

UNIVERSIDADE FEDERAL DE SANTA MARIA
CENTRO DE CIÊNCIAS DA SAÚDE
PROGRAMA DE PÓS-GRADUAÇÃO EM FARMACOLOGIA

Vinícia Garzella Metz

**ESTUDO EXPERIMENTAL DOS EFEITOS DO CANABIDIOL:
POSSÍVEL ESTRATÉGIA PARA O TRATAMENTO DA ADIÇÃO À
ANFETAMINA**

Santa Maria, RS
2022

Vinícia Garzella Metz

**ESTUDO EXPERIMENTAL DOS EFEITOS DO CANABIDIOL: POSSÍVEL
ESTRATÉGIA PARA O TRATAMENTO DA ADIÇÃO À ANFETAMINA**

Tese apresentada ao Curso de Pós-Graduação em Farmacologia, Área de Concentração em Neuropsicofarmacologia e Imunofarmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para a obtenção do título de **Doutora em Farmacologia**.

Orientadora: Prof^a Dr^a Camila Simonetti Pase
Coorientadora: Prof^a Dr^a Marilise Escobar Bürger

Santa Maria, RS
2022

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001

Metz, Vinícia Garzella
ESTUDO EXPERIMENTAL DOS EFEITOS DO CANABIDIOL:
POSSÍVEL ESTRATÉGIA PARA O TRATAMENTO DA ADIÇÃO À
ANFETAMINA / Vinícia Garzella Metz.- 2022.
131 p.; 30 cm

Orientadora: Camila Simonetti Pase
Coorientadora: Marilise Escobar Bürger
Tese (doutorado) - Universidade Federal de Santa
Maria, Centro de Ciências da Saúde, Programa de Pós
Graduação em Farmacologia, RS, 2022

1. Adição 2. Psicoestimulantes 3. Canabinoides 4.
Sistema Dopaminérgico 5. Sistema Endocanabinoide I.
Pase, Camila Simonetti II. Bürger, Marilise Escobar III.
Título.

Sistema de geração automática de ficha catalográfica da UFSM. Dados fornecidos pelo autor(a). Sob supervisão da Direção da Divisão de Processos Técnicos da Biblioteca Central. Bibliotecária responsável Paula Schoenfeldt Patta CRB 10/1728.

Declaro, VINÍCIA GARZELLA METZ, para os devidos fins e sob as penas da lei, que a pesquisa constante neste trabalho de conclusão de curso (Tese) foi por mim elaborada e que as informações necessárias objeto de consulta em literatura e outras fontes estão devidamente referenciadas. Declaro, ainda, que este trabalho ou parte dele não foi apresentado anteriormente para obtenção de qualquer outro grau acadêmico, estando ciente de que a inveracidade da presente declaração poderá resultar na anulação da titulação pela Universidade, entre outras consequências legais.

Vinícia Garzella Metz

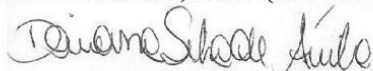
**ESTUDO EXPERIMENTAL DOS EFEITOS DO CANABIDIOL: POSSÍVEL
ESTRATÉGIA PARA O TRATAMENTO DA ADIÇÃO À ANFETAMINA**

Tese apresentada ao Curso de Pós-Graduação em Farmacologia, Área de Concentração em Neuropsicofarmacologia e Imunofarmacologia, da Universidade Federal de Santa Maria (UFSM, RS), como requisito para a obtenção do título de **Doutora em Farmacologia**.

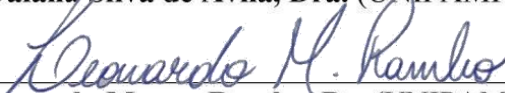
Aprovada em 04 de fevereiro de 2022:



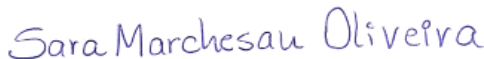
Camila Simonetti Pase, Dra. (Presidente/Orientadora)



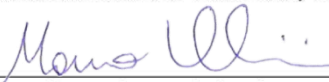
Daiana Silva de Ávila, Dra. (UNIPAMPA)



Leonardo Magno Rambo, Dr. (UNIPAMPA)



Sara Marchesan de Oliveira, Dra. (UFSM)



Mauro Schneider Oliveira, Dr. (UFSM)

Santa Maria, RS
2022

AGRADECIMENTOS

Primeiramente à Deus, pela vida e pela minha história. Pelo seu grandioso e incondicional amor. Por me fazer forte, corajosa e temente a Ti. Por nunca me abandonar e guiar todos os meus caminhos. Graças eu te dou Senhor.

À minha mãe, meu maior exemplo de mulher, mãe, profissional e ser humano. Ao meu pai, amor da minha vida, meu exemplo de bondade, dedicação, trabalho e luta. Vocês são meus pilares de sustentação e o meu refúgio. Obrigada por acreditarem junto comigo nos meus sonhos, me proporcionarem meios de alcançá-los e vibrarem a cada pequena conquista. Em toda a construção do meu ser há inúmeros traços de vocês e da minha criação, o que muito me orgulha. Que eu possa orgulhá-los e honrar seus inúmeros sacrifícios. Obrigada, de todo o meu coração.

Ao meu irmão, por ser um companheiro da vida toda em todas as batalhas. Eu sei que um dia você vai duvidar dos seus sonhos. Eu também duvidei. Contudo, saiba que duvidar não é sinal de fraqueza. Então, quando pensar em desistir, sonhe de novo. E, se eu for motivo de alguma coisa na sua vida, que seja motivo para você não desistir do que ama e almeja. Eu estarei com você sempre.

Às minhas avós, que são os anjos mais lindos do meu céu, por todo o amor que me deram em vida e por agora me cuidarem de onde quer que estejam. E a todos os meus familiares, pelo apoio, palavras de carinho e sustentação.

Meu agradecimento especial à minha orientadora, Prof^ª Camila. Pelo “time” que formamos e que deu mais do que certo. Por acreditar na minha capacidade e ser uma grande incentivadora. Por ter idealizado junto comigo e me ajudado em cada passo do meu projeto. Por toda a orientação prestada, meu muito obrigada. À Prof^ª Marilise, minha coorientadora, por ter aberto as portas do FARMATOX ainda na iniciação científica, me orientando e me capacitado em tudo para que eu chegasse até aqui, minha eterna gratidão. À Prof^ª Judith Homberg, minha coorientadora na Holanda, pela oportunidade, pelo acolhimento em seu grupo de pesquisa e por todo o conhecimento adquirido nesta maravilhosa experiência. Agradeço ainda ao Michel Verheij, que não mediu esforços em me auxiliar e pelo que foi trabalhar ao seu lado durante o doutorado sanduíche.

A todos os colegas do FARMATOX, os quais tive o prazer de conviver e aprender ao longo de toda a minha jornada científica. Especialmente aqueles que se tornaram grandes amigos e que eu levarei para a vida toda, Hecson, Raquel, Caren, Karine, Vel e Lívia. Meu

agradecimento especial à Jéssica, por toda ajuda no desenvolvimento do projeto que originou esta tese.

Aos meus amigos, por viverem esse sonho comigo, mesmo de longe. Por todas as palavras de incentivo e de acolhimento e por compreenderem as necessárias ausências muitas vezes. Suelen e Jonatan, obrigada por terem vivido comigo dias inesquecíveis durante o doutorado sanduíche. Nat, Patrícia, Ju, Nadi, Alan, Vinícius L., Vinícius P., Susana, Fran, Luís Gustavo, Verônica, Aline, Leísa, Matheus e Marcel, obrigada por todo o apoio, vocês são a minha sorte grande.

Aos animais utilizados, meios para a realização deste trabalho, todo o meu respeito e agradecimento.

À Universidade Federal de Santa Maria e o Programa de Pós Graduação em Farmacologia pela minha formação. Sempre grata por todas as oportunidades que essa universidade pública, gratuita e de exímia qualidade me proporcionou. A CAPES, ao Programa CAPES PrInt e a FAPERGS pelo suporte nos meus estudos e para realização deste trabalho.

A todos os que de alguma maneira fizeram parte do meu caminho e torceram por mim... Dedico essa conquista a vocês!

“Segue o teu destino
Rega as tuas plantas
Ama as tuas rosas
O resto é a sombra
De árvores alheias.”

(Fernando Pessoa)

RESUMO

ESTUDO EXPERIMENTAL DOS EFEITOS DO CANABIDIOL: POSSÍVEL ESTRATÉGIA PARA O TRATAMENTO DA ADIÇÃO À ANFETAMINA

AUTORA: Vinícia Garzella Metz

ORIENTADORA: Prof^ª Dr^a Camila Simonetti Pase

COORIENTADORA: Prof^ª Dr^a Marilise Escobar Bürger

A adição à anfetamina (ANF) é uma doença crônica e recidivante caracterizada pela busca e uso compulsivo da droga, apesar de suas consequências prejudiciais. Atualmente, não há tratamento farmacológico eficaz e aprovado para o tratamento dessa importante condição neuropsiquiátrica. Deste modo, a busca por novos alvos terapêuticos que possam favorecer o tratamento da drogadição e reduzir as recorrentes recaídas representa o maior desafio para a cura da condição. O canabidiol (CBD) tem mostrado muitos benefícios no tratamento de doenças que afetam o sistema nervoso central e recentemente, tem sido considerado uma potencial estratégia para o tratamento da adição. Assim, o presente estudo teve por objetivo avaliar os efeitos do CBD sobre parâmetros neuroquímicos e comportamentais de recaída em ratos expostos à ANF, cuja execução envolveu dois protocolos experimentais. No protocolo experimental 1 (PE1), os efeitos do tratamento com CBD sobre parâmetros comportamentais de recaída após condicionamento e recondicionamento com ANF depois da extinção da preferência pela droga foram avaliados. Nesse protocolo, os animais foram inicialmente expostos à ANF (4 mg/kg, i.p) no protocolo de preferência condicionada de lugar (PCL) por 8 dias. Após o teste de PCL, os animais receberam tratamento com CBD (5 ou 10 mg/kg, i.p) durante os 5 dias de abstinência à droga. Na sequência, os animais foram expostos novamente à ANF (recondicionamento) por 3 dias e o teste de recaída foi executado. No protocolo experimental 2 (PE2), o objetivo foi investigar os efeitos do tratamento com CBD sobre parâmetros comportamentais de recaída à ANF induzida por um estímulo estressor. Para isto, os animais foram expostos à ANF (4 mg/kg, i.p) através do protocolo de PCL por 8 dias e sequencialmente tratados com CBD (10 mg/kg, i.p) durante os 5 dias de abstinência da droga. O comportamento de recaída à busca pela droga foi induzido por um protocolo de nado forçado antes do teste de recaída. Em ambos protocolos, os animais também foram submetidos a avaliações comportamentais no teste de campo aberto e labirinto em cruz elevado para análise da atividade locomotora e comportamentos de ansiedade, respectivamente. Além disso, no PE1, o córtex pré-frontal (CPF) e o estriado ventral (EV) foram coletados para a análise de alvos dopaminérgicos através de western blot, enquanto que no PE2, as regiões escolhidas foram a área tegmental ventral (ATV) e o EV para a análise de alvos dopaminérgicos e endocanabinoides. Nossos resultados mostraram que, em ambos os protocolos experimentais, o CBD preveniu a recaída à ANF e diminuiu o comportamento de ansiedade *per se*, também observado nos animais expostos à ANF. No PE1, o CBD restaurou os níveis dos alvos dopaminérgicos (D1R, D2R, DAT e TH), alterados pela exposição à ANF em ambas as regiões cerebrais. Esse mesmo efeito do CBD sobre D1R, D2R e DAT no EV foi observado no PE2. As análises moleculares do PE2 também revelaram que na ATV, o CBD restaurou os níveis de CB1R diminuídos pela exposição à ANF, aumentou os níveis da enzima responsável pela síntese dos endocanabinoides, a NAPE-PLD, e diminuiu os níveis da enzima que faz a degradação destes, a FAAH. Esses achados moleculares nos permitem hipotetizar que o potencial efeito anti-recaída do CBD reflete a capacidade deste em aumentar o tônus endocanabinoide e, então, restaurar o sistema dopaminérgico comprometido pela exposição à ANF. Embora estudos adicionais sejam necessários, o CBD mostra-se como uma alternativa farmacológica promissora para o tratamento da adição por drogas psicoestimulantes como a ANF.

Palavras-chave: Adição, Psicoestimulantes, Canabinoides, Sistema Dopaminérgico, Sistema Endocanabinoide

ABSTRACT

EXPERIMENTAL STUDY OF THE EFFECTS OF CANNABIDIOL: POTENTIAL STRATEGY FOR THE TREATMENT OF AMPHETAMINE ADDICTION

AUTHOR: Vinícia Garzella Metz
ADVISOR: Prof^a Dr^a Camila Simonetti Pase
COADVISOR: Prof^a Dr^a Marilise Escobar Bürger

Amphetamine (AMPH) addiction is a chronic and relapsing disease caused by the compulsive drug seeking and continued use despite harmful consequences. Currently, there is no effective and approved pharmacological treatment for the treatment of this important neuropsychiatric condition. Thus, the search for new therapeutic targets that can favor the treatment of drug addiction and reduce recurrent relapses represents the biggest challenge for the cure of the condition. Cannabidiol (CBD) has shown many benefits in the treatment of diseases that affect the central nervous system and recently, it has been considered a potential strategy for the treatment of addiction. Thus, the present study aimed to evaluate the effects of CBD on neurochemical and behavioral parameters of relapse in rats AMPH-exposed, whose execution involved two experimental protocols. In experimental protocol 1 (EP1), the effects of CBD treatment on behavioral parameters of relapse after conditioning and reconditioning with AMPH after drug preference extinction were evaluated. In that protocol, animals were initially exposed to ANF (4 mg/kg, i.p) in the conditioned place preference (CPP) protocol for 8 days. After the CPP test, the animals received CBD treatment (5 or 10 mg/kg, i.p) during the 5 days of drug abstinence. Subsequently, the animals were exposed again to AMPH (reconditioning) for 3 days and the relapse test was performed. In experimental protocol 2 (EP2), the aim was to investigate the effects of CBD treatment on behavioral parameters of relapse to AMPH induced by a stressor stimulus. For this, the animals were exposed to AMPH (4 mg/kg, i.p) through the CPP protocol for 8 days and sequentially treated with CBD (10 mg/kg, i.p) during the 5 days of drug abstinence. Relapse drug-seeking behavior was induced by a forced swimming protocol prior to relapse testing. In both protocols, the animals were also submitted to behavioral assessments in the open field test and elevated plus maze to analyze locomotor activity and anxiety behaviors, respectively. Furthermore, in EP1, the prefrontal cortex (PFC) and ventral striatum (EV) were collected for the analysis of dopaminergic targets through western blot, while in EP2, the regions chosen were the ventral tegmental area (VTA) and the EV for the analysis of dopaminergic and endocannabinoid targets. Our results showed that, in both experimental protocols, CBD prevented AMPH relapse and decreased anxiety behavior per se, also observed in animals exposed to AMPH. In EP1, CBD restored the levels of dopaminergic targets (D1R, D2R, DAT and TH), altered by AMPH exposure in both brain regions. This same effect of CBD on D1R, D2R and DAT in VE was observed in EP2. Molecular analysis of EP2 also revealed that in VTA, CBD restored CB1R levels lowered by AMPH exposure, increased levels of the enzyme responsible for endocannabinoid synthesis, NAPE-PLD, and decreased levels of the enzyme that degrades them, the FAAH. These molecular findings allow us to hypothesize that the potential anti-relapse effect of CBD reflects its ability to increase endocannabinoid tone and thus restore the dopaminergic system compromised by AMPH exposure. Although further studies are needed, CBD appears to be a promising pharmacological alternative for the treatment of addiction to psychostimulant drugs such as AMPH.

Keywords: Addiction; Psychostimulants; Cannabinoids; Dopaminergic system; Endocannabinoid system.

LISTA DE ILUSTRAÇÕES

REFERENCIAL TEÓRICO

Figura 1 - Estrutura molecular da anfetamina	22
Figura 2 - Mecanismos de ação da anfetamina.....	23
Figura 3 - Vias dopaminérgicas cerebrais	24
Figura 4 – Neurotransmissão dopaminérgica.....	25
Figura 5 – Famílias de receptores de dopamina	26
Figura 6 – Biossíntese e inativação dos endocanabinoides	27
Figura 7 - Estrutura molecular do canabidiol	30
Figura 8 – Alguns dos principais alvos moleculares do canabidiol.....	31

MANUSCRITO CIENTÍFICO I

Figure 1 - Experimental design	73
Figure 2 - Drug preference behavior induced by AMPH observed in CPP paradigm before 5 days of CBD treatment, the extinction period after the last AMPH administration, and drug preference behavior after stress-induced reinstatement of AMPH- CPP..	74
Figure 3 - CBD treatment influence on the locomotor activity in the open field (OF) test after Stress-induced reinstatement of AMPM-CPP.....	75
Figure 4 - CBD treatment influence on anxiety-like behaviors in the elevated plus maze (EPM) test after stress-induced AMPH-CPP.....	76
Figure 5 - CBD treatment influence on dopamine receptor type 1 (D1) levels, dopamine receptor type 2 (D2) levels and on dopamine transporter (DAT) levels in the ventral striatum (VS) after stress-induced reinstatement of AMPH-CPP	77
Figure 6 - CBD treatment influence on on cannabinoid receptor type 1 (CB1) levels, on the enzyme diacylglycerol lipase α (DAGL) levels, on the enzyme monoacylglycerol lipase (MAGL) levels, on the enzyme N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) levels and the enzyme fatty acid amide hydrolase (FAAH) levels) in ventral tegmental area (ATV) after stress-induced reinstatement of AMPH-CPP	78
Figure 7 - Proposed model for CBD influence on endocannabinoid modulation of DA release.....	79

MATERIAL SUPLEMENTAR

Figura 1 – Desenho experimental	109
Figura 2 - Avaliação comportamental de preferência condicionada de lugar induzida por cocaína em ratos SERT +/+ e SERT -/- tratados com reserpina.....	110

LISTA DE ABREVIATURAS E SIGLAS

2-AG	2-araquidonoilglicerol
5-HT	5-hidroxitriptamina (serotonina)
5-HT1A	Receptor de serotonina subtipo 1A
AEA	N-araquidonoiletanolamina
ANF	Anfetamina
ATV	Área tegmental ventral
CBD	Canabidiol
CB1R	Receptor canabinoide tipo 1
CB2R	Receptor canabinoide tipo 2
COMT	Catecol-O-metil-transferase
CPF	Córtex pré-frontal
D1R	Receptor dopaminérgico do tipo D1
D2R	Receptor dopaminérgico do tipo D2
D3R	Receptor dopaminérgico do tipo D3
D4R	Receptor dopaminérgico do tipo D4
D5R	Receptor dopaminérgico do tipo D5
DA	Dopamina
DAGL α	Diacilglicerol lipase alfa
DAT	Transportador de dopamina
eCBs	Endocanabinoides
FAAH	Amida hidrolase dos ácidos graxos
MAO	Monoamina oxidase
MAGL	Monoacilglicerol lipase
MDMA	3,4 - metilenodioximetanfetamina
MOR	Receptor μ -opioide
NA	Noradrenalina
NAc	<i>Nucleus Accumbens</i>
NAPE-PLD	N-acilfosfatidiletanolamina fosfolipase D
NET	Transportador de noradrenalina
PCL	Preferência condicionada de lugar
PKA	Proteína quinase A
SE	Sistema endocanabinoide
SERT	Transportador de serotonina
SNC	Sistema nervoso central
TDAH	Transtorno de déficit de atenção e hiperatividade
TH	Tirosina hidroxilase
TRPV1	Receptor de potencial transitório vaniloide 1
VMAT-2	Transportador vesicular de monoaminas 2

SUMÁRIO

APRESENTAÇÃO	13
1 INTRODUÇÃO	15
2 REFERENCIAL TEÓRICO	19
2.1 ADIÇÃO	19
2.2 RECAÍDA	20
2.3 DROGAS PSICOESTIMULANTES	21
2.4 ANFETAMINA E SEUS EFEITOS SOBRE O SISTEMA NERVOSO CENTRAL	21
2.5 VIA MESOLÍMBICA DOPAMINÉRGICA	24
2.6 O SISTEMA ENDOCANABINOIDE	27
2.7 O PAPEL DOS ENDOCANABINOIDES NA ADIÇÃO	28
2.8 CANABIDIOL: POSSÍVEL ESTRATÉGIA PARA O TRATAMENTO DA ADIÇÃO	30
2.9 MODELO ANIMAL DE DEPENDÊNCIA DE DROGAS	32
3 JUSTIFICATIVA	35
4 OBJETIVOS	37
4.1 OBJETIVO GERAL	37
4.2 OBJETIVOS ESPECÍFICOS	37
5 PRODUÇÃO CIENTÍFICA	39
5.1 ARTIGO CIENTÍFICO	41
5.2 MANUSCRITO CIENTÍFICO	55
6 DISCUSSÃO	89
7 CONCLUSÃO	93
8 MATERIAL SUPLEMENTAR	95
REFERÊNCIAS BIBLIOGRÁFICAS	117

APRESENTAÇÃO

Esta tese está estruturada em seções dispostas da seguinte forma: Introdução, Referencial Teórico, Justificativa, Objetivos, Produção Científica, Discussão, Conclusão, Perspectivas e Referências. Nos itens **INTRODUÇÃO** e **REFERENCIAL TEÓRICO** encontram-se considerações iniciais sobre o tema desenvolvido nesta tese. Os itens **JUSTIFICATIVA** e **OBJETIVOS** dispõem sobre a fundamentação e os objetivos que nortearam este estudo, respectivamente. O item **PRODUÇÃO CIENTÍFICA** é composto por um artigo científico e um manuscrito científico, os quais representam a íntegra dessa tese e contemplam os itens **MATERIAIS E MÉTODOS, RESULTADOS, DISCUSSÃO E REFERÊNCIAS**, sob a formatação das revistas em que se encontram publicados. Ao fim encontram-se os itens **DISCUSSÃO** e **CONCLUSÃO** nos quais há interpretações e comentários gerais dos artigos contidos neste estudo. O item **MATERIAL SUPLEMENTAR** é composto por um resumo expandido relacionado a estudos de doutorado sanduíche no exterior. O item **REFERÊNCIAS** abrange somente às citações apresentadas nos itens **INTRODUÇÃO, REFERENCIAL TEÓRICO** e **DISCUSSÃO**.

1 INTRODUÇÃO

A adição pode ser definida como a perda de controle sobre o uso de drogas ou a busca compulsiva dessas substâncias, apesar das consequências prejudiciais decorrentes (NESTLER, 2001). Doenças, morte, baixa produtividade e crime estão todos associados à dependência de drogas, que, além disso, possui um alto impacto emocional e social (GOLDSTEIN; VOLKOW, 2002). Sendo assim, a adição tem sido objeto de intensa pesquisa científica, tendo em vista a elucidação dos mecanismos neurobiológicos envolvidos nesse distúrbio psiquiátrico e o desenvolvimento de novas alternativas de prevenção e tratamento. Atualmente existem terapias farmacológicas aprovadas para a adição a alguns tipos de drogas, contudo, estas não têm se mostrado suficientes para prevenir efetivamente as altas taxas de recaída após a abstinência (CADTH, 2014).

Psicoestimulantes como as anfetaminas são amplamente utilizados em todo o mundo com fins terapêuticos. No entanto, apresentam alto potencial de abuso dependendo do contexto do seu emprego (WESTOVER; NAOKONEZKY; HALEY, 2008). Alguns derivados anfetamínicos são comumente prescritos na clínica médica para o tratamento do transtorno de déficit de atenção e hiperatividade (TDAH), assim como também são úteis na farmacoterapia da obesidade mórbida e da narcolepsia (BERMAN et al., 2009; CLEGG-KRAYNOK; MCBEAN; MONTGOMERY-DOWNS, 2011). Além disso, também são frequentemente utilizados indevidamente para fins não médicos (JOHNSTON et al. 2017; SAMSHA, 2014). Outras drogas pertencentes a esta classe como o 3,4, metilendioximetanfetamina (MDMA), a metanfetamina e a *d*-anfetamina, são utilizados exclusivamente com caráter abusivo (WESTOVER; NAOKONEZKY; HALEY, 2008). As ANF e seus derivados, juntos, ocupam o segundo lugar como droga mais utilizada de forma abusiva mundialmente (UNODC, 2021). O uso destas substâncias de forma recreacional se deve aos seus efeitos agudos, que incluem aumento da atenção, da autoconfiança, do estado de alerta e de euforia (PHILLIPS; EPSTEIN; PRESTON, 2014). Apesar da alta prevalência de abuso de ANF e de todo o impacto negativo decorrente do uso dessas drogas sobre o indivíduo adicto e a sociedade em geral, não há nenhuma intervenção farmacológica aprovada para o tratamento da adição à ANF (ALTSHULER et al., 2019).

A via dopaminérgica mesolímbica, também conhecida como via da recompensa ou centro do prazer hedônico no cérebro, tem sido evidenciada como o principal alvo das drogas de abuso (KATZUNG et al., 2012; STAHL, 2014). Essa via tem origem na área tegmental ventral, que faz projeções para o *nucleus accumbens*, amígdala, hipocampo e córtex pré-frontal

(FELTENSTEIN; SEE, 2008). De um modo geral, as drogas aditivas agem ativando essa via e desencadeiam diversas consequências neurobiológicas (GOLDSTEIN; VOLKOW, 2002). Já foi demonstrado que o uso crônico de drogas de abuso pode desencadear neuroadaptações a níveis moleculares e celulares. Presume-se que essas mudanças corroboram para o comportamento compulsivo, assim como para a ocorrência de recaídas (CHOU; NARASIMNHAN, 2005).

Embora o sistema dopaminérgico tenha sido considerado o sistema mais importante envolvido nos processos de recompensa do cérebro por décadas (LIU E LI, 2018), evidências recentes sugerem que o sistema endocanabinoide (SE) também tem um papel fundamental na sinalização de eventos recompensadores (MANZANARES et al., 2018; OLIÈRE et al., 2013). Endocanabinóides (eCBs) e receptores de canabinóides são amplamente expressos em áreas cerebrais do sistema mesocorticolímbico e acredita-se que eles podem modular a sinalização dopaminérgica nesta via (EVERETT et al., 2021; LUPICA et al., 2004; MALDONADO E RODRIGUEZ DE FONSECA, 2002; MELIS et al., 2004). Os eCBs também parecem participar da plasticidade sináptica do sistema mesocorticolímbico, necessária para o desenvolvimento das mudanças adaptativas, levando à adição (GERDEMAN et al., 2002; ROBBE et al., 2002). Além disso, foram encontradas neuroadaptações no sistema endocanabinoide após exposição crônica a drogas (PARSONS E HURD, 2015) e a modulação desse sistema tem sido apontada como um alvo potencial para o desenvolvimento de estratégias para o tratamento da adição.

O canabidiol (CBD), um canabinoide exógeno, é o principal composto não psicotomimético da planta *Cannabis sativa* e já demonstrou propriedades ansiolíticas, antipsicóticas, antidepressivas e neuroprotetoras (DOS SANTOS et al., 2017). Os mecanismos moleculares envolvidos nos efeitos induzidos pelo CBD possivelmente se devem à ação desse composto no SE (CAMPOS et al., 2017). Além disso, o CBD parece exercer ações em outros sistemas (ZANELATTI et al., 2010) e tem sido associado à modulação de circuitos neurais envolvidos na adição e comportamentos de busca de drogas (OLIÈRE et al., 2013). Recentemente, o CBD tem sido apontado como uma possível ferramenta farmacológica para o tratamento da adição (CHYE et al., 2019). Alguns estudos pré-clínicos já demonstraram resultados favoráveis e promissores no tratamento da adição por opioides e também por cocaína (DE CARVALHO; TAKAHASHI, 2017; KATSIDONI et al., 2013, REN et al., 2009). Embora a eficácia do tratamento com CBD na adição por ANF vinha sendo pouco explorada (HAY et al., 2018; PARKER et al., 2004), um crescimento no interesse da pesquisa científica nesse sentido tem sido percebido através de um aumento significativo no número de estudos publicados relacionados à essa temática nos dois últimos anos (ANOOSHE et al., 2021; METZ

et al., 2021; NOURI et al., 2021; RAZAVI et al., 2020; SHARIFI et al., 2022; YANG et al., 2020). Esse fato reitera a importância de investigar detalhadamente o potencial terapêutico e os possíveis mecanismos implicados em um tratamento experimental com CBD na prevenção de recaídas por ANF, visto que o mesmo pode surgir como uma nova alternativa de tratamento para esse relevante problema de saúde pública.

2 REFERENCIAL TEÓRICO

2.1 A ADIÇÃO

A adição é um distúrbio neuropsiquiátrico caracterizado pela compulsão e perda do controle sobre o uso de drogas, apesar das graves consequências negativas (GOLDSTEIN; VOLKOW, 2002; NESTLER, 2001). Com causas e consequências fisiológicas, psicológicas e sociais (NESTLER; AGHAJANIAN, 1997), a adição ocasiona quadros de ansiedade, irritabilidade e disforia em situações em que o uso da substância é privado (KOOB; LE MOAL, 2008).

Segundo o último Relatório Mundial sobre Drogas, estima-se que em 2019 cerca de 5,5% da população mundial adulta, isto é, 275 milhões de pessoas fizeram uso de drogas. Além disso, é alarmante o fato de que destes indivíduos, aproximadamente 36 milhões desenvolveram transtornos relacionados ao uso destas substâncias (UNODC, 2021). Vista, atualmente, como um grave problema de saúde pública, a adição despende uma expressiva quantia em gastos públicos, pois envolve altos custos médico-hospitalares e elevados custos sociais relacionados à criminalidade (MC LELLAN, 2017; UNODC; 2021).

Sabe-se que a exposição crônica a drogas de abuso acarreta mudanças significativas a nível molecular e celular no cérebro humano, e presume-se que essas mudanças podem estar subjacentes às anormalidades comportamentais características apresentadas por indivíduos dependentes (NESTLER, 2001). Contudo, os mecanismos neurobiológicos envolvidos nesses processos de neuroadaptações que ocorrem durante a adição não são completamente conhecidos.

Considerada uma doença pela Organização Mundial da Saúde (CID-10: F19), a adição carece de tratamento, porém, seja ele psicológico e/ou farmacológico, está frequentemente atrelado à ocorrência de recaídas (MAJEWSKA, 1996; PAU; LEE; CHAN, 2002). Embora haja um aumento da pesquisa e dos avanços científicos neste segmento, as alternativas farmacológicas desenvolvidas até o momento se restringem a apenas alguns tipos de drogas (VALLOF et al., 2016). Por exemplo, o tratamento farmacológico recomendado para o transtorno por uso de drogas opioides inclui a precipitação da síndrome de abstinência com o antagonista opioide naloxona (LARNEY et al., 2014) e o tratamento de manutenção com agonistas opioides como a buprenorfina e a metadona (BONHOMME et al., 2012; JIANG et al., 2016). A adição por nicotina é tratada através da terapia de reposição da mesma (CADTH, 2014) ou de fármacos que atuam como agonistas dos receptores nicotínicos como a bupropiona

e a varenciclina (EBBERT et al. 2015). Por sua vez, o acamprosato, dissulfiram e a naltrexona são medicamentos aprovados para a dependência alcoólica (NIDA, 2020). É importante salientar que os medicamentos atualmente disponíveis não previnem eficazmente a alta taxa de recaída após a abstinência (CADETH, 2014). Além disso, estes podem produzir efeitos colaterais graves, como a overdose por opioides no tratamento de manutenção com agonistas opioides (BONHOMME et al., 2012). Dessa forma, o desenvolvimento de novas ferramentas para prevenção e tratamento da adição, e especialmente da recaída, são imprescindíveis, considerando os impactos ocasionados por esse fenômeno para o indivíduo e para a sociedade.

2.2 RECAÍDA

É sabido que o uso crônico de drogas pode desencadear alterações citoplasmáticas e em membranas celulares, impactando sobre receptores, transportadores e enzimas (KOOB; SANNA; BLOOM, 1998). Além disso, é também capaz de afetar importantes fatores de transcrição levando a modificações da expressão gênica de proteínas implicadas na transdução de sinal e/ou neurotransmissão. Sendo assim, a exposição recorrente a estas substâncias é capaz de resultar em modificações no funcionamento celular cerebral e em respostas neuronais alteradas (CARLEZON; DUMAN; NESTLER, 2005). Acredita-se que estas neuroadaptações celulares e moleculares persistem mesmo após um longo intervalo de tempo depois da interrupção do uso da droga e corroboram para o comportamento compulsivo, assim como para a ocorrência de recaídas (CHOU; NARASIMNHAN, 2005).

Durante o tratamento da adição, a recaída ao uso de drogas é sem dúvidas o fator clínico mais difícil de ser controlado (KAPLAN; OUDEYER, 2007). Mesmo após um longo período de abstinência e/ou tratamento, a recaída pode ser desencadeada pela exposição à droga em si, através de pistas ambientais associadas à droga ou pelo estresse (SHAHAN; HOPE, 2005). Em virtude à sua complexidade, as estratégias de tratamento precisam abranger vários tipos de intervenções, tendo em vista os elementos biológicos, psicológicos e sociais implicados (KAPLAN E OUDEYER, 2007). A farmacoterapia tem um papel fundamental no tratamento da adição em suas diversas fases, incluindo a prevenção da recaída. Os medicamentos são utilizados principalmente como adjuvantes de tratamentos psicossociais e a escolha dos fármacos empregados nessas circunstâncias depende do tipo específico de droga à qual o indivíduo é adicto (DOUAIHY, 2013; KNEVITZ E BUCCINI, 2018). Contudo, os benefícios dos fármacos atualmente disponíveis para o tratamento da adição a alguns tipos de drogas ainda

são bastante limitados e no que diz respeito à adição a substâncias, como por exemplo os psicoestimulantes, não há farmacoterapia aprovada (KNEVITZ E BUCCINI, 2018).

2.3 DROGAS PSICOESTIMULANTES

Substâncias psicoestimulantes são extensamente utilizadas no mundo todo (UNODC, 2021) e o seu uso se deve aos efeitos proporcionados. O aumento do estado de vigília e a diminuição da sensação de fadiga são alguns de seus principais efeitos quando administrados agudamente ou em doses baixas a moderadas. Ainda, alguns psicoestimulantes parecem provocar uma melhora na cognição a curto prazo nestas circunstâncias. Quando utilizados por longos períodos ou em doses elevadas, provocam sintomas que variam desde aumento da euforia e excitação, até a ocorrência de episódios psicóticos, com delírios e alucinações (BOUTREL; KOOB, 2004; KUMAR, 2008; QU et al. 2008; RANG et al., 2016; VOLKOW et al., 2009). No geral, ainda outros sintomas físicos podem surgir após o uso destas substâncias, como taquicardia, hipertensão, inquietação, tremores e inibição da motilidade gastrointestinal. Em casos mais graves, o uso de psicoestimulantes pode ocasionar insuficiência cardíaca, convulsões e acidente vascular encefálico (CARVALHO et al., 2012; STAHL, 2014). A anfetamina (ANF) e a cocaína são exemplos deste tipo de substâncias, as quais possuem alto potencial aditivo (STAHL, 2014).

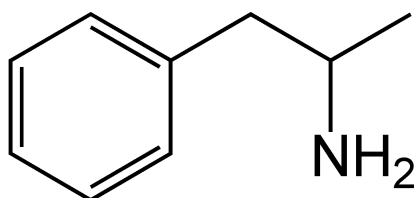
2.4 ANFETAMINA E SEUS EFEITOS SOBRE O SISTEMA NERVOSO CENTRAL

A ANF é uma amina simpaticomimética que desempenha vigorosas ações estimulantes sobre o sistema nervoso central (SNC) (HILAL-DANDA; BRUNTON, 2015). Compreende um anel benzênico com uma cadeia lateral de hidrocarbonetos, além de um grupamento amino possuindo um grupamento alfa-metil em sua estrutura química (Figura 1). Esta estrutura básica é também compartilhada por outros psicoestimulantes do tipo anfetamínicos.

Dependendo do contexto de seu emprego, ou seja, do propósito com o qual são utilizadas, as anfetaminas compõem uma entidade terapêutica, porém também são substâncias passíveis de abuso (LILE; ROSS; NADER, 2005; WESTOVER; NAKONEZKY; HALEY, 2008). Na clínica médica, são utilizadas no tratamento da narcolepsia ou até mesmo como supressores do apetite (anfepramona e femproporex). Além disso, derivados anfetamínicos como o metilfenidato e a lisdexanfetamina são empregados no tratamento do Transtorno do Déficit de Atenção e Hiperatividade (TDAH) (BERMAN et al. 2009; CLEGG-KRAYNOK;

MC BEAN; MONTGOMERY-DOWNS, 2011). Apesar de serem substâncias prescritas, essas ANF também possuem um amplo histórico de abuso (UNOCD, 2021). Já as anfetaminas como a metanfetamina, a d-anfetamina e o 3,4-metilenodioximetanfetamina (MDMA), popularmente conhecida como êcstasy, são utilizados apenas com propósito recreacional e de abuso (HOWARD et al. 2010; TIEGES et al. 2009), sendo que a metanfetamina e o êcstasy são substâncias proibidas, comercializados apenas de forma ilegal. Estima-se que em 2019, 27 milhões de pessoas no mundo tenham feito o uso de ANF e seus derivados de forma abusiva. Além disso, 20 milhões de pessoas fizeram o uso especificamente de êcstasy (UNODC, 2021).

Figura 1 - Estrutura molecular da anfetamina

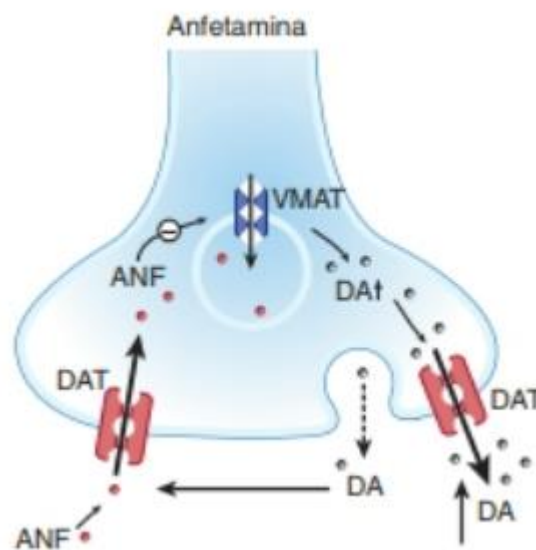


Fonte: (SITTE; FRESSMUTH, 2015).

