UNIVERSIDADE FEDERAL DO PAMPA

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PAPEL DO SISTEMA DOPAMINÉRGICO NOS DÉFICITS DE MEMÓRIA INDUZIDOS PELA PRIVAÇÃO MATERNAL

Uruguaiana
2020

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Dissertação apresentada ao Programa de Pós-Graduação em Bioquímica da Universidade Federal do Pampa como requisito parcial para obtenção do título de Mestre em Bioquímica.

Orientadora: Profa. Dra. Pâmela Billig Mello-Carpes

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APRESENTAÇÃO

O presente trabalho encontra-se dividido 3 partes. Na primeira parte, a sessão INTRODUÇÃO introduz o tema abordado neste documento. Posteriormente, são apresentadas informações atuais sobre o estado da arte dos principais temas abordados na sessão REVISÃO BIBLIOGRÁFICA, que é seguida pela JUSTIFICATIVA do estudo e seus OBJETIVOS.

Os materiais e métodos, bem como os resultados que compõe esta dissertação são apresentados na segunda parte, composta pelo MANUSCRITO CIENTÍFICO apresentado de acordo com as normas da revista ao qual foi submetido.

Na terceira parte da dissertação apresentamos uma breve DISCUSSÃO final, em português, bem como as CONCLUSÕES do estudo e as PERSPECTIVAS FUTURAS. A sessão REFERÊNCIAS BIBLIOGRÁFICAS apresenta as referências incluídas na primeira e na última parte desta dissertação. As referências citadas no manuscrito científico estão listadas ao final do mesmo.

RESUMO

Eventos estressores durante o período neonatal, como a privação maternal (MD, do inglês *maternal deprivation*), levam a déficits de memória que podem persistir durante a vida adulta. Alterações moleculares em estruturas importantes para os processos de memória, tais como o o hipocampo (HP) podem explicar os déficits de memória observados em modelos de MD em roedores. Além disso, existem evidências de que o sistema dopaminérgico, um importante sistema envolvido nos processos de consolidação e persistência da memória, pode ser afetado por este tipo de estresse crônico. Com os experimentos que compõem esta dissertação nós demonstramos que a MD em ratos resulta em déficits da memória de reconhecimento de objetos que são revertidos pela infusão intrahipocampal de dopamina, pela estimulação intra-hipocampal dos receptores dopaminérgicos D1 ou de seus segundos mensageiros, e pela administração periférica de um precussor da dopamina. Por outro lado, a estimulação intra-hipocampal dos receptores dopaminérgicos D5 ou de seus segundos mensageiros não gerou o mesmo efeito. O conjunto de resultados revela o papel do sistema dopaminérgico, especialmente dos receptores do tipo D1, nos déficits de memória relacionados à MD.

Palavras-Chave: Persistência da memória; estresse precoce; dopamina; hipocampo; L-Dopa.

ABSTRACT

Stressful events during the neonatal period, such as maternal deprivation (MD), lead to memory deficits that may persist into adulthood. Molecular changes in structures important for memory processes, such as the hippocampus (HP) may explain the memory deficits observed in rodents' MD models. In addition, there is evidence that this type of chronic stress can affect the dopaminergic system, an important neurotransmitter system involved in memory consolidation and persistence. With the experiments included in this thesis, we demonstrate that MD in rats results in object recognition memory deficits that are reversed by intrahippocampal infusion of dopamine, by intra-hippocampal stimulation of dopaminergic D1 receptors or their second messengers, and by the peripheral administration of a dopamine precursor. On the other hand, intra-hippocampal stimulation of dopaminergic D5 receptors or their second messengers did not have the same effect. The set of results reveals the role of the dopaminergic system, especially of type D1 receptors, in memory deficits related to MD.

Keywords: memory persistence; early life stress; dopamine; hippocampus; L-Dopa

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LISTA DE SIGLAS

Sigla	Definição
BDNF	Fator neurotrófico derivado do cérebro (do inglês *brain-derived neurotrophic factor*)
CaMK II	Proteínoquinase dependente de Ca^{2+}/calmodulina II (do inglês, Ca^{2+}/calmodulin-dependent protein kinase II
cAMP	Adenosina 1', 5'- monofosfato cíclico (do inglês, C*yclic adenosine monophosphat*e)
CNS	Sistema nervoso central (do inglês *central nervous system*).
CREB	Proteínas ligantes ao elemento cAMP (do inglês, *cAMP-response binding protei*n)
EPM	Labirinto em cruz elevado (do inglês elevated plus maze).
ERKs	Proteínoquinases dependentes de estímulos extracelulares (ERK, do inglês *extracellularly responsive kinase*)
HP	Hipocampo
LTM	Memória de longa duração (do inglês *long-term memory*)
MD	Privação maternal (do inglês m*aternal deprivation*).
NS	Sistema nervoso (do inglês, *nervous system)*
OR/RO	Reconhecimento de objetos (do inglês *object regonition).*
PFC	Córtex pré-frontal (do inglês *prefrontal cortex*)
PKA	Proteínoquinase dependente de cAMP (do inglês *Protein Kinase A*)
PKC	Proteínoquinase dependente de Ca^{2} (do inglês *Protein Kinase C*)
PND	Dia pós-natal (do inglês *postnatal day*)
PNS	Sistema nervoso periférico (do inglês *peripheral nervous system*)
STM	Memória de curta duração (do inglês *short-term memory*)
VTA	Área tegmentral ventral (do inglês *ventral tegmental area*)

LISTA DE DROGAS

Droga/sigla	Ação
DH	Cloridrato de dopamina (do inglês *dopamine hydrochloride*) - agonista não seletivo de receptores dopaminérgicos.
SKF 38393	Agonista seletivo de receptores D1/D5.
SCH 23390	Antagonista seletivo de receptores D1/D5.
Sp-cAMP	Estimulador de PKA.
PMA	Estimulador de PKC.
Rp-Camp	Inibidor de PKA.
Gö 6976	Inibidor de PKC.
L-Dopa	Levodopa; Precursor de dopamina

SUMÁRIO

PARTE I

1 INTRODUÇÃO

O estresse durante os primeiros anos de vida pode levar à alterações no desenvolvimento do sistema nervoso central (CNS, do inglês *Central Nervous System*) (TYBOROWSKA *et al.*, 2018). Um exemplo de estresse no início da vida que gera alterações neuroquímicas e estruturais das rede neurais é a privação maternal (MD, do inglês *maternal deprivation*) (BENETTI *et al.*, 2015). Mesmo por curtos períodos (p. ex. durante os dez primeiros dias, 3 horas/dia), a MD pode levar a danos cognitivos que persistem durante a vida adulta em roedores (SOSA *et al.*, 2019; MENEZES *et al.*, 2020).

Diversos mecanismos pelos quais o estresse durante o período neonatal atua vêm sendo propostos. O estresse durante os primeiros dias de vida pode promover modificações anatômicas, tais como maturação anormal de redes neurais (REINCKE E HANGANU-OPATZ, 2017), atrofia ou hipotrofia de estruturas dendríticas em diferentes áreas cerebrais (IVY *et al.*, 2010), além de alterar o número e função de sinapses (SCHMIDT *et al.*, 2011). O desenvolvimento de estruturas envolvidas em funções cognitivas, como o córtex pré-frontal (PFC, do inglês *pre-frontal cortex*) e hipocampo (HP) é especialmente sensível ao estresse durante o período neonatal (REINCKE E HANGANU-OPATZ, 2017). Além disso, foi demonstrado que modelos de estresse neonatal que interrompem a relação mãe-filhote por tempo mais ou menos prolongado levam a alterações na função de diferentes sistemas de neurotransmissores durante a vida adulta, tais como o sistema histaminérgico (BENETTI *et al.*, 2015), colinérgico (BENETTI *et al.*, 2009), serotoninérgico (RENTESI *et al.*, 2013) e glutamatérgico (ROCERI *et al.*, 2002).

Um importante sistema de neurotransmissão é o sistema dopaminérgico. Além de desempenhar um papel em respostas emocionais ao estresse (ZHU *et al.*, 2011), o sistema dopaminérgico está envolvido na consolidação e persistência de diferentes tipos de memórias dependentes do HP e PFC (FURINI *et al.*, 2014). O HP e PFC, por sua vez, recebem aferências dopaminérgicas da área tegmentar ventral (VTA, do inglês *Ventral Tegmentar Area*), da substância nigra (SN), e do *locus coeruleus* (LC) (MCNAMARA E DUPRET, 2017). Sabe-se que a exposição ao estresse durante o período neonatal resulta em prejuízo de aprendizagem durante a vida

adulta, causando alterações na função do HP e PFC (BENETTI *et al.*, 2009); sabe-se, ainda, que a exposição a glicocorticoides no período pré-natal afeta o desenvolvimento do sistema dopaminérgico (PIAZZA E LE MOAL, 1996). Por outro lado, pouco se sabe sobre o envolvimento do sistema dopaminérgico e seus diferentes receptores no dano de memória induzido por estresse durante o período neonatal.

2 REVISÃO BIBLIOGRÁFICA

2.1 Neurodesenvolvimento

2.1.1 Período pré-natal

O processo de desenvolvimento do Sistema Nervoso (NS, do inglês *nervous system*) começa nos primeiros estágios do período embrionário (ANDERSEN, 2003) (Figura 1). Inicialmente, ocorrem divisões mitóticas do zigoto até formar a mórula – esfera sólida composta por células (PALERMO *et al.*, 1994). A divisão celular continua e logo surge uma cavidade no seu interior, chamada blastocele (STILES E JERNIGAN, 2010). A proliferação celular da blástula segue, e no final da primeira semana já está fortemente inserida na parede uterina, passando a se chamar blastocisto (JACOBSON, 2013) (Figura 1A). Nesta ocasião, forma-se uma nova cavidade na porção mais espessa, chamada cavidade aminótica (Figura 1C). Uma estrutura composta por dois folhetos de células separa a cavidade amniótica da blastocele: um folheto mais interno, chamado e *endoderma*, e um folheto mais externo chamado de *ectoderma* (Figura 1B). O *ectoderma* dará origem ao NS (LENT, 2010).

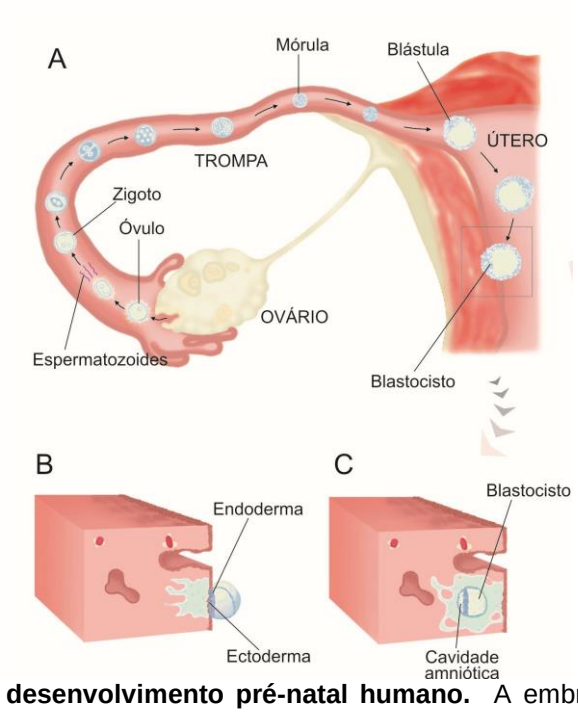

Figura 1. Cronologia do desenvolvimento pré-natal humano. A embriogênese ocorre no útero materno. A. Eventos que ocorrem desde a fecundação até a implementação do blastocisto no útero. B. Implementação do embrião na parede uterina. C. Fase de aparecimento da cavidade aminiótica. Fonte: Adaptada de Lent (2010).

A formação do sistema nervoso central (CNS, do inglês *central nervous system*) inicia com a formação do tubo neural a partir do ectoderma (Figura 2) (STILES E JERNIGAN, 2010). Quando o tubo está prestes a se formar, algumas células se destacam, formando duas lâminas longitudinais conhecidas como *cristas neurais*. O tubo neural irá formar o CNS, enquanto as cristas darão origem ao sistema nervoso periférico (PNS, do inglês *peripheral nervous system*) (STILES E JERNIGAN, 2010).

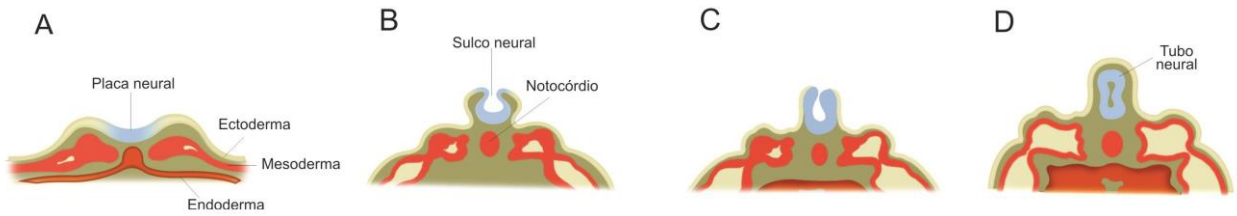

Figura 2. Formação do túbo neural. A formação túbo neural ocorre no primeiro mês de gestação. O fechamento ocorre do centro do tubo para as extremidades cranial e caudal (A – D). Fonte: Adaptada de Lent (2010).

Após o fechamento do tubo neural, a extremidade cranial se dilata, formando 3 vesículas encefálicas primitivas, resultante da intensa proliferação celular (STILES E JERNIGAN, 2010). A vesícula anterior é chamada de *prosencéfalo*; a do meio, *mesencéfalo*; e a posterior, *rombencéfalo (STILES E JERNIGAN, 2010)*. Após este período de formação, as vesículas primitivas subdividem-se. O *prosencéfalo* forma o *telencéfalo* e o *diencéfalo (STILES E JERNIGAN, 2010).* O *mesencéfalo* não se modifica muito. O *rombencéfalo* subdivide-se em *metencéfalo* e *mielencéfalo (STILES E JERNIGAN, 2010)*. Para trás do *mielencéfalo*, o tubo neural continua cilíndrico, transformando-se gradativamente na *medula espinhal primitiva (STILES E JERNIGAN, 2010)*.

A vesícula telencefálica cresce e forma os dois hemisférios cerebrais e os núcleos da base (STILES E JERNIGAN, 2010). A medida em que ocorre o crescimento surgem sulcos e giros que promovem o aumento da superfície do córtex. Dentre as estruturas encefálicas de desenvolvimento mais tardio estão o hipocampo (HP), o córtex e o cerebelo (JACOBSON, 2013).

Ao longo de todo o neurodesenvolvimento as células neurais crescem e diferenciam-se (MCCONNELL E KAZNOWSKI, 1992). A medida que as regiões do tubo neural vão ficando mais espessas, como a região que dará início ao cérebro, novos neurônios deslocam-se ativamente de acordo com predeterminação genética (NADARAJAH *et al.*, 2003). Essa migração é auxiliada por células específicas da glia (STITT *et al.*, 1991). Uma vez que os neurônios atingem seu local de destino eles se arborizam e estabelecem novas conexões (ANDERSEN, 2003), processo que é mediado, em grande parte, pelo fator neurotrófico derivado do cérebro (BDNF, do inglês *brain-derived neurotrophic factor*) (GOLDMAN-RAKIC E BROWN, 1982). Além disso, outros rearranjos morfológicos são iniciados com o objetivos de aumentar a eficiência da transmissão sináptica, tais como morte celular programada ou apoptose (STITT *et al.*, 1991).

2.1.2 Período pós-natal

O neurodesenvolvimento não cessa após o nascimento; ao contrário, o CNS continua a transforma-se até a vida adulta (LEE *et al.*, 2015). Fatores de crescimento desempenham um papel importante na plasticidade sináptica e são essenciais para alterações neuronais associadas ao aprendizado (GOONEY *et al.*, 2002).

A proliferação e migração de células nervosas são estendidas para além do período pré-natal. Durante esse período, há uma interação entre células gliais e neurônios que desempenha um importante papel na organização funcional da circuitaria neural (STILES E JERNIGAN, 2010).,Novos neurônios surgem e migram de zonas periventriculares para o bulbo olfatório, além de serem produzidos no giro denteado do hipocampo (CAYRE *et al.*, 2009). Interessantemente, esses mecanismos de produção e migração de novos neurônios parecem estender-se ao longo da vida adulta (CAYRE *et al.*, 2009). Além de células neuronais, progenitores gliais proliferam-se em regiões subventriculares e migram para outras regiões encefálicas, tais como o córtex, estriado e hipocampo, onde se diferenciam em oligodendrócitos e astrócitos (STILES E JERNIGAN, 2010).

