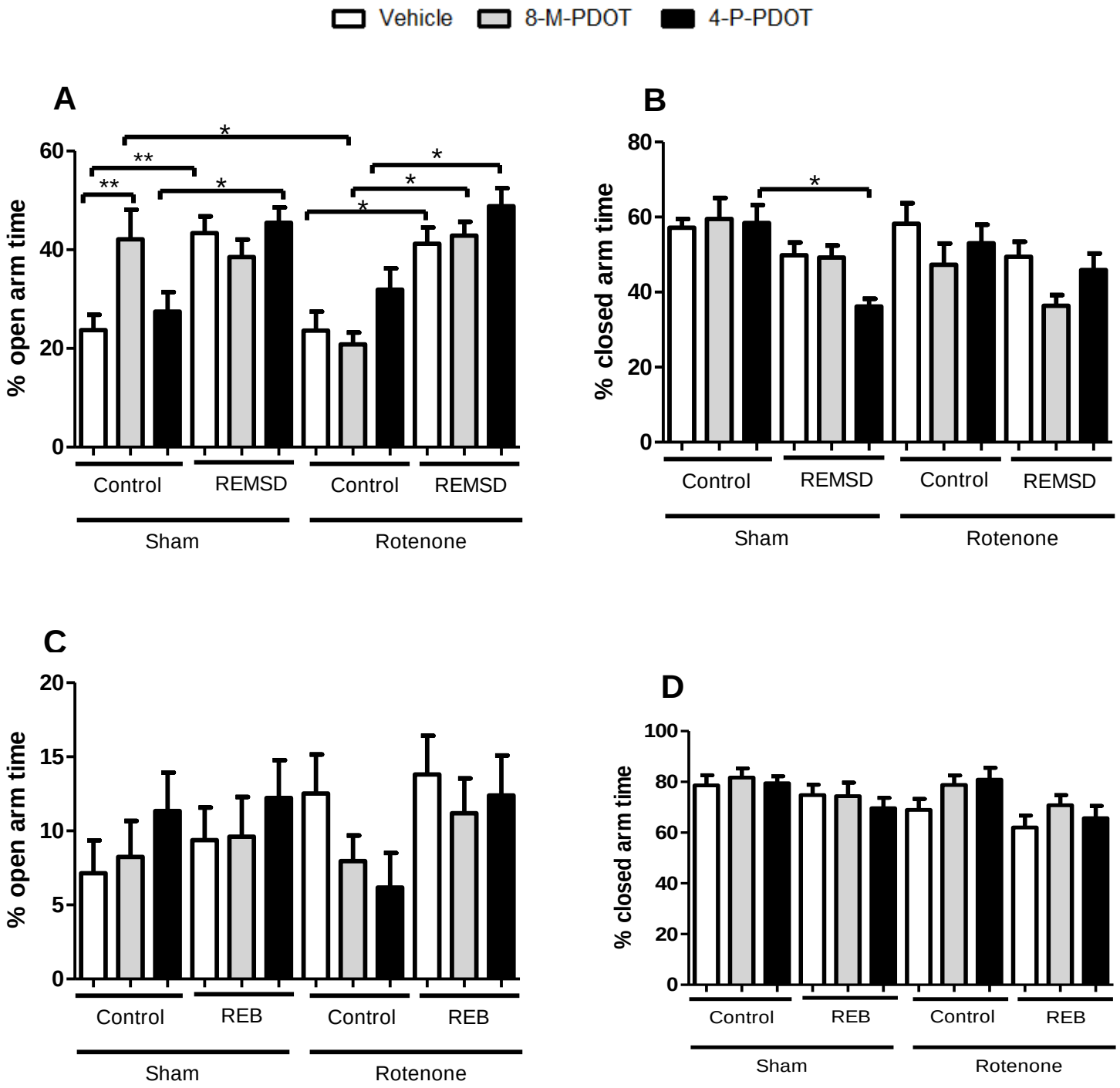

Figure 2. Anxiety-like parameters during plus maze test. **A**. % open arm time after REMSD, **B**. % closed arm time after REMSD, **C**. % open arm time after REB, **D**. % closed arm time after REB. The bars represent the mean ± standard error of the mean. n=15 per group, *P≤0.05, **P≤0.01, ***P≤0.001. Two-way ANOVA followed by the Bonferronipost hoc test.

ANEXO IV

Ministério da Educação
UNIVERSIDADE FEDERAL DO PARANÁ
Setor de Ciências Biológicas
Comissão de Ética no Uso de Animais
(CEUA)

UFPR
UNIVERSIDADE FEDERAL DO PARANÁ

Nº 695

CERTIFICADO

A Comissão de Ética no Uso de Animais (CEUA) do Setor de Ciências Biológicas da Universidade Federal do Paraná, instituído pela PORTARIA Nº 787/03-BL, de 11 de junho de 2003, com base nas normas para a constituição e funcionamento da CEUA, estabelecidas pela RESOLUÇÃO Nº 01/03-BL, de 09 de maio de 2003 e considerando o contido no Regimento Interno da CEUA, **CERTIFICA** que os procedimentos utilizando animais no projeto de pesquisa abaixo especificado, estão de acordo com os princípios éticos estabelecidos pelo Colégio Brasileiro de Experimentação Animal (COBEA) e exigências estabelecidas em "*Guide for the Care and Use of Experimental Animals (Canadian Council on Animal Care)*".

CERTIFICATION

The Ethics Animal Experiment Committee of the Setor de Ciências Biológicas of the Federal University of Paraná, established by the DECREE Nº 787/03-BL on June 11th 2003, based upon the RESOLUTION Nº 01/03-BL from May 9th 2003, and upon the CEUA internal regiment, CERTIFIES that the procedures using animals in the research project specified below are in agreement with the ethical principals established by the Experimental Animal Brazilian Council (COBEA), and with the requirements of the "Guide for the Care and Use of Experimental Animals (Canadian Council on Animal Care)".

PROCESSO: 23075.017829/2013-04 **APROVADO**: 20/06/2013 – R.O. 05/2013

TÍTULO: Análise do papel dos receptores melatoninérgicos MT2 estriatais na regulação do sono no modelo animal de Parkinsonismo induzido por rotenona intranigral

AUTORES: Marcelo de Meira Santos Lima, Ana Carolina Duarte Noseda

DEPARTAMENTO: Fisiologia

Prof. Dr. Aleksander Roberto Zampronio
Coordenador da CEUA