Após ser absorvida rapidamente pelo trato gastrointestinal ou através da mucosa nasal, a ANF atravessa a barreira hematoencefálica e exerce seus poderosos efeitos sobre o SNC (RANG et al. 2016). A ANF atua na liberação de monoaminas, especialmente dopamina e noradrenalina, junto às terminações nervosas cerebrais (GREEN et al., 2003). Isso acontece de várias formas. A ANF é um pseudosubstrato para os transportadores de dopamina (DAT) e transportadores de noradrenalina (NET), e assim, atua como um inibidor competitivo destes transportadores. Ou seja, ela se liga ao mesmo sítio onde as monoaminas se conectam com o transportador e em virtude disso ocorre uma inibição da recaptção de dopamina (DA) e noradrenalina (NA). (KUCZENSKI et al. 1995; RANG et al., 2016; STAHL, 2014; SULZER, 2011). Além disso, a ANF pode entrar no terminal dopaminérgico pré-sináptico via processos de captura ou difusão e interagir com o transportador vesicular de monoaminas 2 (VMAT-2) de modo a inibir a estocagem de DA e NA citoplasmáticas nas vesículas de armazenamento pré-sinápticas. Não obstante, a ANF é transportada para dentro das vesículas de armazenamento pelo VMAT-2 e mobiliza a liberação das monoaminas endógenas estocadas nas vesículas para o citoplasma (RANG et al., 2016). Em concentrações elevadas, a ANF pode ainda inibir a

monoamino-oxidase (MAO), enzima cuja função é degradar as monoaminas citoplasmáticas. Essa redução da degradação das monoaminas, especialmente de DA, aumenta ainda mais as suas concentrações citoplasmáticas (CAO et al. 2016; STAHL, 2014; SULZER, 2011). Essa DA citoplasmática pode sofrer auto-oxidação (HERMIDA-AMEIJEIRAS, 2004), além de também ser capaz de inverter a direção do DAT após sua ligação a esse transportador, liberando DA na sinapse (CAO et al., 2016) (Figura 2).

Figura 2 – Mecanismo de ação da anfetamina



Fonte: (KATSUNG e TREVOR, 2015).

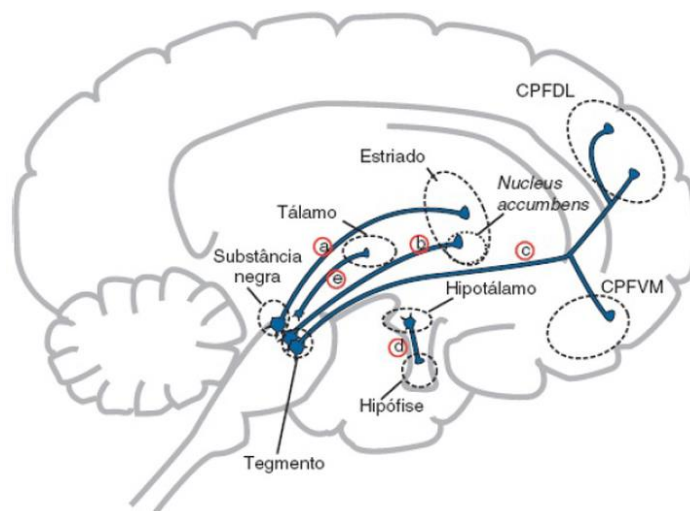
Neurônios dopaminérgicos são encontrados em diversas áreas cerebrais, sendo assim, o aumento dos níveis de monoaminas causado pela ANF pode acarretar diversas consequências (CAO et al., 2016; WISE, 2004). O uso desta substância parece provocar neuroadaptações no sistema mesolímbico-mesocortical, o qual está intimamente relacionado a mecanismos de recompensa, favorecendo assim a adição (HOOKS et al., 1993; ROBINSON; BERRIDGE, 1993).

Entre as neuroadaptações estão modificações da expressão e da sensibilidade de receptores dopaminérgicos (WHITE E WANG, 1984; ACKERMAN E WHITE, 1990; GAO et al., 1998), alterações nos níveis de proteína G (NESTLER et al., 1990; STRIPLIN E KALIVAS, 1997), níveis basais extracelulares de DA aumentados (KALIVAS E DUFFY, 1998), modificações na expressão de tirosina hidroxilase (TH) (SORG et al., 1997), dentre outras.

2.5 VIA MESOLÍMBICA DOPAMINÉRGICA

Drogas psicoativas que acarretam dependência são conhecidas por ativarem a via mesolímbica dopaminérgica, também conhecida como via de recompensa (RANG et al. 2016). Estas substâncias promovem uma liberação de dopamina (DA) de uma forma mais explosiva e prazerosa do que a liberação ocorrida naturalmente no estriado ventral ou *nucleus accumbens* (NAc), região denominada como “centro da recompensa ou centro do prazer hedônico” (STAHL, 2014). A via mesolímbica se origina na área tegmental ventral (ATV), localizada na extremidade do tronco cerebral, e se projeta até o NAc, amígdala, hipocampo e córtex pré-frontal (CPF) (Figura 3) (FELTSTEIN; SEE, 2008).

Figura 3 - Vias dopaminérgicas cerebrais: (a) Via nigroestriatal, (b) Via mesolímbica, (c) Via mesocortical, (d) Via tuberoinfundibular e (e) Via de múltiplos locais, ainda com função não elucidada.

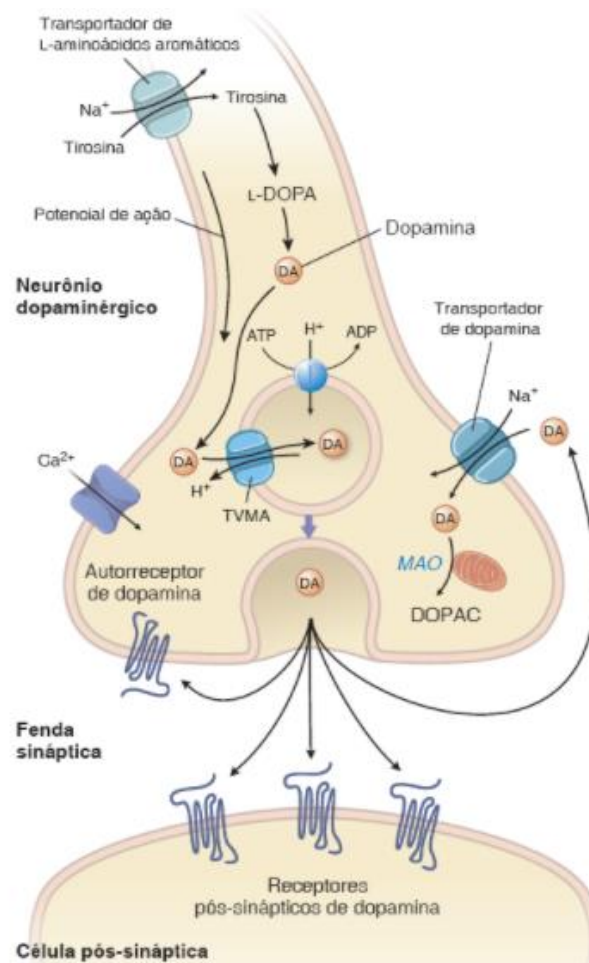


Fonte: (STHAL, 2014).

A DA é um neurotransmissor fundamental na adição (BAIK, 2013). Ela é sintetizada a partir do aminoácido tirosina, o qual, uma vez conduzido para dentro do terminal dopaminérgico através do transportador de tirosina é convertido à DA, através da ação de várias enzimas. A mais importante e também a enzima limitante na síntese de DA é a tirosina hidroxilase (TH). Uma vez sintetizada, a DA é estocada em vesículas pelo transportador VMAT-2, até que seja liberada na sinapse durante a neurotransmissão. Através do transportador

de dopamina (DAT), a DA presente na fenda sináptica pode ser transportada novamente para dentro dos neurônios pré-sinápticos, onde será armazenada em vesículas para futuro uso ou ainda poderá sofrer degradação pela enzima monoaminoxidase (MAO). No meio extracelular, a DA pode ser metabolizada através da enzima catecol-O-metil-transferase (COMT) (RANG et al. 2016) (Figura 4).

Figura 4 - Neurotransmissão dopaminérgica.



Fonte: (STANDAERT e GALANTER, 2008).

Os receptores pré-sinápticos dopaminérgicos do tipo D2 (D2R) são os responsáveis pelo feedback negativo nos terminais pré-sinápticos. Todavia, os receptores pós-sinápticos dopaminérgicos do tipo D1 (D1R), D2R, D3 (D3R), D4 (D4R) e D5 (D5R) regulam a neurotransmissão cada qual com distintas ações. Esses receptores são transmembrana e

acoplados a proteína G, sendo expressos em diferentes quantidades em diversas áreas cerebrais. Os D1R e D5R se ligam a Gs, promovem a estimulação da adenilciclase e ativam a proteína quinase A (PKA). Os demais receptores, D2R, D3R e D4R são acoplados à proteína Gi, sendo assim, ativam canais de potássio, além de inibir os canais de cálcio e a adenilciclase (RANG et al. 2016) (Figura 5).

Figura 5 – Famílias de Receptores de Dopamina: Os cinco subtipos de receptores dopaminérgicos (D1R a D5R) podem ser classificados em duas grandes famílias de receptores.

	Familia do receptor D1		Familia do receptor D2		
Estrutura esquemática					
Sistemas de segundos mensageiros	<ul style="list-style-type: none"> ↑ AMPc (via G_s) ↑ Hidrólise do PIP₂ Mobilização do Ca²⁺ (via IP₃) Ativação da PKC 		<ul style="list-style-type: none"> ↓ AMPc (via G_i) ↑ Correntes de K⁺ ↓ Correntes de Ca²⁺ reguladas por voltagem 		
Distribuição no SNC	D1	D5	D2	D3	D4
	Estriado Neocórtex	Hipocampo Hipotálamo	Estriado Substância negra Hipófise	Tubérculo olfatório <i>Nucleus accumbens</i> Hipotálamo	Córtex frontal Bulbo Mesencéfalo

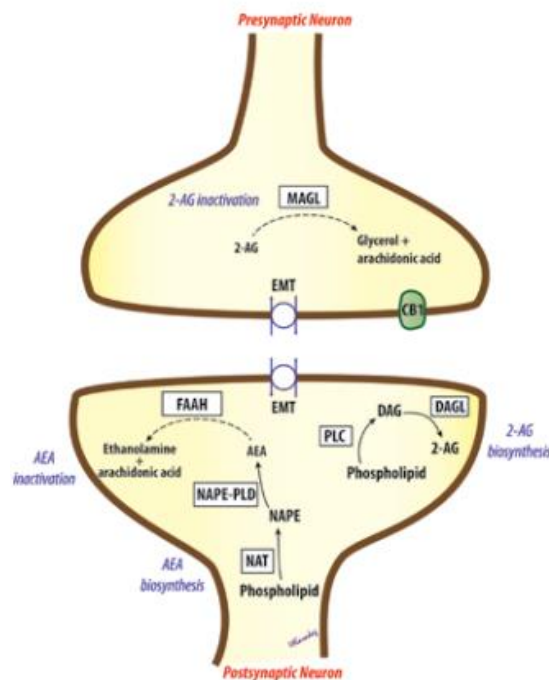
Fonte: (GOLAN, 2014).

A exposição crônica as drogas anfetamínicas é capaz de causar um efeito molecular duradouro e adaptações celulares no sistema dopaminérgico, o que pode contribuir para o uso compulsivo e descontrolado. Os níveis de DA na região do NAc geralmente encontram-se diminuídos durante o período de abstinência de drogas (LINGFORD-HUGHES E NUTT, 2003). Esse estado hipodopaminérgico colabora para o surgimento de alguns sintomas de retirada (ansiedade, comportamento depressivo) sempre observados em indivíduos adictos a psicoestimulantes em período de abstinência, e também na manutenção de comportamentos de uso de drogas (OLIÈRE et al., 2013). Dessa forma, o sistema dopaminérgico é um alvo importante e uma estratégia para o desenvolvimento de tratamentos para a adição (CAO et al. 2016).

2.6 O SISTEMA ENDOCANABINOIDE

O sistema endocanabinoide (SE) é um sistema de sinalização composto por enzimas que sintetizam seus ligantes endógenos – também conhecidos como endocanabinoides (eCBs), receptores que respondem aos eCBs desencadeando cascatas de sinalização intracelular, além de transportadores e enzimas que promovem a síntese e a degradação destes. Dois eCBs, a N-araquidonoiletanolamida (AEA, também conhecida como anandamida) e o 2-araquidonoilglicerol (2-AG) são os mais estudados, entretanto, outros eCBs foram identificados mais recentemente. A AEA e o 2-AG são estruturalmente semelhantes, porém sintetizados e degradados por vias distintas (VOLKOW; HAMPSON; BALER, 2017). Contrariamente a outros neurotransmissores, os eCBs não são sintetizados e armazenados na célula nervosa. Eles são produzidos “sob demanda” a partir de seus precursores, os lipídios de membrana, de maneira dependente de Ca^{2+} (WILSON; KUNOS; NICOLL, 2001; WILSON; E NICOLL, 2001). Foi proposto que a enzima N-acilfosfatidiletanolamina fosfolipase D (NAPE-PDL) possui um papel fundamental na síntese da AEA (OKAMOTO et al., 2007) e que a enzima responsável pela síntese de 2-AG é a diacilglicerol lipase alfa ($\text{DAGL}\alpha$) (TANIMURA et al, 2010) (Figura 6).

Figura 6 – Biossíntese e inativação dos endocanabinoides.



Fonte: (ULUGÖL, 2014).

Após a despolarização dos neurônios pós-sinápticos, os eCBs são liberados na fenda sináptica e se ligam e ativam os receptores canabinoides, que, por sua vez, suprimem a liberação de diferentes neurotransmissores excitatórios e inibitórios (FRIDE, 2005) (Figura 6). Designados tipo 1 (CB1R) e tipo 2 (CB2R), os receptores do SE são acoplados à proteína Gi e possuem diferentes distribuições entre os tecidos. O CB1R é o mais expresso no SNC (PERTWEE et al., 2010; BURNS et al., 2007), sendo encontrado em maior quantidade em regiões como o hipocampo, estriado, córtex e amígdala e em níveis intermediários no cerebelo (ALLEN, 2016; CECCARINI, 2015). Por outro lado, o CB2R tem uma expressão geralmente baixa a nível de SNC, onde é restrito principalmente a microglia, mas é altamente expresso em células e tecidos imunes periféricos (GALIÈGUE et al., 1995).

Após a sua síntese e subsequente liberação, a AEA e o 2-AG são recaptados para dentro dos neurônios novamente onde são metabolizados por enzimas específicas (DURSTEWITZ; SEAMANS, 2008). A AEA pode ser metabolizada pela ação da amida hidrolase dos ácidos graxos (FAAH) (KELLET et al., 2016) e o 2-AG pela monoacilglicerol lipase (MAGL) (VAN DER MEER; VELTHORST, 2015) (Figura 6). De um modo geral, o SE parece estar envolvido na modulação de diversos processos neurobiológicos (VOLKOW; HAMPSON; BALER, 2017). Curiosamente, há um aumento de evidências que apontam a implicação deste sistema no sistema de recompensa e nos processos de adição (OLIÈRE et al., 2013).

2.7 O PAPEL DOS ENDOCANABINOIDES NA ADIÇÃO

Um número crescente de transtornos psiquiátricos, incluindo transtornos por uso de substâncias (GHOSH; BASU, 2015), têm sido relacionados em vários aspectos à desregulação do SE, uma vez que este encontra-se onipresente e desempenha um papel complexo na função cerebral. Estudos prévios observaram neuroadaptações no SE decorrentes da exposição crônica a drogas e têm sugerido que reduções na sinalização desse sistema também podem contribuir para a maior sensibilidade ao estresse e estados negativos de humor na adição (SIDHPURA; PARSONS, 2011). Na verdade, o SE parece ter um papel essencial na regulação de vários processos cognitivos e psicológicos associados à adição como a recompensa, responsividade ao estresse e a plasticidade sináptica relacionada ao uso de drogas (GARDNER, 2005; HEIFETS; CASTILLO, 2009; LUTZ, 2009).

Os eCBs regulam a busca de recompensa por modular a sinalização dopaminérgica na via mesocorticolímbica (WANG; LUPICA, 2014). Apesar do papel dos eCBs ainda permanecer controverso, a maioria dos estudos realizados até o momento indica que esses desencadeiam

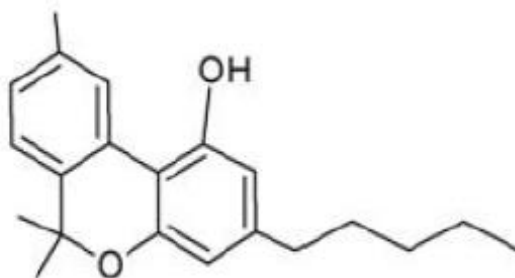
um aumento na liberação de DA após se ligarem ao CB1R (EVERETT et al., 2021; OLESON et al., 2012; SOLINAS et al., 2006). Possíveis mecanismos foram propostos na tentativa de explicar como a ativação do CB1R desencadeia o aumento do disparo e liberação neuronal de DA. São eles: (i) ação estimulatória sobre os neurônios dopaminérgicos; (ii) redução na atividade inibitória e consequente aumento da atividade excitatória (SZABO; SIEMES, 2002). Contudo, acredita-se que a modulação direta de eCBs em neurônios dopaminérgicos via CB1R seja bastante improvável, uma vez que, até onde é sabido, o CB1R não é expresso em neurônios dopaminérgicos (JULIAN et al., 2003). Por outro lado, ganhou força a proposta de atuação por meio de um mecanismo de desinibição. Expressos em neurônios GABAérgicos na ATV, quando os CB1R são ativados, eles supostamente inibem a transmissão GABAérgica nesta região do cérebro, levando a uma diminuição da interrupção inibitória dos neurônios dopaminérgicos (LUPICA; RIEGEL, 2005; SZABO; SIEMES, 2002). Tudo isso culminaria em um aumento na taxa de disparo neuronal e em um aumento na liberação de DA no *nucleus accumbens* (NAc) (MELIS; PISTIS, 2012).

Dessa forma, inicialmente pensava-se que a interrupção da sinalização endocanabinoide poderia contribuir para a diminuição da liberação de DA e que isso poderia impactar positivamente o vício. De fato, alguns estudos mostraram que os antagonistas do CB1R interrompem a sinalização endocanabinoide e diminuem a liberação de DA, que é evocada por drogas aditivas em ratos (CHEER et al., 2004; OLESON et al., 2012). No entanto, devido aos altos riscos clínicos previamente demonstrados por antagonistas do CB1R como o rimonabanto (HILL; GORZALKA, 2004), essa é uma linha de pesquisa que requer grande cautela. Alternativamente, alguns grupos de pesquisa têm investigado a hipótese de que um aumento farmacológico nos níveis de eCBs pode ser um método relativamente seguro de terapia de reposição, especialmente durante a retirada do medicamento. Conforme mencionado anteriormente, acredita-se que nesta fase o aparecimento de sintomas negativos é acompanhado por uma diminuição dos níveis de DA. Assim, a modulação de eCBs poderia normalizar os níveis diminuídos de DA e prevenir efeitos negativos que contribuem para a recaída (MCCUTCHEON et al., 2012; OLESON; CHEER, 2013; ROITMAN et al., 2008). Considerando essa hipótese de que o aumento do tônus endocanabinoide pode produzir efeitos anti-abstinência, o uso de canabinoides exógenos pode vir a ser uma estratégia viável para o tratamento da adição.

2.8 CANABIDIOL: POSSÍVEL ESTRATÉGIA PARA O TRATAMENTO DA ADIÇÃO

O canabidiol (CBD) é um canabinoide exógeno e o principal composto não psicotomimético da planta *Cannabis sativa* (DOS SANTOS et al., 2017) (Figura 7). Nos últimos anos, diversos grupos de pesquisa têm investigado os efeitos do CBD em estudos básicos e clínicos. Os resultados obtidos sugerem que o CBD possui efeitos benéficos altamente promissores na terapêutica de distúrbios neurológicos como epilepsia (DEVINSKY et al., 2014; 2016), esclerose múltipla (GIACOPPO et al., 2015; KOZELA et al., 2011), Parkinson (CHAGAS et al., 2014; ZUARDI et al., 2009) e Alzheimer (CHENG et al., 2014; MARTIN-MORENO et al., 2011). Além disso, há fortes evidências que o CBD melhora a cognição (OSBORNE; SOLOWIJ; WESTON-GREEN, 2017) e a neurogênese (LIPUT et al., 2013; SCHIAVON et al., 2016), além de apresentar efeitos ansiolíticos (BLESSING et al., 2015; MOREIRA; AGUIAR; GUIMARÃES, 2006), antidepressivo (SARTIM; GUIMARAES; JOCA, 2016; LINGE et al., 2016) e antipsicótico (LEWEKE et al., 2016; PERES et al., 2016), o que reforça o seu potencial como estratégia no tratamento de doenças neuropsiquiátricas.

Figura 7 – Estrutura molecular do canabidiol.



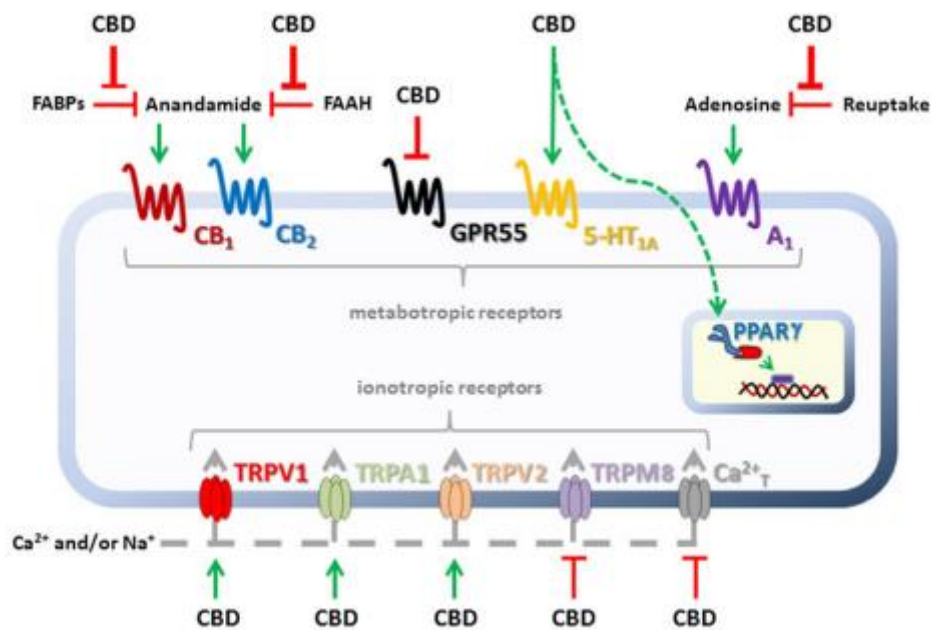
Fonte: (NETZAHUALCOYOTZI-PIETRA, 2009).

No Brasil, até o momento existem 6 medicamentos à base de CBD aprovados pela Agência Nacional de Vigilância Sanitária (ANVISA). Estes podem ser prescritos no caso de estarem esgotadas ou por serem ineficazes as opções terapêuticas disponíveis no mercado brasileiro para determinadas doenças (BRASIL, 2019).

Os mecanismos moleculares do CBD não estão completamente esclarecidos, mas parecem incluir mais de 65 alvos (CAMPOS et al., 2016; IBEAS BIH et al., 2015). Estudos recentes sugerem que o CBD age como um modulador alostérico negativo de CB1R e CB2R

(LAPRAIERI et al., 2015), além de inibir a FAAH e consequentemente aumentar o tônus endocanabinoide (CHAUVET et al., 2015). O CBD também pode agir como um antagonista do receptor GPR55 (RYBERG et al., 2007), agonista parcial do receptor de serotonina 5-HT_{1A} (RUSSO et al., 2005), agonista do receptor de potencial transitório vaniloide do tipo 1 (TRPV1) (BISOGLNO et al., 2001) e modulador alostérico positivo do receptor μ -opioide (MOR) (KATHMANN et al., 2006) (Figura 8). Além disso, o CBD exerce ações em outros sistemas (ZANELATTI et al., 2010) e tem sido associado a modulação de circuitos neurais envolvidos no estabelecimento da adição e em comportamentos de busca de drogas (OLIERE et al., 2013).

Figura 8 – Alguns dos principais alvos moleculares do canabidiol.



Fonte: (LEE et al., 2017)

Diversos pesquisadores têm investigado os efeitos do CBD na saúde física e mental, sendo que um número crescente tem se concentrado em elucidar a sua repercussão no tratamento da adição (PRUD'HOMME et al., 2015). Recentemente, o CBD foi proposto como uma estratégia de tratamento potencialmente eficaz no controle dos transtornos por uso de substâncias (CHYE et al., 2019). Estudos pré-clínicos sugerem que o CBD é capaz de reduzir o desejo e/ou a recaída por algumas substâncias como álcool (VIUDEZ-MARTINEZ et al.,

2018; 2020), opioides (MAHMUD et al., 2017; HURD et al., 2015) e psicoestimulantes (LUJÁN et al., 2018; PARKER et al., 2004). Os efeitos do tratamento com CBD na adição por ANF até então vinham sendo pouco explorados (HAY et al., 2018; PARKER et al., 2004). Especialmente nos últimos dois anos, o CBD parece ter despertado o interesse em pesquisadores da área, os quais têm se concentrado em investigar esse possível potencial. Isso é evidenciado pelo número crescente de estudos envolvendo principalmente a avaliação comportamental dos efeitos de CBD em diversas fases da drogadição por ANF publicados recentemente (ANOOSHE et al., 2021; METZ et al., 2021; NOURI et al., 2021; RAZAVI et al., 2020; SHARIFI et al., 2022; YANG et al., 2020). Os resultados até então obtidos, levam a crer em efeitos promissores desse canabinoide. Sendo assim, avaliar os efeitos comportamentais e celulares e elucidar possíveis mecanismos envolvidos em um tratamento experimental com CBD na prevenção de recidivas por ANF é extremamente relevante, pois pode resultar em uma estratégia farmacológica alternativa para o tratamento de um importante problema de saúde pública.

2.9 MODELO ANIMAL DE DEPENDÊNCIA DE DROGAS

Um número crescente de estudos tem utilizado roedores em diferentes modelos animais a fim de investigar os processos envolvidos na adição, elucidando efeitos comportamentais e neuroquímicos (ARES-SANTOS et al., 2012; MOREIRA-SILVA, 2014). Nesse sentido, a preferência condicionada de lugar (PCL) é um modelo animal comumente utilizado para melhor compreender os fenômenos envolvidos na adição a drogas como as anfetaminas (CRUZ; MARIN; PLANETA, 2008; KUHN et al. 2013; 2015, METZ et al., 2019; 2021; SEGAT et al., 2014; 2016; 2017; 2019).

A PCL é um paradigma clássico baseado no condicionamento estímulo-ambiente e tem sido empregada devido a sua potencial utilidade para testar diferentes estágios da drogadição: dependência, recompensa, extinção e recaída (BARDO; BEVINS, 2000; TZSCHENTKE, 2007). Este protocolo caracteriza-se por envolver a exposição passiva à droga e por apresentar similaridade com o condicionamento clássico de Pavlov (TZSCHENTKE, 1998). Sabe-se que é intrínseco dos seres humanos e outras espécies animais apresentar preferência por alguns lugares em detrimento de outros. Essa preferência pode ocorrer devido a características sensoriais de determinados lugares ou ainda pode ser adquirida como consequência de eventos associados a esses locais (HUSTON et al., 2013; SEGAT et al., 2016).

Os diferentes fenômenos envolvidos na adição são fortemente influenciados por sugestões ambientais, servindo de base para o paradigma de PCL, onde o animal relaciona as pistas ambientais (do lugar) com os efeitos hedônicos da droga (MCCORVY et al. 2011). Na PCL, os animais recebem a droga em um ambiente ou "contexto" com pistas ambientais e uma substância inativa em outro ambiente com sugestões ambientais distintas. Após esse pareamento, os animais, em um estado livre de drogas, têm a opção de se moverem livremente entre os dois ambientes, e a quantidade de tempo em que os mesmos permaneceram no ambiente associado à droga é tomada como um índice de preferência pela mesma. Em outras palavras, uma maior permanência no ambiente pareado com a droga significa que o contexto adquiriu destaque de incentivo, que reflete as propriedades recompensadoras da droga (NAPIER; HERROLD; DE WIT, 2013).

O paradigma de PCL também tem sido utilizado como um modelo animal para acessar os fenômenos de recaída no comportamento de busca de drogas (CALPE-LOPÉZ; GARCÍA-PARDO; AGUILAR, 2019). Para isso, após adquirirem a PCL para um ambiente pareado com a droga, são realizadas várias sessões de extinção. Durante as sessões de extinção, os animais exploram os compartimentos na ausência da droga, de forma que a preferência adquirida seja gradativamente atenuada (YAHYAVI-FIROUZ-ABADI; SEE, 2009). Então, em estudos de recaída, após atingir a extinção da preferência pela droga adquirida anteriormente na PCL, os animais retomam a busca pela substância quando submetidos a uma das seguintes situações: (i) reexposição à droga, (ii) exposição a estímulos estressores, (iii) exposição a estímulos ambientais relacionados à droga (SADEGHZADEH et al., 2015; SHARAM et al., 2003).

Como mencionado, um dos modelos animais utilizados para promover o restabelecimento da PCL é a reexposição à droga. Ou seja, os animais retomam o comportamento extinto de busca de drogas após uma nova exposição à substância (SANCHIS-SEGURA; SPANAGEL, 2006). Dependendo da droga em estudo, essa reintegração pode ser induzida por uma única injeção, a qual contém geralmente uma dose menor do que a utilizada no estabelecimento da PCL (NESBIT; DIAS; PHILLIPS, 2015; SHEN et al., 2014; SCHMEICHEL; BERRIDGE, 2014), ou ainda por uma sequência de injeções da droga que levam a um condicionamento e consequente restabelecimento da PCL (METZ et al., 2019; 2021; SEGAT et al., 2014; 2016; 2017; 2019).

Além disso, vários estudos já demonstraram que o estresse é um dos fatores mais importantes no restabelecimento da recaída à droga após um período de abstinência (KARIMI-HAGHIGHI; HAGHPARAST, 2018; MANTSCH et al., 2016; NYGARD et al., 2016; SANCHIS-SEGURA; SPANAGEL, 2006; SEDKI et al., 2015). Sendo assim, diversos

estímulos estressores têm sido utilizados para promover o restabelecimento da PCL em ratos, dentre os quais destacam-se a exposição do animal ao choque (SHARAM et al., 2003), privação aguda de comida (SEDKI et al., 2015), privação de sono (KARIMI-HAGHIGHI; HAGHPARAST, 2018) e o estresse por natação forçada (HAGHPARAST et al., 2014; MIKAIL et al., 2012), dentre outros.

Por fim, há muito se reconhece que os estímulos ambientais são fatores importantes no comportamento de busca por drogas (DI CHIANO; BLAHA; PHILLIPS, 2001; GOLDBERG, 1976; LE; SHAHAM, 2002). Tendo em vista isso, a exposição a estímulos externos, como por exemplo leves sons ou diferentes tipos de luzes que foram repetidamente emparelhados com a administração da droga podem provocar o restabelecimento da PCL pela substância (ARROYO et al., 1998; WEISS et al., 2001).

3 JUSTIFICATIVA

O uso abusivo de drogas psicoativas como as anfetaminas está cada vez mais difundido na sociedade contemporânea. De particular importância, alguns representantes anfetamínicos lícitos apresentam aplicações clínicas importantes, mas também são utilizados para fins recreacionais, podendo apresentar uso abusivo com o desenvolvimento de adição. Da mesma forma, o uso abusivo de substâncias anfetamínicas ilegais está atrelado à adição. Nesse contexto, indivíduos adictos em processo de tratamento para desintoxicação apresentam frequentes episódios de recaída, evidenciando que a farmacoterapia disponível até o momento é apenas sintomática, não é específica para a situação e não apresenta a eficácia necessária para tratar a causa da doença e evitar o fenômeno de recorrência. O canabidiol (CBD) é um canabinoide exógeno que atua em diferentes sistemas de neurotransmissores envolvidos na adição e exerce propriedades terapêuticas que direta ou indiretamente, poderiam ser úteis no tratamento dos transtornos da drogadição. Estudos experimentais mostraram efeitos promissores do CBD no tratamento do abuso de álcool, cocaína e morfina. Entretanto, os dados disponíveis a respeito da influência do CBD na adição por anfetaminas são limitados e inconclusivos. O grave problema do abuso dessas substâncias em todo o mundo, combinado com a clara necessidade de novas estratégias terapêuticas para tratar a drogadição, como também prevenir os episódios de recaída ao uso da droga justifica a exigência de mais estudos para avaliar o potencial do CBD como uma nova intervenção terapêutica, após sequenciais e necessários estudos clínicos com o referido composto.

4 OBJETIVOS

4.1 OBJETIVO GERAL

Investigar os efeitos do canabidiol (CBD) sobre parâmetros moleculares e comportamentais de recaída em ratos expostos à anfetamina (ANF).

4.2 OBJETIVOS ESPECÍFICOS

- Avaliar o possível efeito terapêutico do CBD na prevenção da recaída por ANF em diferentes modelos experimentais (recondicionamento e restabelecimento por estresse de natação forçada);
- Verificar a influência do tratamento com CBD sobre parâmetros comportamentais de locomoção e ansiedade em animais expostos à ANF;
- Analisar os efeitos do tratamento com CBD sobre alterações decorrentes à exposição à ANF em marcadores moleculares dopaminérgicos e do sistema endocanabinoide em regiões cerebrais envolvidas na drogadição;
- Investigar uma relação causal entre os efeitos moleculares do CBD sobre o sistema dopaminérgico e endocanabinoide e a prevenção dos comportamentos de ansiedade e recaída à ANF.

5 PRODUÇÃO CIENTÍFICA

Os itens Materiais e Métodos, Resultados, Discussão e Referências que fazem parte desta tese estão apresentados sob a forma de um artigo científico e um manuscrito científico, os quais encontram-se dispostos na íntegra de maneira a proporcionar um melhor entendimento do estudo como um todo.

5.1 ARTIGO CIENTÍFICO

Cannabidiol prevents amphetamine relapse and modulates D1- and D2-receptor levels in mesocorticolimbic brain areas of rats

Vinícia Garzella Metz, Jéssica Leandra Oliveira da Rosa, Domenika Rubert Rossato, Laura Hautrive Milanesi, Marilise Escobar Burger, Camila Simonetti Pase.

Periódico: European Neuropsychopharmacology

Status: Publicado (2021)

Disponível em: <https://doi.org/10.1016/j.euroneuro.2021.04.008>

Licença da Elsevier para a utilização deste conteúdo publicado



Cannabidiol prevents amphetamine relapse and modulates D1- and D2-receptor levels in mesocorticolimbic brain areas of rats

Author:

Vinícia Garzella Metz, Jéssica Leandra Oliveira da Rosa, Domenika Rubert Rossato, Laura Hautrive Milanesi, Marilise Escobar Burger, Camila Simonetti Pase

Publication: European Neuropsychopharmacology

Publisher: Elsevier

Date: September 2021

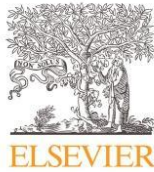
© 2021 Elsevier B.V. and ECNP. All rights reserved.

Journal Author Rights

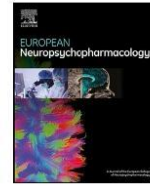
Please note that, as the author of this Elsevier article, you retain the right to include it in a thesis or dissertation, provided it is not published commercially. Permission is not required, but please ensure that you reference the journal as the original source. For more information on this and on your other retained rights, please visit: <https://www.elsevier.com/about/our-business/policies/copyright#Author-rights>

BACK

CLOSE WINDOW



www.elsevier.com/locate/euroneuro



Cannabidiol prevents amphetamine relapse and modulates D1- and D2-receptor levels in mesocorticolimbic brain areas of rats



Vinícia Garzella Metz^a, Jéssica Leandra Oliveira da Rosa^b,
Domenika Rubert Rossato^a, Laura Hautrive Milanesi^a,
Marilise Escobar Burger^{a,*}, Camila Simonetti Pase^{a,c,**}

^aPrograma de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, 97105-900 Santa Maria, RS, Brazil

^bDepartamento de Fisiologia e Farmacologia, Universidade Federal de Santa Maria, RS, Brazil

^cUniversidade Federal do Pampa, Campus Uruguaiana, 97500-970 Uruguaiana, RS, Brazil

Received 13 October 2020; received in revised form 1 March 2021; accepted 11 April 2021

KEYWORDS

Addiction;
Anxiety;
Psychostimulant;
Cannabinoid;
Dopaminergic system

Abstract

Amphetamine (AMPH) is an addictive psychostimulant highly used worldwide and its consumption is related to neurotoxic effects. Currently, there is no pharmacotherapy approved for treating AMPH or other psychostimulant drug addiction. Different studies have shown promising properties of cannabidiol (CBD) for treating many neurological and psychiatric diseases, and recently, CBD is being considered a potential strategy for the treatment of drug addiction disorders. Thus, we investigated possible CBD beneficial effects on relapse symptoms following AMPH re-exposure considering drug relapse is the most difficult clinical factor to control during addiction treatment. Rats received d,l-AMPH (4 mg/kg, i.p.) or vehicle in the conditioned place preference (CPP) paradigm (8 days), when each experimental group was re-assigned to receive CBD at two different doses (5 or 10 mg/kg, i.p.) or control, for 5 days. Subsequently, animals were re-exposed to AMPH-CPP (4 mg/kg, i.p.) for 3 additional days to assess relapse behavior. Besides locomotor and anxiety-like behaviors, dopaminergic molecular parameters were quantified in both prefrontal cortex and ventral striatum. Regarding molecular levels, CBD modulated at basal levels the dopaminergic targets (D1R, D2R, DAT, and TH) in the assessed brain areas, preventing AMPH relapse and decreasing anxiety-like behavior *per se* and in

* Corresponding author.