Outra característica importante durante o período pós-natal é a mielinização dos neurônios. Células progenitoras de oligodendrócitos começam a diferenciar-se e a formar uma membrana que envolve os neurônios próximos (LIN E BERGLES, 2004; STILES E JERNIGAN, 2010), aumentando a velocidade de condução dos neurônios,

além de sintetizarem um número de fatores tróficos que influenciam no tamanho e no diâmetro axonal (MCTIGUE E TRIPATHI, 2008) .

Durante os primeiros dias de vida neurônios dopaminérgicos, noradrenérgicos e glutamatérgicos têm seu crescimento acelerado e ocorre a sua maturação (CAYRE *et al.*, 2009; STILES E JERNIGAN, 2010). Biomarcadores de neurônios monaminérgicos, que incluem as atividades das enzimas tirosina hidroxilase e monoaminoxidase, permitem verificar tais modificações, assim como é possível detectar o aumento dos sítios de recaptação de dopamina e o aumento dos níveis de dopamina (GOLDMAN-RAKIC E BROWN, 1982; BROADDUS E BENNETT, 1990).

Embora mudanças ocorram em todo o cérebro durante os primeiros estágios do neurodesenvolvimento pós-natal, estudos têm demonstrado que mudanças na densidade sináptica ocorrem mais precocemente em níveis subcorticais do que em níveis corticais (ANDERSEN *et al.*, 2000; DERKS *et al.*, 2016). Mudanças anatômicas dessas regiões são acompanhadas de alterações funcionais em regiões corticais e subcorticais *(STILES E JERNIGAN, 2010)*. Por exemplo, as mudanças morfológicas de neurônios dopaminérgicos coincidem com picos aumentados de dopamina no córtex (GOLDMAN-RAKIC E BROWN, 1982). A maturação de áreas cognitivas também obedece a remodelação sináptica e o aumento da conectividade entre neurônios antes da vida adulta. Assim, qualquer alteração ou estresse durante os primeiros anos de vida pode alterar o processo normal do neurodesenvolvimento, principalmente em áreas relacionadas a cognição (HUMPHREYS *et al.*, 2019).

2.1.3 Sistema dopaminérgico

A dopamina é um importante neurotransmissor do CNS, e está envolvida com diversas funções. Além de desempenhar um papel importante no controle do movimento voluntário, o sistema dopaminérgico cria associações com estímulos compensadores, respondendo a estímulos ambientais importantes, motivando comportamentos de recompensa ou punição (BISSONETTE E ROESCH, 2016). Além disso, o sistema dopaminérgico atua na manutenção da memória de trabalho e na regulação de emoções (BISSONETTE E ROESCH, 2016). O fato desse sistema ser crítico para o comportamento animal básico coincide com a observação das implicações que mudanças nessa população neural tem em muitas doenças

psiquiátricas e neurológicas, incluindo a doença de Parkinson, a esquizofrenia e a depressão (WOHLEB *et al.*, 2016).

A dopamina é sintetizada em terminais pré-sinápticos a partir do aminoácido tirosina, oriundo do espaço extracelular, internalizado através de um transportador de tirosina (MEISER *et al.*, 2013). A conversão de tirosina em dopamina é feita pelas enzimas tirosina hidroxilase e dopa descarboxilase (MEISER *et al.*, 2013). A partir de então, a dopamina é empacotada em vesículas sinápticas para posterior liberação na fenda sináptica (MEISER *et al.*, 2013). Além de um transportador de tirosina, o terminal pré-sináptico de neurônios dopaminérgicos contém uma bomba de recaptação de dopamina, denominada DAT (MEISER *et al.*, 2013), de forma que ela pode ser novamente armazenada em vesículas sinápticas (Figura 3).

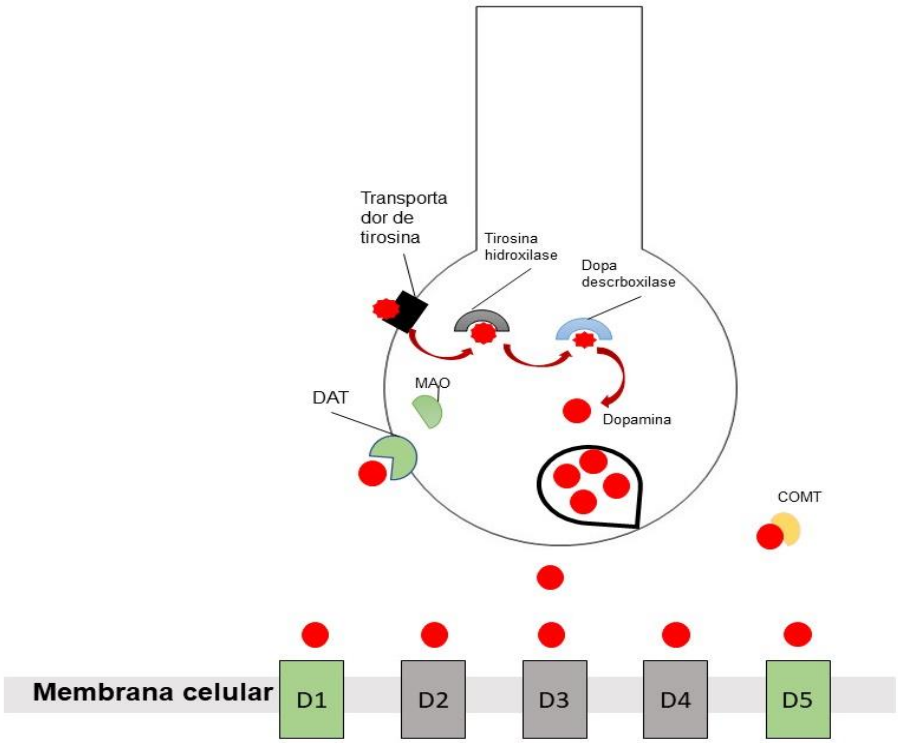

Figura 3. Processos de síntese, liberação, degradação e interação da dopamina com os diferentes receptores. A síntese de dopamina requer a captação e transporte de tirosina do meio extracelular para o meio intracelular por uma proteína transmembrana específica (DAT). A tirosina é convertida em dopamina por uma série de reações enzimáticas, envolvendo a ação das enzimas tirosina hidroxilase e dopa descarboxilase. Posteriormente, a dopamina é empacotada em vesículas sinápticas, e, mediante estímulos específicos pode ser liberada na fenda sináptica e interagir com seus diferentes receptores (D1-D5) na membrana pós-sináptica. A dopamina é degradada por duas enzimas, a monoaminooxidase (MAO) no meio intracelular, e a catecol-O-transferase (COMT), no meio extracelular. Fonte: produzida pelo próprio autor (2020).

A dopamina pode ser degradada tanto no meio intracelular quanto no meio extracelular. A enzima monoaminoxidase (MAO) é responsável pela degradação dopaminérgica no meio intracelular (BEST *et al.*, 2009; MEISER *et al.*, 2013), enquanto a enzima catecol-O-transferase (COMT) é responsável pela degradação da dopamina no meio extracelular (BEST *et al.*, 2009; MEISER *et al.*, 2013) (Figura 3).

Existem basicamente três vias dopaminérgicas encefálicas que exercem diferentes funções (Figura 4). A primeira, conhecida como via mesolímbica, é formada por eferências dopaminérgicas que conectam a área tegmental ventral (VTA, do inglês *ventral tegmantal area*) ao núcleo accumbes, situado no estriado ventral (Figura 4) (LEDONNE E MERCURI, 2017). A via dopaminérgica mesolimbica está relacionada a motivação, o prazer e a recompensa (LEDONNE E MERCURI, 2017), e desempenha um papel importante em comportamentos emocionais como psicose, delírios e alucinações (JAHNG *et al.*, 2010). Assim, estudos vêm demonstrando que o uso de drogas, como anfetaminas e cocaína, causa hiperativação da via dopaminérgica mesolímbica, com consequente aumento da liberação de dopamina, levando a comportamentos do tipo psicóticos (JAHNG *et al.*, 2010; SICILIANO *et al.*, 2015; LEDONNE E MERCURI, 2017).

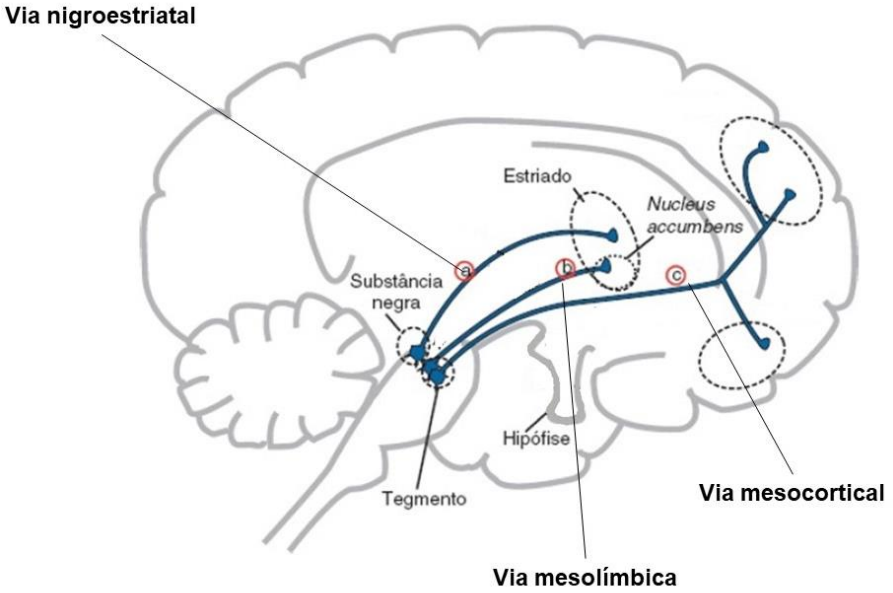

Figura 4 Principais vias dopaminérgicas encefálicas. a) via mesolímbica, projetando-se da área tegemental ventral para o nucleo accumbes; b) via nigroestriatal projetando-se da área tegmental ventral para o estriado; e, c) via mesocortical, projetando-se da área tegemental ventral para o córtex. Fonte: Adaptada de Stahl (2002).

Outra via dopaminérgica importante é a nigroestriatal (Figura 4). Essa via é composta de eferências dopaminérgicas que vão da substância nigra, localizada no mesencéfalo, para o estriado (LEDONNE E MERCURI, 2017), e está relacionada ao controle dos movimentos voluntários (LEDONNE E MERCURI, 2017). A deficiência de dopamina nessa via está relacionada à alterações do movimento, tais como rigidez, acinesia ou bradicinesia, e tremores, que são sintomas característicos da doença de Parkinson (JUNG E BENNETT, 1996).

A via mesocortical projeta eferências dopaminérgicas da VTA para o córtex pré-frontal (PFC, do inglês *prefrontal cortex*) (Figura 4)(LEDONNE E MERCURI, 2017). Aferências dopaminérgicas para o PFC dorsolateral regulam a cognição e as funções executivas (LAPISH *et al.*, 2007). Já aferências para a região ventromedial do PFC controlam as emoções e o afeto (LAPISH *et al.*, 2007; LEDONNE E MERCURI, 2017). Assim, prejuízos cognitivos associados a doenças psiquátricas, tias como esquizofrenia e depressão, podem advir de uma deficiência da atividade da via dopaminérgica mesocortical (MASANA *et al.*, 2011). Redução da atividade dopaminérgica mesocortical também está relacionada a déficit de atenção, problemas de aprendizagem e dificuldades na tomada de decisão (ENGERT E PRUESSNER, 2008).

Após sua liberação na fenda sináptica, a dopamina fica livre para interagir com seus diferentes tipos de receptores. Atualmente, foram identificados 5 subtipos de receptores dopaminérgicos acoplados a proteína G (BEAULIEU E GAINETDINOV, 2011; LEDONNE E MERCURI, 2017). Para efeitos classificatórios, os receptores dopaminérgicos podem ser divididos em duas famílias: (a) família D1, na qual fazem parte os receptores dopaminérgicos D1 e D5; (b) família D2, na qual fazem parte os receptores dopaminérgicos D2, D3, D4 (Figura 3) (BEAULIEU E GAINETDINOV, 2011).

Essas diferentes proteínas receptoras possuem uma distribuição distinta no cérebro. Por exemplo, receptores D1 e D2 têm altos níveis de expressão no estriado, onde desempenham um papel importante no controle motor (BEAULIEU E GAINETDINOV, 2011). Já os receptores D3 estão presentes em maiores níveis no núcleo accumbes e o tubérculo olfatório (SEALFON E OLANOW, 2000; BEAULIEU E GAINETDINOV, 2011). Maiores densidades dos receptores D4 foram encontradas no córtex frontal e tronco encefálico (SEALFON E OLANOW, 2000; BEAULIEU *et al.*, 2015).

Além disso, receptores D1 e D5 são amplamente distribuídos em estruturas encefálicas relacionadas a processos cognitivos, como o hipocampo, e desempenham papel fundamental dos processos de consolidação e persistência da memória (ROSSATO *et al.*, 2009).

O processo de desenvolvimento do sistema dopaminérgico ocorre por uma série de etapas que determinam sua migração, localização, diferenciação, especificação e conectividade, que são reguladas no espaço e no tempo (BISSONETTE E ROESCH, 2016). Essas células são derivadas de células progenitoras localizadas na linha média ventral da placa do tubo neural (ONO *et al.*, 2007). Após uma série de sinalizações que determinam tanto o padrão antero-pôsterior, quanto o padrão dorso-ventral (NAKAMURA E WATANABE, 2005), as células progenitoras diferenciam-se em células dopaminérgicas específicas (BRODSKI *et al.*, 2003). A migração das células precussoras de células dopaminérgicas para seus locais finais resulta no início da expressão do gene Pitx3 (VAN DEN MUNCKHOF *et al.*, 2003). Essa população de neurônios dopaminérgicos normalmente de desenvolve na substância nigra (SNc), no LC e na VTA (VAN DEN MUNCKHOF *et al.*, 2003). Áreas como a VTA e o LC já foram identificadas como fonte de dopamina tanto para o PFC quanto para o HP (KEMPADOO *et al.*, 2016; CASTILLO DÍAZ *et al.*, 2017).

Embora a migração e a diferenciação celular sejam críticas, as conexões do sistema dopaminérgico estabelece determinam quais aspectos funcionais a dopamina é capaz de influenciar. Assim, a maneira e a quantidade de estruturas neurais que os neurônios dopaminérgicos invervam podem desempenhar um papel fundamental em diferentes comportamentos, tais como motivação e cognição (BISSONETTE E ROESCH, 2016). Mudanças em conexões do sistema dopaminérgico têm sido associadas ao uso e dependência de drogas (ROBINSON E KOLB, 2004).

2.2 Aprendizagem e memória

A aprendizagem é definida como *aquisição* de uma informação, ou seja, é a entrada de um evento qualquer nos sistemas neurais ligados à memória (IZQUIERDO, BEVILAQUA, ROSSATO*, et al.*, 2006). A informação pode ser a imagem de um objeto, um som, um acontecimento, uma sequência de movimentos, etc.; esses eventos podem ter origem interoceptiva ou exteroceptiva (IZQUIERDO, BEVILAQUA, ROSSATO*, et*

al., 2006). Já a memória corresponde ao processo de retenção, manutenção e evocação de informação previamente adquiridas pelo CNS (IZQUIERDO, 2002).

Após o processo de *aquisição*, a informação pode ser armazenada por alguns segundos ou até por anos; esse processo é chamado de *retenção* da memória (IZQUIERDO *et al.*, 1998a), e, desta forma, as informações selecionadas ficam (ou não) disponíveis para serem lembradas (IZQUIERDO *et al.*, 1998a). Com o passar do tempo, algumas dessas informações podem desaparecer da memória: é o *esquecimento* (IZQUIERDO, BEVILAQUA E CAMMAROTA, 2006); embora existam outros processos capazes de alterar a evocação, tais como a extinção da memória. Assim, a retenção de uma informação nem sempre é duradoura (IZQUIERDO, BEVILAQUA E CAMMAROTA, 2006).

As memórias podem ser classificadas quanto ao tempo de retenção, podendo ser divididas em: (i) memória de trabalho, na qual a informação é mantida por alguns segundos e é processada basicamente pela atividade elétrica do córtex pré-frontal, não deixando traços bioquímicos; (ii) memória de curta duração (STM, do inglês *short-term memory*), que dura de 1 a 6 horas, depende da atividade de enzimas no hipocampo, córtices entorrinal e perirrinal; e, (iii) memória de longa duração (LTM, do inglês *long-term memory*), que dura de 6 horas até dias, meses e/ou anos, sendo armazenada em diversas áreas do cérebro que sempre incluem o hipocampo e podem abranger a amígdala, o cerebelo, o septo, o estriado e regiões diversas do córtex (IZQUIERDO *et al.*, 1998b).