** Corresponding author at: Universidade Federal do Pampa, Campus Uruguaiana, 97500-970 Uruguaiana, RS, Brazil.
E-mail addresses: marilise.burger@ufsm.br (M.E. Burger), camilapase@unipampa.edu.br (C.S. Pase).

AMPH-CPP animals. The current findings give evidence about CBD-induced AMPH-relapse prevention, which may be linked to dopaminergic mesocorticolimbic system modulation. Although future and clinical studies are needed, our outcomes show that CBD may be a useful alternative to prevent AMPH relapse.

© 2021 Elsevier B.V. and ECNP. All rights reserved.

1. Introduction

Drug addiction is a severe public health problem characterized by compulsive and uncontrolled drug use, resulting in serious medical, psychiatric, and socioeconomic concerns (Cao et al., 2016). Currently, amphetamine-type stimulants, including 3,4-methylenedioxymethamphetamine (MDMA), amphetamine (AMPH), and methamphetamine (METH) are the third most common class of illicit drugs used worldwide (UNODC, 2020). These substances present a high risk of dependence and relapse even after long periods of abstinence (Markou, 2009), however, no approved pharmacological therapy is available for the treatment of addicted individuals (Cao et al., 2016).

Chronic exposure to psychoactive substances may cause molecular and cellular neuroadaptations in brain areas related to the reward system contributing to compulsive and uncontrolled use of addictive drugs (Cao et al., 2016). The mesocorticolimbic dopaminergic circuit is known for its mediation in the addictive drug reinforcement (Marsden, 2006) including AMPH-like drugs, which mechanism involves an increase in the dopaminergic neurotransmission (Koob and Volkow, 2010). Although other systems such as the glutamate system, trace amine system, anti-reward system, and central immune system are also involved in the addiction (Liu and Li, 2018), the dopaminergic signaling modulation is a possible target for the development of pharmacological treatments for drug addiction (Courtney and Ray, 2016).

Cannabidiol (CBD) is the *Cannabis sativa* plant's second most abundant phytocannabinoid component (Mechoulam et al., 2002). Besides, it does not cause psychotomimetic effects (Martin-Santos et al., 2012) and CBD exerts no rewarding or abuse potential (Viudez-Martinez et al., 2019). CBD has stood out in clinical and preclinical studies as a promising compound due to its therapeutic properties when applied for the treatment of several neurological and psychiatric diseases (Black et al., 2019; Crippa et al., 2018). Recently, CBD has caught the researchers' attention, representing a potential strategy for addictive substance use disorders treatment, and to date, preclinical studies had shown promising results in the addiction treatments to different drugs (de Carvalho and Takahashi, 2017; Luján et al., 2018; Viudez-Martinez et al., 2020). CBD presents a multi-target pharmacological profile (Luján et al., 2018) and its mechanism of action is still poorly understood. CBD can act as a GPR55 antagonist (Ryberg et al., 2007), partial agonist to serotonin 5-HT_{1A} receptors (Russo et al., 2005), transient receptor potential vanilloid 1 (TRPV1) agonist (Bisogno et al., 2001), μ -opioid receptor (MOR) positive allosteric modulator (Kathmann et al., 2006) and adenosine uptake inhibitor (Liou et al., 2008). Despite initial controversy about its endocannabinoid targets (Zlebnik and Cheer, 2016), recent

studies indicate that CBD acts as a negative allosteric modulator of type 1 (CB1) and type 2 (CB2) cannabinoid receptors (Laprairie et al., 2015; Tham et al., 2019). In addition, CBD acts as an inhibitor of fatty acid amide hydrolase (FAAH), the main endocannabinoid enzyme, metabolizing anandamide, which is an endocannabinoid and consequently increasing the endocannabinoid tone (Chauvet et al., 2015). CBD still acts in other systems (Zanelati et al., 2010) and has been associated with the modulation of neural circuits involved in addiction and drug-seeking behaviors (Olière et al., 2013), such as the dopaminergic mesocorticolimbic system (Renard et al., 2017).

Although recent studies have demonstrated the CBD beneficial effects in drug addiction treatments, few studies have focused on the impact of this cannabinoid on AMPH addiction (Hay et al., 2018; Karimi-Haghighi and Haghighiparast, 2018; Karimi-Haghighi et al., 2020). Also, there is some evidence that CBD could prevent neuroadaptations induced by psychostimulant drugs (Calpe-López et al., 2019; Murillo-Rodríguez et al., 2011). Regarding this, the current study was performed to evaluate the CBD therapeutic potential on behavioral parameters related to AMPH relapse, besides evaluating the molecular consequences in the dopaminergic mesocorticolimbic brain areas of rats as well.

2. Experimental procedures

2.1. Animals

Forty-two male Wistar rats weighing approximately 100–150 g were used for this study. The experimental protocol started on postnatal day 43 (PND43). This age was selected because it corresponds to the period of the rats' adolescence, which is considered a highly vulnerable period for the development of drug abuse conditions (Teixeira-Gomez et al., 2015). The animals were divided into groups of three (± 1) and housed in Plexiglas cages with food and water ad libitum. They were kept in a room with controlled temperature (22–23 °C) on a 12 h-light/dark cycle.

The experimental procedures were approved by the Research Ethics Committee of the Federal University of Santa Maria (UFSM-8850121118), which is affiliated with the National Council for the Control of Animal Experiments (CONCEA), following international norms of care and animal maintenance.

2.2. Drugs and solution

The d,l-amphetamine (AMPH; Merck, Germany) (4 mg • mL⁻¹/kg), dissolved in saline (0.9% NaCl; AMPH-vehicle) was used to induce conditioned place preference (CPP).

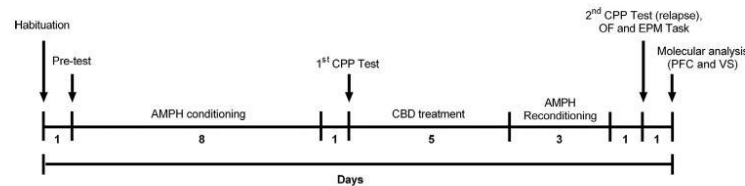


Fig. 1 Experimental design: after basal behavioral evaluation (pre-test), animals were treated with AMPH (4 mg/kg, i.p.) for 8 days, when the 1st behavioral evaluation was performed in the CPP paradigm. Subsequently, animals were treated with CBD (5 or 10 mg/kg, i.p.) for 5 days, followed by three more days of AMPH reconditioning. 24 h after the last AMPH administration, animals were re-exposed to CPP evaluation followed by behavioral and molecular analysis in both PFC and VS. Abbreviations: AMPH: amphetamine; CBD: cannabidiol; CPP: conditioned place preference; EPM: elevated plus maze; OF: open field; PFC: prefrontal cortex; VS: ventral striatum.

The choice of dose and time for both conditioning- and reconditioning-period were standardized in the AMPH-CPP, and used in previous studies of our research group (Segat et al., 2014, 2016, 2017; Metz et al., 2019). Cannabidiol (CBD; STI Pharmaceuticals, United Kingdom) was dissolved in a solution of 2% polysorbate 80 (Tween 80; Sigma-Aldrich, St Louis, MO, USA) and saline to a concentration of 5 or 10 mg · mL⁻¹/kg. For the current study, the rationale for the CBD dose was based on previously published studies performed in animal models of drug addiction (Mahmud et al., 2017; Ren et al., 2009a, 2009b). CBD was administered for 5 days, considering the extinction period of the AMPH preference, which, according to our previous pilot studies, can be observed from the 4th day after the last AMPH exposure. All drugs were intraperitoneally (i.p.) injected at a volume of 1 mL/kg of body weight.

2.3. Experimental protocol

Animals ($n = 42$) were initially designated to two experimental groups ($n = 21$): vehicle (saline - 0.9% NaCl solution, i.p.) and amphetamine (AMPH, 4 mg/kg, i.p.), and exposed to the conditioned place preference (CPP) protocol (as described in 2.4.1 section). After the first CPP test, which was performed to verify AMPH preference, each group was re-assigned and received the CBD treatment at two different doses (CBD 5 or 10 mg/kg, i.p) or control (saline and 2% polysorbate 80, i.p) once a day for five days. This resulted in six final groups ($n = 7$): i) vehicle/control; ii) vehicle/CBD5; iii) vehicle/CBD10; iv) AMPH/control; v) AMPH/CBD5; vi) AMPH/CBD10. Subsequently, animals that were previously conditioned with the drug were re-exposed to the AMPH-CPP paradigm after additional AMPH/vehicle conditioning. Then, the CPP test was performed again to verify AMPH relapse. In sequence, the animals were exposed to the open field test and the elevated plus-maze test for assessing locomotor activity and anxiety-like symptoms, respectively. The behavioral tests were recorded for later assessment. Following 24 h of the last behavioral assessment (Metz et al., 2019; Segat et al., 2017), all animals were anesthetized (isoflurane, the dose to the effect) and euthanized by decapitation. The prefrontal cortex (PFC) and ventral striatum (VS) were dissected according to Paxinos and Watson (2007) and stored at -80 °C freezer for analysis (Fig. 1).

2.4. Behavioral assessments

2.4.1. Conditioned place preference (CPP)

The CPP paradigm is an animal model used to assess the hedonic reinforcement effects of addictive drugs. The CPP device consists of a box with two compartments of equal size ($45 \times 45 \times 50$ cm) and with an equivalent intensity of light but having different environmental stimuli: one compartment has a white floor and striped walls, and the other has a striped floor and smooth white walls. The access to both compartments was via a central compartment ($18 \times 36 \times 50$ cm) separated by manual guillotine doors.

The AMPH-induced CPP was performed according to the following phases: habituation, pre-test, conditioning, CPP test, extinction, reconditioning, and CPP test. On post-natal day (PND) 43, for habituation, the animals were kept for 15 min (min) in each compartment, without access to the neutral compartment. This procedure aimed to exclude exploratory behavior that is common in new environments to avoid misinterpretations on the testing day.

On the following day (PND 44) rats performed the pre-test, where the animal is placed in the central compartment with free access to the entire apparatus for 15 min. The time spent in each compartment was assessed. This test was carried out to determine the innate environmental chamber preference, used to discard the rats who showed strong unconditioned aversion (less than 25% of the session time) or preference (more than 75% of the session time) for any compartment (Vasquez et al., 2006).

On the following 8 days (PND 45–52), animals were conditioned with AMPH for 25 min in the compartment they spent the shortest pre-test time and with vehicle (0.9% NaCl) in the paired compartment, with an interval of 4 h between each administration (Dias et al., 2019; Segat et al., 2018). Vehicle-treated groups received two daily injections of saline solution in both CPP compartments in alternated turns. The CPP test was conducted on PND 53, and the time spent in each compartment was recorded for 15 min. Results express the percentage of time spent by the animals in the compartment associated with AMPH during the abstinence period. Subsequently, additional CPP tests were performed to assess the loss of preference for the drug. The extinction of the preference for AMPH was observed on the fourth day after the last AMPH administration.

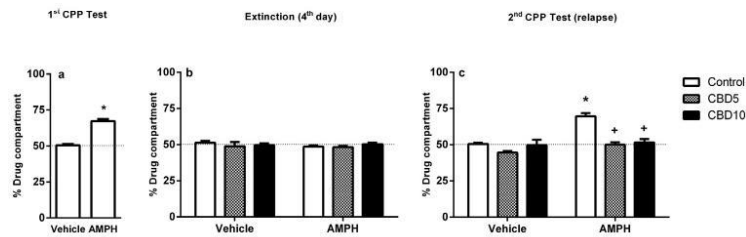


Fig. 2 AMPH-induced preference behavior (AMPH, 4 mg/kg, i.p. for 8 days) observed in the CPP paradigm before CBD treatment (5 and 10 mg/kg, i.p., for 5 days) (Fig 2a), the extinction test on the 4th day after the last AMPH administration (Fig. 2b) and drug preference behavior after AMPH reconditioning (AMPH, 4mg/kg, i.p. for 3 days) (Fig. 2c) ($n=7$). Data are expressed as mean \pm S.E.M. * indicates a significant difference of vehicle/AMPH in the same treatment (control/CBD) ($P<0.05$). + indicates significant difference of treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P<0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol.

CBD treatment started after the first CPP test and lasted for 5 days. After the CBD treatment period, animals were submitted to three more days of the vehicle or AMPH re-conditioning (Dias et al., 2019; Metz et al., 2019; Segat et al., 2016, 2017) in the CPP apparatus, which was followed by an additional drug preference test as described above. Relapse symptoms were quantified by the highest time spent in the drug-paired environment in this second drug exposure (Metz et al., 2019; Segat et al., 2014, 2016)

2.4.2. Open field (OF) task

The open-field (OF) test was used to assess the locomotor activity of rats (Sestakova et al., 2013). Animals were individually placed in the center of an OF arena ($40 \times 40 \times 30$ cm) enclosed by black matte walls and floor divided into squares, as described by Kerr et al. (2005). The number of crossings (horizontal squares crossed) and center crossings (center squares crossed) were quantified for 5 min.

2.4.3. Elevated plus maze (EPM) task

To assess anxiety-like symptoms, animals were subjected to the elevated plus maze (EPM) paradigm. The apparatus consists of a platform elevated 50 cm from the floor. Forty-centimeter-high (40 cm) walls enclose two opposite arms (50×10 cm) while the other two arms have no walls. All arms have a central intersection (10×10 cm). In the present study, we evaluated the time spent in the open arms (sec), open arms entries (number), anxiety index (calculated by the following formula), and total arms entries (number) (Montgomery, 1955):

$$\text{Anxiety index} = 1 - \left(\left(\frac{\text{Open arms time}}{\text{Total time}} \right) + \left(\frac{\text{Open arms entries}}{\text{Total entries}} \right) \right) / 2$$

Anxiety index values range from 0 to 1, where an increase in the index expresses increased anxiety-like behavior (Cohen et al., 2012).

2.5. Molecular assays

Molecular analyses were performed by western blot as described by Dias et al. (2017). Briefly, the membranes were incubated with the primary antibodies (Santa Cruz Biotechnology): anti-dopamine D1 receptor (D1) (1:500, sc-33660), anti-dopamine D2 receptor (D2) (1:500, sc-9113), anti-dopamine transporter (DAT) (1:500, sc-14002), anti-vesicular monoamine transporter 2 (VMAT2) (1:500, sc-374079), anti-tyrosine hydroxylase (TH) (1:500, sc-25269), and β -actin (1:50000; Sigma-Aldrich, St. Louis, USA), followed by the appropriate secondary antibody (Santa Cruz Biotechnology) IgG horseradish peroxidase conjugate. Actin was used as an internal control and data were standardized according to its values.

2.6. Statistical analysis

Power analysis for sample size calculation was performed to determine the balance among the number of subjects, observed size effect, and required alpha value. From this assessment, we calculated the achieved power using G*Power (3.1) and assumed a high effect size ($f = 0,5$) for all analyses. Sample-size of $n = 7$ animals per group was enough to detect a difference between evaluations with 80% of power and 0.05 of significance levels. CPP data before CBD treatment were analyzed by Student's t -test. For other analyses, two-way ANOVA followed by Newman-Keuls test was performed using Statistic® (version 7.0). Values of $P < 0.05$ were considered statistically significant for all comparisons made. GraphPad Prism® (version 6.0) was used to design the figures.

3. Results

3.1. CBD treatment effects on AMPH-conditioned place preference (CPP)

The behavioral assessment performed after AMPH conditioning and before CBD treatment showed that AMPH-conditioned animals remained more time in the drug-

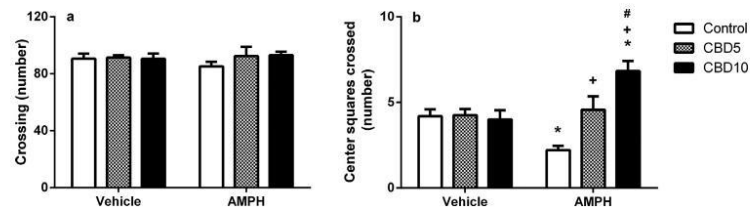


Fig. 3 CBD treatment influence (5 and 10 mg/kg, i.p., for 5 days) on the locomotor activity in the open field (OF) task after AMPH reconditioning (4 mg/kg, i.p., for 3 days). Crossing number (a) and number of central squares crossed (b) were quantified ($n = 7$). Data are expressed as mean \pm S.E.M. * indicates a significant difference of vehicle/AMPH in the same treatment (control/CBD) ($P < 0.05$). + indicates significant differences of treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P < 0.05$). # indicates significant differences of the CBD doses in the same conditioning (vehicle/AMPH) ($P < 0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol.

conditioned compartment in comparison to the vehicle-injected group ($P < 0.05$), which showed no place preference, as expected (Fig. 2a).

On the 4th day after the last AMPH administration, animals showed no place preference in an additional test of CPP, evidencing the extinction of AMPH-preference (Fig. 2b).

Two-way ANOVA of AMPH reconditioning following CBD treatment revealed a main effect of the drug, treatment and treatment \times drug interaction [$F(1,36) = 31.01$, $P < 0.001$; $F(2,36) = 10.37$, $P < 0.001$; $F(2,36) = 14.43$, $P < 0.001$], respectively. Post-hoc comparison showed that both CBD doses were able to prevent AMPH preference after drug reconditioning (Fig. 2c).

3.2. Locomotion parameters assessed in the open-field (OF) task

After AMPH reconditioning, no difference in the locomotor activity among the experimental groups was observed in the OF task (Fig. 3a). Following CBD treatment and AMPH re-exposure, two-way ANOVA of the number of central squares crossed revealed a significant main effect of both treatment and treatment \times drug interaction [$F(2,36) = 8.52$, $P < 0.05$; $F(2,36) = 10.04$, $P < 0.001$], respectively. Considering vehicle-injected animals, the post-hoc comparison showed no differences among the different experimental groups. Concerning AMPH-conditioned animals, AMPH *per se* decreased the number of central squares crossed, in relation to the vehicle group, CBD5 was able to prevent this decrease induced by AMPH and CBD10 increased this behavioral parameter in comparison to both AMPH-control and vehicle-injected groups (Fig. 3b).

3.3. Anxiety-like symptoms assessed in the elevated plus maze (EPM) task

Two-way ANOVA of the EPM revealed a significant main effect of both treatment and treatment \times drug interaction [$F(2,36) = 32.23$, $P < 0.001$; $F(2,36) = 6.58$, $P < 0.05$], respectively, on the time spent in the open arms. Re-

garding the number of open arms entries, a significant main effect of the drug, treatment and treatment \times drug interaction [$F(1,36) = 4.34$, $P < 0.05$; $F(2,36) = 15.17$, $P < 0.001$; $F(2,36) = 7.96$, $P < 0.05$], was observed respectively. A significant main effect of both treatment and treatment \times drug interaction [$F(2,36) = 41.29$, $P < 0.001$; $F(2,36) = 11.94$, $P < 0.001$], respectively, on the anxiety index was also observed.

Post-hoc test revealed that CBD treatment *per se* increased both time spent and entries number in the EPM open arms (Fig. 4a and b). Conversely, AMPH *per se* decreased the time spent and entries number in the open arms, increasing the Anxiety Index, in comparison to the vehicle-injected group (Fig. 4a-c). In addition, AMPH-exposed animals that were subsequently treated with both CBD doses showed increased entries number and time spent in the open arms of the EPM (Fig. 4a and b). Thus, both CBD doses reduced the Anxiety Index in both vehicle and AMPH-conditioned groups (Fig. 4c). There was no statistical difference among experimental groups on the total number of entries in the arms of the EPM (Fig. 4d).

3.4. CBD treatment effects on dopaminergic receptors (D1 and D2) level in both PFC and VS

In PFC, two-way ANOVA revealed a significant main effect of the treatment [$F(2,36) = 4.72$, $P < 0.05$] on D1 level, and a significant main effect of the treatment and treatment \times drug interaction [$F(2,36) = 9.96$, $P < 0.001$; $F(2,36) = 4.33$, $P < 0.05$, respectively] on D2 level. In the VS, two-way ANOVA revealed a significant main effect of the drug and treatment [$F(1,36) = 8.88$, $P < 0.05$; $F(2,36) = 3.56$, $P < 0.05$, respectively] on D1 level, and a significant main effect of the drug [$F(1,36) = 9.33$, $P < 0.05$] on D2 level.

In both evaluated brain areas, the post-hoc comparison showed that AMPH *per se* increased D1 (Fig. 5a and c) and D2 (Fig. 5b and d) levels when compared to vehicle. Besides, both AMPH-conditioned groups that were treated with CBD doses did not show this increase induced by AMPH in PFC (Fig. 5a and b) and VS (Fig. 5c and d) when compared to the control group.

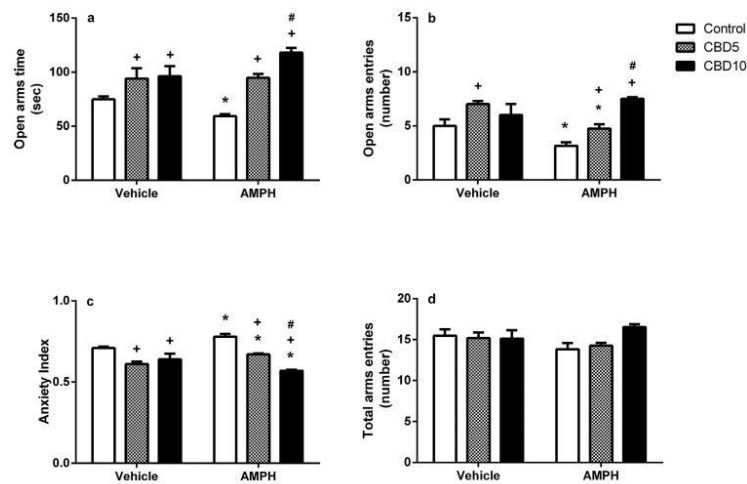


Fig. 4 CBD treatment influence (5 and 10 mg/kg, i.p.) on anxiety-like behaviors in the elevated plus-maze (EPM) task after AMPH reconditioning (4 mg/kg, i.p., for 3 days). The time spent in the open arms (a), number of open arms entries (b), anxiety index (c), and number of total arms entries (d) were quantified ($n = 7$). Data are expressed as mean \pm S.E.M. * indicates a significant difference of vehicle/AMPH in the same treatment (control/CBD) ($P < 0.05$). + indicates significant differences of treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P < 0.05$). # indicates significant differences of CBD doses in the same conditioning (vehicle/AMPH) ($P < 0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol.

3.5. CBD treatment effects on immunoreactivity of dopaminergic targets (DAT, VMAT-2 and TH) levels in both PFC and VS

In the PFC, two-way ANOVA revealed a significant main effect of the treatment on DAT level [$F(2,36) = 5.53, P < 0.05$]. In the VS, two-way ANOVA revealed a significant main effect of both drug and treatment [$F(1,36) = 4.87, P < 0.05$; $F(2,36) = 4.94, P < 0.05$] on DAT; [$F(1,36) = 20.67, P < 0.001$; $F(2,36) = 13.50, P < 0.001$] on VMAT-2 and [$F(1,36) = 8.88, P < 0.05$; $F(2,36) = 15.09, P < 0.001$] on TH levels.

Post-hoc test revealed that AMPH *per se* decreased DAT level in both analyzed brain areas when compared to vehicle (Fig. 6a and d). In AMPH-conditioned animals that were treated with both CBD, doses did not show this decrease induced by AMPH on DAT level in the PFC and VS (Fig. 6a and d, respectively).

There was no statistical difference among the experimental groups on VMAT-2 level in PFC (Fig. 6b). In the VS, the post-hoc test revealed that CBD5 *per se* increased VMAT-2 level which was reduced in AMPH-conditioned animals (Fig. 6e).

In the PFC, there was no statistical difference among experimental groups on TH levels (Fig. 6c). On the other hand, in the VS, the post-hoc comparison showed that CBD5 *per se* increased TH level when compared to control (Fig. 6f). While AMPH conditioning decreased TH levels in relation to the vehicle group, both CBD doses did not show this effect (Fig. 6f).

4. Discussion

In recent years, significant advances to understand the drug addiction pathophysiology instigated the search for innovative strategies to address this serious public health problem. However, there are no approved pharmacotherapies for treating psychostimulant addiction, which includes amphetamine (AMPH) (Calpe-López et al., 2019). Cannabidiol (CBD) has shown multiple therapeutic actions in the treatment of several central nervous system diseases, including epilepsy (Devinsky et al., 2016), anxiety (Blessing et al., 2015), depression (El-Alfy et al., 2010), and Alzheimer's disease (Watt and Karl, 2017). Recently, CBD has been considered for the treatment of addiction disorders. Pre-clinical studies suggested that CBD was able to reduce the craving and/or relapse for some substances, such as alcohol (Viudez-Martínez et al., 2020), opioids (Mahmud et al., 2017; Hurd et al., 2015), and psychostimulants (Karimi-Haghighi and Haghparast, 2018; Karimi-Haghighi et al., 2020; Luján et al., 2018). In this context, the current study provides evidence regarding CBD effectiveness on AMPH-induced behavioral and molecular aspects that were specifically performed through an experimental model, evaluating the relapse to this drug. Our findings show that CBD treatment was able to: (1) prevent AMPH relapse behavior in the animals that previously showed AMPH-conditioned place preference; (2) reduce anxiety-like behaviors following re-exposure to AMPH; and (3) it provides evident protection against AMPH-induced effects, modulating the immunoreactivity of dopaminergic targets in both prefrontal cortex (PFC) and ventral striatum (VS), which are pivotal brain areas involved in drug addiction.

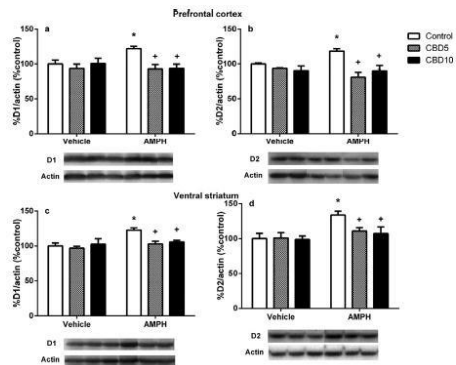


Fig. 5 CBD treatment influence (5 and 10 mg/kg, i.p.) on dopamine receptor type 1 (D1) and dopamine receptor type 2 (D2) levels in both prefrontal cortex (PFC) (a and b) and ventral striatum (VS) (c and d) after AMPH reconditioning (4 mg/kg, i.p., for 3 days) ($n = 7$). Each band in the sequence corresponds to one bar in the Figure. Data are expressed as mean \pm S.E.M. * indicates a significant difference of vehicle/AMPH in the same treatment (control/CBD) ($P < 0.05$). + indicates significant differences of treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P < 0.05$). # indicates significant differences of CBD doses in the same conditioning (vehicle/AMPH) ($P < 0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol.

In this experimental protocol, our focus was to elucidate the influence of two different CBD doses on AMPH relapse, since it is considered the most difficult clinical factor to be controlled following an addiction treatment (Kaplan et al., 2007). In recent years, efforts have been made by our research group (Metz et al., 2019; Segat et al., 2016, 2017, 2018), once the subject is scarce in the global literature, which reiterates the relevance of the outcomes in the current study. The conditioned place preference (CPP) paradigm has been adopted as an important animal model to access the relapse phenomena in drug-seeking behavior (Calpe-López et al., 2019) since this animal model follows a Pavlovian context. In relapse studies, after reaching the extinction of the preference for the drug, the animals recommence the search for the substance when they are re-exposed to the drug, stressors, or related environmental cues (Sadeghzadeh et al., 2015; Sharam et al., 2003). In the experimental model adopted in this study, after the extinction of a previously learned CPP, which concomitantly happened with the treatment period, the exposure to additional conditioning induced the place preference reinstatement (Metz et al., 2019; Segat et al., 2016, 2017, 2018), what is considered a relapse phenomenon. Here, both CBD doses (5 and 10 mg/kg) were able to prevent AMPH relapse in a significant way.

We hypothesized that the CBD effect to prevent AMPH relapse may be a consequence, at least in part, caused by a cannabinoid anxiolytic property, as evidenced by our current findings in the elevated plus maze (EPM) and open-field (OF) tests. Currently, despite not being well established, addiction treatments involve two basic approaches: (1) drug use reduction, minimization of craving, and prevention of

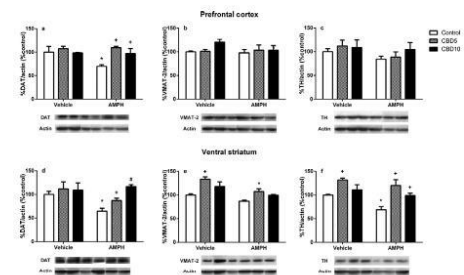


Fig. 6 CBD treatment influence (5 and 10 mg/kg, i.p.) on dopamine transporter (DAT), vesicular monoamine transporter 2 (VMAT-2) and tyrosine hydroxylase (TH) levels in both prefrontal cortex (PFC) (a-c) and ventral striatum (VS) (d-f) after AMPH reconditioning (4 mg/kg, i.p., for 3 days) ($n = 7$). Each band in the sequence corresponds to one bar in the figure. Data are expressed as mean \pm S.E.M. * indicates a significant difference of vehicle/AMPH in the same treatment (control/CBD) ($P < 0.05$). + indicates significant differences of treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P < 0.05$). # indicates significant differences of CBD doses in the same conditioning (vehicle/AMPH) ($P < 0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol.

relapses; (2) treatment with antidepressant and anxiolytic medicines, since anxiety and depression are strongly linked to relapse occurrence (Cao et al., 2016). It is well known that AMPH administration can induce anxiety-like behaviors in rodents (Barr et al., 2010; Vuong et al., 2010), which was once again confirmed by our findings, as observed by the decreasing number of entries and time spent in the open arms of the EPM, resulting in a higher anxiety index. On the other hand, CBD treatment prevented the development of these anxiety-like behaviors in both AMPH and vehicle-injected animals, indicating the CBD anxiolytic activity *per se*. These outcomes are also in line with previous studies, which evidenced CBD anxiolytic properties in clinical and preclinical studies (see review: Blessing et al., 2015). Of special importance, a study of Norris et al. (2016) demonstrated that CBD administration directly within the mesolimbic pathway is able to block anxiety-related behaviors in rats. In this study, the authors suggest that the anxiolytic effects of CBD occur through its functional interactions with serotonergic receptor signaling in the mesolimbic system.

Furthermore, our study included locomotor behavioral assessments in the open field (OF) task, when animals from all experimental groups showed similar locomotor performance. This finding supports our outcomes involving anxiety that were observed in EPM and supports the CPP findings as well, indicating that these behaviors were not a locomotor artifact and that the reduced AMPH-conditioned place preference was related to the non-establishment of CBD-induced drug relapse (Segat et al., 2014, 2016, 2018; Takamatsu et al., 2011). Moreover, AMPH exposure reduced the crossing number in the OF paradigm central zone. The low ambulation in the central zone suggests an anxiety-like behavior (Bernatova et al., 2011; Sestakova et al., 2013) and CBD treatment in both doses prevented the reduction

in this AMPH-induced behavior. These findings corroborate with those found in the EPM test and reinforce the CBD anxiolytic property during the psychostimulant drug withdrawal, as previously mentioned.

CBD's preventive property on AMPH relapse may have a causal relation with the molecular modifications of proteins in the dopaminergic pathway induced by the cannabinoid. It is well known that the dopamine (DA) release induced by drugs such as AMPH, triggers neuroplasticity by causing modifications in the synaptic signaling of the dopaminergic mesocorticolimbic system (Kauer and Malenka, 2007) and Malenka, 2007; Kourrich et al., 2015). Our current study shows the CBD beneficial effect on dopaminergic molecular targets located in the PFC and VS, which are part of the mesocorticolimbic system, also known as the reward pathway. In line with this, AMPH inhibits the DA vesicular reuptake, consequently increasing the neuronal release [see review: Teixeira-Gomes et al., 2015). Among other consequences, excessive AMPH-induced DA levels in the synaptic cleft favor the activation of the dopaminergic receptors, especially of D1 and D2 receptors (D1 and D2), which seem to mediate relapse and drug-seeking behavior mechanisms (Hansen et al., 2002). Our study shows that AMPH-conditioned animals spend more time in the drug-paired compartment, possibly due to increased D1- and D2-receptor immunoreactivity in both evaluated brain areas. Our findings are consistent with previous reports (Metz et al., 2019; Moratalla et al., 2017), which also established a relation between AMPH-induced DA receptors overstimulation and addictive events. On the other hand, the tested doses of CBD were able to prevent relapse behaviors after AMPH re-exposure. These findings are in agreement with the literature, which indicates that CBD administration can reduce addiction behaviors caused by other potentially addictive substances, including psychostimulants. Besides AMPH, CBD also has shown beneficial influence on other amphetamine drugs, such as METH, since it prevented reinstatement of METH-CPP induced by both stimuli: stress and drug-induced (Karimi-Haghighi and Haghparast, 2018; Karimi-Haghighi et al., 2020). Furthermore, CBD also reduced relapse to the METH-seeking behavior in a drug self-administration animal model (Hay et al., 2018). Considering these outcomes and comparing them with ours, these studies support the expectations concerning CBD as a pharmacotherapy to be used to treat drug addiction disorders; however, as such evidence was recently found, the possible molecular mechanisms underlying CBD effects need to be better understood. Here, we observed that CBD prevented D1 and D2 receptors' immunoreactivity increase following AMPH re-exposure. Some studies have shown that D1 agonists could produce AMPH-conditioned place preference, while D1 and D2 antagonists were able to prevent such acquisition (Beninger and Miller, 1998; Young and Geyer, 2010). Moreover, Martin et al. (2008) showed the influence of the endocannabinoid system as a negative modulator of both D1 and D2 receptor-mediated behaviors. In this sense, it has been suggested that endogenous cannabinoids are able to act through a homeostatic mechanism by activating CB1 receptors, which appear to break the dopaminergic function, especially in the striatum (Rodríguez da Fonseca et al., 1994, 1998). Based on this, we could infer that the CBD preventive property on AMPH relapse may also be related to its

ability to modulate dopaminergic receptor levels in brain areas linked to hedonic status or reward, which include PFC and VS.

As previously mentioned, AMPH affects dopaminergic neurotransmission because the DA transport is modified (Volz et al., 2007). Indeed, dopamine transporter (DAT) is responsible for regulating both intracellular and extracellular DA levels, representing a target in AMPH addiction conditions (Raineri et al., 2011, 2012). Furthermore, tyrosine hydroxylase (TH) is the rate-limiting enzyme for DA synthesis, which also plays an essential role in dopaminergic neurotransmission (Ares-Santos et al., 2012). In our study, AMPH decreased DAT immunoreactivity in both PFC and VS, decreasing the TH levels in VS as well. These findings are in agreement with previous literature, in which different preclinical studies have shown that AMPH administration triggers functional abnormalities in the dopaminergic axon terminals, decreasing the DAT and TH immunoreactivity in different brain areas, including the ones studied here (Ares-Santos et al., 2012; Granado et al., 2011; Gou et al., 2015). In contrast, our current study shows that CBD treatment was able to maintain the DAT levels in both PFC and VS, and decreased by AMPH exposure. Regarding the TH, both CBD doses used avoided the decrease in the TH immunoreactivity, however, reduced by AMPH in VS only. Besides, our assays evidenced increased TH and vesicular monoamine transporter 2 (VMAT-2) immunoreactivity *per se* in the VS of CBD5 treated group, and the implications of this result require further studies to support the data presented here. As previously mentioned, dopaminergic neurotransmission is deeply affected by addictive drugs, and alterations in dopaminergic function are evidenced in different stages of the drug addiction. Thus, the maintenance of dopaminergic neurotransmission is a target potentially exploitable therapeutically. The molecular outcomes observed in the current study confirm that the dopaminergic targets disturbed by AMPH were reestablished by CBD treatment, as shown in previous studies. Indeed, Renard et al. (2016) reported attenuation of both sensitization and dopaminergic neuronal activity AMPH-induced by direct injection of CBD in the mesolimbic system. In this way, we may propose that CBD seems to modulate the dopaminergic mesocorticolimbic system to recover the DA homeostasis through poorly understood mechanisms, which could involve DA-metabolism and/or -transporters by direct or indirect actions on this system, and that this modulation exerts a fundamental role on AMPH-relapse blocking. We speculate that the behavioral results found here, such as the potential anti-relapse effect of CBD, may be due to its ability to modulate dopaminergic molecular targets in important brain areas involved in addiction. From our point of view, this interaction between CBD and the dopaminergic system does not occur directly, but is a secondary response due to the action of CBD in the endocannabinoid system. The best understanding of these possible mechanisms will be subject for our further studies

We recognize that from our study we cannot state whether the treatment with CBD reversed the molecular changes underlying the conditioning of AMPH or prevented the molecular neuroadaptations resulting from the re-exposure to AMPH. Recently, several studies have demonstrated an important role of other dopamine receptors such

as D3 and D4 receptors in addiction (Daurio et al., 2020; Boileau et al., 2016). We recognize the limitation of our study by not investigating such relevant targets and we hope to clarify these limitations in further studies. We also emphasize that although the CBD appears to be well tolerated and has few serious known adverse effects (Chesney et al., 2020), additional data about its safety are needed to assess whether the CBD can be used more broadly.

5. Conclusion

In conclusion, CBD seems to be able to regulate dopaminergic targets in pivotal brain areas of the mesocorticolimbic dopaminergic system, subsequently to AMPH exposure. This could be observed in the CBD beneficial effects on AMPH-relapse after drug withdrawal. Although further experimental studies are needed to fully elucidate the CBD's action on the dopaminergic system, our outcomes present findings that may contribute to innovative treatments for psychostimulant drug addiction; however, clinical studies are also required to support such findings.

Role of the funding source

This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and by the Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS). The CAPES and FAPERGS had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Contributors

VM, JR, DR and LM performed the experiment and collected behavioral data. VM and JR performed and analyzed molecular data. VM analyzed data and wrote the manuscript. VM, MB and CP designed the study and interpreted the data. MB and CP reviewed and edited the manuscript. MB and CP were responsible for the funding acquisition. All authors critically reviewed the content and approved the final version for publication.

Declaration of Competing Interest

The authors declare that there are no conflicts of interest.

Acknowledgments

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for the fellowships and financial support and to STI Pharmaceuticals for the donation of cannabidiol. The authors also are grateful to PhD Caren Antoniazzi, PhD

Alexandra Latini and PhD Débora da Luz Scheffer for their contributions to this research.

References

- Ares-Santos, S., Granado, N., Oliva, I., Shea, E.O., Martin, E.D., Colado, M.I., Moratalla, R., 2012. Dopamine D1 receptor deletion strongly reduces neurotoxic effects of methamphetamine. *Neurobiol. Dis.* 45, 810–820.
- Barr, J.L., Renner, K.J., Forster, G.L., 2010. Withdrawal from chronic amphetamine produces persistent anxiety-like behavior but temporally-limited reductions in monoamines and neurogenesis in the adult rat dentate gyrus. *Neuropharmacology* 59 (6), 395–405.
- Beninger, R.J., Miller, R., 1998. Dopamine D1-like receptors and reward related incentive learning. *Neurosci. Biobehav. Rev.* 22, 335–345.
- Bernatova, I., Puzserova, A., Sestakova, N., Mach, M., 2011. Horizontal motor activity of hypertensive rats is associated with level of blood pressure. *Acta Physiol.* 685, 84.
- Bisogno, T., Hanuš, L., Petrocellis, L.D., Tchilibon, S., Ponde, D.E., Brandi, I., Moriello, A.S., Davis, J.B., Mechoulam, R., Di Marzo, V., 2001. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloid VR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. *Br. J. Pharmacol.* 134, 845–852.
- Black, N., Stockings, E., Campbell, G., Tran, L.T., Zagic, D., Hall, W.D., Farrell, M., Degenhardt, L., 2019. Cannabinoids for the treatment of mental disorders and symptoms of mental disorders: a systematic review and meta-analysis. *Lancet Psychiatry* 6 (12), 995–1010.
- Blessing, E.M., Steenkamp, M.M., Manzanares, J., Marmar, C.R., 2015. Cannabidiol as a potential treatment for anxiety disorders. *Neurotherapeutics* 12 (4), 825–836.
- Boileau, I., Payer, D., Rusjan, P.M., Houle, S., Tong, J., McCluskey, T., Wilson, A.A., Kish, S.J., 2016. Heightened dopaminergic response to amphetamine at the D3 dopamine receptor in methamphetamine users. *Neuropsychopharmacology* 41 (13), 2994–3002.
- Calpe-López, C., García-Pardo, M.P., Aguilar, M.A., 2019. Cannabidiol treatment might promote resilience to cocaine and methamphetamine use disorders: a review of possible mechanisms. *Molecule* 24 (14), 2583.
- Cao, D.N., Shi, J.J., Hao, W., Wu, N., Li, J., 2016. Advances and challenges in pharmacotherapeutics for amphetamine-type stimulants addiction. *Eur. J. Pharmacol.* 780, 129–135.
- Chauvet, C., Nicolas, C., Thiriet, N., Lardeux, M.V., Duranti, A., Solinas, M., 2015. Chronic stimulation of the tone of endogenous anandamide reduces cue- and stress-induced relapse in rats. *Int. J. Neuropsychopharmacol.* 18 (1).
- Chesney, E., Oliver, D., Green, A., Sovi, S., Wilson, J., Englund, A., Freeman, T.P., McGuire, P., 2020. Adverse effects of cannabidiol: a systematic review and meta-analysis of randomized clinical trials. *Neuropsychopharmacology* 45 (11), 1799–1806.
- Cohen, H., Liu, T., Kozlovsky, N., Kaplan, Z., Zohar, J., Mathé, A.A., 2012. The neuropeptide Y (NPY)-ergic system is associated with behavioral resilience to stress exposure in an animal model of post-traumatic stress disorder. *Neuropsychopharmacology* 37 (2), 350–363.
- Courtney, K.E., Ray, L.A., 2016. Clinical neuroscience of amphetamine-type stimulants: from basic science to treatment development. *Prog. Brain Res.* 223, 295–310.
- Crippa, J.A., Guimarães, F.S., Campos, A.C., Zuardi, A.W., 2018. Translational investigation of the therapeutic potential of cannabidiol (CBD): toward a new age. *Front. Immunol.* 9, 2009.
- Daurio, A.M., Deschaine, S.L., Modabbernia, A., Leggio, L., 2020. Parsing out the role of dopamine D4 receptor gene (DRD4) on

- alcohol-related phenotypes: a meta-analysis and systematic review. *Addict. Biol.* 25 (3), e12770.
- de Carvalho, C.R., Takahashi, R.N., 2017. Cannabidiol disrupts the reconsolidation of contextual drug-associated memories in Wistar rats. *Addict. Biol.* 22 (3), 742-751.
- Devinsky, O., Marsh, E., Friedman, D., Thiele, E., Laux, L., Sullivan, J., Miller, I., Flamini, R., Wilfong, A., Filloux, F., Wong, M., Tilton, N., Bruno, P., Bluvstein, J., Hedlund, J., Kamens, R., Maclean, J., Nangia, S., Singhal, N.S., Wilson, C.A., Patel, A., Cilio, M.R., 2016. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. *Lancet Neurol.* 15 (3), 270-278.
- Dias, V.T., Vey, L.T., Rosa, H.Z., D'ávila, L.F., Barcelos, R., Burger, M.E., 2017. Could modafinil prevent psychostimulant addiction? An experimental study in rats. *Basic Clin. Pharmacol.* 121 (5), 400-408.
- Dias, V.T., Rosa, H.Z., D'ávila, L.F., Vey, L.T., Barcelos, R., Burger, M.E., 2019. Modafinil reduces amphetamine preference and prevents anxiety-like symptoms during drug withdrawal in young rats: involvement of dopaminergic targets in VTA and striatum. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 92, 199-206.
- El-Alfy, A.T., Ivey, K., Robinson, K., Ahmed, S., Radwan, M., Slade, D., Khan, I., ElSohly, M., Ross, S., 2010. Antidepressant-like effect of delta9-tetrahydrocannabinol and other cannabinoids isolated from *Cannabis sativa* L. *Pharmacol. Biochem. Behav.* 95 (4), 434-442.
- Gou, H., Wen, D., Chunting, M., Li, M., Li, Y., Zhang, W., Liu, L., Cong, B., 2015. Protective effects of cholecystokinin-8 on methamphetamine-induced behavioral changes and dopaminergic neurodegeneration in mice. *Behav. Brain Res.* 283, 87-96.
- Granado, N., Lastres-Becker, I., Ares-Santos, S., Oliva, I., Martín, E., Cuadrado, A., 2011. Nrf2 deficiency potentiates methamphetamine-induced dopaminergic axonal damage and gliosis in the striatum. *Glia* 59, 1850-1863.
- Hansen, J.P., Riddle, E.L., Sandoval, V., Brown, J.M., Gibb, J.W., Hanson, G.R., Fleckenstein, A.E., 2002. Methylene-dioxymethamphetamine decreases plasmalemmal and vesicular dopamine transport: mechanisms and implications for neurotoxicity. *J. Pharmacol. Exp. Ther.* 300, 1093-1100.
- Hay, G.L., Baracz, S.J., Everett, N.A., Roberts, J., Costa, P.A., Arnold, J.C., McGregor, I.S., Cornish, J.L., 2018. Cannabidiol treatment reduces the motivation to self-administer methamphetamine and methamphetamine-primed relapse in rats. *J. Psychopharmacol.* 32 (12), 1369-1378.
- Hurd, Y.L., Yoon, M., Manini, A.F., Hernandez, S., Olmedo, R., Ostman, M., Jutras-Aswad, D., 2015. Early phase in the development of cannabidiol as a treatment for addiction: opioid relapse takes initial center stage. *Neurotherapeutics* 12 (4), 807-815.
- Kaplan, F., Oudeyer, P.Y., Nehaniv, C., Dautenhahn, K., 2007. The Progress-Drive Hypothesis: An interpretation of Early Imitation. *Social and Communication Dimensions*. University Press, New York, Cambridge, pp. 361-377. Models and mechanisms of imitation and social learning: Behavioural.
- Karimi-Haghighi, S., Haghighparast, A., 2018. Cannabidiol inhibits priming-induced reinstatement of methamphetamine in REM sleep deprived rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 82, 307-313.
- Karimi-Haghighi, S., Dargahi, L., Haghighparast, A., 2020. Cannabidiol modulates the expression of neuroinflammatory factors in stress- and drug-induced reinstatement of methamphetamine in extinguished rats. *Addict. Biol.* 25 (2), e12740.
- Kathmann, M., Flau, K., Redmer, A., Tränkle, C., Schlicker, E., 2006. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Naunyn-Schmiedeberg's Arch. Pharmacol.* 372, 354-361.
- Kauer, A., J., Malenka, C., R., 2007. Synaptic plasticity and addiction. *Nature Reviews Neuroscience* 8, 844-858.
- Kerr, D.S., Bevilacqua, L.R., Bonini, J.S., Rossato, J.I., Köhler, C.A., Medina, J.H., Izquierdo, I., Cammarota, M., 2005. Angiotensin II blocks memory consolidation through an AT2 receptor-dependent mechanism. *Psychopharmacology* 179 (3), 529-535.
- Koob, G.F., Volkow, N.D., 2010. Neurocircuitry of addiction. *Neuropsychopharmacology* 35 (1), 217-238.
- Kourrich, S., Calu, D.J., Bonci, A., 2015. Intrinsic plasticity: an emerging player in addiction. *Nat. Rev. Neurosci.* 16 (3), 173-184.
- Laprairie, R.B., Bagher, A.M., Kelly, M.E.M., Denovan-Wright, E.M., 2015. Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *Br. J. Pharmacol.* 172, 4790-4805.
- Liou, G.I., Auchampach, J.A., Hillard, C.J., Zhu, G., Yousufzai, B., Mian, S., Khan, S., Khalifa, Y., 2008. Mediation of cannabidiol anti-inflammation in the retina by equilibrative nucleoside transporter and A2A adenosine receptor. *Investig. Ophthalmol. Vis. Sci.* 49, 5526.
- Liu, J.F., Li, J.X., 2018. Drug addiction: a curable mental disorder? *Acta Pharmacol. Sin.* 39 (12), 1823-1829.
- Luján, M.A., Castro-Zavala, A., Alegre-Zurano, L., Valverde, O., 2018. Repeated Cannabidiol treatment reduces cocaine intake and modulates neural proliferation and CB1R expression in the mouse hippocampus. *Neuropharmacology* 143, 163-175.
- Mahmud, A., Gallant, S., Sedki, F., D'Cunha, T., Shalev, U., 2017. Effects of an acute cannabidiol treatment on cocaine self-administration and cue-induced cocaine seeking in male rats. *J. Psychopharmacol.* 31 (1), 96-104.
- Markou, A., 2009. Accruing preclinical evidence about metabotropic glutamate 5 receptor antagonists as treatments for drug dependence highlights the irreplaceable contributions of animal studies to the discovery of new medications for human disorders. *Neuropsychopharmacology* 34 (4), 817-819.
- Marsden, C.A., 2006. Dopamine: the rewarding years. *Br. J. Pharmacol.* 147, S136-S144.
- Martín, A.B., Fernandez-Espejo, E., Ferrer, B., Gorriti, M.A., Bilbao, A., Navarro, M., Rodriguez de Fonseca, F., Moratalla, R., 2008. Expression and function of CB1 receptor in the rat striatum: localization and effects on D1 and D2 dopamine receptor-mediated motor behaviors. *Neuropsychopharmacology* 33 (7), 1667-1679.
- Martin-Santos, R., Crippa, J.A., Batalla, A., Bhattacharyal, S., Atakan, Z., Borgwardt, S., Allen, P., Seal, M., Langohr, K., Farré, M., Zuardi, A.W., Mc Guire, P.K., 2012. Acute effects of a single, oral dose of d9-tetrahydrocannabinol (THC) and cannabidiol (CBD) administration in healthy volunteers. *Curr. Pharm. Des.* 18, 4966-4979.
- Mechoulam, R., Parker, L.A., Gallily, R., 2002. Cannabidiol: an overview of some pharmacological aspects. *J. Clin. Pharmacol.* 42, 115-195.
- Metz, V.G., Segat, H.J., Dias, V.T., Barcelos, R., Maurer, L.H., Stiebe, J., Emanuelli, T., Burger, M.E., Pase, C.S., 2019. Omega-3 decreases D1 and D2 receptors expression in the prefrontal cortex and prevents amphetamine-induced conditioned place preference in rats. *J. Nutr. Biochem.* 67, 182-189.
- Montgomery, K.C., 1955. The relation between fear induced by novel stimulation and exploratory behavior. *J. Comp. Physiol. Psychol.* 48 (4), 254-260.
- Moratalla, R., Khairnar, A., Simola, N., Granado, N., García-Montes, J.R., Porceddu, P.F., Tizabi, Y., Costa, G., Morelli, M., 2017. Amphetamine-related drugs neurotoxicity in humans and in experimental animals: main mechanisms. *Prog. Neurobiol.* 155, 149-170.
- Murillo-Rodríguez, E., Palomero-Rivero, M., Millán-Aldaco, D., Mechoulam, R., Drucker-Colin, R., 2011. Effects on sleep and dopamine levels of microdialysis perfusion of cannabidiol into the lateral hypothalamus of rats. *Life Sci.* 88, 504-511.
- Norris, C., Loureiro, M., Kramar, C., Zunder, J., Renard, J., Rushlow, W., Laviolette, S.R., 2016. Cannabidiol modulates fear

- memory formation through interactions with serotonergic transmission in the mesolimbic system. *Neuropsychopharmacology* 41 (12), 2839–2850.
- Olière, S., Joliette-Riopel, A., Potvin, S., Jutras-Aswad, D., 2013. Modulation of the endocannabinoid system: vulnerability factor and new treatment target for stimulant addiction. *Front. Psychiatry* 4, 109.
- Paxinos, G., Watson, C., 2007. *The Rat Brain in Stereotaxic Coordinates*, 6th ed. Ne Academic Press, New York.
- Ren, Y., Whittard, J., Higuera-Matas, A., Morris, C.V., Hurd, Y.L., 2009a. Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. *J. Neurosci.* 29 (47), 14764–14769.
- Renard, J., Norris, C., Rushlow, W., Laviolette, S.R., 2017. Neuronal and molecular effects of cannabidiol on the mesolimbic dopamine system: implications for novel schizophrenia treatments. *Neurosci. Biobehav. Rev.* 75, 157–165.
- Raineri, M., Peskin, V., Goitia, B., Taravini, I.R., Giorgeri, S., Urbano, F.J., Bisagno, V., 2011. Attenuated methamphetamine induced neurotoxicity by modafinil administration in mice. *Synapse* 65 (10), 1087–1098.
- Raineri, M., Gonzalez, B., Goitia, B., Garcia-Rill, E., Krasnova, I.N., Cadet, J.L., Urbano, F.J., Bisagno, V., 2012. Modafinil abrogates methamphetamine-induced neuroinflammation and apoptotic effects in the mouse striatum. *PLoS One* 7 (10), e46599.
- Ren, Y., Whittard, J., Higuera-Matas, A., Morris, C.V., Hurd, Y.L., 2009b. Cannabidiol, a non psychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. *J. Neurosci.* 29 (47), 14764–14769.
- Renard, J., Loureiro, M., Rosen, L.G., Zunder, J., de Oliveira, C., Schmid, S., Rushlow, W.J., Laviolette, S.R., 2016. Cannabidiol counteracts amphetamine-induced neuronal and behavioral sensitization of the mesolimbic dopamine pathway through a novel mTOR/p70S6 kinase signaling pathway. *J. Neurosci.* 36 (18), 5160–5169.
- Rodríguez de Fonseca, F., Martín Calderón, J.L., Mechoulam, R., Navarro, M., 1994. Repeated stimulation of D1 dopamine receptors enhances (-)-11-hydroxy-delta 8-tetrahydrocannabinol-dimethyl-heptyl-induced catalepsy in male rats. *NeuroReports* 5 (7), 761–765.
- Russo, E.B., Burnett, A., Hall, B., Parker, K.K., 2005. Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem. Res.* 30, 1037–1043.
- Ryberg, E., Larsson, N., Sjögren, S., Hjorth, S., Hermansson, N.O., Leonova, J., Elebring, T., Nilsson, K., Drmotal, T., Greasley, P.J., 2007. The orphan receptor GPR55 is a novel cannabinoid receptor. *Br. J. Pharmacol.* 152 (7), 1092–1101.
- Sadeghzadeh, F., Babapour, V., Haghparast, A., 2015. Role of dopamine D1-like receptor within the nucleus accumbens in acute food deprivation- and drug priming-induced reinstatement of morphine seeking in rats. *Behav. Brain Res.* 287, 172–181.
- Segat, H.J., Kronbauer, M., Roversi, Kr., Schuster, A.J., Vey, L.T., Roversi, K., Pase, C.S., Antoniazzi, C.T.D., Burger, M.E., 2014. Exercise modifies amphetamine relapse: behavioral and oxidative markers in rats. *Behav. Brain Res.* 262, 94–100.
- Segat, H.J., Martini, F., Barcelos, R.C., Brüning, C.A., Nogueira, C.W., Burger, M.E., 2016. m-Trifluoromethyl-diphenylselenide as a pharmacological tool to treat preference symptoms related to AMPH-induced dependence in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 66, 1–7.
- Segat, H.J., Barcelos, R.C.S., Metz, V.G., Rosa, H.G., Roversi, Kr., Antoniazzi, C.T.D., Vey, L.T., Kronbauer, M., Veit, J.C., Piccolo, J.C., Emanuelli, T., Burger, M.E., 2017. Influence of physical activity on addiction parameters of rats exposed to amphetamine which were previously supplemented with hydrogenated vegetable fat. *Brain Res. Bul.* 135, 69–76.
- Segat, H.J., Metz, V.G., Rosa, H.Z., Dias, V.T., Barcelos, R., Dolci, G.S., Burger, M.E., 2018. Substitution therapy with amphetamine-isotherapeutic attenuates amphetamine toxicological aspects of addiction. *Neurosci. Lett.* 690, 138–144.
- Sestakova, N., Puzserova, A., Kluknavsky, M., Bernatova, I., 2013. Determination of motor activity and anxiety-related behaviour in rodents: methodological aspects and role of nitric oxide. *Interdiscip. Toxicol.* 6 (3), 126–135.
- Shaham, Y., Shalev, U., Lu, L., de Wit, H., Stewart, J., 2003. The reinstatement model of drug relapse: history, methodology and major findings. *Psychopharmacology* 168 (1–2), 3–20.
- Takamatsu, Y., Yamamoto, H., Hagino, Y., Markou, A., Ikeda, K., 2011. The selective serotonin reuptake inhibitor paroxetine, but not fluvoxamine, decreases methamphetamine conditioned place preference in mice. *Curr. Neuropharmacol.* 9 (1), 68–72.
- Teixeira-Gomes, A., Costa, V.M., Feio-Azevedo, R., Bastos, M., Carvalho, F., Capela, J.P., 2015. The neurotoxicity of amphetamines during the adolescent period. *Int. J. Dev. Neurosci.* 41, 44–62.
- Tham, M., Yilmaz, O., Alaverdashvili, M., Kelly, M., Denovan-Wright, E.M., Laprairie, R.B., 2019. Allosteric and orthosteric pharmacology of cannabidiol and cannabidiol-dimethylheptyl at the type 1 and type 2 cannabinoid receptors. *Br. J. Pharmacol.* 176 (10), 1455–1469.
- UNODC - United Nations Office for Drug Control and Crime Prevention. *World Drug Report, 2020.*
- Vasquez, V., Penit-Soria, J., Durand, C., Besson, M.J., Giros, B., Daugé, V., 2006. Brief early handling increases morphine dependence in adult rats. *Behav. Brain Res.* 170, 211–218.
- Viudez-Martínez, A., García-Gutiérrez, M.S., Medrano-Relinque, J., Navarrón, C.M., Navarrete, F., Manzanares, J., 2019. Cannabidiol does not display drug abuse potential in mice behavior. *Acta Pharmacol. Sin.* 40 (3), 358–364.
- Viudez-Martínez, A., García-Gutiérrez, M.S., Manzanares, J., 2020. Gender differences in the effects of cannabidiol on ethanol binge drinking in mice. *Addict. Biol.* 25 (3), e12765.
- Volz, T.J., Hanson, G.R., Fleckenstein, A.E., 2007. The role of the plasmalemmal dopamine and vesicular monoamine transporters in methamphetamine-induced dopaminergic deficits. *J. Neurochem.* 101, 883–888.
- Vuong, S.M., Oliver, H.A., Scholl, J.L., Oliver, K.M., Forster, G.L., 2010. Increased anxiety-like behavior of rats during amphetamine withdrawal is reversed by CRF2 receptor antagonism. *Behav. Brain Res.* 208 (1), 278–281.
- Watt, G., Karl, T., 2017. In vivo evidence for therapeutic properties of cannabidiol (CBD) for Alzheimer's disease. *Front. Pharmacol.* 8, 20.
- Young, J.W., Geyer, M.A., 2010. Action of modafinil—increased motivation via the dopamine transporter inhibition and D1 receptors? *Biol. Psychiatry* 67, 784–787.
- Zanelati, T.V., Biojone, C., Moreira, F.A., Guimarães, F.S., Joca, S.R., 2010. Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. *Br. J. Pharmacol.* 159 (1), 122–128.
- Zlebnik, N.E., Cheer, J.F., 2016. Drug-induced alterations of endocannabinoid-mediated plasticity in brain reward regions. *J. Neurosci.* 36 (40), 10230–10238.

5.2 MANUSCRITO CIENTÍFICO

Cannabidiol treatment prevents drug reinstatement and the molecular alterations evoked by amphetamine on receptors and enzymes from dopaminergic and endocannabinoid systems in rats

Vinícia Garzella Metz, Jéssica Leandra Oliveira da Rosa, Domenika Rubert Rossato, Marilise Escobar Burger, Camila Simonetti Pase

Periódico: European Neuropsychopharmacology

Status: Submetido para Publicação

Cannabidiol treatment prevents drug reinstatement and the molecular alterations evoked by amphetamine on receptors and enzymes from dopaminergic and endocannabinoid systems in rats

Vinícia Garzella Metz^a, Jéssica Leandra Oliveira da Rosa^a, Domenika Rubert Rossato^a, Marilise Escobar Burger^a, Camila Simonetti Pase^{ab*}

^aPrograma de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, RS, Brazil.

^bUniversidade Federal do Pampa, Campus Uruguaiana, RS, Brazil.

*Corresponding author:

Camila Simonetti Pase, Ph.D.
Universidade Federal do Pampa
97500-970, Uruguaiana, RS, Brazil
Tel. +55-55-3413-4321
E-mail: camilapase@unipampa.edu.br

Vinícia Garzella Metz, M.Sc.
Physiology and Pharmacology Department
Health Sciences Center
Graduation Program of Pharmacology
97105-900, Santa Maria, RS, Brazil
Tel. +55-55-3220-8676
E-mail: vinicia_metz@hotmail.com

Metz, VG: vinicia_metz@hotmail.com
da Rosa, JLO: gessica10leandra@hotmail.com
Rossato, DR: domenikarossato@outlook.com
Burger, ME: marilise.burger@ufsm.br
Pase, CS: camilapase@unipampa.edu.br

Abstract

In psychostimulant drug addiction, relapse is the most concerning outcome to be managed, considering there is no approved treatment for this neuropsychiatric condition. Here, we investigated the effects of the CBD treatment on the relapse behavior triggered by stress, after being submitted to the amphetamine (AMPH)-induced conditioned place preference (CPP) in rats. To elucidate the mechanisms of action underlying the CBD treatment, we evaluated the neuroadaptations on dopaminergic and endocannabinoid targets in the ventral striatum (VS) and ventral tegmental area (VTA) of the brain. Animals received d,l-AMPH (4 mg/kg, i.p.) or vehicle in the CPP paradigm for 8 days. Following the first CPP test, animals were treated with CBD (10 mg/kg, i.p) or its vehicle for 5 days and subsequently submitted to forced swim stress protocol to induce AMPH-CPP relapse. Behavioral findings showed that CBD treatment prevented AMPH-reinstatement, also exerting anxiolytic activity. At the molecular level, in the VTA, CBD restored the CB1R levels decreased by AMPH-exposure, increased NAPE-PLD, and decreased FAAH levels. In the VS, the increase of D1R and D2R, as well as the decrease of DAT levels induced by AMPH were restored by CBD treatment. The current outcomes evidence a substantial preventive action of the CBD on the AMPH-reinstatement evoked by stress, also involving neuroadaptations in both dopaminergic and endocannabinoid systems in brain areas closely involved in the addiction. Although further studies are needed, these findings support the therapeutic potential of CBD in AMPH-relapse prevention.

Keywords: Addiction; Conditioned place preference; Psychostimulants; Ventral striatum; Ventral tegmental area.

1 Introduction

Drug addiction is a chronic disorder characterized by compulsive seeking and loss of control over substance use despite serious negative consequences ensue (Koob and Volkow, 2009). Amphetamine-type psychostimulant drugs, such as amphetamine (AMPH), 3,4-methylenedioxymethamphetamine (MDMA), and methamphetamine (METH) are highly

addictive, and therefore widely abused (UNODC, 2020). In recent decades, the development of new treatments for psychostimulant addiction has been the focus of several studies. However, no specific pharmacological therapy had shown effectiveness in alleviating abstinence symptoms (e.g. craving and anxiety) and preventing relapse (Cao et al., 2016).

The mesocorticolimbic system, also known as the reward system, plays an important role in drug addiction. The use of drugs such as AMPH increases dopaminergic neurotransmission in this pathway (Maldonado, 2003) being able to activate reward symptoms, and this response is related to the drug rewarding effect (Di Chiara et al., 2004). Furthermore, repeated exposure to these substances can cause transient and persistent adaptations in brain areas related to the mesocorticolimbic system (Robinson and Berridge, 2003) and the adaptive changes seem to corroborate for compulsive and uncontrolled use of drugs, as well as for the relapse occurrence (Chou and Narasimham, 2005).

Although the dopaminergic system has been considered the most important system involved in brain reward processes for decades (Liu and Li, 2018), recent evidence suggests that the endocannabinoid system (ECS) also has a fundamental role in the signaling of rewarding events (Manzanares et al., 2018; Olière et al., 2013). Endocannabinoids (eCBs) and cannabinoid receptors are extensively expressed in brain areas of the mesocorticolimbic system and it is believed that they can modulate the dopaminergic signaling in this pathway (Everett et al., 2021; Lupica et al., 2004; Maldonado and Rodriguez de Fonseca, 2002; Melis et al., 2004). The eCBs also seem to participate in the mesocorticolimbic system synaptic plasticity, which is required for the development of the adaptive changes, leading to drug addiction (Gerdeman et al., 2002; Robbe et al., 2002). Also, neuroadaptations in the endocannabinoid system after chronic exposure to drugs were found (Parsons and Hurd, 2015) and the modulation of this system has been pointed as a potential target for the development of strategies for drug addiction treatment.

Cannabidiol (CBD) is a phytocannabinoid constituent of the *Cannabis sativa* plant devoid of addictive effects (Mechoulan et al., 2002; Viudez-Martínez et al., 2019). CBD has multiple mechanisms of action, and its pharmacology is not completely understood. It acts on several receptors and systems including the ECS (Pertwee, 2008), serotonergic (Campos and Guimarães, 2008; Russo et al., 2005), and opioid (Kathmann et al., 2006) systems, among others (see review: Bonaccorso et al., 2019). Within the ECS, CBD has a low affinity for cannabinoid type 1 (CB1R) and 2 (CB2R) receptors (Zlebnik and Cheer, 2016), although exerting a negative modulation effect on both receptors (Ligresti et al., 2016). Furthermore,

CBD decreases hydrolysis of the endocannabinoid anandamide (Arachidonylethanolamine, AEA) mediated by fatty acid amide hydrolase (FAAH) (Ligresti et al, 2016; Campos et al., 2012). Recently, CBD emerged as a promising compound and a new pharmacological strategy for a wide range of disorders (Ligresti et al., 2016), with both human and pre-clinical studies showing its potential as a therapeutic tool in the treatment of various psychiatric diseases including anxiety, depression, and psychosis (Bonaccorso et al., 2019; Campos et al., 2012). Currently, CBD has drawn the scientific community's attention due to its possible beneficial effects on substance use disorders treatment (see review: Chye et al., 2019).

Pre-clinical studies investigating the CBD use on animal models of addiction showed possibilities to reduce the behavioral and molecular manifestations of maladaptive neuroplasticity underlying drug addiction. For instance, CBD reduced the consumption and relapse to ethanol (Viudez-Martínez et al., 2017), craving and relapse to heroin (Ren et al., 2009) as well as reward and withdrawal symptoms to morphine (Bhargava, 1976; Katsidoni et al., 2013). In addition, CBD administration reduced the intake (Weiss and Gonzales-Cuevas, 2019) and prevented reinstatement of cocaine-seeking (Gonzales-Cuevas et al., 2018). Current evidence, including studies from our research group, has demonstrated potential anti-relapse effects of CBD on amphetamine addiction (Karimi-Haghighi and Haghparast, 2018; Karimi-Haghighi et al., 2020; Metz et al., 2021). Nonetheless, the information available on the possible neural mechanisms by which CBD acts and reduces drug use behaviors are still very scarce. The explanation regarding these processes would provide a consistent understanding of existing data and could consolidate the promise of CBD as a treatment for drug addiction.

In order to expand upon this research line, the current study aimed to evaluate the behavioral effects of CBD treatment on the stress-induced reinstatement of AMPH-CPP. Moreover, to explore a possible mechanism underlying the CBD effects, we also evaluated the molecular modifications on the dopaminergic and endocannabinoid targets in the rats' brain areas of reward.

2 Materials and Methods

2.1 Animals

Twenty-four male *Wistar* rats (Universidade Federal de Santa Maria - UFSM, RS, Brazil) weighing 120-150g were used for this study. The animals were 40-days old, which is

considered by many studies as the adolescence period in rodents, and a highly vulnerable period for developing drug addiction (Teixeira-Gomes et al., 2015). The animals were kept in Plexiglas cages with free access to water and food in a room with controlled temperature (22 ± 1 °C) and on a 12-h light/dark cycle. All procedures were performed according to the Animal Ethics Committee (UFMS-8850121118) guidelines, affiliated to the National Council for the Control of Animal Experimentations (CONCEA), following international standards of care and animal maintenance. The importance of current animal experiments in brain research is described in Homberg et al. (2021).

2.2 Drugs and solutions

The d, l-amphetamine (AMPH; Merck, Germany) ($4 \text{ mg} \cdot \text{mL}^{-1} / \text{kg}$) was dissolved in saline (0.9% NaCl; AMPH-vehicle) and was used to induce conditioned place preference (CPP). The chosen dose and time for the conditioning period were standardized in the AMPH-CPP and used in previous studies from our research group (Metz et al., 2019; 2021; Segat et al., 2016; 2017). Cannabidiol (CBD; STI Pharmaceuticals, UK) ($10 \text{ mg} \cdot \text{mL}^{-1} / \text{kg}$) was dissolved in a solution of 2% polysorbate 80 (Tween 80; Sigma-Aldrich, St Louis, MO, USA) and saline. CBD dose was based on a previously published study from our research group (Metz et al., 2021). CBD was administered for 5 days, considering the extinction period of the AMPH preference, which, according to our previous study, can be observed 4 days after the last AMPH exposure (Metz et al., 2021). Animals received CBD 30 minutes before the extinction test (Mahmud et al., 2017). All drugs were intraperitoneally (i.p.) injected at a volume of 1 ml/kg of body weight.

2.3 Experimental protocol

At the postnatal day (PND) 40, the rats ($n=24$) were assigned to two experimental groups: vehicle (received saline – 0.9% NaCl solution, i.p., $n=12$) and amphetamine (AMPH) (received AMPH, 4 mg/kg , i.p., $n=12$) and were exposed to the conditioned place preference (CPP) protocol (as described below). Following the first CPP test, which was performed to verify AMPH preference, half of each experimental group received the cannabidiol treatment (CBD, 10 mg/kg , i.p) or control (saline and 2% polysorbate 80, i.p) once a day for five consecutive days. This resulted in four final groups ($n=6$): i) vehicle/control; ii) vehicle/CBD; iii) AMPH/control; v) AMPH/CBD. During the CBD treatment period, additional CPP tests

were performed to assess the loss of preference for the drug. Twenty-four hours after the last treatment, animals were submitted to forced swim stress protocol to induce the reinstatement of extinguished AMPH-CPP (as described below). Then, in sequence, a CPP test was conducted. Moreover, for assessing the locomotor activity and anxiety-like symptoms, the animals were tested on the open field and elevated plus-maze tests, respectively. On the next day, the rats were anesthetized (isoflurane, the dose to the effect), euthanized by decapitation, the brains were extracted and the ventral striatum (VS) and ventral tegmental area (VTA) were dissected according to Paxinos and Watson (2007) for molecular analysis (Figure 1).

2.4 Behavioral assessments

2.4.1 Conditioned place preference (CPP)

The CPP apparatus consists of a box with two compartments of equal size ($45 \times 45 \times 50$ cm) and an equivalent intensity of light incidence but having different environmental stimuli: one compartment has a white floor and striped walls, and the other one has a striped floor and smooth white walls. Both compartments were accessible through a central compartment ($18 \times 36 \times 50$ cm) separated by manual guillotine doors.

Initially, on the post-natal day (PND) 40, the animals were placed for 15 min in each compartment, except the neutral compartment, for habituation. The purpose of this procedure was to exclude exploratory behavior that is common in new environments to avoid misinterpretations.

One day after habituation (PND 41), the pre-test was performed. This phase consists of placing the animal into the central compartment allowing free access to the entire apparatus for 15 min and the time spent in each compartment was recorded. This procedure aimed to determine the innate environmental chamber preference. Rats that showed strong unconditioned aversion (less than 25% of the session time) or preference (more than 75% of the session time) for any compartment were discarded (Vasquez et al., 2006).

Following this, the conditioning phase was carried out for 8 days (PND 42–49). The animals received AMPH and were conditioned for 25 min in the compartment they spent the shortest time in the pre-test (Carlezon et al., 2002). After a 4-hour interval, they received the vehicle (0.9% NaCl) and were confined in the opposite compartment. Vehicle-treated groups received two daily injections of NaCl solution in both compartments of the CPP in alternated

turns. On PND 50, the CPP test was conducted (without drug/vehicle administration). The time spent in each compartment was assessed for 15 min and results express the percentage of time spent by the animals in the compartment associated with AMPH during the abstinence period (Metz et al., 2019; 2021; Segat et al., 2016).

CBD treatment was performed from PND 51 to PND 55. Additional CPP tests were assessed during this phase to evaluate the drug preference extinction, which was observed on the fourth day (PND54) of CBD treatment.

After the treatment period (PND 56), animals were submitted to forced swim stress (FSS) protocol to induce the reinstatement of preference for AMPH (Hagharast et al., 2014; Mikail et al., 2012). The forced swim stress (FSS) apparatus consists of a plastic cylindrical tank measuring 50 cm height, 30 cm width, which was filled up with 30 cm of clean tap water (23–27 °C). The rats performed individually a 6-min forced swim. After, they were dried with towels and returned to their home cages for at least 10 min before the reinstatement test (Hagharast et al., 2014; Mikail et al., 2012). Next, an additional CPP test was performed to assess relapse. Symptoms of reinstatement preference were quantified by the longer time spent in the drug-paired environment after exposure to the FSS protocol.

2.4.2 *Open field (OF) test*

Animals were individually placed in the center of an OF arena (40 × 40 × 30 cm) enclosed by black matte walls and floor divided into squares, as described by Kerr et al. (2005). The number of crossings (horizontal squares crossed) was quantified for 5 min and used to measure the locomotor activity.

2.4.3 *Elevated plus maze (EPM) test*

The anxiety-like behavior was assessed in the elevated plus-maze (EPM) paradigm. The apparatus consists of a platform elevated 50 cm above the floor. Forty-centimeter-high walls enclose two opposite arms (50 × 10 cm) while the other two arms had no walls. All arms are connected by a central intersection (10 × 10 cm). The time spent in the closed arms (sec), the time spent in the open arms (sec), the total arm entries (number), the frequency of head-dipping (number), and anxiety index (calculated by the following formula) were evaluated for 5 min (Montgomery, 1955):

$$\text{Anxiety index} = 1 - \left(\left(\frac{\text{Open arms time}}{\text{Total time}} \right) + \left(\frac{\text{Open arms entries}}{\text{Total entries}} \right) \right) / 2$$

Anxiety index values range from 0 to 1, where an increase in the index expresses increased anxiety-like behavior (Cohen et al., 2012).

2.5 Molecular assays

Molecular analyses were performed by western blot as described by Dias et al. (2017). Briefly, the membranes were incubated with the primary antibodies (Santa Cruz Biotechnology): anti-dopamine D1 receptor (D1R) (1:500, sc-33660), anti-dopamine D2 receptor (D2R) (1:500, sc-9113), anti-dopamine transporter (DAT) (1:500, sc-14002), anti-cannabinoid CB1 receptor (CB1R) (1:250, sc-293419), anti-diacylglycerol lipase α (DAGL α) (1:500, sc-390409), anti-monoacylglycerol lipase (MAGL) (1:500, sc-398942), anti-N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) (1:500, sc-514372), anti-fatty acid amide hydrolase (FAAH) (1:250, sc-100739), anti- β -actin (1:50000; Sigma-Aldrich, St. Louis, USA), followed by the appropriate secondary antibody (Santa Cruz Biotechnology) IgG horseradish peroxidase conjugate. Actin was used as an internal control and data were standardized according to its values.