Além disso, as memórias podem ser classificadas quanto ao conteúdo em memória declarativas ou explícitas, e memórias implícitas ou procedurais. As memórias declarativas podem ser subdivididas em (i) memórias episódicas, que se referem a eventos, e, (ii) memórias semânticas, que se referem a fatos e conhecimentos (i.e., conhecimento de história, geografia, física) (IZQUIERDO *et al.*, 1998b). As memórias procedurais são também chamadas de memórias de procedimento e referem-se à habilidades motoras adquiridas (i.e., andar de bicicleta, nadar, tocar violão) (IZQUIERDO, 2002) .

Embora haja uma classificação das memórias, tanto em humanos quanto em animais, há uma sobreposição entre os diferentes tipos e subtipos. Assim, uma memória declarativa pode ser de curta ou longa duração; um aprendizado episódico pode fazer parte de uma memória semântica, etc. Em animais, a memória explícita,

por exemplo, pode ser classificada em dois subtipos: memórias não associativas; e memórias associativas. Assim, enquanto as tarefas de reconhecimento de objetos (OR, do inglês *object recognition*) e labirinto aquático de Morris (MWM, do inglês Morris' water maze) avaliam o aprendizado associativo, a habituação ao campo aberto (OF, o inglês o*pen field*) avalia a memória não associativa, por exemplo (IZQUIERDO *et al.*, 1998a; IZQUIERDO, 2002; QUILLFELDT, 2015) (Figura 5).

Figura 5 Principais tarefas de avaliação do aprendizado e memória em roedores. De forma didática, a memória em animais pode ser dividida em memória explicita e implícita e ambas podem ocorrer por associação ou não. Fonte: Adaptada de Quillfeldt (2015) .

Em nível celular, a consolidação da memória declarativa em LTM é dada pela estimulação de neurônios hipocampais glutamatérgicos (IZQUIERDO *et al.*, 1998a; RIEDEL *et al.*, 2003). Contudo, outros neurotransmissores, tais como acetilcolina, noradrenalina e dopamina muitas vezes são moduladores essenciais de neurônios glutamatérgicos (IZQUIERDO *et al.*, 1998a; CASTILLO DÍAZ *et al.*, 2017). Existem 3 tipos de receptores glutamatérgicos: os receptores N-metil-D-aspartato (NMDA); os receptores ácido α-amino-3-hidroxi-5-metil-4-isoxazolepropiônico (AMPA); e os receptores metabotrópicos.

No princípio do processo de consolidação da memória, a entrada de Na^+ e Ca^{2+}, causada pela estimulação dos receptores do tipo NMDA hipocampais, é somada ao Ca^{2+} intracelular e ativa a proteinoquinase dependente de

Ca^{2+}/calmodulina II (CaMKII, do inglês, *Ca^{2+}/calmodulin-dependent protein kinase II*), enzima que promove a fosforilação de proteínas na membrana (IZQUIERDO *et al.*, 1998b) (Figura 6). O influxo de Ca^{2+} também ativa a proteínoquinase dependente de adenosina 1',5'- monofosfato cíclico (PKA, do do inglês *protein kinase cAMP) (IZQUIERDO et al., 1998b).* A ativação da proteínoquinase dependente de cálcio (PKC, do inglês *protein kinase Ca^{2+} -dependent*) soma efeitos aos das proteínoquinases dependentes de estímulos extracelulares (ERK, do inglês *extracellularly responsive kinase*) (IZQUIERDO, 2002). Assim, tanto as subunidades catalíticas da PKA e PKC quanto das ERKs podem migrar até o núcleo e ativar fatores de transcrição de proteínas ligantes ao elemento cAMP (CREB, do inglês *cAMP-response binding protein*) (IZQUIERDO, BEVILAQUA, ROSSATO*, et al.*, 2006) (Figura 6). Evidências demonstram que a fosforilação de CREB é crucial para a formação da LTM (IZQUIERDO, 2002; IZQUIERDO, BEVILAQUA, ROSSATO*, et al.*, 2006).

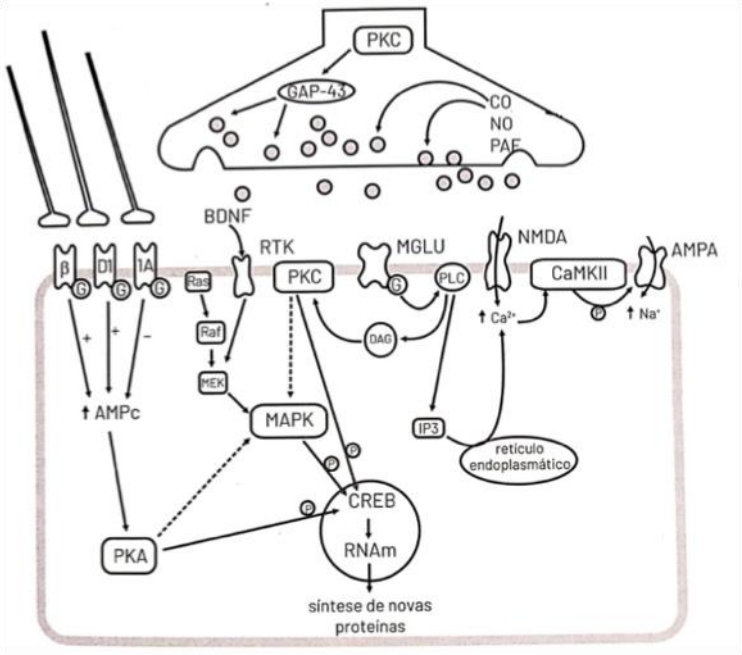

Fig. 6. Mecanismos intracelulares do processo de consolidação da memória. O processo de consolidação da memória requer a ativação de receptores glutamatérgicos NMDA e AMPA. O influxo de Ca^{2+} ativa enzimas intracelulares que culminam na forforilação de CREB e síntese de proteínas, como o BDNF. Fonte: Adaptado de Quevedo (2019).

2.3 Sistema dopaminérgico e os processos de consolidação e persistência da memória

Ao contrário do processo de consolidação da memória, que já foi amplamente estudado, o processo de persistência ainda não é totalmente compreendido. Embora

a persistência de uma memória dependa fortemente do seu processo de consolidação, mecanismos específicos relacionados à persistência têm sido estudados (ROSSATO *et al.*, 2009). Nas primeiras horas (6 a 24 horas) a persistência da memória depende da síntese do fator neurotrófico derivado do cérebro (BDNF, do inglês *Brain Derived Neurotrophic Fator)* no hipocampo, processo que é modulado por vias dopaminérgicas (GRANADO *et al.*, 2008).

Após a liberação da dopamina na fenda sináptica, este neurotransmissor pode interagir com famílias de receptores dopaminérgicos do tipo I (D1) ou do tipo II (D2) no membrana do neurônio pós-sináptico (HANSEN E MANAHAN-VAUGHAN, 2014). A família D1 é subdividida em receptores D1 e D5, ambos presentes em estruturas cerebrais relacionadas a funções cognitivas, como o hipocampo e o córtex pré-frontal (FURINI *et al.*, 2014). Embora esses dois tipos de receptores metabotrópicos sejam ativados pela interação com a dopamina, ativam diferentes segundos mensageiros: enquanto os receptores D1 usam a adenilato ciclase como o segundo mensageiro, modulando a PKA, os receptores D5 podem ativar o sistema fosfatidilinositol-3-quinase (PI3K) e modulam a atividade da PKC (FELDER *et al.*, 1989; FURINI *et al.*, 2014) (Figura 7). Tanto PKA quanto PKC estão envolvidas na consolidação de diferentes tipos de memórias (IZQUIERDO *et al.*, 2008; MICHEL *et al.*, 2011).

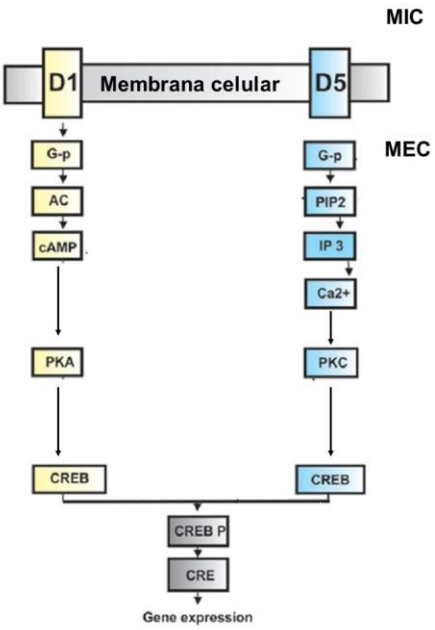

Fig. 7. Cascata bioquímica desencadeada pela estimalação dos receptores dopaminérgicos D1 e D5. Enquanto a estimulação dos receptores dopaminérgicos D1 regula a atividade da proteína quinase A (PKA) através do aumento do cAMP, a estimulação dos receptores dopaminérgicos D5

regula a atividade da proteína quinase C (PKC), via fosfolipase C. Ambas as enzimas são importantes para o processo de consolidação e persistência da memória. MIC: Meio intracelular; MEC: Meio extracelular. Fonte: produzida pelo próprio autor (2020).

Um fator importante para a persistência da LTM é o nível de alerta emocional, principalmente durante a fase inicial da sua formação. Em situações de alerta, neurônios dopaminérgicos da VTA são ativados, culminando na liberação de dopamina na região CA1 do hipocampo (MCNAMARA E DUPRET, 2017). Estudos prévios têm demonstrado que a persistência LTM é dependente desta circuitaria e que depende da estimulação dos receptores dopaminérgicos da família D1 (D1 e D5), levando à síntese de BDNF no hipocampo (KUPPERS E BEYER, 2001; GRANADO *et al.*, 2008).

Rossato *et al.* (2009) demonstraram mecanismos de persistência da memória desencadeado no hipocampo 12h após a aquisição da informação (ROSSATO *et al.*, 2009). Nessa fase, a ativação de neurônios dopaminérgicos da VTA culmina na liberação de dopamina da região CA1 do hipocampo (ROSSATO *et al.*, 2009). Uma vez liberada na sinapse, a dopamina age sobre receptores dopaminérgicos D1/D5 (ROSSATO *et al.*, 2009). A ativação desses receptores leva à rápida síntese e liberação de BDNF no hipocampo, resultando em um fortalecimento das sinapses hipocampais que participam da recente consolidação dessa memória e sua persistência (ROSSATO *et al.*, 2009). Além disso, Eckel-Mahan *et al.* (2008) demonstraram um mecanismo cíclico também envolvido na persistência da memória, que consiste no aumento da atividade das proteínoquinases dependentes de estímulos extracelulares (ERKs, do inglês *extracellular signal-regulated kinase*) e da proteínoquinase dependente Ca^{2+}/calmodulina (CaMKII, do inglês *Ca^{2+}/calmodulin-dependent protein kinase II*) no hipocampo a cada 12h após a aprendizagem (ECKEL-MAHAN *et al.*, 2008).

Assim, tanto o mecanismo que envolve a ativação de neurônios dopaminérgicos da VTA, com consequente aumento de BDNF, quanto a ativação do sistema circadiano que regula os níveis de CaMKII e ERKs, são iniciados no hipocampo 12 horas após a aquisição da informação; os dois processos determinam a persistência das memórias.

2.4 Estresse neonatal e prejuízos da aprendizagem e memória

O estresse pode ser definido como o desequilíbrio homeostático que pode ser desencadeado por uma ameaça ao bem-estar (ULRICH-LAI E HERMAN, 2009). Os eventos estressores podem ter origem interoceptiva (p. ex. alteração da osmolalidade) ou exteroceptiva (p. ex. ameaça de um predador) (ULRICH-LAI E HERMAN, 2009). Essas informações desencadeiam a ativação de circuitos neurais e neuroendócrino específicos, gerando uma resposta fisiológica para a manutenção as homeostasia (ULRICH-LAI E HERMAN, 2009). Nesse sentido, o sistema nervoso autônomo desencadeia respostas imediatas à exposição ao estresse por meio do sistema nervoso simpático e parasimpático (ULRICH-LAI E HERMAN, 2009), levando a uma restauração rápida da homeostase (p. ex. controle barorreflexo da pressão arterial). Por outro lado, a ativação do eixo hipotálamo-pituitária-adrenal (HPA) culmina no aumento dos níveis de glicocorticoides plasmáticos que são relativamente lentos na manutenção da homeostase (VAN BODEGOM *et al.*, 2017).

Embora o estresse tenha um valor fisiológico na manutenção da homeostase, o estresse por um período prolongado pode acarretar em mudanças importantes, principalmente no cérebro (VAN BODEGOM *et al.*, 2017). Por exemplo, o hipocampo, uma importante estrutura relacionada a formação da memória e a avaliação do contexto, tem grande quantidade de receptores de glicocorticoides, cuja estimulação crônica pode levar à prejuízos cognitivos (DASKALAKIS *et al.*, 2015).

Desta forma, o estresse durante o período neonatal pode levar à mudanças importantes no desenvolvimento do CNS (HUMPHREYS *et al.*, 2019). Estudos vêm demonstrando que o estresse nos primeiros dias de vida pode levar ao aumento do hormônio liberador de corticotropina (HLC) e dos níveis plasmáticos de glicocorticóidos (DASKALAKIS *et al.*, 2015; YANG *et al.*, 2015). Consequentemente, ocorrem alterações neuroquímicas em diferentes estruturas da rede neural (YANG *et al.*, 2015). Estas alterações estão relacionadas a transtornos psiquiátricos, como esquizofrenia e depressão, alterações comportamentais e prejuízos cognitivos na vida adulta (YANG *et al.*, 2015; MENEZES *et al.*, 2017).

Para estudar os efeitos do estresse durante os primeiros dias de vida, diversos modelos animais de privação maternal têm sido pesquisados. Alguns deles são modelos de estresse agudo, onde os animais filhotes são separados de suas genitoras por um período de 24h, geralmente no 9º dia pós-natal (PND, do inglês

postnatal day) (RENTESI *et al.*, 2013). Ao ser separado do contato materno por um período prolongado, o filhote é privado de estímulos térmicos, nutricionais e sensoriais; assim, a separação por um longo período de horas pode desencadear comportamento do tipo depressivo e ansioso (RENTESI *et al.*, 2013). Por outro lado, modelos de estresse neonatal que utilizam curtos períodos de ausência materna (por exemplo, 3h por dia durante os 10 primeiros dias) não promovem alterações na atividade locomotora, exploratória e tampouco estão relacionados a comportamentos do tipo ansioso ou depressivo (MENEZES *et al.*, 2017; MENEZES *et al.*, 2020). Contudo, esses modelos causam déficit de aprendizagem e memória que persistem na vida adulta (NEVES *et al.*, 2015; MENEZES *et al.*, 2020).

Os déficits cognitivos observados em animais que passaram por protocolos de MD têm sido relacionados com alterações morfofuncionais de estruturas relacionados a processos cognitivos, tais como hipocampo, amígdala e PFC, que são estruturas especialmente sensíveis ao estresse neonatal (NEVES *et al.*, 2015). Estudos recentes têm demonstrado que a MD leva a uma alteração do equilíbrio oxidativo tanto no PFC quanto no HP, além de diminuit os níveis de BDNF no HP (NEVES *et al.*, 2015; MENEZES *et al.*, 2020). Além disso, a exposição a glicocortícóides no período neonatal afeta o desenvolvimento do sistema dopaminérgico e a expressão e a função dos receptores D1 e D5 em diferentes estruturas do cérebro, tais como o córtex pré-límbico e o estriado (MAJCHER-MAŚLANKA *et al.*, 2017).

Corroborando com os achados supracitados, estudos têm demonstrado que o estresse no período do neurodesenvolvimento pode afetar o desenvolvimento do sistema dopaminérgico (SANDI E HALLER, 2015; DAHOUN *et al.*, 2019) e a exposição a traumas durante os primeiros anos de vida pode levar aumento da liberação de dopamina no núcleo accumbes (NAc) (SANDI E HALLER, 2015). A hiperatividade da via VTA-NAc está relacionada a mudanças comportamentais, como o isolamento social (FRANCIS *et al.*, 2015), por outro lado, a privação de cuidado maternais durante os primeiros anos de vida está relacionada à diminuição da conectividade VTA-hipocampo e à déficits cognitivos (VALENTI *et al.*, 2011; MARUSAK *et al.*, 2017). Além disso, o dano do sistema dopaminérgico relacionado à situações de estresse ou trauma está associado ao desenvolvimento de doenças psiquiátricas como a depressão e esquizofrenia (JAHNG *et al.*, 2010; DAHOUN *et al.*, 2019).