2.6 Statistical analysis

All the results from behavioral, biochemical, and molecular assays were expressed as the mean \pm standard error of the mean (S.E.M.). CPP data before CBD treatment were analyzed by Student's *t*-test. Repeated-measures ANOVA was performed for the analysis of the extinction period and reinstatement test and Newman–Keuls post-hoc was used to obtain differences between the different treatment groups. For other analyses, two-way ANOVA followed by Newman–Keuls test was performed using Statistic® (version 7.0). Values of *P* < 0.05 were considered statistically significant for all comparisons made. GraphPad Prism® (version 6.0) was used to design the figures.

3 Results

3.1 CBD treatment prevented stress-induced reinstatement of AMPH-CPP.

Behavioral assessment performed before starting CBD treatment (1st CPP test) showed that AMPH-conditioned rats remained a longer time in the drug-conditioned place in comparison to the vehicle group ($P < 0.05$), which showed no place preference (Fig. 2a).

Two-way ANOVA for repeated measures revealed a significant main effect of the drug, treatment and time on the drug preference [$F(1, 20) = 152.79, P < 0.001$; $F(1, 20) = 4.83, P < 0.05$; $F(1, 20) = 12.45, P < 0.001$, respectively]. Also, two-way ANOVA for repeated measures revealed a significant interaction of time with drug [$F(1, 20) = 14.72, P < 0.001$], treatment [$F(1, 20) = 7.28, P < 0.001$], and drug \times treatment [$F(1, 20) = 3.93, P < 0.001$].

Post hoc analysis showed that there was a significant decrease of drug preference on the 4th day of extinction compared to the 1st day, indicating the extinction of AMPH-CPP. As represented in Fig. 2b, AMPH-CPP showed no difference among experimental groups in the extinction test, thus confirming the drug preference extinction on the 4th day after the last AMPH administration. The drug preference in the reinstatement test was significantly higher compared to the extinction test, indicating the reinstatement of CPP. In addition, post-hoc comparison showed that CBD treatment was able to prevent stress-induced reinstatement of AMPH-CPP.

3.2 AMPH exposure and CBD treatment did not affect locomotor activity in the open field (OF) task.

After stress-induced reinstatement of AMPH-CPP, the locomotor activity observed in the OF task showed no difference among the experimental groups (Fig. 3).

3.3 CBD treatment reduced anxiety parameters assessed in the elevated plus-maze (EPM) test.

Two-way ANOVA of the EPM revealed a significant main effect of the drug, treatment, and treatment \times drug interaction on the time spent in the closed arms [$F(1, 20) = 8.41, 46.27$ and $10.91, P < 0.05$, respectively] and on the time spent in the open arms [$F(1, 20) = 5.00, 427.72$; and $28.11, P < 0.05$, respectively]. Two way ANOVA of the EPM also revealed a significant main effect of treatment and treatment \times drug interaction on the head dipping frequency [$F(1, 20) = 8.07, 4.78, P < 0.05$, respectively] and on the anxiety index [$F(1, 20) = 41.49$ and $5.15, P < 0.05$, respectively].

Newman-Keuls test revealed that CBD treatment *per se* decreased the time spent in the closed arms and increased the time spent in the open arms of the EPM (Fig. 4a and 4b). On the other hand, AMPH *per se* increased the time spent in the closed arms, decreased time spent and head dipping frequency in the EPM open arms when compared to the vehicle-injected group (Fig. 4a, 4b, and 4d). However, AMPH-exposed animals that were subsequently treated with CBD showed decreased time spent in the closed arms and an increase in the time spent and head dipping frequency in the open arms (Fig. 4a, 4b, and 4d). There was no statistical difference among experimental groups on the total number of entries in both arms in the EPM test (Fig. 4c). Furthermore, CBD treatment reduced the anxiety index in both vehicle and AMPH-conditioned groups (Fig. 4e).

3.4 CBD treatment modified the dopamine-receptor (D1R and D2R) and -transporter (DAT) immunoreactivity in the ventral striatum (VS).

Two-way ANOVA of dopaminergic markers revealed a significant main effect of the drug, treatment, and treatment x drug interaction on D1R level [$F(1, 20) = 17.92$; 11.08 and 13.24, $P < 0.05$, respectively], as well as a significant main effect of drug and treatment on D2R level [$F(1, 20) = 6.53$ and 4.76, $P < 0.05$, respectively] and a significant main effect of the drug on DAT level [$F(1, 20) = 8.55$, $P < 0.05$].

Newman-Keuls test showed that AMPH exposure increased both D1R (Fig. 5a) and D2R (Fig. 5b) immunoreactivity in the VS when compared to the vehicle group. In contrast, AMPH-conditioned animals further treated with CBD did not show this increase induced by AMPH (Fig. 5a and 5b). In addition, AMPH exposure was able to reduce DAT levels when compared to vehicle, but the immunoreactivity of this transporter was preserved by CBD treatment in the AMPH-exposed rats (Fig. 5c).

3.5 CBD treatment modified cannabinoid receptor type 1 (CB1R), as well as the enzymes diacylglycerol lipase α (DAGL), monoacylglycerol lipase (MAGL), N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD), and fatty acid amide hydrolase (FAAH) immunoreactivity in the ventral tegmental area (VTA).

Two-way ANOVA revealed a significant main effect of the drug, treatment and treatment \times drug interaction on DAGL levels [$F(1, 20) = 26.84$; 281.47 and 10.55, $P < 0.05$,

respectively] and a significant main effect of the treatment on CB1R, MAGL and FAAH immunoreactivity [$F(1, 20) = 14.95; 20.99$ and $26.84, P < 0.05$, respectively]. Two-way ANOVA also revealed a significant main effect of both treatment and treatment \times drug interaction on NAPE-PLD immunocontent [$F(1,20) = 480.74, P < 0.05; F(1,20) = 18.92, P < 0.05$, respectively].

Neuman-Keuls test revealed that AMPH *per se* decreased CB1R and NAPE-PLD levels (Fig. 6a and 6d), increasing FAAH levels (Fig. 6e) when compared to the vehicle group. CBD treatment *per se* increased DAGL and NAPE-PLD levels (Fig. 6b and 6d) and decreased MAGL and FAAH immunoreactivity (Fig. 6c and 6e). In animals exposed to AMPH, CBD treatment was able to restore CB1R levels (Fig. 6a), which were decreased by AMPH exposure. In addition, CBD treatment increased DAGL and NAPE-PLD immunocontent in comparison to both AMPH-conditioned and vehicle-treated groups (Fig. 6b and 6d) and decreased MAGL and FAAH levels in comparison to AMPH-conditioned animals (Fig. 6c and 6e).

4 Discussion

Here we investigated the effects of cannabidiol (CBD) treatment on stress-induced reinstatement of amphetamine (AMPH) conditioned place preference (CPP) in rats. Our findings showed that: i) CBD exerted relevant benefits on the animal behavior, being able to prevent AMPH-CPP reinstatement induced by a stressor. ii) CBD presented promising effects in relapse-promoting conditions, such as anxiety. iii) CBD can interact and stimulate significant changes in dopaminergic and endocannabinoid targets in both ventral striatum (VS) and ventral tegmental area (VTA). These brain areas are closely involved in addiction processes, where CBD promoted maintenance of the cellular homeostasis even after AMPH exposure. Taken together, these observations provide evidence that supports a therapeutic potential for CBD, especially when the addiction is associated with AMPH-type psychostimulants.

CBD treatment effects on AMPH reinstatement behavior

In the current study, AMPH-exposed animals showed drug-reinstatement behavior in the CPP paradigm after exposure to forced swimming stress while CBD treatment prevented AMPH-CPP reinstatement induced by this stressor. This potential anti-reinstatement effect demonstrated by CBD treatment is consistent and reinforces our previous data when CBD administration prevented drug relapse induced by AMPH re-exposure (Metz et al., 2021). Also,

these outcomes confirm other findings, showing that CBD is also effective in preventing relapse by other AMPH-type psychostimulants drugs in different experimental models (Hay et al., 2018; Karimi-Haghighi et al., 2020; Metz et al., 2021). Taken together, these studies report the therapeutic potential of the CBD to treat psychostimulants drug addiction, thus reiterating the importance of the continuance and advances in scientific research in this regard. Furthermore, to validate the experimental model, we evaluated the locomotor activity of the animals in the open field (OF) task, which results showed no differences in locomotor behavior among the experimental groups. This data supports the assertion that the behavioral results found in the CPP test are indeed due to the effects underlying the treatment with CBD and are not related to locomotor artifacts (Metz et al., 2019; Segat et al., 2016; Takamatsu et al., 2011).

Anxiolytic properties of CBD and its impact on AMPH reinstatement

In humans and rodents, anxiety is an important factor involved in establishing and maintaining drug addiction, including AMPH. It was reported that 30–40% of drug-addicted individuals also suffer from depression or anxiety disorder (Conway et al., 2006). After a prolonged exposure and subsequent detoxification, anxiety can be one of the main elements that trigger drug relapse (Biala et al., 2009). Here, we observed that AMPH administration followed by exposure to a stressor stimulus triggered anxiogenic behaviors in the animals when they were subjected to the EPM task. Our findings are in agreement with previous experimental studies showing AMPH-induced anxiety-like behaviors (Barr et al., 2010; Metz et al., 2021; Vuong et al., 2010). Moreover, clinical and preclinical evidence has supported the anxiolytic effects of CBD, especially as a treatment for anxiety disorders (see review: Blessing et al., 2015). Our current outcomes also showed that CBD treatment was able to prevent anxiety-like behaviors in AMPH-exposed animals, but considering this anxiolytic effect was also observed in the animals not exposed to AMPH, it allowed us to attribute the anxiolytic activity to CBD *per se*. These findings are compatible with previous findings from our research group, when CBD showed anxiolytic properties *per se*, but also prevented anxiety-like behaviors in AMPH-reconditioned animals (Metz et al., 2021). Other researchers also evidenced that CBD produced anti-anxiety-like effects in rats in the context of cocaine and crack addiction (Gonzalez-Cuevas et al., 2018; Luján et al., 2018). We hypothesized that the anxiolytic properties of CBD can contribute, at least in part, to its anti-reinstatement effect, since anxiety symptoms substantially contribute to the relapse occurrence.

CBD effects on maladaptive dopaminergic molecular changes underlying AMPH exposure

In addition to the behavioral effects previously discussed, the current study also evidenced the molecular changes underlying AMPH exposure. We believe that these neural adaptations are closely related to the behavior modifications also observed in the animals. The immunoreactivity of dopaminergic targets in the VS showed that AMPH-conditioning followed by the exposure to forced swimming stress triggered the increase of dopaminergic receptors 1 (D1R) and 2 (D2R) levels in this brain region when compared to the group not exposed to the drug. D1R- and D2R are recognized for playing pivotal roles in the addiction processes as they mediate relapse and drug-seeking behavior mechanisms (Hansen et al., 2002). Besides us, other authors have recently documented a relation between the overstimulation of D1R and D2R induced by AMPH and addictive behaviors (Metz et al., 2019; 2021; Moratalla et al., 2017), supporting the outcomes shown here. Possibly the overstimulation of these receptors, which occurs due to the high levels of extracellular DA available after the AMPH administration, can cause neuroadaptations and persistent modifications in their immunoreactivity. Also, we showed the impact of AMPH exposure on DA transporter (DAT) levels. As seen in previous studies (Ares-Santos et al., 2012; Granado et al., 2012; Gou et al., 2015), exposure to AMPH triggered a decrease in DAT immunoreactivity in the VS. Likewise, DAT is a relevant dopaminergic target in AMPH addiction, once this transporter is responsible for both intracellular and extracellular DA levels maintenance (Raineri et al., 2011; 2012), whereas, AMPH acts blocking this primary DAT function (Volz et al., 2007). Considering our current outcomes, CBD treatment preserved this dopaminergic target in the animals that were previously exposed to AMPH. This finding is consistent as we found similar results in an earlier study (Metz et al., 2021). We speculate that this maintenance of dopaminergic targets and possible restoration of dopaminergic tone could be behind the beneficial behavioral effects of CBD during AMPH reinstatement. In this sense, an important study by Renard et al. (2016) demonstrated that CBD administration into the mesolimbic system decreased the AMPH-induced sensitization, thus affecting the dopaminergic neuronal activity.

CBD influence on endocannabinoid modulation of DA release

Different studies demonstrated that endocannabinoids (eCBs) are capable of modulating the mesolimbic dopaminergic pathways (Everett et al., 2021; Lupica et al., 2004; Su and Zhao,

2017) by triggering the DA release incrementing a cannabinoid type 1 receptor (CB1R)-dependent manner (Everett et al., 2021; Oleson et al., 2012; Solinas et al., 2006). Some research groups have investigated the hypothesis that a pharmacological increase in eCBs levels could be a relatively safe method of replacement therapy, especially during drug withdrawal, given that it is believed that at this stage, the presence of negative symptoms is accompanied by a decrease in DA levels. Thus, modulation of eCBs could normalize DA levels decreased and prevent negative effects that contribute to relapse (McCutcheon et al., 2012; Oleson and Cheer, 2013; Roitman et al., 2008).

Our hypothesis (Fig. 7) is in line with the idea that the increase in endocannabinoid tone can produce anti-abstinence effects and thus corroborate to prevent the occurrence of AMPH-CPP reinstatement. Our outcomes pointed out that AMPH exposure triggered persistent disturbances by decreasing CB1R immunoreactivity. This is in accordance with previously published data showing that exposure to crack and cocaine also triggers a decrease in the CB1R expression in mesolimbic brain areas (Areal et al., 2015). In addition, we observed that AMPH exposure decreased N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) and increased FAAH levels. NAPE-PLD and FAAH are responsible for the synthesis and degradation of AEA, respectively (Blankman and Cravatt, 2013). Changes in the eCB levels are known to reflect changes in the enzyme expression involved in their metabolism. A decrease in AEA levels may be a consequence of reduced synthesis by NAPE-PLD or improved degradation by FAAH (Smaga et al., 2017). Taken together, these findings seem to provide evidence that the AMPH impaired the endocannabinoid neurotransmission, at least concerning AEA. Regarding eCBs 2-AG, we found no significant changes underlying AMPH exposure in the immunoreactivity of its synthesis and degradation enzymes, diacylglycerol lipase α (DAGL), and monoacylglycerol lipase (MAGL), respectively. Conversely, CBD treatment provided an increase in NAPE-PLD, as well as a reduction in FAAH levels in both experimental groups exposed or not to AMPH. In this context, changes in these enzymes are associated with a possible increase in AEA levels (Di Marzo, 2008). Moreover, CBD was able to maintain the CB1R levels in AMPH exposed animals. Finally, we also observed that CBD treatment increased DAGL and decreased MAGL levels, regardless of AMPH exposure. Given what other studies have similarly reported (Smaga et al., 2017), we can infer that the CBD's influence on these enzymes resulted in increased levels of the 2-AG. All of these data allow us to hypothesize that the CBD treatment is capable of triggering a general increase in endocannabinoid tone. This idea is in line with our hypothesis about the potential anti-reinstatement effect of the CBD,

which reflects the ability of this cannabinoid to increase endocannabinoid tone and subsequently restore a dopaminergic system compromised by the AMPH administration.

Although we investigated important dopaminergic and endocannabinoid targets in VS and VTA, we recognized that our study had some limitations. In the current study, we were unable to quantify DA and its derivatives levels as well as measure the levels of the main eCBs. We hope to be able to clarify these limitations in future studies.

5 Conclusion

In summary, our results provide consistent evidence to support the CBD potential in preventing AMPH reinstatement. Furthermore, the absence of abuse potential combined with its good tolerability in general highlights CBD as a promising candidate to be used as pharmacotherapy in psychostimulant drug use disorders. Additional preclinical and future clinical studies are needed to better understand the interactions between endocannabinoid and dopaminergic systems, as well as the potential of CBD in the context of drug addiction.

6 Declarations

6.1 Authors contributions

Vinícia Garzella Metz: Conceptualization, Formal analysis, Investigation; Writing - Original Draft, Writing - Review & Editing; **Jéssica Leandra Oliveira da Rosa:** Investigation; **Domenika Rubert Rossato:** Investigation; **Marilise Escobar Burger:** Conceptualization, Resources, Writing - Review & Editing, Project administration, and Funding acquisition; **Camila Simonetti Pase:** Conceptualization, Formal analysis, Resources, Writing - Review & Editing, Project administration, and Funding acquisition.

6.2 Conflicts of interest

The authors declare that there are no conflicts of interest.

6.3 Ethics approval

The experimental procedures were approved by the Research Ethics Committee of Universidade Federal de Santa Maria (UFSM-8850121118), which is affiliated to the National Council for the Control of Animal Experiments (CONCEA), following international norms of care and animal maintenance.

6.4 Funding

This study was financed by the and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS).

6.5 Acknowledgements

The authors are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES), and Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) for the fellowships and financial support and to STI Pharmaceuticals for the donation of cannabidiol.

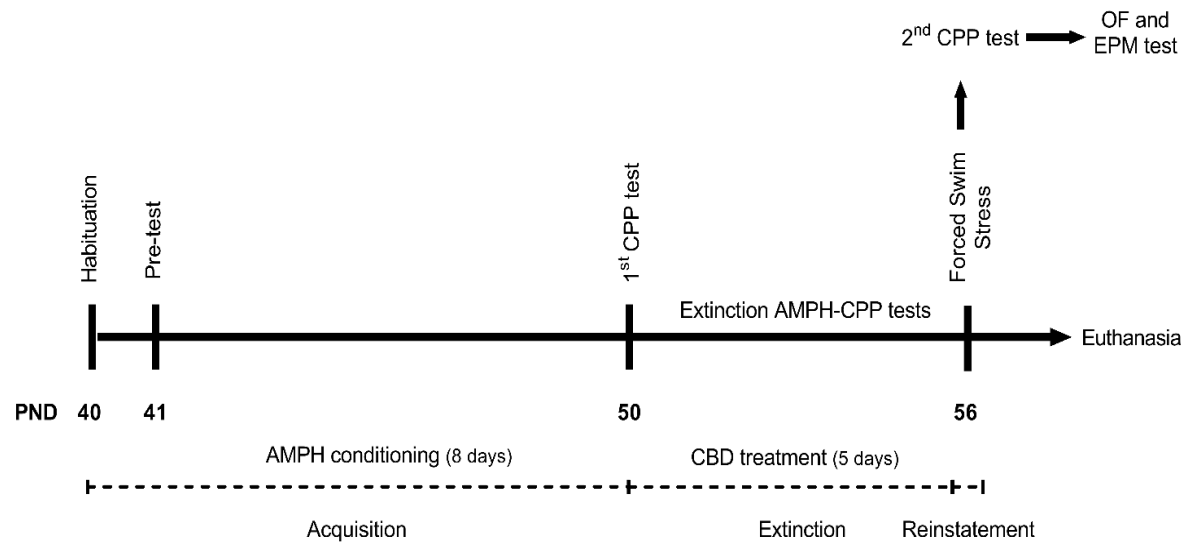


Fig.1 Experimental design: after basal behavioral evaluation (pre-test), animals were treated with AMPH (4 mg/kg, i.p.) for 8 days, when the 1st behavioral evaluation was performed in the CPP paradigm. Subsequently, animals were treated with CBD (10 mg/kg, i.p.) for 5 days. During the CBD treatment period, additional CPP tests were performed to assess the extinction of AMPH-CPP. Twenty-four hours after the last treatment dose, animals were submitted to forced swim stress protocol to induce the reinstatement of extinguished AMPH-CPP and in sequence, a CPP test was conducted. OF and EPM tests were also performed, followed by molecular analysis in both VS and VTA. Abbreviations: AMPH: amphetamine; CBD: cannabidiol; CPP: conditioned place preference; OF: open field; EPM: elevated plus maze; VS: ventral striatum; VTA: tegmental ventral area.

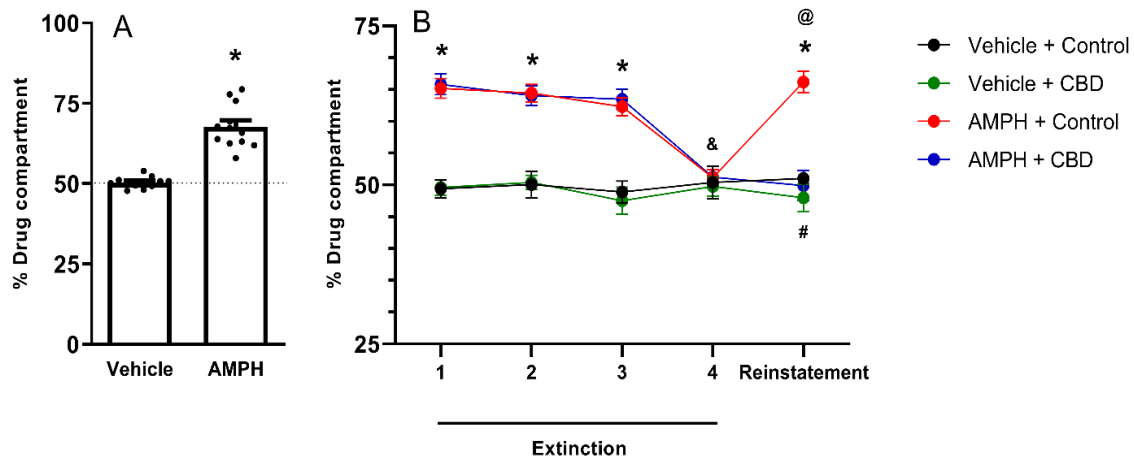


Fig. 2 Drug preference behavior induced by AMPH (4 mg/kg, i.p. for 8 days) observed in CPP paradigm before 5 days of CBD (10 mg/kg, i.p.) treatment (Fig. 2a), the extinction period after the last AMPH administration, and drug preference behavior after stress-induced reinstatement of AMPH-CPP (Fig. 2b). (n=6). Data are expressed as mean \pm S.E.M. * indicates a significant difference of conditioning (vehicle/AMPH) in the same treatment (control/CBD) ($P < 0.05$). # indicates a significant difference of the treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P < 0.05$). & indicates significant difference compared to day 1 of extinction ($P < 0.05$). @ indicates a significant difference compared to day 4 of extinction ($P < 0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol; CPP: conditioned place preference.

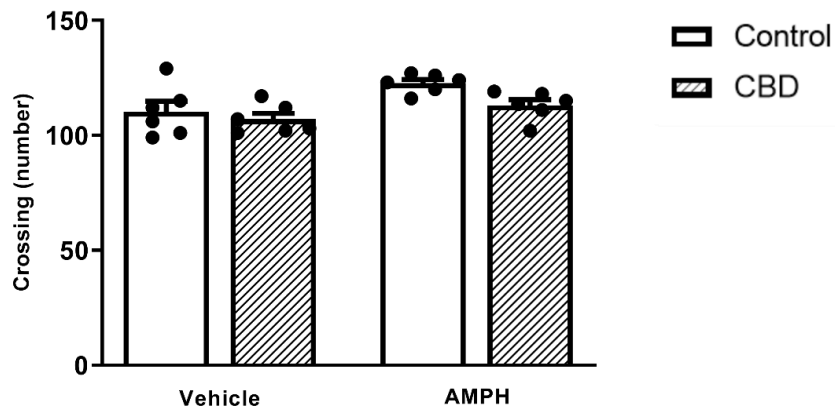


Fig. 3 CBD treatment influence (10 mg/kg, i.p., for 5 days) on the locomotor activity in the open field (OF) test after stress-induced reinstatement of AMPH-CPP. Crossing number was quantified. (n=6). Data are expressed as mean \pm S.E.M. * indicates a significant difference of conditioning (vehicle/AMPH) in the same treatment (control/CBD) ($P < 0.05$). # indicates significant differences of the treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P < 0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol.

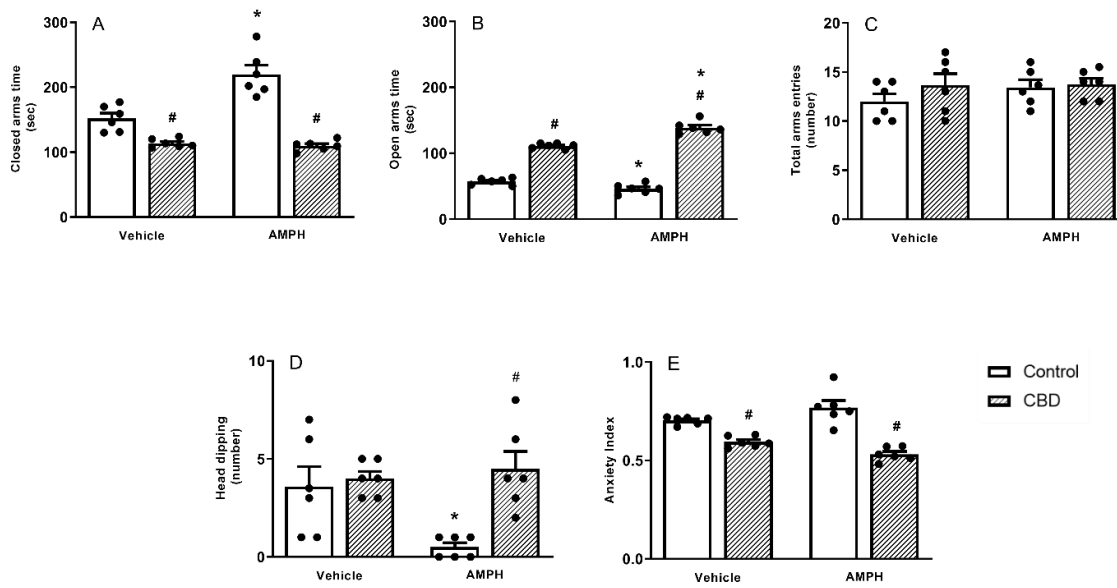


Fig. 4 CBD treatment influence (10 mg/kg, i.p.) on anxiety-like behaviors in the elevated plus-maze (EPM) test after stress-induced reinstatement of AMPH-CPP. The time spent in the closed arms (Fig. 4a), the time spent in the open arms (Fig. 4b), the number of total arms entries (Fig. 4c), the number of head dipping (Fig. 4d), and the anxiety index (Fig. 4e) were quantified. (n=6). Data are expressed as mean \pm S.E.M. * indicates a significant difference of conditioning (vehicle/AMPH) in the same treatment (control/CBD) ($P < 0.05$). # indicates significant differences of the treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P < 0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol.

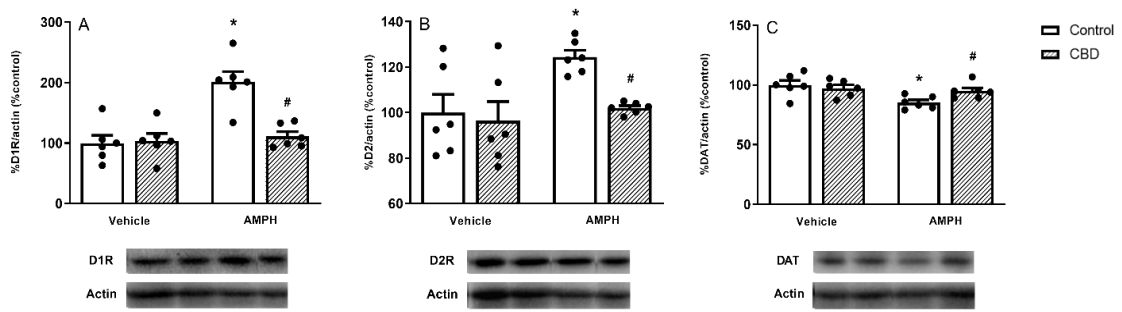


Fig. 5 CBD treatment influence (10 mg/kg, i.p.) on dopamine receptor type 1 (D1) levels (Fig. 5a), dopamine receptor type 2 (D2) levels (Fig. 5b) and on dopamine transporter (DAT) levels (Fig. 5c) in ventral striatum (VS) after stress-induced reinstatement of AMPH-CPP. (n=6). Each representative band in the sequence corresponds to one bar in the Figure. Data are expressed as mean \pm S.E.M. * indicates a significant difference of conditioning (vehicle/AMPH) in the same treatment (control/CBD) ($P < 0.05$). # indicates significant differences of the treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P < 0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol.

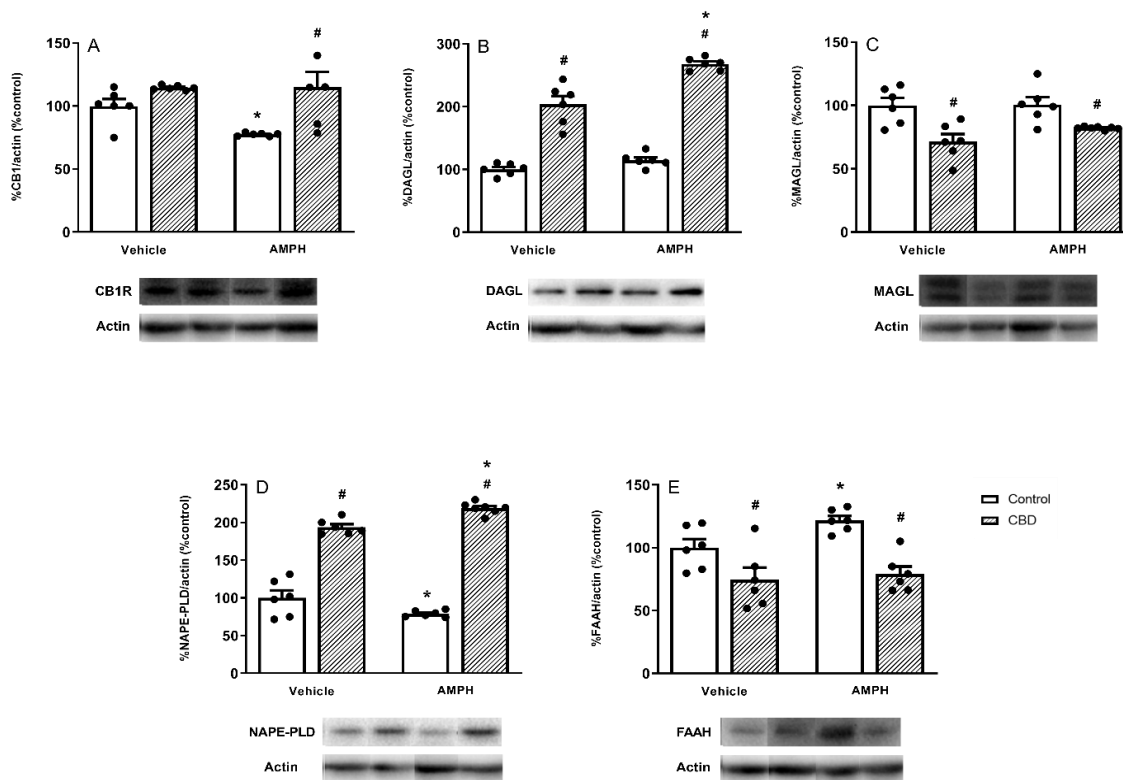


Fig. 6 CBD treatment influence (10 mg/kg, i.p.) on cannabinoid receptor type 1 (CB1) levels (Fig. 6a), on the enzyme diacylglycerol lipase α (DAGL) levels (Fig 6b), on the enzyme monoacylglycerol lipase (MAGL) levels (Fig 6c), on the enzyme N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D (NAPE-PLD) levels (Fig. 6d) and the enzyme fatty acid amide hydrolase (FAAH) levels (Fig. 6e) in ventral tegmental area (ATV) after stress-induced reinstatement of AMPH-CPP. (n=6). Each representative band in the sequence corresponds to one bar in the Figure. Data are expressed as mean \pm S.E.M. * indicates a significant difference of conditioning (vehicle/AMPH) in the same treatment (control/CBD) ($P < 0.05$). # indicates significant differences of the treatment (control/CBD) in the same conditioning (vehicle/AMPH) ($P < 0.05$). Abbreviations: AMPH: amphetamine; CBD: cannabidiol.

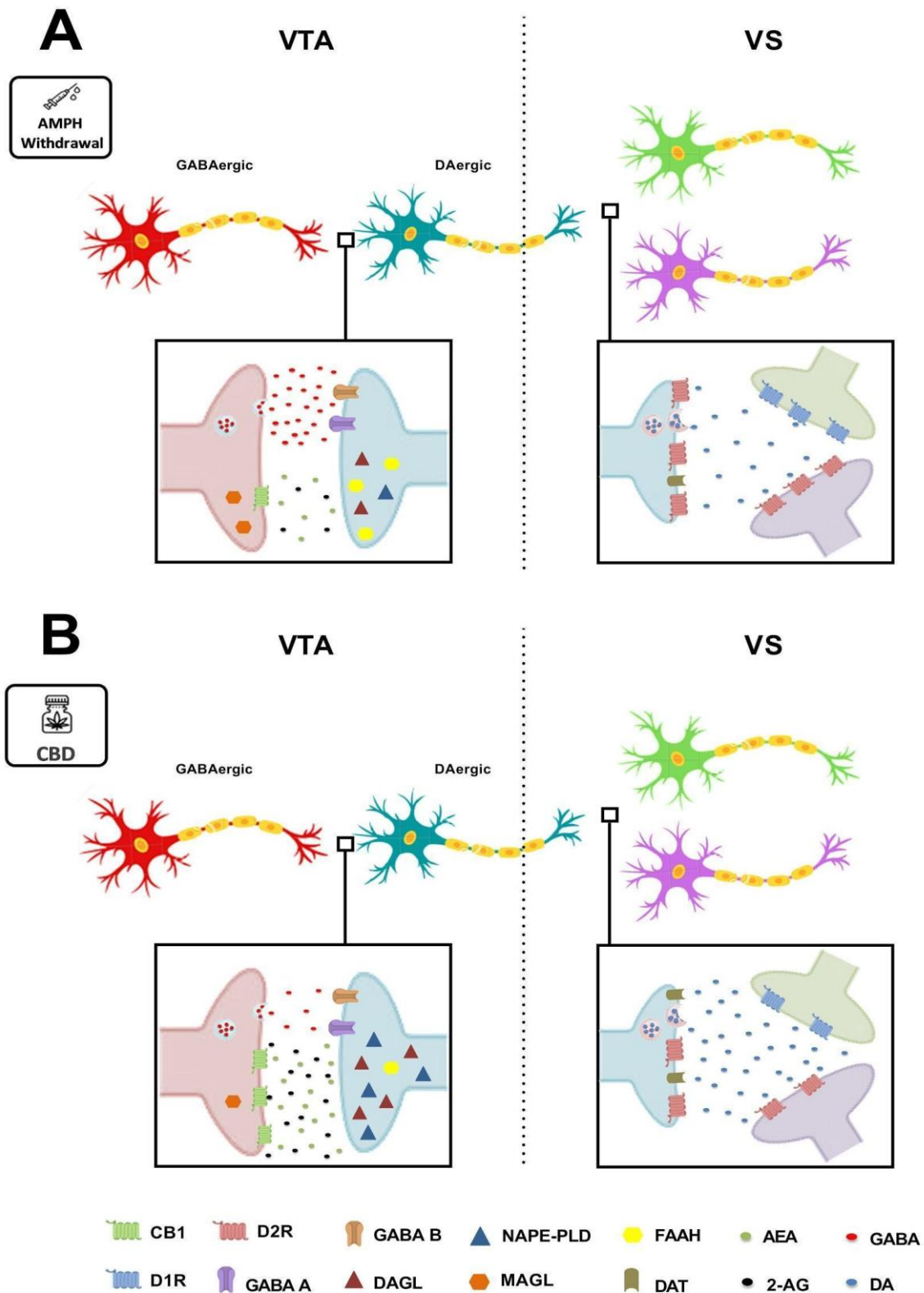


Fig. 7 Proposed model for CBD influence on endocannabinoid modulation of DA release. (A) Exposure to drugs of abuse such as AMPH causes maladaptive changes in the reward system. During the withdrawal phase of the drug, a decrease in endocannabinoid tone in VTA is observed, which triggers an increase in the inhibitory effects exerted by GABAergic neurons in

this brain area and culminates in a decrease in the release of DA in the VS. This hypodopaminergic state can be directly related to the development of negative symptoms and facilitate the occurrence of relapse episodes. (B) CBD increases endocannabinoid tonus by inhibiting the degradation and reuptake of eCBs. Expressed in GABAergic neurons in the VTA, when CB1 receptors are activated by eCBs, they inhibit GABAergic transmission in this brain area, triggering a decrease in the inhibitory interruption of dopaminergic neurons. It leads to an increase in the release of DA in the VS. Possibly, a restoration of baseline DA levels in VS could prevent the development of negative symptoms and contribute to relapse prevention.

Abbreviations: 2-AG: 2-arachidonoylglycerol; AEA: N-arachidonoyl ethanolamine (anandamide); AMPH: amphetamine; CB1: cannabinoid receptor type 1; CBD: cannabidiol; D1R: dopaminergic receptor type 1; D2R: dopaminergic receptor type 2; DA: dopamine; DAT: dopamine transporter; DAGL: diacylglycerol lipase; eCBs: endocannabinoids; FAAH: fatty acid amide hydrolase; GABA: γ -Aminobutyric acid; GABA A: γ -Aminobutyric acid type A receptor; GABA B: γ -Aminobutyric acid type B receptor; MAGL: monoacylglycerol lipase ; NAPE-PLD: N-acyl-phosphatidylethanolamine-hydrolyzing phospholipase D; VS: ventral striatum; VTA: ventral tegmental area.