3 JUSTIFICATIVA

Eventos estressores durante os primeiros dias de vida muitas vezes geram consequências que persistem ao longo da vida, tanto em humanos quanto em animais (MARROCCO *et al.*, 2019). Observações neurobiológicas importantes relacionaram o desenvolvimento de esquizofrenia ao estresse neonatal; evidências iniciais indentificaram aumento da expressão de genes relacionado à esquizofrenia em casos de partos com histórico de prematuridade, pré-eclâmpsia e infecção intrauterina (MURRAY *et al.*, 2017). Além disso, a exposição ao estresse crônico durante os primeiros anos de vida leva a uma série de alterações microgliais, produzindo mudanças estruturais e funcionais que suscitam sintomas depressivos e ansiosos (WOHLEB *et al.*, 2016).

Os crescentes casos de pessoas que desenvolvem transtornos psiquiátricos na fase adulta estão, muitas vezes, ligados a consequências do impacto a curto e longo prazo de eventos estressores durante o período neonatal. Em animais, o modelo de privação maternal (MD) em ratos tem se demonstrado interessante para o estudo do tema (ELLENBROEK *et al.*, 1998). Um destes modelos é realizado durante os dez primeiros dias (3 horas/dia), e representa um importante modelo de estresse que culmina em alterações da função cognitiva, como o déficit de memória (MENEZES *et al.*, 2020), além de ser uma forma de mimetizar o estresse ocorrido no inicio da vida em crianças (BENETTI *et al.*, 2009).

Estudos utilizando modelos de MD têm demonstrado que ocorre um desequilíbrio do sistema dopaminérgico em diferentes regiões do cérebro, tais como o estriado, o córtex pré-frontal e a amígdala (LLORENTE *et al.*, 2010; MAJCHER-MAŚLANKA *et al.*, 2017). O sistema dopaminérgico é uma via que desempenha um papel importante em respostas emocionais ao estresse (HIRANO *et al.*, 2007; ZHU *et al.*, 2011). Além disso, é importante para o processo de consolidação e persistência da memória (ROSSATO *et al.*, 2009). Assim, torna-se importante que pesquisas sejam realizadas para a compreensão do impacto de um evento estressor durante a fase de desenvolvimento sobre o sistema dopaminérgico e suas funções, proporcionando o crescente conhecimento sobre essa temática e ampliando as possibilidades para futuras terapêuticas nesta área.

4 OBJETIVOS

4.1 Objetivo geral

O objetivo geral deste estudo é investigar o envolvimento do sistema dopaminérgico e seus diferentes receptores no déficit de memória induzido pela privação maternal em ratos.

4.2 Objetivos específicos

Os objetivos específicos deste estudo incluem:

- Investigar se a estimulação não seletiva dos receptores dopaminérgicos reverte os déficits de memória de OR induzidos pela MD;

- Investigar se a estimulação dos receptores dopaminérgicos D1/D5, reverte os déficits na memória de OR induzidos pela MD;

- Investigar se o bloqueio dos receptores dopaminérgico D1/D5 promove déficits na memória de OR;

- Investigar se a estimulação do segundo mensageiro dos receptores D1, PKA, reverte os déficits na memória de OR induzidos pelo bloqueio de receptores D1/D5;

- Investigar se a estimulação do segundo mensageiro dos receptores D5, PKC, reverte os déficits na memória de OR induzidos pelo bloqueio de receptores D1/D5;

- Investigar se a estimulação do segundo mensageiro dos receptores D1, PKA, reverte os déficits na memória de OR induzidos pela MD;

- Investigar se a estimulação do segundo mensageiro dos receptores D5, PKC, reverte os déficits na memória de OR induzidos pela MD;

- Investigar se a administração periférica sistêmica do precursor da dopamina L-Dopa é capaz de reverter os déficits na memória de OR induzidos pela MD.

PARTE II

5 Manuscrito científico submetido à revista *Neurobiology of the Learning and Memory*

ON THE ROLE OF THE DOPAMINERGIC SYSTEM IN THE MEMORY DEFICITS INDUCED BY MATERNAL DEPRIVATION

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ON THE ROLE OF THE DOPAMINERGIC SYSTEM IN THE MEMORY DEFICITS INDUCED BY MATERNAL DEPRIVATION

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Abstract

Previous researches showed that maternal deprivation (MD) leads to memory deficits that persists until adulthood. The hippocampus, an important brain structure involved in memory processes, receives dopaminergic afferences from other brain areas that modulate memory. Here we demonstrated that MD results in object recognition memory deficits that are reverted by intra-hippocampal stimulation of D1-dopaminergic receptor and peripheral administration of a dopamine precursor.

Key-words: memory persistence; early life stress; dopamine; hippocampus; L-Dopa.

1. INTRODUCTION

Neural development is affected by early life stress (Tyborowska et al., 2018). In this period of life, the stress leads to hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis (Marais et al., 2008; van Bodegom et al., 2017). The increase in corticotropin releasing hormone (CRH) levels activates the pituitary to release corticotropin, which stimulates the adrenal cortex to release corticosterone (Ulrich-Lai and Herman, 2009). When increased, corticosterone can disrupt neural architecture in different brain regions related to cognitive processes, such as hippocampus and prefrontal cortex (Humphreys et al., 2019; McEwen et al., 2016; Ohta et al., 2014).

The hippocampus and the prefrontal cortex are critical for learning and memory processes, and the mechanisms by which neonatal stress, such as maternal deprivation (MD), affects different types of memory have been widely studied. Critical periods of maternal absence could lead to mnemonic impairment in hippocampal-dependent behavioral tasks (Menezes et al., 2017), which are related to hippocampal and prefrontal cholinergic alterations (Benetti et al., 2009) and oxidative stress [6, 24]. In addition, studies shown that early life stress exerts effects at brain cellular

level (Marrocco et al., 2019), alters immune and endocrine functions (Amini-Khoei et al., 2019; Rice et al., 2008), decreases hippocampal neurotrophines levels, as brain-derived neurotrophic factor (BDNF) (Menezes et al., 2017; Neves et al., 2015), and alters different neurotransmitters systems, such as cholinergic (Markovic et al., 2014), histaminergic (Benetti et al., 2015), serotoninergic (Li et al., 2013; Ohta et al., 2014), noradrenergic (Kalpachidou et al., 2016), and dopaminergic (Jahng et al., 2010).

The dopaminergic system plays an important role in emotional responses to stress (Hirano et al., 2007; Segovia et al., 2008). There are evidences showing that exposure to glucocorticoids in pre and postnatal periods affects the development of the dopaminergic system and suggesting that the increase of sensitivity to drugs of abuse could be related to interactions between prenatal stress, glucocorticoids and dopaminergic neurons (Piazza and Le Moal, 1996; Rentesi et al., 2013). Dopaminergic receptors (DR) are widely distributed in brains regions. Especially the D1-family is expressed in hippocampus and prefrontal cortex and its activation is required for memory consolidation and persistence (Castillo Díaz et al., 2017; Furini et al., 2014).

The D1-family is composed of D1 and D5 receptors. Although these two types of metabotropic receptors are activated by interaction with dopamine, they activate different second messengers: whereas D1-receptors use adenylate cyclase as the second messenger, modulating protein kinase A (PKA), D5-receptors activate the phosphatidylinositol-3 kinase (PI3K), modulating protein kinase C (PKC) activity (Sahu et al., 2009; Undieh, 2010). Both PKA and PKC are involved in the consolidation of different types of memories (Furini et al., 2014; Izquierdo et al., 2006). The dopaminergic system also plays an important role in the consolidation and persistence of different memory types, that are dependent on brain structures such as hippocampus (Izquierdo et al., 1998).

Although there is some studies about the mechanisms underline the MD-induced memory deficits, little is known about the role of the D1-family of dopaminergic receptors. Here, we demonstrated that MD causes memory deficits, which are revered by stimulation of hippocampal dopaminergic system and by the peripheral injection of a dopamine precursor. Additionally, using behavioral

pharmacology approaches, we demonstrated that D1 receptors, but not D5, are essential to memory consolidation and persistence.

2. MATERIALS AND METHODS

2.1. Animals

Pregnant female Wistar rats were obtained from the Central Vivarium of the Federal University of Santa Maria (RS/Brazil). All animals were kept in a 12h light/12h dark cycle (light phase started at 7h), with controlled temperature (23 ± 2ºC) and air humidity (60 ± 5%). The pregnant female rats were individually housed with sawdust bedding with food and water available ad libitum. The day of delivery was considered day 0. At postnatal day 1 (PND-1), the MD protocol was initiated with half of the pups and lasted until PND-10. Animals were weaned at 21 days of age (PND-21) and were housed 4 male per cage in regular cages. Only the males were used in the following experiments; the females were donated to other studies in progress. All experiments were conducted in accordance with the principles of laboratory animal care (National Research Council Committee for the Update of the Guide for the and Use of Laboratory, 2011) and were approved by the Institutional Animal Care and Use Committee of the Local Institution (Protocol #032/2018).

The male rats were randomly divided into different groups at PND-21 (n = 7-10/group), according to 5 subsequent studies, as described below:

Study 1: Effects of intra-hippocampal dopamine infusion on memory deficits induced by maternal deprivation

(i) Control, in which the rats were submitted to behavioral tests, and immediately after the training session in object recognition task (OR) received an intra-hippocampal infusion of vehicle;

(ii) MD, in which the rats were submitted to the MD protocol and to the behavioral tests, and immediately after the OR training received an intra-hippocampal infusion of vehicle;

(iii) DH, in which the rats were submitted to behavioral tests, and immediately after the OR training received an intra-hippocampal infusion of dopamine hydrochloride (DH), a non-selective agonist dopaminergic receptor;

(iv) MD + DH, in which the rats were submitted to the MD protocol and to the behavioral tests, and immediately after the OR training session received an intra-hippocampal infusion of DH.

Study 2: Effects of D1-family receptors activation on memory deficits induced by maternal deprivation

(i) Control, in which the rats were submitted to behavioral tests, and immediately after the training session in OR task received an intra-hippocampal infusion of vehicle;

(ii) MD, in which the rats were submitted to the MD protocol and to the behavioral tests, and immediately after the OR training received an intra-hippocampal infusion of vehicle;

(iii) SKF 38393, in which the rats were submitted to behavioral tests, and immediately after the OR training session received an intra-hippocampal infusion of SKF 38393, a D1/D5 agonist;

(iv) MD + SKF 38393, in which the rats were submitted to the MD protocol and the behavioral tests, and immediately after the OR training session received an intra-hippocampal infusion of SKF38393.

Study 3: D1-family receptors involvement on memory consolidation and persistence

(i) SCH 23390, in which the rats were submitted to behavioral tests, and immediately after the training session in OR task received an intra-hippocampal infusion of SCH 23390, a D1/D5 antagonist;

(ii) SCH 23390 + Sp-cAMP, in which the rats were submitted to behavioral tests, and immediately after the OR training received an intra-hippocampal infusion of SCH 23390 + Sp-cAMP, a PKA stimulator (PKA is a second messenger of D1 receptors);

(iii) SCH 23390 + PMA, in which the rats were submitted to behavioral tests, and immediately after the OR training received an intra-hippocampal infusion of SCH 23390 + PMA, a PKC stimulator (PKC is a second messenger of D5 receptors).

Study 4: Involvement of D1 and D5 receptors on memory deficits induced by maternal deprivation

(i) MD + Sp-cAMP, in which the rats were submitted to the MD protocol and to the behavioral tests, and immediately after the OR training received an intra-hippocampal infusion of Sp-cAMP;

(ii) MD + PMA, in which the rats were submitted to the MD protocol and to the behavioral tests, and immediately after the OR training received an intra-hippocampal infusion of PMA;

(iii) MD + SKF 39393 + Rp-cAMP, in which the rats were submitted to the MD protocol and to the behavioral tests, and immediately after the OR training received an intra-hippocampal infusion SKF 39393 + Rp-Camp (Rp-Camp is a PKA blocker);

(iv) MD + SKF 39393 + Gö 6976, in which the rats were submitted to the MD protocol and to the behavioral tests, and immediately after the OR training received an intra-hippocampal infusion SKF 39393 + Gö 6976 (Gö 6976 is a PKC blocker).

Study 5: Effects of peripheral administration of L-Dopa on memory deficits induced by maternal deprivation

(i) Control, in which the rats were submitted to behavioral tests, and 30 minutes before the OR training session received an intraperitoneal infusion of vehicle;

(ii) MD, in which the rats were submitted to the MD protocol and to the behavioral tests, and 30 minutes before the OR training received an intraperitoneal infusion of vehicle;

(iii) L-Dopa, in which the rats were submitted to behavioral tests, and 30 minutes before the OR training received an intraperitoneal infusion of L-Dopa (L-Dopa is a dopamine precursor);

(iv) MD + L-Dopa, in which the rats were submitted to the MD protocol and to the behavioral tests, and 30 minutes before the OR training received an intraperitoneal infusion of L-Dopa.

In adulthood (PND-90), all the animals were summited to surgical procedure, memory task, drugs' infusion and/or behavioral control tasks, according to the groups' allocation. The experimental design is summarized in figure 1.

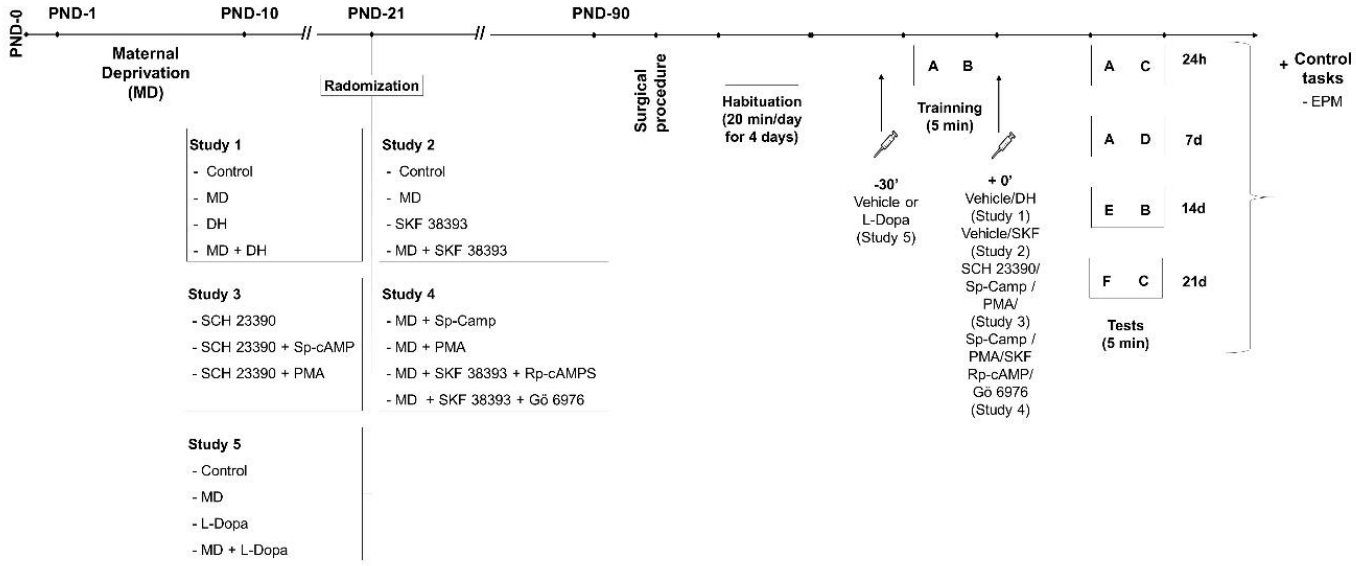

Figure 1. General experimental design. Delivery was considered PND-0. From PND-1 to PND-10 the animals were submitted to maternal deprivation (MD) protocol. In PND-21 the animals were weaned and randomly divided into different groups, depending on the study. The studies were conducted from PND-90. After stereotaxic surgery, the animals were subjected to behavioral experiments. All animals were habituated and trained in the OR task. In studies 1-4, the animals received an intrahippocampal infusion of different drug(s) or saline immediately after the OR training session. In study 5, the animals received intraperitoneal L-Dopa injection 30 minutes before the OR training session. The OR test sessions were performed 24h (to assess OR memory consolidation), 7, 14, and 21 days after the training session (to assess OR memory persistence). A control behavioral test to verify anxiety levels (Elevated Plus Maze - EPM) was performed in all test days (24h, 7, 14 and 21 days).

2.2. Maternal Deprivation (MD) protocol

Pregnant rats were kept in their individual boxes until the delivery day (considered day zero). Rats from MD groups were submitted to MD protocol, that consisted of removing the mother from the residence box to other room for the period of 3h per day from PND-1 to PND-10 (Benetti et al., 2009). The pups were maintained in their home cage, without any manipulation. While the mothers were absent the room temperature was increased to 32ºC to compensate for the absence of the mother's body heat. At the conclusion of each daily deprivation session, the mothers were returned to their home boxes. The control rats remained in their resident boxes together with their mothers during the first ten days of life. On PND-21, all the animals were weaned, and the males were maintained in groups of 4 in plastic boxes with food and water available ad libitum.

2.3. Surgical procedure and drugs delivery

To the infusion of the drugs or vehicle, in studies 1 to 4 cannulas were implanted bilaterally in the CA1 region of the hippocampus in the rats. The rats were anesthetized with ketamine and xylazine (i.p., 75 mg/kg and 10 mg/kg respectively) and 27 gauge guide cannulae were implanted by means of stereotactic surgery to the CA1 region of the dorsal hippocampus (AP = - 4,2; LL = ± 3.0, DV = – 2.0 mm) according to the coordinates of the anatomy atlas Paxinos e Watson (Paxinos and Watson, 2013). The cannulae were fixed with dental cement. The post-surgical recovery time was 4 days.

For drug infusion, a 30-gauge cannulae were fitted into the guide cannulae, going 1.0 mm deeper to access CA1 on hippocampus. Infusions (1 µl/side) in the CA1 region of the dorsal hippocampus were performed for a period of 60 seconds using an infusion pump, and the cannulas were left in place for an additional 60 seconds to minimize backflow. The drugs infusions were performed immediately after the OR training session. Cannula placements were verified postmortem: 2–4 h after the last behavioral test a 4% methylene-blue solution was infused at the same volume used in the experiments, and the extension of the dye 30 min thereafter was taken as an indication of the presumable diffusion of the vehicle or drug previously given to each animal.

2.4. Drugs and reagents

The drugs were diluted in DMSO or saline (vehicle), and the doses administered in the CA1 region of the hippocampus were based on the literature (Bernabeu et al., 1997; Bonini et al., 2005; Fiorenza et al., 2012; Quevedo et al., 2004; Rossato et al., 2009; Vargas et al., 2019; Wang et al., 2017; Yang and Lee, 1993): Dopamine hydrochloride (DH), 1 µg/µL; SKF 38393, 12.5 µg/µL; SCH 23390, 1.5µg/µL; Go 6976, 1.7ng/µL; Rp-cAMPS, 0.5 µg/µL; PMA, 0.5 µg/µL; Sp-cAMP, 0.5µg/µL; L-Dopa, 25 mg/Kg of body weight. Considering the design of studies 1 to 4, drugs or vehicle infusion (1 µL/side) was performed using guide cannulas previously implanted in the CA1 region of the dorsal hippocampus by stereotactic surgery (Bernabeu et al., 1997; Bonini et al., 2005; Fiorenza et al., 2012; Quevedo et al., 2004; Rossato et al., 2009; Vargas et al., 2019; Yang and Lee, 1993). In study 5, L-Dopa was injecting intraperitoneally, as previously described (Wang et al., 2017).

2.5. Object recognition (OR) task

The training and test in object recognition (OR) task were carried out in a wooden box with the front in transparent glass (50 x 50 x 50 cm) (Ennaceur and Delacour, 1988). Object recognition is based on the animal's natural tendency to explore the new things/objects in a known environmental context. Firstly, the animals were habituated to the OR apparatus, for this, during 4 consecutive days, the animals were allowed to freely explore the environment (box) for 20 minutes per day. On the 5th day (training session), the animals were placed again in the apparatus with two different objects for free exploration for 5 min.

The long-term memory consolidation test session was performed at 24 h after the training session. The memory persistence tests were performed 7, 14 and 21 days after the training session. In each test session, a novel and a familiar object were available for 5 min of exploration. The time spent exploring each object was recorded and expressed as a percentage of total exploration time (Mello-Carpes and Izquierdo, 2013). The objects were made of metal or plastic, and were previously tested, so that animals had no preference for any of them. Exploration was defined as touch or sniff with the nose and/or front paws the objects. To avoid olfactory preferences, objects and apparatus were cleaned with 70% alcohol after testing each animal.

2.6. Control behavioral task

In order to evaluate the animals' general behavior and to ensure that the infusion of drugs or other procedure did not impair these behaviors, altering the results of the OR test, we evaluate some control parameters: OR total exploration time in training and testing sessions, as an indicator of locomotion and exploration (supplementary material 1), and anxiety (supplementary material 2); the anxiety was evaluated in the same day of memory tests.

To evaluate the state of anxiety the animals were submitted to the Elevated Plus Maze (EPM). For this test, the animals were placed in the center of the apparatus and during the 5-minute session, the number of entries in the open and closed arms of the EPM and the time spent on open and closed arms were recorded (Pellow et al., 1985).

2.7. Statistical analyses

The objects' exploration time in the OR task was converted to a percentage of the total exploration time and the one sample Student t-test was used to compare the percentage of the total exploration time spent on each object with a theoretical mean (50%). The total exploration time during the OR training and the EPM results were analyzed using ANOVA.

All data were expressed as mean ± standard deviation (SD). The differences were considered statistically significant when $P < 0.05$.

3. RESULTS

3.1. Intra-hippocampal dopamine infusion reverts the OR memory deficit related to MD

To investigate whether the dopaminergic system is involved on memory deficits induced by MD we infused a non-selective dopamine agonist, dopamine hydrochloride (DH), into the CA1 region of the hippocampus. During the OR training session, as expected, rats from all groups explored each of the objects for about 50% of the total exploration time ($t(6) = 1.155$, $P = 0.2922$, for Control; $t(9) = 0.1508$, $P = 0.8835$, for MD; $t(6) = 1.982$, $P = 0.0948$, for DH; $t(9) = 1.096$, $P = 0.3015$, for MD + DH; Fig. 2A express the mean of all groups).

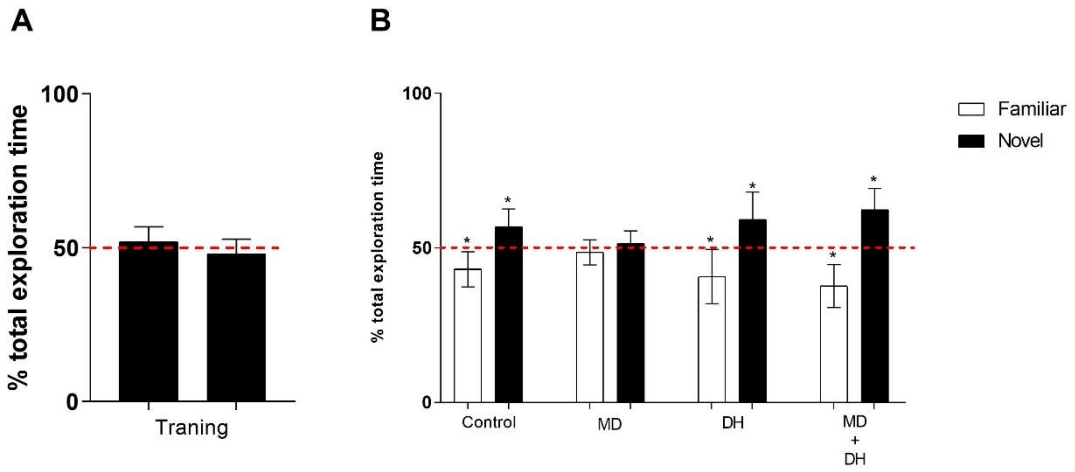

Figure 2. Maternal deprivation causes object recognition (OR) memory deficits. Dopamine hydrochloride infusion into the CA1 region of the hippocampus reverses this deficit. A. In the OR task training session the animals of the different groups were exposed to two novel objects for 5 min, and explored about 50% of the total exploration time of each object. **B.** In the consolidation test session, the animals were exposed to the familiar object and to a novel object. The MD group was not able to differentiate in the novel from the familiar object, but the MD rats that received dopamine hydrochloride infusion after the OR training session were. Data are expressed as mean ± SD of the percentage of the total exploration time; * $P < 0.05$ in the one sample Student t-test, considering a theoretical mean of 50%; n = 7-10 per group. MD = maternal deprivation rats; DH = dopamine hydrochloride.

Rats from the control group explored significantly more than 50% of the total exploration time of the novel object on 24h test ($t(6) = 3.227$; $P = 0.018$; Fig. 2B), which demonstrates preserved memory. Rats from the MD group spent a percentage similar to 50% of the total exploration time exploring the familiar and the novel object on test day ($t(9) = 1.11$; $P = 0.293$; Fig. 2B), which suggests OR memory deficit. Hippocampal infusion of DH maintained the memory on non-deprived rats ($t(6) = 2.78$; $P = 0.032$; Fig. 2B) and was able to reverse OR memory deficit induced by MD, since the rats from the MD+DH group explored significantly more than 50% of the total exploration time the novel object ($t(9) = 5.59$; $P = 0.0003$; Fig. 2B).

3.2. D1 family receptors stimulation promotes memory persistence and reverts the memory deficits related to MD

To investigate the effects of dopaminergic receptor D1/D5 stimulation, we infused a selective D1-family dopamine receptors agonist (SKF 38393) in the rats' hippocampus. As expected, during the training session rats of all groups explored each of the objects for about 50% each ($t(6) = 0.03204$, $P = 0.9755$; for control; $t(8) = 0.6850$, $P = 0.5127$, for MD; $t(7) = 0.2308$, $P = 0.8241$, for SKF 38393; $t(8) = 0.6355$, $P = 0.5428$, for MD + SKF 38393; Fig. 3A express the mean of all groups).

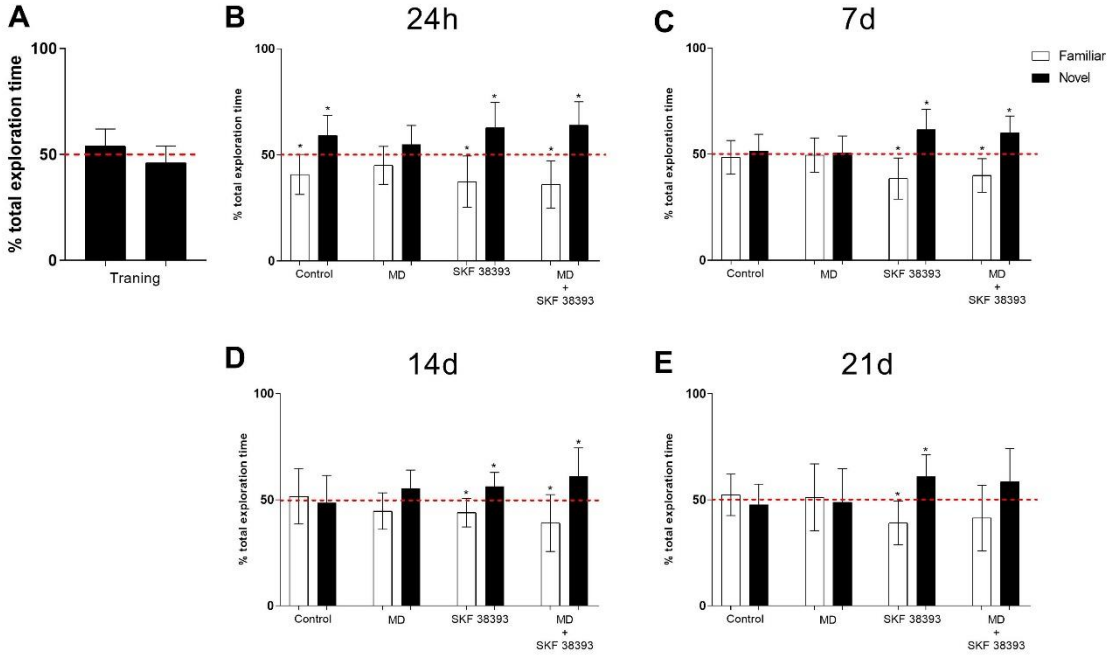

Figure 3. MD causes object recognition (OR) memory deficits. Hippocampal SKF 38393 infusion reverts these deficits and promotes persistence memory. A. In the OR training session the animals from different groups were exposed two novel objects for 5 min and explored about 50% of the total exploration time of each object. B. The long-term memory consolidation test was performed 24h after training. The MD group was not able to differentiate in the novel from the familiar object, and the SKF 38393 infusion immediately after training reverts this deficit. The long-term memory persistence tests were performed 7 (C), 14 (D) and 21 (E) days after the training. The control and MD groups were unable to distinguish the novel from the familiar objects on 7, 14 and 21 days' tests. SKF 38393 promoted memory persistence in control animals, as verified on tests performed 7, 14 and 21 days after training. The MD group that received SKF 38393 infusion after the OR training session explored the novel object significantly more than 50% of the total exploration time on tests performed 7 and 14 days after the OR training. Data are expressed as mean ± SD of the percentage of the total exploration time; * $P < 0.05$ in the one sample Student t-test, considering a theoretical mean of 50%; n = 7-9 per group. MD = maternal deprivation rats; SKF 38393 = D1/D5-stimulator.

On the 24h LTM consolidation test, animals from control group explored significantly more than 50% of the total exploration time of the novel object ($t(6) = 2.577$, $P = 0.0419$), which demonstrates a preserved memory (Fig. 3B - Control). The MD group spent about 50% of the total exploration time exploring the familiar and the novel object ($t(8) = 1.647$, $P = 0.1381$), which suggests OR memory deficit (Fig. 3B – MD). SKF 38393 and MD + SKF 38393 groups explored significantly more than 50% of the total exploration time the novel object (SKF 38393: $t(7) = 2.961$, $P = 0.0211$; MD+SKF 398393: $t(8) = 3.765$, $P = 0.0055$), which demonstrates a preserved memory (Fig. 3B – SKF; MD+SKF).

On memory persistence tests, the animals from the control group explored similarly the two objects on 7th ($t(6) = 0.4916$, $P = 0.6405$; Fig. 3C), 14th ($t(6) = 0.3255$, $P = 0.7558$; Fig 3D), and 21st test days ($t(6) = 0.6437$, $P = 0.5436$; Fig. 3E), which was expected, suggesting a normal physiological forgetfulness of recognition memory. As expected, since they did not presented memory consolidation, the MD group spent a similar percentage of time exploring the two objects on 7th day ($t(8) = 0.1687$, $P = 0.8702$; Fig 3C), 14th day ($t(8) = 1.873$, $P = 0.0979$; Fig 3D), and 21st test day ($t(8) = 0.2041$, $P = 0.8440$; Fig 3E). However, animals from SKF 38393 group explored significantly more than 50% of the total exploration time the novel object on the 7th ($t(7) = 3,364$, $P = 0.0120$; Fig 3C),14th day ($t(7) = 2.574$, $P = 0.0368$; Fig 3D), and 21st test day ($t(7) = 3.015$, $P = 0.0195$; Fig 3E), demonstrating memory persistence. Additionally, the animals from MD+SKF 38393 group spent more time exploring the novel object on 7th ($t(8) = 3.811$, $P = 0.0052$; Fig 3C) and 14th test day ($t(8) = 2.459$, $P = 0.0394$; Fig 3D), but not on 21st test day ($t(8) = 1.676$, $P = 0.1322$; Fig 3E).

3.3. D1 receptor, but not D5 receptor activation is necessary to memory consolidation and persistence

To investigate if D1, or D5, or both receptors, are involved on OR memory consolidation, we promoted the stimulation of PKA (second messenger D1 receptor) or PKC (second messenger of D5 receptor) after the blockade of these two receptors (by the infusion of a selective dopamine antagonist D1/D5, SCH 23390). To do this,

the SCH infusion was associated or not with a hippocampal injection of Sp-cAMP (PKA-stimulator) or PMA (PKC-stimulator).

As expected, rats from all groups explored each of the objects for about 50% of the total exploration time during the OR training ($t(7) = 1.598$, $P = 0.1541$; for SCH 23390; $t(7) = 0.03221$, $P = 0.9752$, for SCH 23390 + Sp-cAMP; $t(6) = 2.154$, $P = 0.0747$, for SCH 23390 + PMA; Fig. 4A express the mean of all groups).