References

- Alger, B.E., Kim, J., 2011. Supply and demand for endocannabinoids. *Trends Neurosci.* 34, 304–15. <https://doi.org/10.1016/j.tins.2011.03.003>
- Areal, L.B., Rodrigues, L.C., Andrich, F., Moraes, L.S., Cicilini, M.A., Mendonça, J.B., Pelicão, F.S., Nakamura-Palacios, E.M., Martins-Silva, C., Pires, R.G., 2015. Behavioural, biochemical and molecular changes induced by chronic crack-cocaine inhalation in mice: The role of dopaminergic and endocannabinoid systems in the prefrontal cortex. *Behav. Brain Res.* 1, 290:8-16. <https://doi.org/10.1016/j.bbr.2015.04.036>
- Ares-Santos, S., Granado, N., Oliva, I., Shea, E.O., Martin, E.D., Colado, M.I., Moratalla, R., 2012. Dopamine D1 receptor deletion strongly reduces neurotoxic effects of methamphetamine. *Neurobiol. Dis.* 45, 810–20. <https://doi.org/10.1016/j.nbd.2011.11.005>
- Barr, J.L., Renner, K.J., Forster, G.L., 2010. Withdrawal from chronic amphetamine produces persistent anxiety-like behavior but temporally-limited reductions in monoamines and neurogenesis in the adult rat dentate gyrus. *Neuropharmacology* 59(6), 395–405. <https://doi.org/10.1016/j.neuropharm.2010.05.011>
- Bhargava, H.N., 1976. Effect of some cannabinoids on naloxone-precipitated abstinence in morphine-dependent mice. *Psychopharmacol. (Berl)* 49, 267–70. <https://doi.org/10.1007/BF00426828>
- Biala, G., Kruk, M., Budzyska, B., 2009. Effects of the cannabinoid receptor ligands on anxiety-related effects of d-amphetamine and nicotine in the mouse elevated plus maze test. *J. Physiol. Pharmacol.* 60(2), 113–122.
- Blankman, J.L., Cravatt, B.F., 2013. Chemical probes of endocannabinoid metabolism. *Pharmacol. Rev.* 65, 849–871. <https://doi.org/10.1124/pr.112.006387>
- Blessing, E.M., Steenkamp, M.M., Manzanares, J., Marmar, C.R., 2015. Cannabidiol as a Potential Treatment for Anxiety Disorders. *Neurotherapeutics* 12(4), 825–836. <https://doi.org/10.1007/s13311-015-0387-1>
- Bonaccorso, S., Ricciardi, A., Zangani, C., Chiappini, S., Schifano, F., 2019. Cannabidiol (CBD) use in psychiatric disorders: A systematic review. *Neurotoxicology* 74, 282–298. <https://doi.org/10.1016/j.neuro.2019.08.002>
- Bystrowska, B., Smaga, I., Frankowska, M., Filip, M., 2014. Changes in endocannabinoid and N-acylethanolamine levels in rat brain structures following cocaine self-administration and extinction training. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 50, 1–10. <https://doi.org/10.1016/j.pnpbp.2013.12.002>
- Campos, A.C., Guimarães, F.S., 2008. Involvement of 5HT1A receptors in the anxiolytic-like effects of cannabidiol injected into the dorsolateral periaqueductal gray of rats. *Psychopharmacology (Berl)* 199, 223–30. <https://doi.org/10.1007/s00213-008-1168-x>
- Campos, A.C., Moreira, F.A., Gomes, F.V., Del Bel, E.A., Guimaraes, F.C., 2012. Multiple

mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders. *Philos. Trans. Biol. Sci.* 367, 3364–3378. <https://doi.org/10.1098/rstb.2011.0389>

Cao, D.N., Shi, J.J., Hao, W., Wu, N., Li, J., 2016. Advances and challenges in pharmacotherapeutics for amphetamine-type stimulants addiction. *Eur. J. Pharmacol.* 780, 129–135. <https://doi.org/10.1016/j.ejphar.2016.03.040>

Carlezon Jr, W.A., Pliakas, A.M., Parow, A.M., Detke, M.J., Cohen, B.M., Renshaw, P.F., 2002. Antidepressant-like effects of cytidine in the forced swim test in rats. *Biol. Psychiatry* 51, 882–9. [https://doi.org/10.1016/s0006-3223\(01\)01344-0](https://doi.org/10.1016/s0006-3223(01)01344-0)

Cheer, J.F., Wassum, K.M., Heien, M.L., Phillips, P.E., Wightman, R.M., 2004. Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. *J. Neurosci.* 24, 4393–4400. <https://doi.org/10.1523/JNEUROSCI.0529-04.2004>

Chou, I., Narasimham, K., 2005. Neurobiology of addiction. *Nat. Neurosci.* 8 (11), 1427.

Chye, Y., Christensen, E., Solowij, N., Yücel, M., 2019. The Endocannabinoid System and Cannabidiol's Promise for the Treatment of Substance Use Disorder. *Front. Psychiatry* 10, 63. <https://doi.org/10.3389/fpsy.2019.00063>

Cohen, H., Liu, T., Kozlovsky, N., Kaplan, Z., Zohar, J., Mathé, A.A., 2012. The neuropeptide Y (NPY)-ergic system is associated with behavioral resilience to stress exposure in an animal model of post-traumatic stress disorder. *Neuropsychopharmacology* 37(2), 350–363. <https://doi.org/10.1038/npp.2011.230>

Conway, K.P., Compton, W., Stinson, F.S., Grant, B.F., 2006. Lifetime comorbidity of DSM-IV mood and anxiety disorders and specific drug use disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions. *J. Clin. Psychiatry*, 67(2), 247–257. <https://doi.org/10.4088/jcp.v67n0211>

Devane, W.A., Hanus, L., Breuer, A., Pertwee, R.G., Stevenson, L.A., Griffin, G., Gibson, D., Mandelbaum, A., Etinger, A., Mechoulam, R., 1992. Isolation and structure of a brain constituent that binds to the cannabinoid receptor. *Science* 258, 1946–1949. <https://doi.org/10.1038/nn.2219>

Dias, V.T., Vey, L.T., Rosa, H.Z., D'Avila, L.F., Barcelos, R., Burger, M.E., 2017. Could Modafinil Prevent Psychostimulant Addiction? An Experimental Study in Rats. *Basic Clin. Pharmacol.* 121(5), 400–408. <https://doi.org/10.1111/bcpt.12821>

Di Chiara, G., Bassareo, V., Fenu, S., De Luca, M.A., Spina, L., Cadoni, C., Acquas, E., Carboni, E., Valentini, V., Lecca, D., 2004. Dopamine and drug addiction: the nucleus accumbens shell connection. *Neuropharmacology* 47 Suppl 1, 227–41. <https://doi.org/10.1016/j.neuropharm.2004.06.032>

Di Marzo, V., 2008. Targeting the endocannabinoid system: to enhance or reduce? *Nat. Rev. Drug Discov.* 7, 438–455. <https://doi.org/10.1038/nrd2553>

Everett, T.J., Gomez, D.M., Hamilton, L.R., Oleson, E., 2021. Endocannabinoid modulation of dopamine release during reward seeking, interval timing, and avoidance. *Progr.*

Neuropsychopharmacol. Biol. Psychiatry 104, 110031.

<https://doi.org/10.1016/j.pnpbp.2020.110031>

Gerdeman, G.L., Ronesi, J., Lovinger, D.M., 2002. Postsynaptic endocannabinoid release is critical to long-term depression in the striatum. *Nat. Neurosci.* 5(5), 446-51.

<https://doi.org/10.1038/nn832>

Gonzalez-Cuevas, G., Martin-Fardon, R., Kerr, T.M., Stouffer, D. G., Parsons, L. H., Hammell, D. C., Banks, S. L., Stinchcomb, A. L., Weiss, F. 2018. Unique treatment potential of cannabidiol for the prevention of relapse to drug use: preclinical proof of principle.

Neuropsychopharmacology 43(10), 2036-2045. <https://doi.org/10.1038/s41386-018-0050-8>

Gou, H., Wen, D., Chunling, M., Li, M., Li, Y., Zhang, W., Liu, L., Cong, B., 2015.

Protective effects of cholecystinin-8 on methamphetamine-induced behavioral changes and dopaminergic neurodegeneration in mice. *Behav. Brain Res.* 283, 87–96.

<https://doi.org/10.1016/j.bbr.2015.01.028>

Granado, N., Lastres-Becker, I., Ares-Santos, S., Oliva, I., Martin, E., Cuadrado, A., 2011.

Nrf2 deficiency potentiates methamphetamine-induced dopaminergic axonal damage and gliosis in the striatum. *Glia* 59, 1850–63. <https://doi.org/10.1002/glia.21229>

Haghparast, A., Fatahi, Z., Alamdary, S.Z., Reisi, Z., Khodagholi, F., 2014. Changes in the levels of p-ERK, p-CREB, and c-fos in rat mesocorticolimbic dopaminergic system after morphine-induced conditioned place preference: The role of acute and subchronic stress. *Cell. Mol. Neurobiol.* 34, 277–288. <https://doi.org/10.1007/s10571-013-0011-z>

Hansen, J.P., Riddle, E.L., Sandoval, V., Brown, J.M., Gibb, J.W., Hanson, G.R.,

Fleckenstein, A.E., 2002. Methylenedioxymethamphetamine decreases plasmalemmal and vesicular dopamine transport: mechanisms and implications for neurotoxicity. *J. Pharmacol. Exp. Ther.* 300, 1093–100. <https://doi.org/10.1124/jpet.300.3.1093>

Hay, G.L., Baracz, S.J., Everett, N.A., Roberts, J., Costa, P.A., Arnold, J.C., McGregor, I.S., Cornish, J.L., 2018. Cannabidiol treatment reduces the motivation to self-administer methamphetamine and methamphetamine-primed relapse in rats. *J. Psychopharmacol.* 32(12), 1369–1378. <https://doi.org/10.1177/0269881118799954>

Homberg, J.R., Adan, R.A.H., Alenina, N., Asiminas, A., Bader, M., et al., 2021. The continued need for animals to advance brain research. *Neuron* 109 (15), 2374-2379.

<https://doi.org/10.1016/j.neuron.2021.07.015>

Karimi-Haghighi, S., Haghparast, A., 2018. Cannabidiol inhibits priming-induced reinstatement of methamphetamine in REM sleep deprived rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 82, 307–313.

<https://doi.org/10.1016/j.pnpbp.2017.08.022>

Karimi-Haghighi, S., Dargahi, L., Haghparast, A., 2020. Cannabidiol modulates the expression of neuroinflammatory factors in stress- and drug-induced reinstatement of methamphetamine in extinguished rats. *Addict. Biol.* 25(2), e12740.

<https://doi.org/10.1111/adb.12740>

- Kathmann, M., Flau, K., Redmer, A., Tränkle, C., Schlicker, E., 2006. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. *Arch. Pharmacol.* 372, 354–61. <https://doi.org/10.1007/s00210-006-0033-x>
- Katsidoni, V., Anagnostou, I., Panagis, G., 2013. Cannabidiol inhibits the reward-facilitating effect of morphine: involvement of 5-HT1A receptors in the dorsal raphe nucleus. *Addict. Biol.* 18, 286–96. <https://doi.org/10.1111/j.1369-1600.2012.00483.x>
- Kerr, D.S., Bevilaqua, L.R., Bonini, J.S., Rossato, J.I., Köhler, C.A., Medina, J.H., Izquierdo, I., Cammarota, M., 2005. Angiotensin II blocks memory consolidation through an AT2 receptor-dependent mechanism. *Psychopharmacology* 179(3), 529–535. <https://doi.org/10.1007/s00213-004-2074-5>
- Koob, G.F., Volkow, N.D., 2009. Neurocircuitry of addiction. *Neuropsychopharmacology* 35 (1), 217–238. <https://doi.org/10.1038/npp.2009.110>
- Ligresti, A., De Petrocellis, L., Di Marzo, V., 2016. From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology. *Physiol. Rev.* 96 (4), 1593–1659. <https://doi.org/10.1152/physrev.00002.2016>
- Liu, J.F., Li, J.X., 2018. Drug addiction: a curable mental disorder? *Acta Pharmacol. Sin.* 39 (12), 1823–1829. <https://doi.org/10.1038/s41401-018-0180-x>
- Luján, M.A., Castro-Zavala, A., Alegre-Zurano, L., Valverde, O., 2018. Repeated Cannabidiol treatment reduces cocaine intake and modulates neural proliferation and CB1R expression in the mouse hippocampus. *Neuropharmacology*, 143, 163–175. <https://doi.org/10.1016/j.neuropharm.2018.09.043>
- Lupica, C.R., Riegel, A.C., Hoffman, A.F., 2004. Marijuana and cannabinoid regulation of brain reward circuits. *Br. J. Pharmacol.* 143, 227–34. <https://doi.org/10.1038/sj.bjp.0705931>
- Mahmud, A., Gallant, S., Sedki, F., D'Cunha, T., & Shalev, U., 2017. Effects of an acute cannabidiol treatment on cocaine self-administration and cue-induced cocaine seeking in male rats. *Journal of psychopharmacology (Oxford, England)*, 31(1), 96–104. <https://doi.org/10.1177/0269881116667706>
- Maldonado, R., Rodriguez de Fonseca, F., 2002. Cannabinoid addiction: behavioral models and neural correlates. *J. Neurosci.* 22, 3326–31. <https://doi.org/10.1523/JNEUROSCI.22-09-03326.2002>
- Maldonado, R., 2003. The neurobiology of addiction. *J. Neural Transm. Suppl.* 1–14. https://doi.org/10.1007/978-3-7091-0541-2_1
- Manzanares, J., Cabañero, D., Puente, N., García-Gutiérrez, M.S., Grandes, P., Maldonado, R., 2018. Role of the endocannabinoid system in drug addiction. *Biochem. Pharmacol.* 157, 108–121. <https://doi.org/10.1016/j.bcp.2018.09.013>
- McCutcheon, J.E., Ebner, S.R., Loriaux, A.L., Roitman, M.F., 2012. Encoding of aversion by dopamine and the nucleus accumbens. *Front. Neurosci.* 6, 137.

<https://doi.org/10.3389/fnins.2012.00137>

Mechoulam, R., Ben-Shabat, S., Hanus, L., Ligumsky, M., Kaminski, N.E., Schatz, A.R., Gopher, A., Almog, S., Martin, B.R., Compton, D.R., 1995. Identification of an endogenous 2-monoglyceride, present in canine gut, that binds to cannabinoid receptors. *Biochem. Pharmacol.* 50, 83–90. [https://doi.org/10.1016/0006-2952\(95\)00109-d](https://doi.org/10.1016/0006-2952(95)00109-d)

Mechoulam, R., Parker, L.A., Gallily, R., 2002. Cannabidiol: an overview of some pharmacological aspects. *J. Clin. Pharmacol.* 42, 11–19S. <https://doi.org/10.1002/j.1552-4604.2002.tb05998.x>

Melis, M., Pisitis, M., Perra, S., Muntoni, A.L., Pillolla, G., Gessa, G.L., 2004. Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors. *J. Neurosci.* 24, 53–62. <https://doi.org/10.1523/JNEUROSCI.4503-03.2004>

Metz, V.G., Segat, H.J., Dias, V.T., Barcelos, R., Maurer, L.H., Stiebe, J., Emanuelli, T., Burger, M.E., Pase, C.S., 2019. Omega-3 decreases D1 and D2 receptors expression in the prefrontal cortex and prevents amphetamine-induced conditioned place preference in rats. *J. Nutr. Biochem.* 67, 182–189. <https://doi.org/10.1016/j.jnutbio.2019.02.007>

Metz, V.G., da Rosa, J.L.O., Rossato, D.R., Milanese, L.H., Burger, M.E., Pase, C.S., 2021. Cannabidiol prevents amphetamine relapse and modulates D1- and D2-receptor levels in mesocorticolimbic brain areas of rats. *Eur. Neuropsychopharmacol.* 50, 23–33. <https://doi.org/10.1016/j.euroneuro.2021.04.008>

Mikail, H.G., Dalla, C., Kokras, N., Kafetzopoulos, V., Papadopoulou-Daifoti, Z., 2012. Sertraline behavioral response associates closer and dose-dependently with cortical rather than hippocampal serotonergic activity in the rat forced swim stress. *Physiol. Behav.* 107, 201–206. <https://doi.org/10.1016/j.physbeh.2012.06.016>

Montgomery, K.C., 1955. The relation between fear induced by novel stimulation and exploratory behavior. *J. Comp. Physiol. Psychol.* 48(4), 254–260. <https://doi.org/10.1037/h0043788>

Moratalla, R., Khairnar, A., Simola, N., Granado, N., García-Montes, J.R., Porceddu, P.F., Tizabi, Y., Costa, G., Morelli, M., 2017. Amphetamine-related drugs neurotoxicity in humans and in experimental animals: Main mechanisms. *Prog. Neurobiol.* 155, 149–70. <https://doi.org/10.1016/j.pneurobio.2015.09.011>

Oleson, E.B., Beckert, M.V., Morra, J.T., Lansink, C.S., Cachope, R., Abdullah, R.A., Loriaux, A.L., Schettters, D., Pattij, T., Roitman, M.F., 2012. Endocannabinoids shape accumbal encoding of cue-motivated behavior via CB1 receptor activation in the ventral tegmentum. *Neuron* 73 (2), 360–373. <https://doi.org/10.1016/j.neuron.2011.11.018>

Oleson, E.B., Cheer, J.F., 2013. On the role of subsecond dopamine release in conditioned avoidance. *Frontiers in Decision Making* 7, 96. <https://doi.org/10.3389/fnins.2013.00096>

Olière, S., Joliette-Riopel, A., Potvin, S., Jutras-Aswad, D., 2013. Modulation of the endocannabinoid system: vulnerability factor and new treatment target for stimulant

addiction. *Front. Psychiatry* 4, 109. <https://doi.org/10.3389/fpsy.2013.00109>

Parsons, L.H., Hurd, Y.L., 2015. Endocannabinoid signalling in reward and addiction. *Nat. Rev. Neurosci.* 16, 579–94. <https://doi.org/10.1038/nrn4004>

Paxinos, G., Watson, C., 2007. *The rat brain in stereotaxic coordinates*. 6th ed. New York: New Academic Press.

Pertwee, R.G., 2008. The diverse CB1 and CB2 receptor pharmacology of three plant cannabinoids: delta9-tetrahydrocannabinol, cannabidiol and delta9-tetrahydrocannabivarin. *Br. J. Pharmacol.* 153, 199–215. <https://doi.org/10.1038/sj.bjp.0707442>

Raineri, M., Peskin, V., Goitia, B., Taravini, I.R., Giorgeri, S., Urbano, F.J., Bisagno, V., 2011. Attenuated methamphetamine induced neurotoxicity by modafinil administration in mice. *Synapse* 65(10), 1087–1098. <https://doi.org/10.1002/syn.20943>

Raineri, M., Gonzalez, B., Goitia, B., Garcia-Rill, E., Krasnova, I.N., Cadet, J.L., Urbano, F.J., Bisagno, V., 2012. Modafinil abrogates methamphetamine-induced neuroinflammation and apoptotic effects in the mouse striatum. *PloS one* 7(10), e46599. <https://doi.org/10.1371/journal.pone.0046599>

Ramesh, D., Ross, G.R., Schlosburg, J.E., Owens, R.A., Abdullah, R.A., Kinsey, S.G., Long, J.Z., Nomura, D.K., Sim-Selley, L.J., Cravatt, B.F., Akbarali, H.I., Lichtman, A.H., 2011. Blockade of endocannabinoid hydrolytic enzymes attenuates precipitated opioid withdrawal symptoms in mice. *J. Pharmacol. Exp. Ther.* 339, 173–185. <https://doi.org/10.1124/jpet.111.181370>

Ramesh, D., Gamage, T.F., Vanuytsel, T., Owens, R.A., Abdullah, R.A., Niphakis, M.J., Shea-Donohue, T., Cravatt, B.F., Lichtman, A.H., 2013. Dual inhibition of endocannabinoid catabolic enzymes produces enhanced anti withdrawal effects in morphine-dependent mice. *Neuropsychopharmacology* 38, 1039–1049. <https://doi.org/10.1038/npp.2012.269>

Ren, Y., Whittard, J., Higuera-Matas, A., Morris, C.V., Hurd, Y.L., 2009. Cannabidiol, a nonpsychotropic component of cannabis, inhibits cue-induced heroin seeking and normalizes discrete mesolimbic neuronal disturbances. *J. Neurosci.* 29, 14764–9. <https://doi.org/10.1523/JNEUROSCI.4291-09.2009>

Renard, J., Loureiro, M., Rosen, L.G., Zunder, J., de Oliveira, C., Schmid, S., Rushlow, W.J., Laviolette, S.R., 2016. Cannabidiol Counteracts Amphetamine-Induced Neuronal and Behavioral Sensitization of the Mesolimbic Dopamine Pathway through a Novel mTOR/p70S6 Kinase Signaling Pathway. *J. Neurosci.* 36(18), 5160–5169. <https://doi.org/10.1523/JNEUROSCI.3387-15.2016>

Robbe, D., Kopf, M., Remaury, A., Bockaert, J., Manzoni, O.J., 2002. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc. Natl. Acad. Sci. USA* 99(12), 8384–8. <https://doi.org/10.1073/pnas.122149199>

Robinson, T.E., Berridge, K.C., 2003. Addiction. *Annu. Rev. Psychol.* 54, 25–53. <https://doi.org/10.1146/annurev.psych.54.101601.145237>

- Roitman, M.F., Wheeler, R.A., Wightman, R.M., Carelli, R.M., 2008. Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli. *Nature Publishing Group* 1376–1377. <https://doi.org/10.1038/nm.2219>
- Russo, E.B., Burnett, A., Hall, B., Parker, K.K., 2005. Agonistic properties of cannabidiol at 5-HT_{1a} receptors. *Neurochem. Res.* 30, 1037–43. <https://doi.org/10.1007/s11064-005-6978-1>
- Segat, H.J., Martini, F., Barcelos, R.C., Brüning, C.A., Nogueira, C.W., Burger, M.E., 2016. m-Trifluoromethyl-diphenyldiselenide as a pharmacological tool to treat preference symptoms related to AMPH-induced dependence in rats. *Prog. Neuropsychopharmacol. Biol. Psychiatry* 66, 1–7. <https://doi.org/10.1016/j.pnpbp.2015.11.002>
- Segat, H.J., Barcelos, R.C., Metz, V.G., Rosa, H.Z., Roversi, K., Antoniazzi, C., Vey, L.T., Kronbauer, M., Veit, J.C., Piccolo, J.C., Emanuelli, T., Burger, M.E., 2017. Influence of physical activity on addiction parameters of rats exposed to amphetamine which were previously supplemented with hydrogenated vegetable fat. *Brain Res. Bull.* 135, 69–76. <https://doi.org/10.1016/j.brainresbull.2017.09.013>
- Smaga, I., Jastrzębska, J., Zaniewska, M., Bystrowska, B., Gawliński, D., Faron-Górecka, A., Broniowska, Z., Miszkiel, J., Filip, M., 2017. Changes in the Brain Endocannabinoid System in Rat Models of Depression. *Neurotox. Res.* 31, 421–435. <https://doi.org/10.1007/s12640-017-9708-y>
- Solinas, M., Justinova, Z., Goldberg, S.R., Tanda, G., 2006. Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. *J. Neurochem.* 98 (2), 408–419. <https://doi.org/10.1111/j.1471-4159.2006.03880.x>
- Su, H., Zhao, M., 2017. Endocannabinoid mechanism in amphetamine-type stimulant use disorders: A short review. *J. Clin. Neurosci.* 46, 9–12. <https://doi.org/10.1016/j.jocn.2017.08.042>
- Sugiura, T., Kondo, S., Sukagawa, A., Nakane, S., Shinoda, A., Itoh, K., Yamashita, A., Waku K., 1995. 2-arachidonoylglycerol: a possible endogenous cannabinoid receptor ligand in brain. *Biochem. Biophys. Res. Commun.* 215, 89–97. <https://doi.org/10.1006/bbrc.1995.2437>
- Takamatsu, Y., Yamamoto, H., Hagino, Y., Markou, A., Ikeda, K., 2011. The Selective Serotonin Reuptake Inhibitor Paroxetine, but not Fluvoxamine, Decreases Methamphetamine Conditioned Place Preference in Mice. *Curr. Neuropharmacol.* 9(1), 68–72. <https://doi.org/10.2174/157015911795017236>
- Teixeira-Gomes, A., Costa, V.M., Feio-Azevedo, R., Bastos, M., Carvalho, F., Capela, J.P., 2015. The neurotoxicity of amphetamines during the adolescent period. *Int. J. Dev. Neurosci.* 41, 44–62. <https://doi.org/10.1016/j.ijdevneu.2014.12.001>
- UNODC – United Nations Office for Drug Control and Crime Prevention, 2020. World Drug Report.

Vasquez, V., Penit-Soria, J., Durand, C., Besson, M.J., Giros, B., Daugé, V., 2006. Brief early handling increases morphine dependence in adult rats. *Behav. Brain Res.* 170, 211–8.

<https://doi.org/10.1016/j.bbr.2006.02.022>

Viudez-Martinez, A., Garcia-Gutierrez, M.S., Navarron, C.M., Morales-Calero, M.I., Navarrete, F., Torres-Suarez, A.I., 2017. Cannabidiol reduces ethanol consumption, motivation and relapse in mice. *Addict Biol.* 23, 154–64. <https://doi.org/10.1111/adb.12495>

Viudez-Martínez, A., García-Gutiérrez, M.S., Medrano-Relinque, J., Navarrón, C.M., Navarrete, F., Manzanares, J., 2019. Cannabidiol does not display drug abuse potential in mice behavior. *Acta Pharmacol Sin* 40(3), 358–364. <https://doi.org/10.1038/s41401-018-0032-8>

Volz, T.J., Hanson, G.R., Fleckenstein, A.E., 2007. The role of the plasmalemmal dopamine and vesicular monoamine transporters in methamphetamine-induced dopaminergic deficits. *J. Neurochem.* 101, 883–8. <https://doi.org/10.1111/j.1471-4159.2006.04419.x>

Vuong, S.M., Oliver, H.A., Scholl, J.L., Oliver, K.M., Forster, G.L., 2010. Increased anxiety-like behavior of rats during amphetamine withdrawal is reversed by CRF2 receptor antagonism. *Behav. Brain Res.* 208(1), 278–281. <https://doi.org/10.1016/j.bbr.2009.11.036>

Weiss, F., Gonzalez-Cuevas, G., 2019. Unique treatment potential of cannabidiol for the prevention of relapse to drug use. *Neuropsychopharmacol.* 44, 229. <https://doi.org/10.1038/s41386-018-0218-2>

Zlebnik, N.E., Cheer, J.F., 2016. Beyond the CB1 receptor: is cannabidiol the answer for disorders of motivation? *Annu. Rev. Neurosci.* 39, 1–17. <https://doi.org/10.1146/annurev-neuro-070815-014038>

6 DISCUSSÃO

Considerando os resultados apresentados na íntegra desta tese, o presente estudo fornece evidências dos efeitos benéficos do tratamento com canabidiol (CBD) sobre aspectos comportamentais de recaída por anfetamina (ANF). Os efeitos do tratamento com CBD foram avaliados por meio de dois modelos experimentais distintos de recaída por ANF. No protocolo experimental I, a recaída foi induzida pela reexposição dos animais à droga, através de um protocolo de condicionamento (SEGAT et al., 2014; 2016; 2018; METZ et al., 2019). Por outro lado, no protocolo experimental II, utilizamos um modelo de indução de recaída por um estímulo estressor (HAGAPARASH et al., 2014; MIKHAIL et al., 2012). O CBD preveniu a recaída por ANF em ambos os protocolos experimentais. Isto reafirma a relevância dos nossos achados e evidencia o CBD como uma promissora estratégia farmacológica no tratamento da adição a drogas psicoestimulantes como a ANF.

Da mesma maneira, avaliamos parâmetros de ansiedade no labirinto em cruz elevado. A ansiedade é um fator importante envolvido no estabelecimento e manutenção da dependência de drogas. Estima-se que cerca de 30-40% dos indivíduos adictos também apresentam depressão ou transtornos de ansiedade (CONWAY et al., 2006). Além disso, após uma exposição prolongada e subsequente desintoxicação, a ansiedade pode ser um dos principais fatores que desencadeiam a recaída (BIALA et al., 2009). Em nosso estudo, o CBD *per se*, assim como o tratamento com CBD antes do restabelecimento da preferência, exerceu influências benéficas sobre os sinais semelhantes à ansiedade, em ambos protocolos experimentais. Esses resultados estão de acordo com estudos prévios que evidenciaram propriedades ansiolíticas do CBD (BLESSING et al., 2015). Nós hipotetizamos que o efeito anti-recaída à ANF causado pelo tratamento com CBD, pode ser, pelo menos em parte, devido às propriedades ansiolíticas deste canabinoide.

Nossos estudos também incluíram avaliações comportamentais no teste de campo aberto, onde os animais de todos os grupos experimentais apresentaram atividade locomotora similar. Esses achados reafirmam que nossos resultados observados no labirinto em cruz elevada e também nos testes de recaída observados no paradigma de preferência condicionada de lugar não são um artefato locomotor.

Além disso, após análises moleculares dos tecidos cerebrais dos animais submetidos ao protocolo experimental I, evidenciamos um efeito protetor do CBD contra as alterações celulares induzidas pela ANF. Os resultados moleculares mostraram que os alvos dopaminérgicos alterados pela exposição à ANF (D1R, D2R, DAT, VMAT-2 e TH) foram

restaurados pelo tratamento com CBD. Nossos achados vão de encontro com um estudo anterior, o qual relatou que a administração direta de CBD no sistema mesolímbico foi capaz de atenuar a sensibilização e a atividade neuronal dopaminérgica induzida por ANF (RENARD et al., 2016). Dessa forma, nós propusemos que o CBD poderia modular o sistema dopaminérgico de modo a recuperar a homeostase alterada pela ANF, através de mecanismos ainda mal compreendidos, mas que possivelmente poderiam envolver ações diretas ou indiretas sobre esse sistema. Por fim, ainda, essa ação do CBD sobre o sistema dopaminérgico no córtex pré-frontal e no estriado ventral, que são regiões cerebrais intimamente relacionadas à adição (STHAL, 2014), seria a responsável pelos efeitos comportamentais anti-recaída observados no estudo. Interessantemente, um estudo recém publicado mostrou que a administração de antagonistas dos receptores D1R e D2R inibiram os efeitos benéficos do CBD na preferência condicionada de lugar induzida por metanfetamina (MET) (SHARIFI et al., 2022). Esse estudo sugere que o CBD exerce suas ações benéficas frente à adição por MET, pelo menos em parte, por meio destes dois subtipos de receptores dopaminérgicos, o que coincide com a nossa hipótese.

Tendo em vista os resultados encontrados no protocolo experimental I e o aumento de evidências que apontam o envolvimento do sistema endocanabinoide (SE) no sistema de recompensa (OLIÉRE et al., 2013) como um modulador da sinalização dopaminérgica (WANG; LUPICA, 2014), desenvolvemos o protocolo experimental II. O objetivo desse segundo estudo foi avaliar os efeitos do CBD frente a parâmetros comportamentais de recaída por ANF induzida por estresse de natação forçada, assim como, avaliar alvos dopaminérgicos e endocanabinóides em regiões cerebrais envolvidas na adição. Isso porque acreditamos que os efeitos desencadeados pelo CBD sobre alvos dopaminérgicos podem ser devidos à sua ação já conhecida sobre o SE.

A exposição a drogas de abuso causa mudanças mal adaptativas no sistema de recompensa, tanto a nível dopaminérgico quanto endocanabinoide. Bystrowska e colaboradores (2014) mostraram que há uma diminuição nos níveis de anandamida (AEA) nas regiões mesolímbicas durante a fase de extinção da preferência por cocaína. Aqui, embora não tenhamos sido capazes de mensurar os níveis de eCBs, nossos resultados evidenciaram que a exposição à ANF desencadeou distúrbios persistentes no SE ao diminuir a imunorreatividade do CB1R. Além disso, observamos que a exposição à ANF diminuiu a N-acil-fosfatidiletanolamina-fosfolipase D (NAPE-PLD) e aumentou os níveis da amida hidrolase dos ácidos graxos (FAAH). NAPE-PLD e FAAH são as enzimas responsáveis pela síntese e degradação de AEA, respectivamente (BLANKMAN E CRAVATT, 2013). Alterações nos

níveis de eCB são conhecidos por refletir mudanças na expressão da enzima envolvida em seu metabolismo. Uma diminuição nos níveis de AEA pode ser uma consequência da síntese reduzida por NAPE-PLD ou degradação melhorada por FAAH (SMAGA et al., 2017). Tomados em conjunto, esses achados parecem fornecer evidências de que a ANF prejudicou a neurotransmissão endocanabinoide, pelo menos no que diz respeito à AEA. Dessa forma, nós hipotetizamos que essa diminuição do tônus endocanabinoide na área tegmental ventral (ATV) durante a fase de retirada da droga, desencadeia um aumento dos efeitos inibitórios exercidos pelos neurônios GABAérgicos nessa região do cérebro e culmina na diminuição da liberação de DA no estriado ventral (EV). Estudos anteriores já demonstraram que os níveis de DA se encontram diminuídos na região do *Nucleus accumbens* durante o período de abstinência de drogas (LINGFORD-HUGHES E NUTT, 2003) e que esse estado hipodopaminérgico pode estar diretamente relacionado ao desenvolvimento de sintomas de retirada, como ansiedade e comportamento depressivo, os quais facilitam a ocorrência de episódios de recaída (OLIERE et al., 2013).

Uma vez que o CBD atua como um inibidor da FAAH, sua administração desencadeia um aumento nos níveis de AEA e é capaz de provocar um aumento do tônus endocanabinoide no geral (CAMPOS et al., 2012; LIGESTRI et al., 2016). Em nosso estudo, nós mostramos que o tratamento com CBD aumentou os níveis de NAPE-PLD, bem como a diminuição dos níveis de FAAH, tanto *per se*, quanto em animais previamente expostos à ANF. Nesse contexto, alterações nessas enzimas podem estar associadas a um possível aumento nos níveis de AEA (DI MARZO, 2008). Além disso, o CBD também foi capaz de manter os níveis de CB1R em animais expostos à ANF. Por fim, também observamos que o tratamento com CBD aumentou os níveis de diacilglicerol lipase α (DAGL) e diminuiu os níveis de monoacilglicerol lipase (MAGL), enzimas responsáveis pela síntese e degradação de outro importante eCBs, o 2-araquidonoilglicerol (2-AG). Dessa maneira, nós acreditamos que a ativação dos receptores CB1R expressos em neurônios GABAérgicos na ATV, após esse aumento do tônus endocanabinoide desencadeado pelo CBD, levam a uma inibição da neurotransmissão GABAérgica nessa região cerebral. Isso culminaria em uma diminuição da interrupção inibitória dos neurônios dopaminérgicos, resultando em um aumento da liberação de DA no estriado ventral (EV). De fato, nossos resultados mostraram que o tratamento com CBD restaurou os níveis dos receptores D1R e D2R e de outros alvos dopaminérgicos nessa região do cérebro. Por fim, nós acreditamos que uma restauração dos níveis basais de DA no EV pelo CBD poderia prevenir o desenvolvimento de sintomas negativos e contribuir para a prevenção de recaídas, como evidenciado pelos nossos achados comportamentais. Em consonância com

isso, outros grupos de pesquisa também têm explorado o aumento do tônus endocanabinoide como ferramenta para restaurar o estado hipodopaminérgico encontrado durante a fase de abstinência da droga, a fim de prevenir efeitos de abstinência e a consequente ocorrência de recaídas (MCCUTCHEON et al., 2012; OLESON E CHEER, 2013; ROITMAN et al., 2008).

Em resumo, nossos resultados mostraram um potencial efeito benéfico do CBD na prevenção da recaída por ANF em diferentes modelos experimentais. A nível molecular, nós evidenciamos que o CBD foi capaz de restabelecer a homeostase dopaminérgica e endocanabinoide alterada pela exposição à ANF. Nós atribuímos isso a um efeito indireto deste canabinoide, uma vez que, hipotetizamos que os efeitos desencadeados pelo CBD sobre alvos dopaminérgicos podem ser devidos à sua ação sobre o SE. Nós acreditamos que a ação do CBD no SE pode culminar em uma restauração do estado hipodopaminérgico encontrado em animais expostos à ANF. Por fim, essa regulação da homeostase dopaminérgica poderia ser a responsável pelos efeitos anti-recaída do CBD aqui apresentados.

7 CONCLUSÃO

Tomados em conjunto, os resultados apresentados nesta tese nos permitem concluir que:

- O tratamento com canabidiol (CBD) exerce efeitos benéficos na prevenção da recaída à anfetamina (ANF).
- O CBD também apresenta evidente propriedade ansiolítica, a qual possivelmente pode estar atrelada à capacidade anti-recaída aqui demonstrada pelo canabinoide.
- Os achados moleculares nos permitem hipotetizar que o potencial efeito anti-recaída do CBD reflete a capacidade deste em aumentar o tônus endocanabinóide e, posteriormente, restaurar o sistema dopaminérgico comprometido pela exposição à ANF.
- Embora estudos pré-clínicos adicionais e estudos clínicos futuros são necessários para melhor compreender a ação deste canabinoide e as interações entre os sistemas endocanabinóide e dopaminérgico, o CBD parece ser um candidato promissor para ser usado como farmacoterapia na dependência de drogas psicoestimulantes.

8 MATERIAL SUPLEMENTAR

RESUMO EXPANDIDO

Cocaína: Interação com recaptção ou liberação de monoaminas?

Vinícia Garzella Metz^a, Nienke van der Berg^b, Camila Simonetti Pase^a, Marilise Escobar Bürger^a, Michel Verheij^c, Judith Homberg^c

O presente resumo expandido é fruto das atividades desenvolvidas durante o período de Doutorado Sanduíche no Exterior no Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen, Nijmegen, Holanda (Fevereiro a Julho de 2021).

Cocaína: Interação com a recaptção ou liberação de monoaminas?

Vinícia Garzella Metz^a, Nienke van der Berg^b, Camila Simonetti Pase^a, Marilise Escobar Bürger^a, Michel Verheij^c, Judith Homberg^c

a Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria, RS, Brazil.

b HAS University of Applied Sciences, s'-Hertogenbosch, The Netherlands

c Department of Cognitive Neuroscience, division of Molecular Neurogenetics, Donders Institute for Brain, Cognition and Behaviour, Radboud University Nijmegen Medical Centre, Nijmegen, The Netherlands

RESUMO

O presente estudo teve por objetivo avaliar um possível novo mecanismo de ação da cocaína (COC). Nós hipotetizamos que a COC exerce seus efeitos hedônicos através da liberação de monoaminas das vesículas de armazenamento pré-sináptico, além do já conhecido bloqueio dos transportadores pré-sinápticos de monoaminas. Para isso, conduzimos um experimento utilizando ratos nocautes para o transportador de serotonina (SERT) a fim de investigar se a cocaína é capaz de induzir seus efeitos recompensadores mesmo na ausência de SERT, e se esses efeitos poderiam ser bloqueados por um agente que esgota as vesículas de armazenamento monoaminérgico. Animais selvagens (SERT +/+) e nocautes para o transportador de serotonina (SERT -/-) foram inicialmente submetidos ao protocolo de preferência condicionada de lugar (PCL), onde receberam uma administração diária de cocaína (10 mg/kg, i.p.) ou solução salina (0,9% NaCl, i.p.) nos dias 2, 4, 6 e 8. Durante os dias 3, 5, 7 e 9 do condicionamento, os mesmos animais receberam uma administração diária de salina. Imediatamente após o último condicionamento, no dia 9, os animais receberam reserpina (RES), um agente depletor de vesículas de armazenamento monoaminérgico, na dose de 1 mg/kg (i.p) ou seu veículo. O teste de PCL foi realizado 24 horas após a injeção de RES. Nossos resultados mostraram que os animais SERT -/- apresentaram comportamento de preferência condicionada de lugar induzido por COC. Ainda, estes mesmos animais mostraram ser mais suscetíveis aos efeitos comportamentais da COC em comparação aos animais SERT +/+, fato esse evidenciado por maior aumento na frequência de entradas, distância percorrida e na velocidade do animal no compartimento do aparato de PCL pareado com a droga. Além disso, a depleção das vesículas de armazenamento monoaminérgico pela RES impediu o estabelecimento da preferência pelo psicoestimulante. Tomadas em conjunto, nossas descobertas sugerem que a RES impediu a PCL induzida por COC ao interferir nas propriedades de reforço do psicoestimulante, por ter promovido a depleção das vesículas de armazenamento monoaminérgico. Embora mais estudos sejam necessários, a manipulação de vesículas de armazenamento pode servir como um novo alvo para o desenvolvimento de uma terapia potencial para a adição à COC.

Palavras-chaves: drogadição; psicoestimulantes; serotonina; SERT; vesículas de armazenamento monoaminérgico.

1. INTRODUÇÃO

A adição à cocaína (COC) é uma doença crônica e recidivante caracterizada pela busca e uso compulsivo da droga, apesar de suas consequências prejudiciais (VOLKOW et al., 2016). Estudos epidemiológicos mostraram que a cocaína é a segunda droga ilícita mais consumida na Europa (OEDT, 2016) e nos Estados Unidos (CBHSQ, 2015). Atualmente, não existem medicamentos eficazes e aprovados para o tratamento da adição à COC (CZOTY et al., 2016), o que reitera a importância da pesquisa científica nesse sentido, visando a elucidação completa da patofisiologia da drogadição à psicoestimulantes e o desenvolvimento de estratégias terapêuticas inovadoras para o tratamento deste grave transtorno psiquiátrico.

A dopamina (DA) é conhecida por mediar os efeitos da COC (KOOB, 1996; KOOB E VOLKOW, 2016; VOLKOW E MORALES, 2015; VOLKOW, KOOB E MCLELLAN, 2016), porém evidências recentes sugerem que a serotonina (5-HT) também desempenha um papel importante nos efeitos neuroquímicos e comportamentais da COC (FILIP et al., 2010; HOMBERG et al., 2008; NONKES et al., 2011; VERHEIJ et al., 2014; 2016). Por exemplo, a administração de COC aumenta, enquanto a retirada de COC diminui os níveis extracelulares de DA e 5-HT no *nucleus accumbens* (ANDREWS E LUCKI, 2001; PARSONS, KOOB E WEISS, 1995; 1996; TENEUD et al., 1996; VERHEIJ et al., 2014). Ademais, além dos receptores acumbais dopaminérgicos, os receptores acumbais serotoninérgicos mostraram mediar a atividade locomotora induzida por COC e a recompensa de COC (FERGUSON, MITCHELL E NEUMAIER, 2008; FILIP E CUNNINGHAM, 2002; FILIP et al., 2002; HERGES E TAYLOR, 2000; KATSIDONI, APAZOGLU E PANAGIS, 2011; MCMAHON, FILIP E CUNNINGHAM, 2001; PRZEGALINSKI et al., 2002).

É sabido que a COC exerce seus efeitos por aumentar os níveis de monoaminas extracelulares ao bloquear os transportadores responsáveis pela recaptação destas (NAH E GU, 2016). No entanto, a resposta comportamental e a ingestão de COC não é reduzida em animais e humanos que possuem níveis reduzidos de transportadores de recaptação de monoaminas (ENOCH et al., 2011; GERRA et al., 2007; GUINDALINI et al., 2006; HOMBERG et al., 2008; ROCHA et al., 1998; SORA et al., 1998; VERHEIJ et al., 2018). Além disso, alguns estudos evidenciaram que um desafio agudo de COC ainda resultou em um aumento nos níveis extracelulares de DA e 5-HT no *nucleus accumbens* de

animais sem transportadores de DA e 5-HT (CARBONI et al., 2001; GAINETDINOV, SOTNIKOVA e CARON, 2002; SORA et al., 2001; VERHEIJ et al., 2014).

Essas descobertas sugerem que um mecanismo diferente da inibição da recaptação de DA e 5-HT pode contribuir para a recompensa e efeitos aditivos da COC. Curiosamente, em 1977, Scheel-Krüger e colaboradores mostraram que a resposta comportamental à COC também depende da presença de vesículas de armazenamento monoaminérgico (SCHEEL-KRÜGER et al., 1977). Essas observações levaram à hipótese de que a COC libera DA e 5-HT na fenda sináptica através do esgotamento de vesículas de armazenamento pré-sinápticas. Sendo assim, o objetivo deste estudo foi elucidar um possível novo mecanismo de ação da COC, comprovando que esta exerce seus efeitos recompensadores através da liberação de DA e 5-HT das vesículas de armazenamento monoaminérgico, além do já descrito bloqueio dos transportadores de dopamina (DAT) e de serotonina (SERT).

2. METODOLOGIA

2.1 ANIMAIS

Neste estudo foram utilizados 24 ratos Wistar machos adultos SERT $-/-$ e 24 ratos Wistar machos adultos selvagens (SERT $+/+$), provenientes do Centro Animal da Radboud University, Nijmegen, Holanda. Os ratos SERT $-/-$ são ratos nocaute para o transportador de serotonina, os quais foram gerados segundo Homberg et al. (2008). O experimento seguiu as leis e diretrizes institucionais, nacionais e internacionais de cuidados animal e bem-estar.

2.2 PROTOCOLO EXPERIMENTAL

Os ratos SERT $+/+$ (n=24) e SERT $-/-$ (n=24) foram expostos ao protocolo de preferência condicionada de lugar (PCL), conforme descrito detalhadamente no item a seguir. Inicialmente, ambos os animais SERT $+/+$ e SERT $-/-$, foram divididos randomicamente em dois grupos experimentais, os quais receberam durante a fase de condicionamento, nos dias 2, 4, 6 e 8, uma administração diária de cocaína (10 mg/kg, i.p., n=16) (HOMBERG et al., 2008) ou solução salina (0,9% NaCl, i.p., n=8) e imediatamente foram confinados em um dos compartimentos do aparato de PCL por 40

minutos (Fig 1.) Durante os dias 3, 5, 7 e 9 do condicionamento, os mesmos animais receberam uma administração diária de salina, seguida de 40 minutos de exposição ao compartimento não pareado com o psicoestimulante. Imediatamente após o último condicionamento, no dia 9, os animais foram redistribuídos e receberam reserpina, um agente depletor de vesículas de armazenamento monoaminérgico, na dose de 1 mg/kg (i.p) (VERHEIJ et al., 2011) ou seu veículo (solução salina 0,9% NaCl + 0,5% ácido acético, i.p.), dando origem aos seguintes grupos experimentais:

- i) SERT +/+ : Salina + Veículo (n=8)
- ii) SERT +/+ : Cocaína + Veículo (n=8)
- iii) SERT +/+ : Cocaína + Reserpina (n=8)
- iv) SERT -/- : Salina + Veículo (n=8)
- v) SERT -/- : Cocaína + Veículo (n=8)
- vi) SERT -/- : Cocaína + Reserpina (n=8)

O teste de PCL foi realizado 24 horas após a injeção de reserpina. Na sequência, os animais foram anestesiados (isoflurano, dose ao efeito) e eutanasiados por decapitação.

2.3 ANÁLISES COMPORTAMENTAIS

2.3.1 Preferência Condicionada de Lugar

A preferência condicionada de lugar (PCL) é um modelo animal usado para avaliar os efeitos hedônicos de drogas que causam dependência. Ele consiste no pareamento repetido em um compartimento com um estímulo específico (drogas), enquanto no compartimento oposto ocorre o pareamento com um estímulo neutro.

O aparato de PCL consiste em uma caixa dividida em dois compartimentos, os quais apresentam pistas ambientais distintas. Um dos compartimentos da caixa, possui piso preto e paredes quadriculadas, enquanto o outro compartimento possui piso preto e paredes listradas. Estes encontram-se conectados por um terceiro compartimento neutro. A caixa foi colocada em uma sala silenciosa e em ambas as câmaras de condicionamento e no espaço central, uma luz branca fraca (20–30 lx) foi acesa. O cronograma da PCL é composto pelas seguintes fases:

Habituação: Os animais foram colocados durante 15 minutos em cada compartimento da caixa de preferência, exceto na área neutra, no dia 0. Tal procedimento foi realizado para a familiarização do animal ao ambiente.

Pré-teste: No dia seguinte à habituação (dia 1), os ratos foram colocados no centro da área neutra com livre acesso aos compartimentos e tiveram seu tempo de permanência em cada compartimento registrado durante 15 minutos (FUKUSHIRO et al., 2007). Os animais que demonstraram forte aversão incondicionada (menos de 25% do tempo) ou preferência (mais de 75% do tempo) por um dos compartimentos foram descartados (VAZQUEZ et al., 2006).

Condicionamento: Durante a fase de condicionamento, nos dias 2, 4, 6 e 8, os animais receberam uma administração diária de cocaína (10mg/kg, i.p.) ou solução salina (0,9% NaCl), conforme o grupo experimental, e foram imediatamente confinados por 40 minutos no compartimento em que permaneceram menor tempo durante o pré-teste. Nos dias 3, 5, 7 e 9, os mesmos animais receberam uma injeção diária de solução salina, seguida de 40 minutos de exposição ao compartimento não pareado com o psicoestimulante (adaptado de HOMBERG Et al., 2008).

Teste de PCL: O teste de PCL foi conduzido 24 horas após o término da fase de condicionamento. Os animais tiveram acesso livre aos compartimentos durante 15 minutos (FUKUSHIRO et al., 2007), sendo quantificado, o número de entradas, o tempo de permanência, a distância percorrida, assim como a velocidade do animal em cada compartimento

2.4 ANÁLISE ESTATÍSTICA

Primeiramente, a influência do condicionamento no genótipo foi analisada através de ANOVA de duas vias seguido pelo teste Post-hoc de Newman-Keuls. Da mesma forma, a influência do tratamento com reserpina sobre o genótipo em animais condicionados com COC foi analisada através de ANOVA de duas vias seguido pelo teste Post-hoc de Newman-Keuls. As comparações entre o grupo salina+veículo e o grupo COC+reserpina, em ambos os genótipos, foram analisadas através de teste-t de Student. O software Statistica 12 foi utilizado para a realização de todas as análises. Os valores de $P < 0,05$ foram considerados estatisticamente significativos em todas as comparações realizadas. Para a confecção dos Gráficos foi utilizado o programa GraphPad Prism ® (versão 9). Os dados foram expressos em média \pm erro padrão.

3. RESULTADOS

3.1 AVALIAÇÃO COMPORTAMENTAL DE PREFERÊNCIA CONDICIONADA DE LUGAR INDUZIDA POR COCAÍNA EM RATOS SERT $+/+$ E SERT $-/-$ TRATADOS COM RESERPINA

Tempo gasto no compartimento pareado com a droga

ANOVA de duas vias entre o condicionamento e o genótipo revelou efeito principal do condicionamento [$F(1,20) = 29.88, P < 0,01$] no tempo gasto no compartimento pareado com a droga. Além disso, a ANOVA de duas vias entre o genótipo e o tratamento em animais do mesmo condicionamento, revelou efeito do tratamento [$F(1,20) = 20.51, P < 0,01$] neste mesmo parâmetro.

O teste *post-hoc* de Newman-Keuls revelou que os animais de ambos os genótipos (SERT $+/+$ e SERT $-/-$) condicionados com cocaína (COC) permaneceram mais tempo no compartimento condicionado com a droga em comparação ao grupo condicionados com salina, que não mostrou preferência, como esperado (Figura 2a). Inversamente, em ambos os genótipos, o tratamento com o reserpina (RES) diminuiu o tempo que os animais condicionados com COC permaneceram no compartimento da droga (Figura 2a).

Frequência de entradas no compartimento pareado com a droga

ANOVA de duas vias entre o condicionamento e o genótipo revelou efeito principal do condicionamento [$F(1,20) = 9.58, P < 0,01$] e do genótipo [$F(1,20) = 12.49, P < 0,01$] na frequência de entradas no compartimento pareado com a droga. Além disso, ANOVA de duas vias entre o genótipo e o tratamento em animais do mesmo condicionamento, revelou efeito do genótipo [$F(1,20) = 11.95, P < 0,01$] e do tratamento [$F(1,20) = 30.92, P < 0,01$] neste mesmo parâmetro.

A comparação *post-hoc* mostrou que os animais SERT $-/-$ condicionados com COC entraram mais vezes no compartimento pareado com a droga em comparação com os animais condicionados com salina do mesmo genótipo (Figura 2b). Além disso, essa maior suscetibilidade ao efeito da droga parece estar relacionada ao genótipo, uma vez que, esse mesmo grupo experimental apresentou diferença estatística em comparação com os animais SERT $+/+$ condicionados com COC (Figura 2b). Por fim, uma diminuição

no número de entradas no compartimento da droga foi observada nos animais condicionados com COC e posteriormente tratados com RES em ambos os genótipos em comparação com animais do mesmo condicionamento e que foram tratados com VEIC (Figura 2b).

Distância percorrida no compartimento pareado com a droga

ANOVA de duas vias entre o condicionamento e o genótipo revelou efeito principal do condicionamento [$F(1,20) = 32.97, P < 0,01$] e do genótipo [$F(1,20) = 6.58, P < 0,05$] na distância percorrida no compartimento pareado com a droga. Além disso, ANOVA de duas vias entre o genótipo e o tratamento em animais do mesmo condicionamento, revelou um efeito do genótipo [$F(1,20) = 6.23, P < 0,05$], do tratamento [$F(1,20) = 41.47, P < 0,01$] e da interação genótipo x tratamento [$F(1,20) = 6.94, P < 0,05$] neste mesmo parâmetro.

O teste *post-hoc* de Newman-Keuls mostrou que os animais de ambos os genótipos condicionados com COC apresentaram um aumento na atividade locomotora em comparação com os animais condicionados com salina, o que foi evidenciado pela distância percorrida no compartimento pareado com a droga (Figura 2c). Além disso, esse aumento foi significativamente maior nos animais SERT^{-/-} em comparação aos SERT^{+/+}, apontando mais uma vez, uma possível maior suscetibilidade deste genótipo aos efeitos da droga (Figura 2c). Por sua vez, o tratamento com RES, tanto em animais SERT^{+/+} quanto em SERT^{-/-} condicionados com COC, desencadeou uma diminuição da distância percorrida, em comparação com animais do mesmo condicionamento e que foram tratados com VEIC (Figura 2c).

O teste T de Student entre os grupos salina+veículo e COC+reserpina em ambos os genótipos (SERT^{+/+} e SERT^{-/-}) não apresentou diferença significativa ($P < 0,911$ e $P < 0,911$, respectivamente), o que evidencia que a RES não prejudicou a atividade locomotora dos animais.

Velocidade dos animais no compartimento pareado com a droga

ANOVA de duas vias entre o condicionamento e o genótipo revelou efeito principal do genótipo [$F(1,20) = 12.82, P < 0,01$] e da interação condicionamento x genótipo [$F(1,20) = 9.26, P < 0,01$] na velocidade dos animais no compartimento pareado

com a droga. Além disso, a ANOVA de duas vias entre o genótipo e o tratamento em animais do mesmo condicionamento revelou um efeito do genótipo [$F(1,20) = 19.59, P < 0,01$], do tratamento [$F(1,20) = 6.77, P < 0,05$] e da interação genótipo x tratamento [$F(1,20) = 5.80, P < 0,05$] neste mesmo parâmetro.

A velocidade dos animais SERT -/- condicionados com COC foi aumentada no compartimento da droga em comparação ao grupo SERT -/- condicionado com salina. Uma diferença significativa entre esse mesmo grupo em comparação aos animais SERT +/+ condicionados com COC também foi evidenciada, revelando um maior efeito da droga neste genótipo (Figura 2d). Inversamente, o tratamento com RES diminuiu a velocidade de animais SERT -/- condicionados com COC em comparação com o grupo de animais do mesmo condicionamento e que foi tratado com VEIC.

O teste T de Student entre os grupos salina+veículo e COC+reserpina em ambos os genótipos (SERT +/+ e SERT -/-) não apresentou diferença significativa ($P < 0,125$ e $P < 0,332$, respectivamente), o que evidencia que a RES não prejudicou a velocidade e consequentemente a atividade locomotora dos animais.

4. DISCUSSÃO

Este estudo teve como objetivo avaliar um novo mecanismo de ação da cocaína (COC). Nós hipotetizamos que a COC exerce seus efeitos de recompensa através da liberação de monoaminas das vesículas de armazenamento pré-sináptico, além do já conhecido mecanismo de bloqueio dos transportadores de monoaminas. Para isso, conduzimos um experimento utilizando ratos com a ausência do transportador de serotonina (SERT), a fim de investigar se a cocaína é capaz de induzir seus efeitos recompensadores mesmo na ausência de SERT e se esses efeitos poderiam ser bloqueados por um agente que esgota as vesículas de armazenamento monoaminérgico. Este foi realmente o caso, uma vez que nossas descobertas mostraram que: (i) animais SERT -/- apresentaram comportamento de preferência condicionada de lugar (PCL) induzido por COC; (ii) estes mesmos animais parecem ser mais suscetíveis aos efeitos comportamentais da COC em comparação aos animais que naturalmente expressam esse transportador (SERT +/+); (iii) a depleção das vesículas de armazenamento monoaminérgico pela reserpina impediu o estabelecimento da preferência pelo psicoestimulante.

Como mencionado previamente, embora a dopamina (DA) seja conhecida como a grande responsável por mediar os efeitos da COC (KOOB, 1996; KOOB E VOLKOW, 2016; VOLKOW E MORALES, 2015; VOLKOW, KOOB E MCLELLAN, 2016), uma série de estudos recentes têm demonstrado que a serotonina (5-HT) também possui um papel relevante nos efeitos comportamentais e neuroquímicos da COC (FILIP et al., 2010; HOMBERG et al., 2008; NONKES et al., 2011; VERHEIJ et al., 2014; 2016). Até então, sabia-se que o aumento dos níveis de monoaminas na fenda sináptica devido ao bloqueio dos transportadores responsáveis pela recaptação dessas, seria o mecanismo através do qual a COC exerce os seus efeitos (NAH e GU, 2016). Contudo, diversos estudos nos últimos anos revelaram que diferentemente do que se esperava, o consumo de COC, assim como a sua resposta comportamental não é reduzida, tanto em humanos quanto em animais que apresentam níveis reduzidos de transportadores de recaptação de monoaminas (ENOCH et al., 2011; GERRA et al., 2007; GUINDALINI et al., 2006; HOMBERG et al., 2008; ROCHA et al., 1998; SORA et al., 1998; VERHEIJ et al., 2018).

Em humanos, a expressão e a função de SERT reduzidas (HEILS et al., 1996; HEINS et al., 200; LESCH et al., 1996; PEZAWAS et al., 2005) predispõe o aumento da presença de estados emocionais negativos (LESCH et al., 1996; OSHER, HAMER E BENJAMIN, 2000) e o aumento do consumo de drogas psicoestimulantes (ENOCH et al., 2011; GERRA et al., 2007; MARTIN-SANTOS et al., 2010). Esses achados clínicos são consistentes com estudos realizados em ratos nocautes para SERT, os quais mostraram que a inativação genética desse transportador aumenta não apenas o comportamento relacionado à ansiedade (KALUEFF et al., 2010; OSHER, HAMER E BENJAMIN, 2000), mas também a auto-administração de ecstasy e COC (HOMBERG et al., 2008; OAKLY et al., 2014). Nossos achados vão de encontro à esses estudos anteriores, uma vez que também demonstramos que os animais SERT $-/-$, apresentaram PCL induzida por COC, resultado esse evidenciado por um aumento do tempo de permanência, da frequência de entradas, da distância percorrida e também da velocidade do animal no compartimento pareado com a droga no teste da PCL. Embora o tempo despendido no compartimento da droga pelos animais SERT $-/-$ não tenha sido diferente dos animais SERT $+/+$, interessante, em todos os outros parâmetros (frequência de entradas, distância percorrida e velocidade), os animais nocautes para SERT mostraram-se mais suscetíveis aos efeitos da droga psicoestimulante. Também de acordo com os nossos resultados, Homberg e colaboradores (2008) e Sora e colaboradores (1998)

mostraram, respectivamente, que a COC aumenta a atividade locomotora e a PCL mais fortemente em animais geneticamente modificados que não expressam SERT.

Nossas descobertas, sustentadas por todas essas evidências anteriores, sugerem que um mecanismo diferente da inibição da recaptação de DA e 5-HT pode mediar os efeitos aditivos e recompensadores da COC. Em 1977, Scheel-Krüger e colaboradores já haviam demonstrado que os efeitos comportamentais da COC também dependiam da presença das vesículas de armazenamento monoaminérgico. Esses indícios, em conjunto, nos levaram à hipótese de que a COC também pode ser capaz de liberar monoaminas na fenda sináptica através do esgotamento do conteúdo de vesículas de armazenamento pré-sináptico. A fim de testar essa hipótese, aqui nós administramos reserpina (RES), um agente depletor dessas vesículas de armazenamento, em animais condicionados com COC, 24 horas antes do teste de PCL. A RES é um alcaloide que apresenta alta afinidade para o transportador vesicular de monoamina 2 (VMAT-2). Sendo assim, esta é capaz de se ligar ao transportador e bloquear a ligação das monoaminas a esse, inibindo o armazenamento vesicular e impedindo a liberação destas na fenda sináptica (COLPAERT, 1987; BURGER et al., 2005; FUENTES et al., 2007). Após o tratamento com RES, as vesículas de armazenamento monoaminérgico são conhecidas por ficarem vazias e os níveis extracelulares de catecolaminas diminuídos (COLLIVER et al., 2000; DAHLSTROM et al., 1965; GONG et al., 2003; POTHOS et al., 1998; WAGNER, 1985). Uma vez impedidas de serem armazenadas dentro das vesículas e então acumuladas no citosol do neurônio pré-sináptico, esses neurotransmissores são rapidamente metabolizados pela enzima monoamino-oxidase (MAO), o que causa uma redução também dos níveis intracelulares dessas catecolaminas (METZGER et al., 2002). O nosso estudo mostrou que a administração de RES em animais condicionados com COC, 24 horas antes do teste de PCL impediu os efeitos de recompensadores da droga psicoestimulante, tanto em animais SERT +/+ quanto em SERT -/-. Isso foi evidenciado pela ausência de PCL, o que foi constatado pelo tempo que os animais permaneceram no compartimento da droga e também pela frequência de entradas no mesmo. Esses resultados vão de encontro a nossa hipótese, dado que, nós mostramos que os efeitos recompensadores da COC são observados mesmo em animais com a ausência de SERT, e que então, as vesículas de armazenamento monoaminérgico parecem desempenhar um papel fundamental nesse sentido. Ao que tudo indica, o esgotamento das vesículas de armazenamento pré-sináptico pode ter sido o grande responsável pela ausência dos efeitos recompensadores da COC.

Além disso, a administração de RES também impediu o aumento da atividade locomotora visto em animais condicionados com COC e que receberam apenas veículo. Nós constatamos isso ao observar uma diminuição da distância percorrida pelos animais SERT +/+ e SERT -/- e também pela diminuição da velocidade dos animais SERT -/-. Diferentemente do que se pode inicialmente pensar, esses resultados não se devem a um artefato locomotor ocasionado pela administração de RES, uma vez que não houve diferença estatística em relação à estes parâmetros quando comparados os grupos condicionados com salina e que receberam veículo e os grupos de ambos os genótipos que foram condicionados com a droga e tratados com RES. De fato, é sabido que altas doses de RES podem inibir o comportamento motor, aumentando a rigidez muscular (JURNA, 1976). Contudo, esse desempenho motor prejudicado é tipicamente observado após a administração intraperitoneal de 10 mg/kg de RES ou mais (JOHNELS, 1983; JOHNELS et al., 1978; SOUTHWICK E ANDERSON, 1981; WAGNER E ANDERSON, 1982). Sendo assim, os efeitos de 1 mg/kg de RES observados no presente estudo não podem ser explicados por desempenho motor prejudicado.

Tomadas em conjunto, nossas descobertas sugerem que a RES impediu a PCL induzida por COC ao interferir nas propriedades de reforço do psicoestimulante, por ter promovido a depleção das vesículas de armazenamento monoaminérgico. Dado que a 5-HT extracelular também pode modular a recompensa (BARI et al., 2010; FILIP E CUNNINGHAM, 2002; FILIP et al., 2002; UHL, HALL E SORA, 2002), a manipulação de vesículas de armazenamento pode servir como um novo alvo para o desenvolvimento de uma terapia potencial para a adição à cocaína.

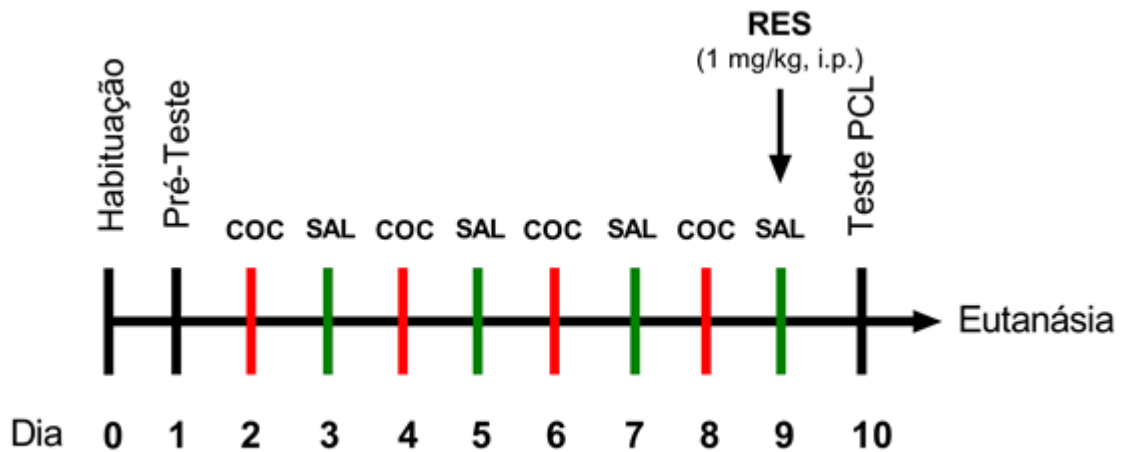


Figura 1: Desenho experimental. Após a habituação e a avaliação comportamental basal (pré-teste), os animais de ambos os genótipos (SERT $+/+$ e SERT $-/-$) foram condicionados com COC (10mg/kg, i.p.) nos dias 2, 4, 6 e 8. Durante os dias 3, 5, 7 e 9, os mesmos animais foram condicionados com SAL (0,9% NaCl, i.p.). Após o término do condicionamento, no 9º dia, os ratos receberam uma injeção de RES (1 mg/kg, i.p.). 24 horas depois o teste de PCL foi realizado e na sequência os animais foram eutanasiados e os tecidos cerebrais coletados para posteriores análises *ex vivo*. Abreviaturas: COC: cocaína; PCL: preferência condicionada de lugar; RES: reserpina; SAL: salina; SERT $+/+$ animais selvagens; SERT $-/-$: animais nocautes para o transportador de serotonina.

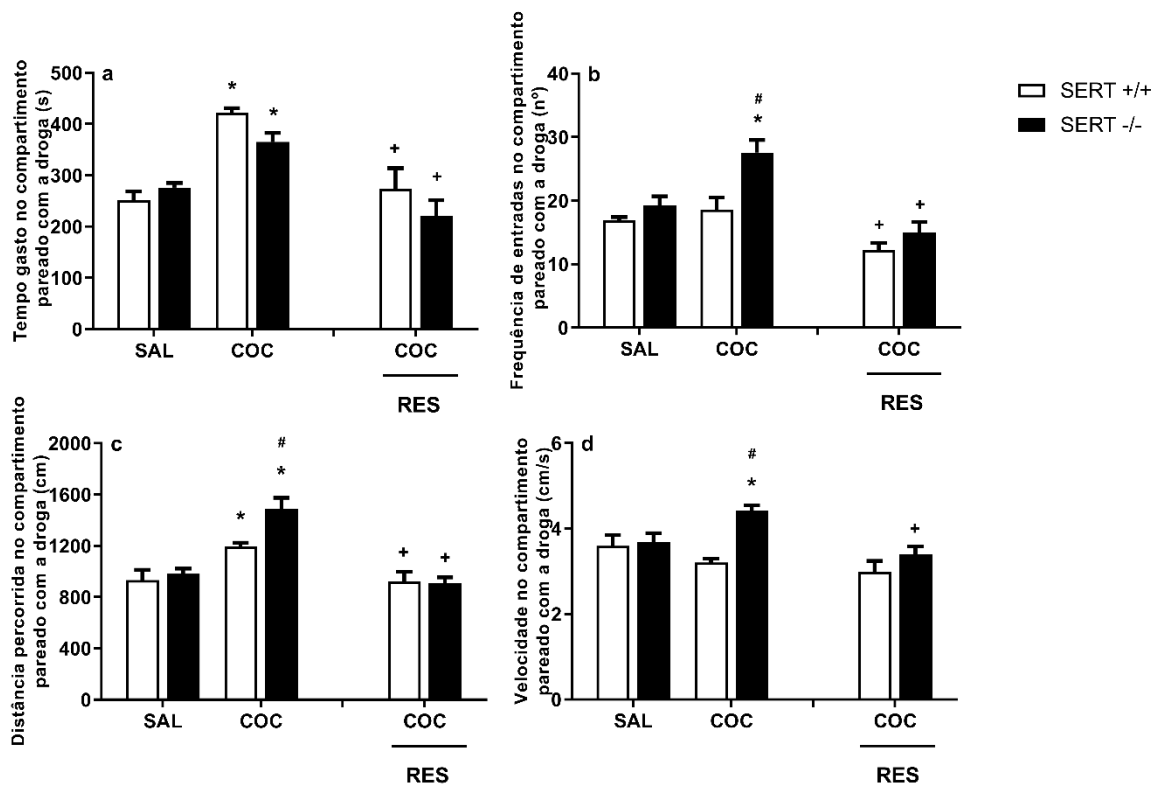


Figura 2: Avaliação comportamental de preferência condicionada de lugar induzida por cocaína em ratos SERT +/+ e SERT -/- tratados com reserpina. Os dados foram expressos em média \pm erro. * Indica diferença significativa entre o condicionamento (SAL/COC) no mesmo genótipo (SERT +/+ / SERT -/-) ($P < 0,05$); # Indica diferença significativa entre os genótipos (SERT +/+ / SERT -/-) no mesmo condicionamento (SAL/COC); ($P < 0,05$) + Indica diferença significativa entre os tratamentos (VEIC/RES) no mesmo condicionamento (SAL/COC) e no mesmo genótipo (SERT +/+ / SERT -/-) ($P < 0,05$). Abreviações: COC: cocaína; RES: reserpina; SAL: salina; SERT +/+: animais selvagens; SERT -/-: animais nocaute para o transportador de serotonina; VEIC: veículo.

REFERÊNCIAS

- ANDREWS, C.M., LUCKI, I. **Effects of cocaine on extracellular dopamine and serotonin levels in the nucleus accumbens.** *Psychopharmacology (Berl)* 155, 221-229, 2001.
- BARI, A. et al. **Serotonin modulates sensitivity to reward and negative feedback in a probabilistic reversal learning task in rats.** *Neuropsychopharmacology* 35, 1290-1301, 2010.
- BÜRGER, M. E. et al. **Acute reserpine and subchronic haloperidol treatments change synaptosomal brain glutamate uptake and elicit orofacial dyskinesia in rats.** *Brain Res*, v. 1031, n. 2, p. 202-10, 2005.
- CARBONI, E. et al. **Cocaine and amphetamine increase extracellular dopamine in the nucleus accumbens of mice lacking the dopamine transporter gene.** *J Neurosci*. 21, RC141-RC144, 2001.
- CENTER FOR BEHAVIORAL HEALTH STATISTICS AND QUALITY. **Behavioral Health Trends in the United States: Results from the 2014 National Survey on Drug Use and Health.** (HHS Publication No. SMA 15-4927, NSDUH Ser. H-50. 64, 2015.
- COLLIVER, T.L. et al. **VMAT-Mediated changes in quantal size and vesicular volume.** *J Neurosci* 20:5276–82, 2000.
- COLPAERT, F. C. **Pharmacological characteristics of tremor, rigidity and hypokinesia induced by reserpine in rat.** *Neuropharmacology*, v. 26, n. 9, p. 1431-40, 1987.
- CZOTY, P.W.; STOOPS, W.W.; RUSH, C.R. **Evaluation of the development of medications for cocaine use disorder: a review of translational preclinical, human laboratory, and clinical trial research.** *Pharmacol. Rev.* 68, 533–562, 2016.
- DAHLSTROM, A.; FUXE, K.; HILLARP, N.A. **The site of action of reserpine.** *Acta Pharmacol Toxicol Copenh* 22:277–92, 1965.
- EUROPEAN MONITORING CENTRE FOR DRUGS AND DRUG ADDICTION. **European Drug Report 2016: Trends and Developments, European Monitoring of Drugs and Drugs Addiction.** 2016.
- ENOCH, M.A. et al. **Functional genetic variants that increase synaptic serotonin and 5-HT₃ receptor sensitivity predict alcohol and drug dependence.** *Mol Psychiatry* 16, 1139-1146, 2011.
- FERGUSON, S.M., MITCHELL, E.S., NEUMAIER, J.F. **Increased expression of 5-HT₆ receptors in the nucleus accumbens blocks the rewarding but not psychomotor activating properties of cocaine.** *Biol Psychiatry* 63, 207-213, 2008.

FILIP, M.; CUNNINGHAM, K.A. **Serotonin 5-HT(2C) receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine.** *Pharmacol Biochem Behav* 71, 745-756, 2002.

FILIP, M. et al. **Effects of serotonin (5-HT) (1B) receptor ligands, microinjected into accumbens subregions, on cocaine discrimination in rats.** *Naunyn Schmiedebergs Arch Pharmacol* 366, 226-234, 2002.

FILIP, M. et al. **Behavioral evidence for the significance of serotonergic (5-HT) receptors in cocaine addiction.** *Addict Biol* 15, 227- 249, 2010.

FUENTES, P. et al. **Inhibition of VMAT-2 and DT-diaphorase induce cell death in a substantia nigra-derived cell line--an experimental cell model for dopamine toxicity studies.** *Chem Res Toxicol*, v. 20, n. 5, p. 776-83, 2007.

FUKUSHIRO, D. F. et al. **Haloperidol (but not ziprasidone) withdrawal enhances cocaine-induced locomotor activation and conditioned place preference in mice.** *Progr. Neuro Psychopharmacol. Biol Psychiatry*, v. 31, p. 867-872, 2007.

GAINETDINOV, R.R.; SOTNIKOVA, T.D.; CARON, M.G. **Monoamine transporter pharmacology and mutant mice.** *Trends Pharmacol Sci* 23, 367-373, 2002.

GERRA, G. et al. **Perceived parenting behavior in the childhood of cocaine users: relationship with genotype and personality traits.** *Am J Med Genet* 144B, 52-57, 2007.

GONG, L.W. et al. **Secretory vesicles membrane area is regulated in tandem with quantal size in chromaffin cells.** *J Neurosci* 23: 7917–21, 2003.

GUINDALINI, C. et al. **A dopamine transporter gene functional variant associated with cocaine abuse in a Brazilian sample.** *Proc Natl Acad Sci USA* 103, 4552-4557, 2006.

HAN, D.D.; GU, H.H. **Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs.** *BMC Pharmacol* 6, 6, 2006.

HERGES, S.; TAYLOR, D.A. **Involvement of 5-HT(3) receptors in the nucleus accumbens in the potentiation of cocaine-induced behaviours in the rat.** *Br J Pharmacol* 131, 1294-1302, 2000.

HEILS, A. et al. **Allelic variation of human serotonin transporter gene expression.** *J Neurochem.* 66:2621–2624, 1996.

HEINZ, A. et al. **A relationship between serotonin transporter genotype and in vivo protein expression and alcohol neurotoxicity.** *Biol Psychiatry.* 47:643–649, 2000.

HOMBERG, J.R. et al. **Adaptations in pre- and postsynaptic 5-HT1A receptor function and cocaine supersensitivity in serotonin transporter knockout rats.** *Psychopharmacology (Berl)* 200, 367-380, 2008.

HOMBERG, J. et al. **Action of cocaine involves depletion of dopaminergic and serotonergic storage vesicles.** bioRxiv 651505, 2019.

JOHNELS, B. **Reserpine-induced rigidity in rats: drug effects on muscle tone from corpus striatum and nucleus accumbens.** Pharmacol Biochem Behav 19:463–70, 1983.

JOHNELS, B.; STEG, G.; UNGERSTEDT, U. **A method for mechanographical recording of muscle tone in the rat: the effect of some antiparkinsonian drugs on rigidity induced by reserpine.** Brain Res 140:177–81, 1978.