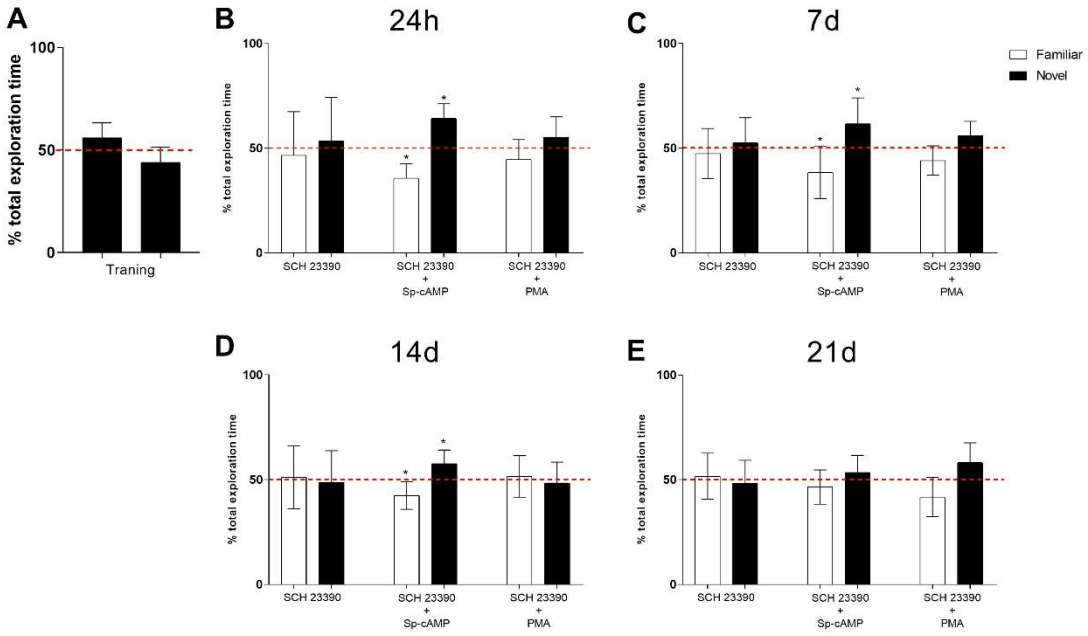

Figure 4. D1, but not D5, dopaminergic receptor activation is necessary to OR memory consolidation and persistence. A. In the OR task training session the animals of different groups were exposed two to novel objects for 5 min and explored about 50% of the total exploration time each object. B. The long-term OR memory consolidation test was performed 24h after training. The SCH 23390 group was not able to differentiate in the novel from the familiar object, and Sp-cAMP, but not PMA, reverts this memory deficit. The OR memory persistence was tested 7 (C), 14 (D) and 21 (E) days after the OR training. The SCH 23390 group that received Sp-cAMP was able to differentiate the novel from the familiar object 7 and 14 days after the training session. Data are expressed as mean ± SD of the percentage of the total exploration time; *$P < 0.05$ in the one sample Student t-test, considering a theoretical mean of 50%; n = 7-8 per group. SCH 23390 = D1/D5-blocker; Sp-cAMP = PKA-stimulator (second messenger of D1 receptor); PMA = PKC-stimulator (second messenger of D5 receptor).

On the 24h LTM consolidation test, animals from SCH 23390 group spent about 50% of the total exploration time exploring the familiar and the novel object

(t(7) = 0.4584, P = 0.6605, Fig. 4B), which suggests OR memory impairment. Animals that received SCH + Sp-cAMP explored the novel object for more than 50% of the total exploration time (t(7) = 5.869, P = 0.0006, Fig. 4B). However, rats that received SCH + PMA spent about 50% of the total exploration time exploring the familiar and the novel object (t(6) = 1.491, P = 0.1865, Fig. 4B).

On the OR memory persistence tests, animals from SCH 23390 group explored for about 50% of the total exploration time each object during the tests performed on 7th (t(7) = 0.6154, P = 0.5578; Fig. 4C), 14th (t(7) = 0.2213, P = 0,8312; Fig. 4D) and on 21st day (t(7) = 0.4318, P = 0.6789; Fig. 4E). The animals that received SCH + Sp-cAMP showed memory persistence on 7th day (t(7) = 2.652, P = 0.0328; Fig. 4C) and on 14th day (t(7) = 3.058, P = 0.0223; Fig. 4D), but not on 21st day (t(7) = 1.593, P = 0.1552; Fig. 4E). The animals that received SCH 23390 + PMA spent a percentage similar to 50% of the total exploration time exploring each object on 7th (t(6) = 2.231, P = 0.0672; Fig. 4C), 14th (t(6) = 0.4133, P = 0.6938; Fig. 4D) and on 21st test day (t(6) = 2.336, P = 0.0581; Fig. 4E).

3.4. D1, but not D5, receptor activation can reverse the memory deficits related to MD

To investigate if the stimulation of D1 or D5 receptors, or both, is able to revert the memory deficits related to MD, we infused Sp-cAMP (PKA-stimulator) or PMA (PKC-stimulator) in the hippocampus of MD rats. In addition, we assess whether stimulation of D1/D5 receptors, by SKF 38390, associated with the block of D1 second messengers by Rp-cAMP (PKA-blocker), or associated with the block of D5 second messengers by Gö 6976 (PKC-blocker) is able to reverse the induced memory deficit by DM.

As expected, rats from all groups explored each of the objects for about 50% of the total exploration time during the training session (t(7) = 1.468, P = 0.1856; for MD + Sp-cAMP; t(6) = 2.136, P = 0.0766, for MD + PMA; t(6) = 1.189, P = 0.2795, for MD + SKF 38393 + Rp-cAMP; t(6) = 0.001198, P = 0.9991, for MD + SKF 38393 + Gö 6976; Fig. 5A express the mean of all groups).

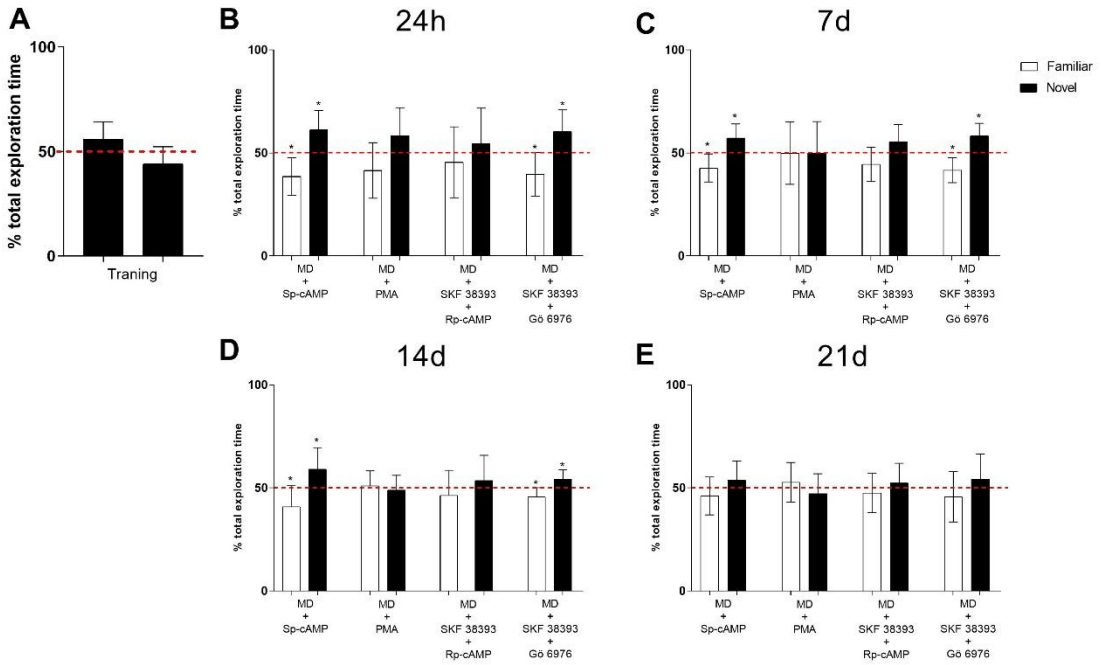

Figure 5. D1, but not D5, receptor activation can reverse the memory deficits related to MD. A. In the OR task training session the animals of the different groups were exposed two novel objects for 5 min and explored about 50% of the total exploration time each object. B. The OR long-term memory consolidation test was performed 24h after training. The MD group that received the Sp-cAMP was able to differentiate the novel from the familiar object; the MD group that received the PMA was not. The MD group that received the SKF 38393 associated with Rp-cAMP was unable to distinguish the novel from the familiar object, but de MD group that received the SKF 38393 associated with Gö 6976 was. The OR memory persistence tests were performed 7 (C), 14 (D) and 21 (E) days after the OR training. The animals from MD group that received Sp-cAMP explored the novel object more than 50% of the total exploration time on the testes performed 7 and 14 days after the training session. The MD group that received SKF 38393 associated to Rp-cAMP was not able to differentiate the two objects, but the MD group that received SKF 38393 associated to Gö 6976 was. *P < 0.05 in the one sample Student t-test, considering a theoretical mean of 50%; n = 7-8 per group. MD = maternal deprivation rats; SKF 38393 = D1/D5-stimulator; Sp-cAMP = PKA-stimulator (second messenger of D1 receptor); PMA = PKC-stimulator (second messenger of D5 receptor); Rp-cAMP = PKA-blocker; Gö 6976 = PKC-blocker.

On the 24h LTM consolidation test, animals from MD + Sp-cAMP group explored significantly more than 50% of the total exploration time the novel object ($t(7) = 3.513$, $P = 0.0098$; Fig. 5B). On the other hand, animals form MD + PMA group explored about 50% of the total exploration time each object ($t(7) = 0.1183$, $P = 0.1183$; Fig. 5B). The stimulation of D1/D5 receptors (by SKF hippocampal infusion) does not reverse the memory deficit induced by MD when associated with

PKA-blocker (Rp-cAMP) ($t(6) = 0.6933$, $P = 0.5140$, Fig. 5B), but reverses when associated to PKC-blocker (Gö 6976) ($t(6) = 2.605$, $P = 0.0404$, Fig. 5B).

On the OR memory persistence tests, animals from MD + Sp-cAMP group explored significantly more the novel object on 7th day ($t(7) = 3.039$, $P = 0.0189$; Fig. 5C), and on 14th day ($t(7) = 2.474$, $P = 0.0426$; Fig. 5D), but not on 21st day ($t(7) = 1.194$, $P = 0.2714$; Fig. 5E). The animals from MD + PMA group did not show memory persistence on 7th day ($t(7) = 0.01432$, $P = 0.9890$; Fig. 5C), 14th day ($t(7) = 0.4223$, $P = 0.6855$; Fig. 4D) and 21st day ($t(7) = 0.7993$, $P = 0.4504$; Fig. 4E). Either, no memory persistence was observed in MD animals that received SKF 38393 associated to Rp-cAMP (PKA-blocker) (7th day: $t(7) = 1.766$, $P = 0.1279$, Fig. 5C; 14th day: $t(7) = 0.7896$, $P = 0.4598$, Fig. 5D; 21st day: $t(7) = 0.6621$, $P = 0.5325$, Fig. 5E). MD rats that received SKF 38393 + Gö explored significantly more than 50% of the total exploration time the novel object on 7th day ($t(6) = 3.622$, $P = 0.0111$; Fig. 5C), and 14th day ($t(6) = 2.545$, $P = 0.0438$; Fig. 5D), but not on 21st day ($t(6) = 0.9312$, $P = 0.3877$; Fig. 5E).

3.5. The administration of a dopamine precursor reverts de memory deficits induced by MD

To investigate if the administration of a dopamine precursor is able to reverse the OR memory deficits related to MD, we administrated L-Dopa intraperitoneally 30 minutes before the OR training session.

As expected, rats from all groups explored each of the objects for about 50% of the total exploration time during the training session ($t(6) = 0.03204$, $P = 0.9755$, for Control; $t(8) = 0.685$, $P = 0.5127$, for MD; $t(7) = 0.2308$, $P = 0.8241$, for L-Dopa; $t(8) = 0.909$, $P = 0.3899$, for MD + L-Dopa; Fig. 6A express the mean of all groups).

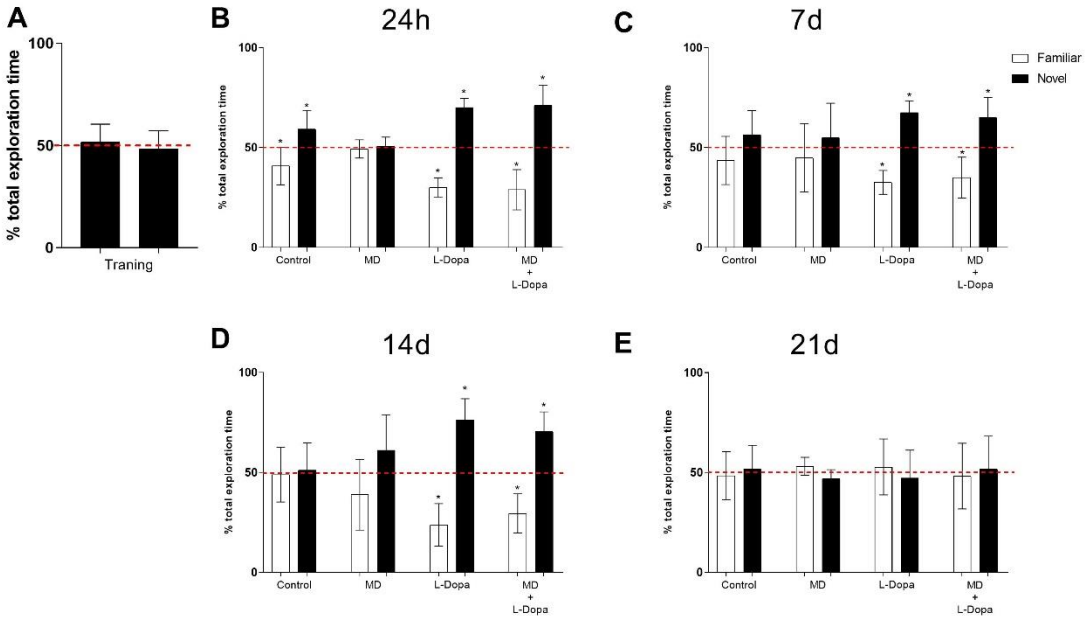

Figure 6. The administration of a dopamine precursor (L-dopa) reverts the memory deficits induced by MD. A. In the OR task training session the animals of the different groups were exposed two novel objects for 5 min and explored about 50% of the total exploration time each object. B. The OR long-term memory consolidation test was performed 24h after training. The MD group was not able to differentiate in the novel from the familiar object; the L-Dopa infusion 30 minutes before the OR training reverts this deficit. The long-term OR memory persistence tests were performed 7 (C), 14 (D) and 21 (E) days after the training. The animals from control group explored for a similar percentage of time the two objects 7, 14 and 21 days. The control and MD group were unable to distinguish the novel from the familiar object on 7, 14 and 21 days tests. L-Dopa promoted memory persistence for 14 days in control rats. The MD group that received L-Dopa before OR training session explored more than 50% of the total exploration time the novel object on the tests performed 7 and 14 days after the OR training. Data are expressed as mean ± SD of the percentage of the total exploration time; *P < 0.05 in the one sample Student t-test, considering a theoretical mean of 50%; n = 7-9 per group. MD = maternal deprivation rats; L-Dopa = dopamine precursor.

On the 24h LTM consolidation test, animals from Control and L-Dopa groups explored significantly more than 50% of the total exploration time the novel object (Control: $t(6) = 2.577$, $P = 0.0419$; L-Dopa: $t(9) = 13.34$, $P < 0.0001$; Fig. 6B), which demonstrates a preserved memory. However, the MD group spent a percentage of time similar to 50% of the total exploration time exploring each object on the 24h test day ($t(9) = 0.4491$, $P = 0.6640$; Fig. 6B) which suggests OR memory deficit. The L-Dopa administration was able to reverses MD induced memory deficit, since the MD rats that received L-Dopa explored significantly more than 50% of the total exploration time the novel object ($t(9) = 6.638$, $P < 0.0001$; Fig. 6B).

On OR memory persistence tests, the animals from the Control group explored similarly the two objects on 7th ($t(9) = 1.603$, $P = 0.1475$; Fig. 6C), 14th ($t(9) = 0.8094$, $P = 0.8094$; Fig 6D), and 21st tests day ($t(8) = 0.4037$, $P = 0.6970$; Fig. 6E), which suggests physiological forgetfulness. As expected, the MD group spent similarly percentage of time exploring the two objects in all test days (7th: $t(9) = 0.9414$, $P = 0.3711$, Fig 6C; 14th: $t(9) = 1.99$, $P = 0.0778$, Fig 6D; 21st: $t(9) = 2.166$, $P = 0.0585$; Fig 6E). Animals from L-Dopa group explored significantly more than 50% of the total exploration time the novel object on the 7th ($t(9) = 9.205$, $P < 0.0001$; Fig 6C) and on 14th day ($t(9) = 7.815$, $P < 0.0001$; Fig 6D), but not 21st day ($t(9) = 0.6299$, $P = 0.05444$; Fig 6E). The animals from MD + L-Dopa group spent more than 50% of the total exploration time exploring the novel object on 7th ($t(9) = 4.652$, $P = 0.0012$; Fig 6C) and 14th day ($t(9) = 6.623$, $P < 0.0001$; Fig 6D), but not on 21st day ($t(9) = 0.3368$, $P = 0.7440$; Fig 6E).

3.6. The procedures did not cause any alteration on control behavioral parameters

MD, intrahippocampal drug infusions or L-Dopa injection did not cause any alterations on the exploratory and locomotor behavioral observed in OR total exploration time ($P > 0.05$ in all tests and groups' comparison; supplementary material 1, tables 1-5).

In the same way, the procedures (MD, intrahippocampal drug infusions or L-Dopa intraperitoneal injection did not cause any alterations in the anxiety, since no differences in the number of entrances or time spent in the closed arms over 5 minutes of each EPM session were detected ($P > 0.05$ in all tests and groups' comparison; supplementary material 2, tables 1-5).

4. DISCUSSION

Here we demonstrate that intrahippocampal infusion of dopamine hydrochloride (DH), a non-selective dopaminergic receptor agonist, immediately after the OR learning is able to reverse the memory deficit induced by MD, evidencing the involvement of the dopaminergic system in the MD-related mnemonic dysfunction. Furthermore, the selective D1/D5 dopamine receptor stimulation modulates and improves memory, since that the intrahippocampal infusion of SKF 38393 promotes

memory persistence in control rats for 21 days, and, in MD rats, SKF reverts memory deficit and promotes memory persistence for 14 days after the training session.

It was already demonstrated that early life events, as MD, could impact the brain development, altering neurotransmitter systems related to emotional and cognitive responses, such as the dopaminergic system (Majcher-Maślanka et al., 2017). Studies have shown that stress in the neonatal period may affect the expression of dopaminergic receptors D1, D2, D3, and D5 (Amiri et al., 2016; Majcher-Maślanka et al., 2017), impairing spatial (Aisa et al., 2009; Fabricius et al., 2008) and aversive memory (Chocyk et al., 2014; Fabricius et al., 2008). These results corroborate with our findings, demonstrating that MD rats present disruption on the dopaminergic system.

The activation of the dopaminergic pathway that culminates on hippocampus dopamine release regulates the expression of BDNF, an important protein for the establishment of neural plasticity and LTM consolidation 12h after the leaning (Bekinschtein et al., 2007). In addition, our data demonstrate that the improvement of OR memory appears to be mediated by activation of D1 receptors in the dorsal hippocampus. Recently, Vargas et al. (2019), demonstrated that the persistence of OR memory depends on the activation of the dopaminergic system immediately after learning. The authors showed that intrahippocampal dopamine infusion immediately after a learning session promotes memory persistence (Vargas et al., 2019). Taken together, these results demonstrate that dopamine plays an important role in LTM persistence.

The hippocampus is an important structure involved in memory formation and consolidation, and it receives dopaminergic inputs of ventral tegmental area (VTA) and locus coeruleus (LC) (Kempadoo et al., 2016; Murty et al., 2016). After released, dopamine can act on two distinct groups of metabotropic receptors: D1-like receptors (D1 and D5), and D2-like receptors (D2, D3,, and D4). Although these two types of receptors are present in the hippocampus, D1-like receptors are widely expressed (Ariano et al., 1997; Ciliax et al., 2000), while D2-like are sparse in this region (Gangarossa et al., 2012). In addition, D1-like and D2-like receptors involve different biochemical activation cascades. While D1 and D5 receptors are positively coupled to adenylyl-cyclase (AC) and phosphoinositide responses (Monsma Jr et al., 1990;

Undieh, 2010), D2, D3, and D4 receptors seems to be negatively coupled to AC (Enjalbert and Bockaert, 1983; Kebabian and Greengard, 1971).

Considering that SKF stimulates both D1 and D5, we used a pharmacological strategy to differentiate the role of D1 and D5 receptors on memory deficits related to MD. D1 dopaminergic receptors activate adenylyl- cyclase and regulate the PKA activity, while D5 dopaminergic receptors activates the inositol-3-kinase system and regulate the PKC activity (Undieh, 2010). In our experiments, first, we showed that SCH 23390, a D1/D5 dopamine receptor antagonist, avoids memory consolidation in control rats. Then we demonstrated that the pharmacological activation of the D1 second messenger, PKA, but not the activation of the D5 second messenger, PKC, was able to reverse memory deficits induced by the block of D1/D5 receptors. In the same way, the stimulation of the PKA, but not PKC, was able to reverse the memory deficits induced by MD and promote memory persistence. Corroborating with our findings, Granado et al. (2008) show that the D1 dopaminergic receptors are critical for long-term potentiation (LTP) in the hippocampus (Granado et al., 2008). Both memory consolidation and persistence are based on hippocampal synaptic plasticity mechanisms mediated by LTP.

Lastly, we show that the stimulation of the dopaminergic system by peripheral administration of the dopamine precursor L-Dopa reverses the memory impairment related to MD. Our results are in line with a previous study demonstrating that L-Dopa administration is able to recover CA1 synaptic plasticity, restores hippocampal postsynaptic density and avoid memory deficit in an Alzheimer's disease model (Nobili et al., 2017), a neurodegenerative disease that causes damage to dopaminergic neurons of VTA and can lead to memory impairment (Nobili et al., 2017). Thus, this may explain why peripheral stimulation of the dopaminergic system by L-Dopa, a well-known drug used to treat Parkinson's disease, reverses memory deficits and promotes memory persistence in animals submitted to MD. However, additional studies will be needed to elucidate whether L-Dopa increase dopamine levels directly in the hippocampus, or leads to activation of dopaminergic neurons in VTA, indirectly stimulating this structure. Nevertheless, here we provide new evidence that L-Dopa may represent an alternative to future therapy for MD-induced memory deficit.

So, we present evidences that support the idea that D1 receptors could be more important to memory consolidation and persistence than D5. Our results reinforce the findings of previous studies about the involvement of dopaminergic dysfunctions on memory deficit induced by maternal deprivation (Menezes et al., 2017; Neves et al., 2015), provides new evidence that the D1 dopaminergic receptors are involved in these deficits, and demonstrates that the pharmacological manipulation of the dopaminergic system can influence OR learning.

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Authors contributions

Conceived and designed the experiments: B.H.S.N., P.B.M.C.; Performed theexperiments: B.H.S.N., G.P.D.R.B., A.C.S.R., S.S.P., G.M.,P.M.S.; Contributedto data analysis and interpretation writing and reviewing of the manuscript: B.H.S.N., P.B.M.C.; Approved the final manuscript version: B.H.S.N., G.P.D.R.B., A.C.S.R., S.S.P., G.M.,P.M.S. P.B.M.C.

SUPPORTING INFORMATION 1

Table 1. On study 1, MD and/or intrahippocampal drug infusions did not alter the exploratory activity in the Object Recognition (OR) training and testing sessions (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

		Control	MD	DH	MD + DH	*P Value*
Training						
	Total exploration time (s)	43.63 ± 20.26	57.5 ± 16.32	44.33 ± 15.95	41.7 ± 12.68	0.7845
24h test						
	Total exploration time (s)	45.25 ± 17.69	50.4 ± 10.61	43.11 ± 22.41	37.6 ± 19.92	0.4741

Table 2. On study 2, MD and/or intrahippocampal drug infusions did not alter the exploratory activity in the Object Recognition (OR) training and testing sessions (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

		Control	MD	SKF 38393	MD + SKF 38393	*P Value*
Trainning						
	Total exploration time (s)	86.0 ± 32.3	76.33 ± 32.64	74.75 ± 15.56	66.11 ± 24.07	0.5480
24h test						
	Total exploration time (s)	76.57 ± 25.01	84.78 ± 22.22	76.75 ± 23.61	69.89 ± 24.84	0.6297
7d test						
	Total exploration time (s)	80.29 ± 17.69	63.11 ± 8.992	75.5 ± 18.67	55.0 ± 30.35	0.0762
14d test						
	Total exploration time (s)	64.57 ± 23.87	52.78 ± 9.782	52.63 ± 13.61	54.89 ± 15.69	0.4435
21d test						
	Total exploration time (s)	72.86 ± 19.43	61.33 ± 22.99	72.88 ± 31.37	55.22 ± 16.69	0.3336

Table 3. On study 3, MD and/or intrahippocampal drug infusions did not alter the exploratory activity in the Object Recognition (OR) training and testing sessions (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

		SCH 23390	SCH 23390 + Sp-cAMP	SCH 23390 + PMA	*P Value*
Trainning					
	Total exploration time (s)	66.38 ± 27.43	84.88 ± 28.18	77.29 ± 21.44	0.3794
24h test					
	Total exploration time (s)	71.0 ± 32.51	75.38 ± 5.85	61.86 ± 25.6	0.5550
7d test					
	Total exploration time (s)	68.0 ± 21.71	60.88 ± 25.1	76.57 ± 14.0	0.3733
14d test					
	Total exploration time (s)	61.0 ± 19.73	60.88 ± 26.73	48.86 ± 17.42	0.4877
21d test					
	Total exploration time (s)	59.63 ± 20.45	66.25 ± 19.72	60.43 ± 18.1	0.7655

Table 4. On study 4 MD and/or intrahippocampal drug infusions did not alter the exploratory activity in the Object Recognition (OR) training and testing sessions (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

		MD + Sp-cAMP	MD + PMA	MD + SKF 38393 + Rp-cAMP	MD + SKF 38393 + Gö 6976	*P Value*
Trainning						
	Total exploration time (s)	95.88 ± 15.86	76.88 ± 26.36	76.57 ± 13.81	71.43 ± 13.33	0.0714
24h test						
	Total exploration time (s)	76.38 ± 18.63	47.75 ± 20.38	67.86 ± 19.58	63.71 ± 30.1	0.1028
7d test						
	Total exploration time (s)	79.0 ± 21.18	59.25 ± 24.71	64.86 ± 12.25	65 ± 8.406	0.1963
14d test						
	Total exploration time (s)	68.5 ± 26.08	55.75 ± 19.51	66.43 ± 23.99	52.86 ± 14.28	0.4253
21d test						
	Total exploration time (s)	75.75 ± 17.93	62 ± 26.95	63.0 ± 9.381	63.43 ± 8.979	0.3937

Table 5. On study 5, L-Dopa and saline intraperitonial injection, as well as MD, did not alter the exploratory activity in the Object Recognition (OR) training and testing sessions (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

		Control	MD	L-Dopa	MD+L-Dopa	*P Value*
Trainning						
	Total exploration time (s)	56.0 ± 23.39	49 ± 22.14	56.1 ± 26.81	60.0 ± 29.97	0.8137
24h test						
	Total exploration time (s)	82.7 ± 16.63	56.5 ± 24.61	72.5 ± 31.42	60.7 ± 31.44	0.1362
7d test						
	Total exploration time (s)	64.4 ± 25.55	48.0 ± 27.21	63 ± 20.43	78.8 ± 25.78	0.0691
14d test						
	Total exploration time (s)	53.1 ± 17.67	38.1 ± 22.1	49.3 ± 13.64	65.8 ± 38.19	0.1129
21d test						
	Total exploration time (s)	53.8 ± 19.47	44.0 ± 18.00	68.0 ± 14.96	63.0 ± 26.14	0.0648

SUPPORTING INFORMATION 2

Table 1. On study 1, drug or vehicle intrahippocampal infusion, as well as MD, did not alter anxiety behavior in the Elevated Plus Maze task (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

		Control	MD	DH	MD + DH	*P Value*
24h						
	Elevated Plus Maze					
	Time in closed arms (s)	179.0 ± 37.84	170.9 ± 51.32	200.2 ± 51.45	178.2 ± 57.14	0.5778
	Entrances in closed arms (n)	5.87 ± 1.126	5.50 ± 2.014	5.55 ± 2.007	5.10 ± 2.378	0.9198

Table 2. On study 2, drug or vehicle intrahippocampal infusion, as well as MD, did not alter anxiety behavior in the Elevated Plus Maze task (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

			Control	MD	SKF 38393	MD + SKF 38393	*P Value*
24h							
	Elevated Plus Maze						
		Time in closed arms (s)	78.00 ± 41.26	90.33 ± 38.53	112.3 ± 32.88	130.00 ± 54.21	0.0910
		Entrances in closed arms (n)	6.14 ± 2.47	8.00 ± 3.08	7.75 ± 2.31	7.44 ± 1.94	0.4940
7d							
	Elevated Plus Maze						
		Time in closed arms (s)	120.00 ± 54.62	120.4 ± 43.39	138.5 ± 35.20	191.5 ± 79.74	0.1084
		Entrances in closed arms (n)	7.71 ± 2.98	8.88 ± 2.93	8.87 ± 2.74	6.55 ± 3.77	0.3696
14d							
	Elevated Plus Maze						
		Time in closed arms (s)	105.00 ± 53.21	151.2 ± 61.82	141.8 ± 57.70	171.2 ± 74.66	0.1661
		Entrances in closed arms (n)	6.28 ± 2.69	8.00 ± 2.24	8.37 ± 2.92	7.77 ± 2.53	0.4452
21d							
	Elevated Plus Maze						
		Time in closed arms (s)	138.4 ± 52.43	138.9 ± 53.35	138.3 ± 70.15	152.3 ± 34.45	0.9497
		Entrances in closed arms (n)	6.14 ± 2.61	8.11 ± 2.71	7.37 ± 3.11	6.77 ± 3.45	0.6035

Table 3. On study 3 drug(s) or vehicle intrahippocampal infusion, as well as MD, did not alter anxiety behavior in the Elevated Plus Maze task (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

			SCH 23390	SCH 23390 + Sp - cAMP	SCH 23390 + PMA	*P Value*
24h						
	Elevated Plus Maze					
		Time in closed arms (s)	84.75 ± 37.38	94.25 ± 57.08	82.0 ± 30.22	0.8476
		Entrances in closed arms (n)	6.37 ± 2.13	5.12 ± 2.03	6.85 ± 1.86	0.2478
7d						
	Elevated Plus Maze					
		Time in closed arms (s)	109.3 ± 48.44	124.8 ± 50.62	146.0 ± 33.56	0.3193
		Entrances in closed arms (n)	7.25 ± 3.19	8.0 ± 1.19	9.14 ± 2.26	0.3223
14d						
	Elevated Plus Maze					
		Time in closed arms (s)	130.1 ± 54.09	117.6 ± 50.44	154.1 ± 50.06	0.4101
		Entrances in closed arms (n)	8.50 ± 3.96	8.25 ± 3.01	8.57 ± 3.10	0.9813
21d						
	Elevated Plus Maze					
		Time in closed arms (s)	115.6 ± 41.57	122.1 ± 52.62	160.7 ± 53.11	0.2062
		Entrances in closed arms (n)	5.85 ± 2.47	7.87 ± 2.10	7.85 ± 2.85	0.2338

Table 4. On study 4 drug(s) or vehicle intrahippocampal infusion, as well as MD, did not alter anxiety behavior in the Elevated Plus Maze task (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

			MD + Sp - cAMP	MD + PMA	MD + SKF 38393 + Rp - cAMP	MD + SKF 38393 + Gö 6976	*P Value*
24h	Elevated Plus Maze						
		Time in closed arms (s)	75.0 ± 40.41	95.13 ± 45.08	75.86 ± 29.89	69.0 ± 44.05	0.6179
		Entrances in closed arms (n)	5.62 ± 2.82	5.87 ± 1.80	6.71 ± 2.56	6.14 ± 2.73	0.8558
7d	Elevated Plus Maze						
		Time in closed arms (s)	119.9 ± 55.31	120.6 ± 44.23	113.9 ± 69.75	107.0 ± 53.38	0.9624
		Entrances in closed arms (n)	8.37 ± 1.50	9.50 ± 2.97	8.28 ± 1.97	7.0 ± 1.29	0.1684
14d	Elevated Plus Maze						
		Time in closed arms (s)	128.9 ± 48.94	155.1 ± 60.24	115.3 ± 57.85	105.1 ± 42.91	0.3085
		Entrances in closed arms (n)	9.87 ± 2.85	9.12 ± 3.68	8.0 ± 3.51	8.71 ± 2.92	0.7325
21d	Elevated Plus Maze						
		Time in closed arms (s)	123.3 ± 56.01	147.2 ± 58.94	153.4 ± 60.71	186.7 ± 65.37	0.2335
		Entrances in closed arms (n)	7.75 ± 2.49	8.0 ± 2.72	6.14 ± 2.73	6.57 ± 2.50	0.4639

Table 5. On study 5 L-Dopa or saline intraperitonial injection, as well as MD, did not alter anxiety behavior in the Elevated Plus Maze task (P > 0.05; one-way ANOVA). The data are expressed as mean ± SD (n = 7-10/group).

			Control	MD	L-Dopa	MD + L-Dopa	*P Value*
24h	Elevated Plus Maze						
		Time in closed arms (s)	99.6 ± 26.68	91.9 ± 17.99	119.1 ± 25	105.3 ± 19.43	0.0680
		Entrances in closed arms (n)	7.2 ± 2.741	7 ± 2.708	6 ± 1.944	6.1 ± 2.685	0.7239
7d	Elevated Plus Maze						
		Time in closed arms (s)	93.3 ± 31.32	108.9 ± 45.58	113.5 ± 16.47	104.6 ± 29.36	0.5501
		Entrances in closed arms (n)	7.5 ± 3.1	7.8 ± 2.781	8.6 ± 2.675	9.2 ± 3.19	0.3911
14d	Elevated Plus Maze						
		Time in closed arms (s)	98.3 ± 23.12	110.7 ± 44.67	114 ± 46.73	88 ± 32.72	0.4109
		Entrances in closed arms (n)	8.8 ± 1.549	7.3 ± 3.401	7.6 ± 1.578	9.5 ± 3.567	0.2370
21d	Elevated Plus Maze						
		Time in closed arms (s)	105.3 ± 24.19	93.6 ± 20.77	108.6 ± 17.9	99.1 ± 21.65	0.4122
		Entrances in closed arms (n)	7.0 ± 2.667	6.1 ± 2.424	8.3 ± 2.452	5.9 ± 1.524	0.1577

PARTE III

6 DISCUSSÃO

Esta dissertação buscou investigar o envolvimento do sistema dopaminérgico no déficit de memória induzido pela privação maternal (MD, do inglês *maternal deprivation*). Inicialmente buscou-se investigar o papel dos diferentes receptores dopaminérgicos no prejuízo cognitivo relacionado à MD. Assim, foi realizada a estimulação farmacológica não seletiva dos diferentes receptores dopaminérgicos através da infusão intra-hipocampal de um agonista dopaminérgico não seletivo. Neste mesmo sentido, utilizamos refinados protocolos farmacológico-comportamentais para estudar o envolvimento dos diferentes tipos de receptores dopaminérgicos no déficit de memória relacionado à MD. Ao final, buscou-se investigar se a estimulação periférica do sistema dopaminérgico, através da administração intraperitonial de um precursor da dopamina (L-Dopa), é capaz de reverter o déficit de memória induzido pela MD.

Inicialmente, nossos resultados demonstram que a infusão de um agonista não seletivo dos diferentes receptores dopaminérgicos, o cloridrato de dopamina (DH, do inglês *dopamine hydrochloride*), na região CA1 do hipocampo imediatamente após o aprendizado da tarefa Reconhecimento de Objetos (OR, do inglês *object recognition*) é capaz de reverter o déficit de memória induzido pela MD. O resultado do primeiro estudo confirmou a hipótese de que o sistema dopaminérgico seria afetado pelo o estresse neonatal, já que a infusão da DH reverteu os déficits induzidos pela MD. Estudos prévios já demonstravam que um evento estressor durante o período de desenvolvimento afeta a função do sistema dopaminérgico em diferentes regiões do encéfalo, tais como córtex pré-frontal (PFC, do inglês *prefrontal cortex*), área tegmentar ventral (VTA, do inglês *ventral tegmental area*) e substância nigra (SN), aumentando o risco do desenvolvimento de doenças psiquiátricas como a depressão e a esquizofrenia (DALLE E MABANDLA, 2018; NOVICK *et al.*, 2018; DAHOUN *et al.*, 2019). Ademais, nós demonstramos o envolvimento do sistema dopaminérgico no déficit de memória relacionado a MD.

A estimulação dos diferentes receptores dopaminérgicos culmina na ativação de diferentes cascatas bioquímicas intracelulares. Os efeitos são produzidos através da interação da dopamina com o receptores dopaminérgicos acoplados a proteína G (GARAU *et al.*, 1978). Inicialmente, os receptores dopaminérgicos foram divididos em duas grandes classes: receptores do tipo D1 e do tipo D2 (GARAU *et al.*, 1978; KEBABIAN E CALNE, 1979). Contudo, técnicas moleculares permitiram a distinção de membros de famílias de receptores dopaminérgicos, resultando em uma reorganização em subfamílias de receptores. Assim, a família de receptores dopamiérgicos D1 foi subdividida em receptores D1 e D5 (SEEMAN, 1980; HANSEN E MANAHAN-VAUGHAN, 2014), enquanto a família D2 foi subdividida em receptores D2, D3 e D4 (SEEMAN, 1980).

Uma vez que o efeito da dopamina hipocampal na formação da memória de longa duração (LTM, do inglês *long-term memory*) é mediado principalmente pela estimulação de receptores dopaminérgicos da família D1 (receptores D1/D5), nós investigamos o papel desses receptores no déficit de memória causado pela MD. Assim, nossos resultados confirmam resultados prévios de que a infusão intrahipocampal de um agonista seletivo de receptores dopaminérgicos D1/D5, o SKF 38393, promove a persistência da memória de longa duração em ratos controle (ROSSATO *et al.*, 2009). Além disso, demonstramos que a estimulação seletiva destes receptores dopaminérgicos reverte os déficits da memória de OR e promove a sua persistência por até 14 dias após a aprendizagem.

Já está bem estabelecido na literatura que o estresse neonatal, como a MD, pode afetar a expressão dos diferentes receptores dopaminérgicos (AMIRI *et al.*, 2016; MAJCHER-MAŚLANKA *et al.*, 2017). Por exemplo, Majcher-Maślanka *et al.* (2017) demonstraram uma diminuição dos níveis do RNAm para receptores dopaminérgicos D5, acompanhada de uma diminuição dos níveis de RNAm para receptores dopaminérgicos D2, no córtex pré-límbico de ratos submetidos ao estresse neonatal em animais submetidos ao estresse pré-natal. Esses resultados demonstram que o estresse durante o período crítico do neurodesenvolvimento altera a expressão de receptores em áreas encefélicas ligadas à cognição, levando ao prejuízdo de memória espacial (FABRICIUS *et al.*, 2008; AISA *et al.*, 2009) e aversiva (CHOCYK *et al.*, 2014).

A estimulação de receptores dopaminérgicos D1/D5 é importante para a formação e persistência da LTM (ROSSATO *et al.*, 2009). Embora esses dois receptores sejam estimulados pela dopamina, ativam segundos mensageiros diferentes: enquanto os receptores D1 regulam a atividade da proteínoquinase A (PKA, do inglês *protein kinase A*), os receptores D5 regulam a atividade da proteínoquinase C (PKC, do inglês *protein kinase C*) (FELDER *et al.*, 1989; FURINI *et al.*, 2014). Tanto PKA quanto PKC estão envolvidas na consolidação de diferentes tipos de memórias (IZQUIERDO *et al.*, 2008).

Uma vez que ainda não são disponibilizados fármacos que estimulem separadamente os receptores dopaminérgicos D1 ou D5, usamos uma estratégia farmacológica para diferenciar o papel dos diferentes receptores através da estimulação/inibição de segundos mensageiros. Primeiro, demonstramos que o bloqueio de receptores dopaminérgicos pelo antagonista seletivo D1/D5, o SCH 23390, evita a consolidação da memória em ratos controle. Em seguinda, associado ao bloqueio de receptores D1/D5, utilizamos um estimulador do segundo mensageiro dos receptores D1, PKA. Demonstramos que além de reverter os déficts de memória induzido pelo bloqueio de receptores D1/D5, a estimulação de PKA promoveu a persistência da memória. Tal resultado não foi observado quando utilizamos um estimulador do segundo mensageiro dos receptores D5, a PKC.

Da mesma forma, a estimulação de PKA, mas não de PKC, foi capaz de reverter os déficits de memória induzidos pela MD e promover a persistência da memória nestes animais. Corroborando com nossos achados, Granado *et al.* (2008) demostraram que os receptores dopaminérgicos D1 são críticos para a potencialização a longo prazo[1] (LTP, do inglês *long-term potentiation*) no hipocampo (GRANADO *et al.*, 2008). Tanto a consolidação da memória quanto a persistência são baseadas nos mecanismos de plasticidade sináptica do hipocampo mediados por LTP.

[1] Potenciação a longo prazo, ou LTP é um aumento da resposta pós-sináptica que pode persistir por horas, dias ou semanas após a estimulação repetitiva de um neurônio pré-sináptico e tem sido considera a base neurobiológica da consolidação da memória de longa duração.

Por fim, mostramos que a estimulação do sistema dopaminérgico pela administração periférica do precursor da dopamina L-Dopa reverte o comprometimento da memória relacionado à DM. Nossos resultados estão alinhados com um estudo anterior que demonstra que a administração de L-Dopa é capaz de recuperar a plasticidade sináptica de CA1, restaurar a densidade pós-sináptica do hipocampo e evitar o déficit de memória em um modelo de doença de Alzheimer (NOBILI *et al.*, 2017), uma doença neurodegenerativa que causa danos aos neurônios dopaminérgicos da ATV e pode levar à perda de memória (NOBILI *et al.*, 2017). Assim, isso pode explicar por que a estimulação periférica do sistema dopaminérgico pelo fármaco L-Dopa, um conhecido medicamento usado para tratar a doença de Parkinson em humanos, reverte os déficits de memória e promove a persistência da memória em animais submetidos à DM. Estudos adicionais ainda são necessários para elucidar se a L-Dopa aumenta os níveis de dopamina diretamente no hipocampo ou leva à ativação de neurônios dopaminérgicos na ATV, estimulando indiretamente essa estrutura. De qualquer forma, neste trabalho fornecemos novas evidências de que a L-Dopa pode representar uma alternativa à terapia futura para o déficit de memória induzido por MD.

7 CONCLUSÃO

Com base em nossos dados podemos concluir que:

I. O bloqueio dos receptores dopaminérgico D1/D5 promove déficits na consolidação da memória de OR;

II. A estimulação dos receptores dopaminérgicos D1 reverte os déficits na memória de OR induzidos pela MD;

III. A estimulação do segundo mensageiro dos receptores D1, PKA, reverte os déficits na memória de OR induzidos pelo bloqueio de receptores D1/D5 e pela MD;

IV. A inativação do segundo mensageiro dos receptores D1, PKA, promove déficits na memória de OR;

V. A estimulação dos receptores dopaminérgicos D5, não reverte os déficits na memória de OR induzidos pela MD;

VI. A estimulação do segundo mensageiro dos receptores D5, PKC, não reverte os déficits na memória de OR induzidos pelo bloqueio de receptores D1/D5 e pela MD;

VII. A inativação do segundo mensageiro dos receptores D5, PKC, não promove déficits na memória de OR;

VIII. A administração periférica sistêmica do precursor da dopamina L-Dopa é capaz de reverter os déficits na memória de OR induzidos pela MD.

Tomados em conjuntos, nossos dados fornecem evidências que sustentam a ideia de que os receptores D1 podem ser mais importantes para a consolidação e persistência da memória do que o D5. Além disso, fornecem novas evidências de que os receptores dopaminérgicos D1 estão envolvidos nos déficits da memória de reconhecimento induzidos pela MD e demonstram que a manipulação farmacológica do sistema dopaminérgico pode influenciar a consolidação e persistência da memória de OR.

8 PERSPECTIVAS FUTURAS

Os resultados dessa dissertação confirmaram hipóteses prévias levantadas pela literatura científica especializada. A partir da confirmação de que o sistema dopaminérgico e seus diferentes receptores está envolvido nos déficits de memória causados pela privação maternal em tarefas de memória dependentes do hipocampo, novas perguntas surgiram no sentido de compreender se o estresse neonatal pode afetar outras estruturas encefálicas que participam do processo de modulação da memória.

A partir do conjunto de resultados apresentados, perspectivas futuras envolvem a continuidade dos experimentos nesta linha, com o início do doutoramento junto ao Grupo de Pesquisa em Fisiologia da Universidade Federal do Pampa, tendo como objetivos:

(i) Investigar os efeitos da privação maternal na morfologia e função de neurônios noradrenérgicos do *locus coeruleus;*

(ii) Investigar os efeitos da privação maternal na morfologia e função de neurônios dopaminérgicos da área tegmental ventral;

(iii) Investigar os efeitos da estimulação do *locus coeruleus* no déficit de memória induzido pela privação maternal;

(iv) Investigar os efeitos da estimulação da área tegmental ventral no déficit de memória induzido pela privação maternal.

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10 ANEXOS

a. Carta de aprovação da Comissão de Ética no Uso de Animais (CEUA)

MINISTÉRIO DA EDUCAÇÃO
FUNDAÇÃO UNIVERSIDADE FEDERAL DO PAMPA
(Lei nº 11.640, de 11 de janeiro de 2008)

Pró-Reitoria de Pesquisa, Pós-Graduação e Inovação (PROPPI)

COMISSÃO DE ÉTICA NO USO DE ANIMAIS - CEUA

Fone: (55)3911-0200, E-mail: ceua@unipampa.edu.br

CERTIFICADO DE APROVAÇÃO DE PROTOCOLO PARA USO DE ANIMAIS EM PESQUISA

Número do protocolo da CEUA: 032/2018

Título: Envolvimento do sistema dopaminérgico e seus diferentes receptores no déficit de memória induzido pela deprivação maternal

Data da aprovação: 10/09/2018

Período de vigência do projeto: 10/09/2020

Pesquisadores(a): Pâmela Billig Mello Carpes

Campus: Uruguaiana

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CEUA

Finalidade	**( ) Ensino (X) Pesquisa**
Espécie/Linhagem/Raça	Ratos Wistar
Nº de animais	169
Peso/Idade	3 – 4 meses/500g nascimento até 4 meses/até 400g
Sexo	Fêmeas e machos
Origem	Biotério Central da UFSM

Comissão de Ética em Uso de Animais

Profª. Drª. Cátia Aline Veiverberg
Coordenadora Substituta CEUA/UNIPAMPA

b. Normas da revista *Neurobiology of Learning and Memory*

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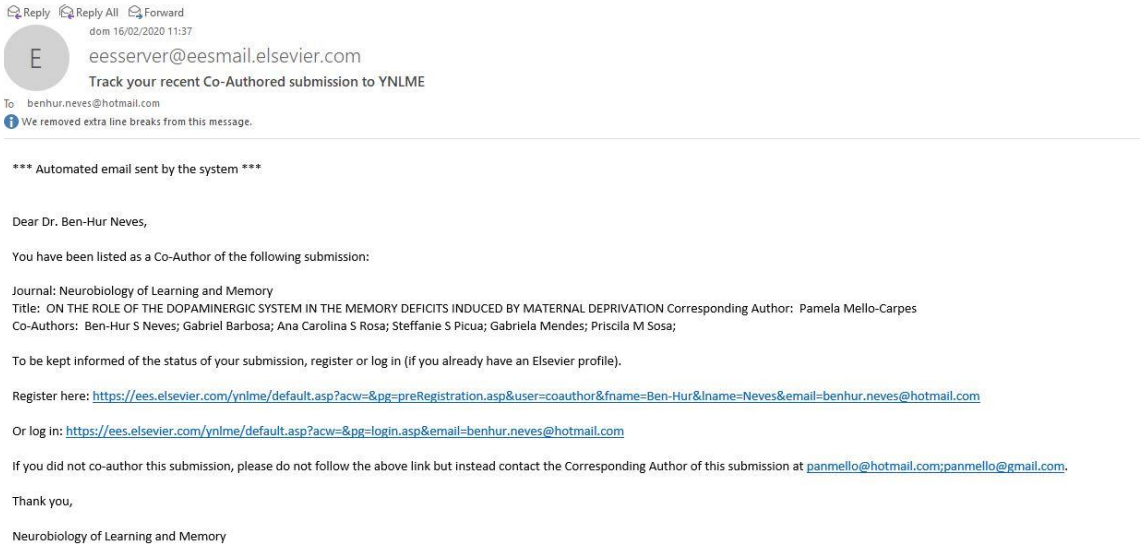

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