JURNA, I. **Striatal monoamines and reserpine and chlorpromazine rigidity.** Pharmacol Ther 2:113–28, 1976.

KALUEFF, A.V. et al. **Conserved role for the serotonin transporter gene in rat and mouse neurobehavioral endophenotypes.** Neurosci Biobehav Rev. 34:373–386, 2010.

KATSIDONI, V.; APAZOGLU, K.; PANAGIS, G. **Role of serotonin 5-HT_{2A} and 5-HT_{2C} receptors on brain stimulation reward and the reward-facilitating effect of cocaine.** Psychopharmacology (Berl) 213, 337-354, 2011.

KOOB, G.F. **Hedonic valence, dopamine and motivation.** Mol Psychiatry 1, 186-189. 2, 1996.

KOOK, G.F.; VOLKOW, N.D. **Neurobiology of addiction: a neurocircuitry analysis.** Lancet Psychiatry 3, 760-773, 2016.

LESCH, K.P. et al. **Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region.** Science. 274:1527–1531, 1996.

MARTIN-SANTOS, R. et al. **5-HTTLPR polymorphism, mood disorders and MDMA use in a 3-year follow-up study.** Addict Biol.15:15–22, 2010.

MCMAHON, L.R.; FILIP, M.; CUNNINGHAM, K.A. **Differential regulation of the mesoaccumbens circuit by serotonin 5-hydroxytryptamine (5-HT)_{2A} and 5-HT_{2C} receptors.** J Neurosci 21, 7781-7787, 2001.

METZGER, R. R. et al. **Inhibitory effect of reserpine on dopamine transporter function.** Eur J Pharmacol, v. 456, n. 1-3, p. 39-43, 2002.

NONKES, L.J. et al. **The interplay between brain 5-hydroxytryptamine levels and cocaine addiction.** Behav Pharmacol 22, 723-738, 2011.

OAKLY, A.C. et al. **A genetic deletion of the serotonin transporter greatly enhances the reinforcing properties of MDMA in rats.** Mol Psychiatry. 19:534–535, 2014.

OSHER, Y.; HAMER, D.; BENJAMIN, J. **Association and linkage of anxiety-related traits with a functional polymorphism of the serotonin transporter gene regulatory region in Israeli sibling pairs.** Mol Psychiatry. 5:216–219, 2000.

- PARSONS, L.H.; KOOB, G.F.; WEISS, F. **Serotonin dysfunction in the nucleus accumbens of rats during withdrawal after unlimited access to intravenous cocaine.** *J Pharmacol Exp Ther* 274, 1182-1191, 1995.
- PARSONS, L.H.; KOOB, G.F.; WEISS, F. **Extracellular serotonin is decreased in the nucleus accumbens during withdrawal from cocaine self-administration.** *Behav Brain Res* 73, 225-228, 1996.
- PEZAWAS, L. et al. **5-HTTLPR polymorphism impacts human cingulate-amygdala interactions: a genetic susceptibility mechanism for depression.** *Nat Neurosci*. 8:828–834, 2005.
- POTHOS, E.N.; DAVILA, V.; SULZER, D. **Presynaptic recording of quanta from midbrain dopamine neurons and modulation of the quantal size.** *J Neurosci* 18:4106–18, 1998.
- PRZEGALINSKI, E. et al. **Effects of 5-HT1B receptor ligands microinjected into the accumbal shell or core on the cocaine-induced locomotor hyperactivity in rats.** *J Physiol Pharmacol* 53, 383-394, 2002.
- ROCHA, B.A. et al. **Cocaine self-administration in dopamine-transporter knockout mice.** *Nat Neurosci* 1, 132-137, 1998.
- SCHEEL-KRÜGER, J. et al. **Cocaine: discussion on the role of dopamine in the biochemical mechanism of action.** In: *Advances in behavioral biology*, Volume 1, E.H. Ellinwood Jr and M.M. Kilbey, eds. (New York: Plenum press), pp. 373-407, 1977.
- SORA, I. et al. **Cocaine reward models: conditioned place preference can be established in dopamine- and in serotonin-transporter knockout mice.** *Proc Natl Acad Sci USA* 95, 7699-7704, 1998.
- SORA, I. et al. **Molecular mechanisms of cocaine reward: combined dopamine and serotonin transporter knockouts eliminate cocaine place preference.** *Proc Natl Acad Sci USA* 98, 5300-5305, 2001.
- SOUTHWICK, S.; ANDERSON, R.J. **Antagonism of reserpine rigidity without inducing sedation.** *Psychopharmacology* 74:29–32, 1981.
- TENEUD, L.M. et al. **Systemic and local cocaine increase extracellular serotonin in the nucleus accumbens.** *Pharmacol Biochem Behav* 53, 747-752, 1996.
- UHL, G.R.; HALL, F.S.; SORA, I. **Cocaine, reward, movement and monoamine transporters.** *Mol Psychiatry* 7, 21-26, 2002.
- VASQUEZ, V. et al. **Brief early handling increases morphine dependence in adult rats.** *Behav Brain Res* 170:211–8, 2006.

VERHEIJ, M.M.; COOLS, A.R. **Reserpine differentially affects cocaine-induced behavior in low and high responders to novelty.** *Pharmacol Biochem Behav.* 98(1):43-53, 2011.

VERHEIJ, M.M. et al. **Reduced cocaine-induced serotonin, but not dopamine and noradrenaline, release in rats with a genetic deletion of serotonin transporters.** *Eur Neuropsychopharmacol* 24, 1850-1854, 2014.

VERHEIJ, M.M. et al. **Median and Dorsal Raphe Serotonergic Neurons Control Moderate Versus Compulsive Cocaine Intake.** *Biol Psychiatry* 83, 1024-1035, 2018.

VOLKOW, N.D.; MORALES, M. **The Brain on Drugs: From Reward to Addiction.** *Cell* 162, 712-725, 2015.

VOLKOW, N.D.; KOOB, G.F.; MCLELLAN, A.T. **Neurobiologic advances from the brain disease model of addiction.** *N. Engl. J. Med.* 374, 363–371., 2016.

WAGNER, B.H.; ANDERSON, R.J. **Prevention of reserpine rigidity by alpha-2 adrenergic antagonists.** *Pharmacol Biochem Behav* 16:731–5, 1982

WAGNER, J.A. Structure of catecholamine secretory vesicles from PC12 cells. *J Neurochem* 45:1244–53, 1985.

REFERÊNCIAS

- ACKERMAN, J. M., WHITE, F. J. A10 somatodendritic dopamine autoreceptor sensitivity following withdrawal from repeated cocaine treatment. **Neurosci. Lett.**, v. 117, p. 181-187, 1990.
- ALLEN INST. BRAIN SCI. **Allen Brain Atlas**. Seattle, WA: Allen Inst, 2016.
- ALTSHULER, R.D. et al. The protein kinase C β -selective inhibitor, enzastaurin, attenuates amphetamine-stimulated locomotor activity and self-administration behaviors in rats. **Psychopharmacol**, v. 236, p. 3231, 2019.
- ANOOSHE, M. et al. **Cannabidiol efficiently suppressed the acquisition and expression of methamphetamine-induced conditioned place preference in the rat**. Behavioural brain research, v. 404, p. 113158, 2021.
- ANTONIAZZI, C.T.D. et al. Influence of neonatal tactile stimulation on amphetamine preference in young rats: parameters of addiction and oxidative stress. **Pharmacol Biochem Behav**, v. 124, p. 341–349, 2014.
- ARES-SANTOS, S. et al. Dopamine D1 receptor deletion strongly reduces neurotoxic effects of methamphetamine. **Neurobiol. Dis.** v. 45, p. 810–20, Feb. 2012.
- ARROYO, M.; MARKOU, A.; ROBBINS, T.W.; EVERITT, B.J. Acquisition, maintenance and reinstatement of intravenous cocaine self-administration under a second-order schedule of reinforcement in rats: effects of conditioned cues and continuous access to cocaine. **Psychopharmacol.**, v. 140 p. 331-344, 1998.
- BAIK, J.H. Dopamine Signaling in reward-related behaviors. **Front Neural Circuits**. v. 7, p. 152, Oct. 2013.
- BARDO, M.T.; BEVINS, R.A. Conditioned place preference: what does it add to our preclinical understanding of drug reward? **Psychopharmacology**, v. 153, p. 31–43, Dec. 2000.
- BERMAN, S. M. et al. Potential adverse effects of amphetamine treatment on brain and behavior: a review. **Mol. Psychiatry**, v. 14, p. 123-142, Feb. 2009.
- BIALA, G.; KRUK, M.; BUDZYNSKA, B. **Effects of the cannabinoid receptor ligands on anxiety-related effects of d-amphetamine and nicotine in the mouse elevated plus maze test**. J. Physiol. Pharmacol. v. 60(2), p. 113–122, 2009.
- BISOGNO, T. et al. Molecular targets for cannabidiol and its synthetic analogues: effect on vanilloidVR1 receptors and on the cellular uptake and enzymatic hydrolysis of anandamide. **Br J Pharmacol.**, v. 134, p. 845–52, 2001.
- BLANKMAN, J.L.; CRAVATT, B.F. **Chemical probes of endocannabinoid metabolism**. Pharmacol. Rev. v. 65, p. 849–871, 2013.

- BLESSING, E.M et al. Cannabidiol as a potential treatment for anxiety disorders. **Neurotherapeutics**, v. 12, p. 825–36, 2015.
- BONHOMME, J. et al. Opioid addiction and abuse in primary care practice: a comparison of methadone and buprenorphine as treatment options. **J Natl Med Assoc.**, v.104, p. 342–50, 2012.
- BOUTREL, B.; KOOB, G.F. What keeps us awake: the neuropharmacology of stimulants and wakefulness-promoting medications. **Sleep**, v. 27, p. 1181-1194, 15 Sep. 2004.
- BRASIL. Ministério da Saúde. Agência Nacional de Vigilância Sanitária (ANVISA). Resolução da Diretoria Colegiada – RDC nº 327, de 9 de dezembro de 2019, dispõe sobre os procedimentos para concessão da Autorização Sanitária para a fabricação e a importação, bem como estabelece requisitos para a comercialização, prescrição, a dispensação, o monitoramento e a fiscalização de produtos de Cannabis para fins medicinais. Diário Oficial da União, Poder Executivo, Brasília, DF, 11 de dez. 2019.
- BURNS, H.D. et al. MK-9470, a positron emission tomography (PET) tracer for in vivo human PET brain imaging of the cannabinoid-1 receptor. **PNAS**, v. 104, p. 9800–5, 2007.
- BYSTROWSKA, B. et al. **Changes in endocannabinoid and N-acyl ethanolamine levels in rat brain structures following cocaine self-administration and extinction training.** *Prog. Neuropsychopharmacol. Biol. Psychiatry* v. 50, p. 1–10, 2014.
- CADTH - Canadian Agency for Drugs and Technologies in Health. Nicotine Replacement Therapy for Smoking Cessation or Reduction: A Review of the Clinical Evidence. **CADTH Report in Brief 2014.** Ottawa, 2014.
- CALPE-LÓPEZ, C.; GARCÍA-PARDO, M.P.; AGUILAR, M.A. Cannabidiol treatment might promote resilience to cocaine and methamphetamine use disorders: a review of possible mechanisms. **Molecule**, v. 24 (14), p. 2583, 2019.
- CAMPOS, A.C. et al **Multiple mechanisms involved in the large-spectrum therapeutic potential of cannabidiol in psychiatric disorders.** *Philos. Trans. Biol. Sci.* v. 367, p. 3364–3378, 2012.
- CAMPOS, A.C. et al. Cannabidiol, neuroprotection and neuropsychiatric disorders. **Pharmacol Res.**, v. 112, p. 119–27, 2016.
- CAMPOS, A.C. et al. Plastic and Neuroprotective Mechanisms Involved in the Therapeutic Effects of Cannabidiol in Psychiatric Disorders. **Front Pharmacol** v. 8, p. 269, 2017.
- CAO, D.N. et al. Advances and challenges in pharmacotherapeutics for amphetamine-type stimulants addiction. **Eur. J. Pharmacol.** v. 780, p. 129-135, Jun. 2016.
- CARLEZON, W.A.; DUMAN R.S.; NESTLER R.J. The many faces of CREB. **Trends neurosci.** v. 8, n. 4, p. 436-445, Aug. 2005.
- CARVALHO, M. et al. Toxicity of amphetamines: an update. **Archi. Toxicol.** v. 86, p. 1167-1231, Aug. 2012.

CASTRO, A.A. et al. Lithium and valproate prevent olfactory discrimination and short-term memory impairments in the intranasal 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) rat model of Parkinson's disease. **Behav Brain Res.**, v. 229, p. 208–215, 2012.

CECCARINI, J. et al. MK-9470 PET measurement of cannabinoid CB1 receptor availability in chronic cannabis users. **Addict Biol.** v. 20, p. 357–67, 2015.

CHAGAS, M.H. et al. Effects of cannabidiol in the treatment of patients with Parkinson's disease: an exploratory double-blind trial. **J Psychopharmacol.**, v. 28, p. 1088–98, 2014.

CHAUVET, C. et al. Chronic stimulation of the tone of endogenous anandamide reduces cue- and stress-induced relapse in rats. **Int J Neuropsychopharmacol**, v. 18(1), 2015

CHEER J.F.; WASSUM, K.M.; HEINEN, M.L.; PHILLIPS, P.E; WIGHTMAN, R.M. Cannabinoids enhance subsecond dopamine release in the nucleus accumbens of awake rats. **J. Neurosci.**, v. 24, p. 4393–4400, 2004.

CHENG, D. et al. Long-term cannabidiol treatment prevents the development of social recognition memory deficits in Alzheimer's disease transgenic mice. **J Alzheimers Dis.** v. 42, p. 1383–96, 2014.

CHOU, I.; NARASIMHAM, K. Neurobiology of addiction. **Nat. neurosci.** v. 8, n. 11, p. 1427, 2005.

CHYE, Y. et al. The Endocannabinoid System and Cannabidiol's Promise for the Treatment of Substance Use Disorder. **Front Psy**, v. 10, p. 63, 2019

CLEGG-KRAYNOK, M.M.; MC BEAN, A.L.; MONTGOMERY-DOWNS, H.E. Sleep quality and characteristics of college students who use prescription psychostimulants nonmedically. **Sleep Med.**, v. 12, p. 598–602, Jun. 2011.

COHEN, H. et al. The impact of environment factors in determining post-exposure responses in isogenic strains of mice: can genetic predisposition explain phenotypic vulnerability?. **Int J Neuropsychopharmac**, v. 11, p. 331–349, 2007.

CONWAY, K.P. et al. **Lifetime comorbidity of DSM-IV mood and anxiety disorders and specific drug use disorders: results from the National Epidemiologic Survey on Alcohol and Related Conditions.** *J. Clin. Psychiatry*, v. 67(2), p. 247–257, 2006.

CRUZ, F.C.; MARIN, M.T.; PLANETA, C.S. The reinstatement of amphetamine-induced place preference is long-lasting and related to decreased expression of AMPA receptors in the nucleus accumbens. **Neurosci**, v. 151, p. 313–9, Jan. 2008.

DE CARVALHO, C.R.; TAKAHASHI, R.N. Cannabidiol disrupts the reconsolidation of contextual drug-associated memories in Wistar rats. **Addict Biol.**, v. 22, p. 742–751, 2017.

DEVINSKY, O. et al. Cannabidiol: pharmacology and potential therapeutic role in epilepsy and other neuropsychiatric disorders. **Epilepsia** v. 55, p. 791–802, 2014.

DEVINSKY, O. et al. Cannabidiol in patients with treatment-resistant epilepsy: an open-label interventional trial. **Lancet Neurol.**, v. 15, p. 270–8, 2016.

DI CIANO, P.; BLAHA, C.D.; PHILLIPS, A.G. Changes in dopamine efflux associated with extinction, CS-induced and d-amphetamine-induced reinstatement of drug-seeking behavior by rats. **Behav. Brain Res.**, v. 120, p. 147–158, 2001.

DI MARZO, V. **Targeting the endocannabinoid system: to enhance or reduce?** *Nat. Rev. Drug Discov.* v. 7, p. 438–455, 2008.

DOS SANTOS, R.G. et al. Cannabidiol for the Treatment of Drug Use Disorders. **Handbook of Cannabis and Related Pathologies**, v. 97, p. 939–946, 2017.

DOUAIHY, A. B; KELLY, T. M; SULLIVAN, C. Medications for Substance Use Disorders. **Social Work in Public Health**, n. 28, p. 264–78, 2013.

DURSTEWITZ, D.; SEAMANS, J.K. The dual-state theory of prefrontal cortex dopamine function with relevance to catechol-*O*-methyltransferase genotypes and schizophrenia. **Biol. Psychiatry**, v. 64, p. 739–49, 2008.

EBBERT, J.O. Effect of varenicline on smoking cessation through smoking reduction: a randomized clinical trial. **JAMA**, v. 313, p. 687–94, 2015.

EVERETT, T.J.; GOMEZ, D.M.; HAMILTON, L.R.; OLESON, E.B. Endocannabinoid modulation of dopamine release during reward seeking, interval timing, and avoidance. **Progr. Neuropsychopharmacol Biol Psychiatry**, v. 104, p. 110031, 2021.

FELTENSTEIN, M.W.; SEE, R.E. The neurocircuitry of addiction: an overview. **Br J Pharmacol**, v. 154, p. 261–74, 2008.

FRIDE, E. Endocannabinoids in the central nervous system: from neuronal networks to behavior. **Curr Drug Targets CNS Neurol Disord**, v. 4, p. 633–42, 2005.

FUKUSHIRO, D. F. et al. Haloperidol (but not ziprasidone) withdrawal enhances cocaine-induced locomotor activation and conditioned place preference in mice. **Progr. Neuro Psychopharmacol. Biol Psychiatry**, v. 31, p. 867–872, 6 Feb. 2007.

GALIÈGUE, S. et al. Expression of central and peripheral cannabinoid receptors in human immune tissues and leukocyte subpopulations. **Eur. J. Biochem.**, v. 232, p. 54–61, 1995.

GAO, W. Y. et al. Alterations in baseline activity and quinpirole sensitivity in putative dopamine neurons in the substantia nigra and ventral tegmental area after withdrawal from cocaine pretreatment. **Neuropsychopharmacol.** v. 18, p. 222–23, 1998.

GARDNER, E.L. Endocannabinoid signaling system and brain reward: emphasis on dopamine. **Pharmacol Biochem Behav**, v. 81, p. 263–84, 2005.

GERDEMAN, G.L.; RONESI, J.; LOVINGER, D.M. **Postsynaptic endocannabinoid release is critical to long-term depression in the striatum.** *Nat. Neurosci.* v. 5(5), p. 446–51, 2002.

GHOSH, A., BASU, D. Cannabis and psychopathology: the meandering journey of the last decade. **Indian J. Psychiatry** v. 57, p. 140–49, 2015.

GIACOPPO, S. et al. Purified Cannabidiol, the main non-psychoactive component of Cannabis sativa, alone, counteracts neuronal apoptosis in experimental multiple sclerosis. **Eur Ver Med Pharmacol Sci.**, v. 19, p. 4906–19, 2015.

GOLAN, D.E. et al. **Princípios de Farmacologia. A base fisiopatológica da Farmacologia.** Editora Guanabara Koogan, 3ª Edição, 2014.

GOLDBERG, S.R. Stimuli associated with drug injections as events that control behavior. **Pharmacol. Rev.**, v. 27, p. 325–340, 1976.

GOLDSTEIN, R.Z.; VOLKOW, N.D. Drug addiction and its underlying neurobiological basis: neuroimaging evidence for the involvement of the frontal cortex. **Am J Psychiatry**, v. 159, p. 1642–1653, 2002.

GREEN, A.R. et al. **The pharmacology and clinical pharmacology of 3,4 methylenedioxymethamphetamine (MDMA, ecstasy) and its derivatives.** **Pharm. Rev.** v. 55, p. 463–508, 2003.

HAGHPARAST, A. et al. Changes in the levels of p-ERK, p-CREB, and c-fos in rat mesocorticolimbic dopaminergic system after morphine-induced conditioned place preference: The role of acute and subchronic stress. **Cel Molec Neurobiol**, v. 34, p. 277–288, 2014.

HAY, G. L. et al. Cannabidiol treatment reduces the motivation to self-administer methamphetamine and methamphetamine-primed relapse in rats. **J Psychopharmacol**, v. 32(12), p. 1369–1378, 2018.

HEIFETS, B.D.; CASTILLO, P.E. Endocannabinoid signaling and long-term synaptic plasticity. **Annu Rev Physiol**, v. 71, p. 283–306, 2009.

HENDERSON, N.D. et al. QTL analyses of multiple behavioral measures of anxiety in mice. **Behav. Genet.**, v. 34, p. 267–93, 2004.

HERMIDA-AMEIJEIRAS, A. et al. Autoxidation and MAO-mediated metabolism of dopamine as a potential cause of oxidative stress: role of ferrous and ferric ions. **Neurochem. Int.** v. 45, p. 103–116, Jul. 2004.

HILAL-DANDAN, R.; BRUNTON, L.L. **Manual de Farmacologia e Terapêutica de Goodman & Gilman**, 2ª edição, AMGH, 2015.

HILL, M.N.; GORZALKA, B.B. The endocannabinoid system and the treatment of mood and anxiety disorders. **CNS Neurol. Disord. Drug Targets**, v. 8, p. 451–8, 2009.

HLAVACOVA, N., BAKOS, J., JEZOVA, D. Eplerenone, a selective mineralocorticoid receptor blocker, exerts anxiolytic effects accompanied by changes in stress hormone release. **J Psychopharmacol**, v. 24, p. 779–786, 2010.

HOOKS, M.S. et al. Environmental and pharmacological sensitization: effects of repeated administration of systemic and intra-nucleus accumbens cocaine. **Psychopharmacol.**, v. 111, p. 109–116, Apr. 1993.

HOWARD, P. et al. Psychostimulants. **J. Pain Symptom Manag.** v. 40, p. 789–795, 2010.

HURD, Y.L. et al. Early Phase in the Development of Cannabidiol as a Treatment for Addiction: Opioid Relapse Takes Initial Center Stage. **Neurotherapeutics**, v. 12(4), p. 807–815, 2015.

HUSTON, J.P. What's conditioned in conditioned place preference? **Trends Pharmacol. Sci.** v. 34, n. 3, p. 162–166, Mar. 2013.

IBEAS BIH, C. et al. Molecular targets of cannabidiol in neurological disorders. **Neurotherapeutics.**, v. 12, p. 699–730, 2015.

JIANG, H. et al. Dose, plasma level, and treatment outcome among methadone patients in Shanghai, China. **Neurosci Bull.**, v. 32, p. 538–44, 2016.

JOHNSTON, L. et al. Monitoring the Future national survey results on drug use, 1975–2016: Overview, key findings on adolescent drug use. **Ann Arbor, MI: Institute for Social Research, The University of Michigan; 2017.**

JULIAN, M.D.; MARTIN, A.B.; CUELLAR, B.; RODRIGUEZ DE FONSECA, F.; NAVARRO, M.; MORATALLA, R.; GARCIA-SEGURA, L.M. Neuroanatomical relationship between type 1 cannabinoid receptors and dopaminergic systems in the rat basal ganglia. **Neuroscience**, v. 119, p. 309–318, 2003.

KALIVAS, P. W., DUFFY, T. Repeated cocaine administration alters extracellular glutamate levels in the ventral tegmental area. **J. Neurochem.** v. 70, p. 1497–1502, 1998.

KAPLAN, F.; OUDEYER, P.-Y. The progress-drive hypothesis: an interpretation of early imitation. In: Nehaniv, C., Dautenhahn, K. (Eds.), **Models and Mechanisms of Imitation and Social Learning: Behavioural, Social and Communication Dimensions.** University Press, New York, Cambridge, p. 361–377, 2007.

KARIMI-HAGHIGHI, S.; HAGHPARAST, A. Cannabidiol inhibits priming-induced reinstatement of methamphetamine in REM sleep deprived rats. **Progr. Neuropsychopharmacol. Biol. Psychiatry**, v. 82, p. 307–313, 2018.

KATHMANN, M. et al. Cannabidiol is an allosteric modulator at mu- and delta-opioid receptors. **Naunyn-Schmiedeberg's Arch Pharmacol.**, v. 372, p. 354–361, 2006.

KATSIDONI, V. et al. Cannabidiol inhibits the reward-facilitating effect of morphine: involvement of 5-HT_{1A} receptors in the dorsal raphe nucleus. **Addict. Biol.**, v. 18, p. 286–296, 2013.

KATZUNG, B.G. **Basic & Clinical Pharmacology.** 12th Edition. – Lange Medical Publications, San Francisco, 2012.

KATZUNG, B.G. **Basic & Clinical Pharmacology**. 13th Edition. – Lange Medical Publications, San Francisco, 2015.

KELLEY, M.E. et al. Marijuana use in the immediate 5-year premorbid period is associated with increased risk of onset of schizophrenia and related psychotic disorders. **Schizophr. Res.**, v. 171, p. 62–67, 2016.

KNEVITZ, M.F.; BUCCINI, D.F. Psicofármacos no tratamento da dependência química: uma revisão. **Revista Interdisciplinar de Estudos em Saúde**, v. 7 (1), p. 205, 2018.

KOOB, G. F.; LE MOAL, M. Addiction and the brain antireward system. *Annu. Rev. Psychol.*, v. 59, p. 29-53, 2008.

KOZELA, E. et al. Cannabidiol inhibits pathogenic T cells, decreases spinal microglial activation and ameliorates multiple sclerosis-like disease in C57BL/6 mice. **Br J Pharmacol.**, v. 163, p. 1507–19, 2011.

KUCZENSKI, R. et al. Hippocampus norepinephrine, caudate dopamine and serotonin, and behavioral responses to the stereoisomers of amphetamine and methamphetamine. **J. Neurosci.** v. 15, p. 1308-1317, Feb. 1995.

KUHN, F.T. et al. Influence of trans fat and omega-3 on the preference of psychostimulant drugs in the first generation of young rats. **Pharmacol. Biochem. Behav.** v. 110, p. 58–65, Sep. 2013.

KUHN, F.T. et al. Cross-generational trans fat consumption favors self-administration of amphetamine and changes molecular expressions of BDNF, DAT, and D1/D2 receptors in the cortex and hippocampus of rats. **Neurotoxic. Res.** v. 28, p. 319–331, Nov. 2015.

KUMAR, R. Approved and investigational uses of modafinil: an evidence-based review. **Drugs**, v. 68, p. 1803-1839, 2008.

LAPRAIRIE, R.B. et al. Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. **Br J Pharmacol**, v. 172, p. 4790–4805, 2015.

LARNEY, S. et al. A systematic review and meta-analysis of naltrexone implants for the treatment of opioid dependence. *Drug Alcohol Rev.*, v. 33, p. 115–28, 2014.

LE, A.; SHAHAM, Y. Neurobiology of relapse to alcohol in rats. **Pharmacol. Ther.**, v. 94, p. 137–156, 2002.

LEE, J. et al. **Cannabidiol regulation of emotion and emotional memory processing: relevance for treating anxiety-related and substance abuse disorders.** *British journal of pharmacology*, v. 174(19), p. 3242–3256, 2017.

LIGRESTI, A.; DE PETROCELLIS, L.; DI MARZO, V. **From phytocannabinoids to cannabinoid receptors and endocannabinoids: pleiotropic physiological and pathological roles through complex pharmacology.** *Physiol. Rev.* v. 96 (4), p. 1593–1659, 2016.

LILE, J.A.; ROSS, J.T.; NADER, M.A. A comparison of the reinforcing efficacy of 3,4-methylenedioxymethamphetamine (MDMA, "ecstasy") with cocaine in rhesus monkeys. **Drug and Alcohol Dependence**, v. 78, p. 135-140, May 2005.

LINGE, R. et al. Cannabidiol induces rapid-acting antidepressant-like effects and enhances cortical 5-HT/glutamate neurotransmission: role of 5-HT_{1A} receptors. **Neuropharmacology**, v. 103, p. 16–26, 2016.

LINGFORD-HUGHES, A.; NUTT, D. **Neurobiology of addiction and implications for treatment**. The British journal of psychiatry: the journal of mental science, v. 182, p. 97–100, 2003.

LIPUT, D.J. et al. Transdermal delivery of cannabidiol attenuates binge alcohol-induced neurodegeneration in a rodent model of an alcohol use disorder. **Pharmacol Biochem Behav.**, v. 111, p. 120–7, 2013.

LIU, J. F.; LI, J. X. **Drug addiction: a curable mental disorder?** Acta Pharmacol Sin v. 39(12), p. 1823–1829, 2018.

LUJÁN, M.Á. et al. Repeated Cannabidiol treatment reduces cocaine intake and modulates neural proliferation and CB1R expression in the mouse hippocampus. **Neuropharmacology**, v. 143, p. 163–175, 2018.

LUPICA, C.R.; RIEGEL, A.C.; HOFFMAN, A.F. **Marijuana and cannabinoid regulation of brain reward circuits**. Br. J. Pharmacol. v. 143, p. 227–34, 2004.

LUPICA, C.R.; RIEGEL, A.C. Endocannabinoid release from midbrain dopamine neurons: a potential substrate for cannabinoid receptor antagonist treatment of addiction. **Neuropharmacology**, v. 48, p. 1105–1116, 2005.

LUTZ, B. Endocannabinoid signals in the control of emotion. **Curr Opin Pharmacol**, v. 9, p. 46–52, 2009.

MAHMUD, A. et al. Effects of an acute cannabidiol treatment on cocaine self-administration and cue-induced cocaine seeking in male rats. **J Psychopharmacol**, v. 31, p. 96–104, 2017.

MAJEWSKA, M.D. Cocaine addiction as a neurological disorder: implications for treatment. **NIDA Res Monogr.**, v. 163, p. 1-26, 1996.

MALDONADO, R.; RODRIGUEZ DE FONSECA, F. **Cannabinoid addiction: behavioral models and neural correlates**. J. Neurosci. v. 22, p. 3326–31, 2002.

MANTSCH, J.R.; BAKER, D.A.; FUNK, D.; LE, A.D.; SHAHAM, Y. Stress-induced reinstatement of drug seeking: 20 years of progress. **Neuropsychopharmacol.**, v. 41, p. 335–356, 2016.

MANZANARES, J. et al. **Role of the endocannabinoid system in drug addiction**. Biochem Pharmacol. v. 157, p. 108-121, 2018.

MARTIN-MORENO, A.M. et al. Cannabidiol and other cannabinoids reduce microglial activation in vitro and in vivo: relevance to Alzheimer's disease. **Mol Pharmacol.**, v. 79, p. 964–73, 2011.

MAZOR, A. et al. Gender-related qualitative differences in baseline and post-stress anxiety responses are not reflected in the incidence of criterion-based PTSD-like behaviour patterns. **World J Biol Psy**, v.10, p.856–869, 2007.

MCCORVY, J.D. et al. A 5-HT (2C) receptor antagonist potentiates a low dose amphetamine-induced conditioned place preference. **Neurosci. Lett.** v. 505, p. 10–3, Nov. 2011.

MCLELLAN, A. T. Substance misuse and substance use disorders: why do they matter in healthcare? **Transactions of the American Clinical and Climatological Association**, v. 128, p. 112-130, 2017.

MCCUTCHEON, J.E.; EBNER, S.R.; LORIAUX, A.L.; ROITMAN, M.F. Encoding of aversion by dopamine and the nucleus accumbens. **Front Neurosci.**, v. 6, p. 137, 2012.

Melis, M. et al. **Endocannabinoids mediate presynaptic inhibition of glutamatergic transmission in rat ventral tegmental area dopamine neurons through activation of CB1 receptors.** *J. Neurosci.* v. 24, p. 53–62, 2004.

MELIS, M.; PISTIS, M. Hub and switches: endocannabinoid signalling in midbrain dopamine neurons. **Philos Trans R Soc Lond B Biol Sci.**, v. 367, p. 3276–3285, 2012.

METZ, V.G. et al. Omega-3 decreases D1 and D2 receptors expression in the prefrontal cortex and prevents amphetamine-induced conditioned place preference in rats. **J Nut Biochem.**, v. 67, p. 182–189, 2019.

METZ, V.G. et al. Cannabidiol prevents amphetamine relapse and modulates D1- and D2-receptor levels in mesocorticolimbic brain areas of rats. **Eur. Neuropsychopharmacol.**, v. 50, p. 23–33, 2021.

MIKAIL, H. G. et al. Sertraline behavioral response associates closer and dose-dependently with cortical rather than hippocampal serotonergic activity in the rat forced swim stress. **Physiol Behav**, v. 107, p. 201–206, 2012.

MONTGOMERY, K. The relation between fear induced by novel stimulation and exploratory behavior. **J. Comp. Physiol.** v. 48, p. 254–260, 1995.

MOREIRA, F.A.; AGUIAR, D.C.; GUIMARAES, F.S. Anxiolytic-like effect of cannabidiol in the rat Vogel conflict test. **Prog Neuropsychopharmacol Biol Psychiatry**, v. 30, p.1466–71, 2006.

MOREIRA-SILVA, D. Stress abolishes the effect of previous chronic ethanol consumption on drug place preference and the mesocorticolimbic brain pathway. *Alcoholism: Clinical and Experimental Research*, v. 38, n.5, p. 1227-1236, 2014.

NAPIER, T.C.; HERROLD, AA; DE WIT, H. Using conditioned place preference to identify relapse prevention medications. **Neurosci Biobehav Rev.**, v. 37(9 Pt A), p. 2081-2086, 2013.

NESBIT, M.O.; DIAS, C.; PHILLIPS, A.G. The effects of d-gavadine on conditioned place preference with d-amphetamine or food reward. **Behav Brain Res**, v. 321, p. 223–231, 2015.

NESTLER, E. J. et al. Chronic cocaine treatment decreases levels of the G protein subunits $G_{i\alpha}$ and $G_{o\alpha}$ in discrete regions of rat brain. **J. Neurochem.** v. 55, p. 1079-1082, 1990.

NESTLER, E. J.; AGHAJANIAN G. K. Molecular and cellular basis of addiction. **Science**, v. 278, p. 58-63, Oct. 1997.

NESTLER, E.J. Molecular basis of long-term plasticity underlying addiction. **Nat Rev Neurosci**, v. 2, p. 119–129, 2001.

NETZAHUALCOYOTZI-PIETRA et al. La marihuana y el sistema endocanabinoide: De sus efectos recreativos a la terapéutica. **Rev Biomed.** v. 20, p. 128-153, 2009.

NIDA – National Institute on Drug Abuse. Principles of Effective Treatment. **National Institute on Drug Abuse website** 18 Sep. 2020.

NOURI, K. et al. **Involvement of Hippocampal D1-Like Dopamine Receptors in the Inhibitory Effect of Cannabidiol on Acquisition and Expression of Methamphetamine-Induced Conditioned Place Preference.** *Neurochemical research*, v. 46(8), p. 2008–2018, 2021.

NYGARD, S.K.; HOURGUETTES, N.J.; SOBCZAK, G.G.; CARLEZON, W.A.; BRUCHAS, M.R. Stress-induced reinstatement of nicotine preference requires dynorphin/kappa opioid activity in the basolateral amygdala. **J. Neurosci.**, v. 36, p. 9937–9948, 2016.

OKAMOTO, Y. et al. Biosynthetic pathways of the endocannabinoid anandamide. **Chem Biodivers**, v. 4, p. 1842–57, 2007.

OLESON, E.B. et al. Endocannabinoids shape accumbal encoding of cue-motivated behavior via CB1 receptor activation in the ventral tegmentum. **Neuron**, v.73, p. 360–73, 2012.

OLESON, E.B; CHEER, J.F. On the role of subsecond dopamine release in conditioned avoidance. **Frontiers in Decision Making**, 2013.

OLIÈRE, S. et al. Modulation of the endocannabinoid system: vulnerability factor and new treatment target for stimulant addiction. **Front Psych.**, v. 4:129, p. 1-21, 2013.

OSBORNE, A.L.; SOLOWIJ, N.; WESTON-GREEN, K. A systematic review of the effect of cannabidiol on cognitive function: relevance to schizophrenia. **Neurosci Biobehav Rev.**, v. 72, p. 310–24, 2017.

PARSONS, L.H.; HURD, Y.L. **Endocannabinoid signalling in reward and addiction.** *Nat. Rev. Neurosci.* v. 16, p. 579–94, 2015.

- PARKER, L.A. et al. Effect of low doses of D9-tetrahydrocannabinol and cannabidiol on the extinction of cocaine-induced and amphetamine-induced conditioned place preference learning in rats. **Psychopharmacol**, v. 175, p. 360–366, 2004.
- PAU, C.W.H.; LEE, T.M.C.; CHAN, S.F.F. The impact of heroin on frontal executive functions. **Arch. Clin. Neuropsychol.** v. 17, p. 663-670, Oct. 2002.
- PAXINOS G.; WATSON, C. **The Rat Brain in Stereotaxic Coordinates**, sixth ed. Ne Academic Press, New York, 2007.
- PERES, F.F. et al. Cannabidiol, among other cannabinoid drugs, modulates prepulse inhibition of startle in the SHR animal model: implications for schizophrenia pharmacotherapy. **Front Pharmacol.**, v. 7, p. 303, 2016.
- PERTWEE, R.G. et al. et al. International Union of Basic and Clinical Pharmacology. LXXIX. Cannabinoid receptors and their ligands: beyond CB1 and CB2. **Pharmacol. Rev.**, v. 62, p. 588–631, 2010.
- PHILLIPS KA, EPSTEIN DH, PRESTON KL. Psychostimulant addiction treatment. **Neuropharmacol**, v. 87, p. 150-60, 2011.
- PRUD'HOMME et al. Cannabidiol as an Intervention for Addictive Behaviors: A Systematic Review of the Evidence. **Substance Abuse: Research and Treatment** v. v. 9, p. 33–38, 2015.
- QU, W.M. et al. Dopaminergic D1 and D2 receptors are essential for the arousal effect of modafinil. **J. Neurosci.**, v. 28, p. 8462-8469, Aug. 2008.
- RANG, H.P. et al. **Rang and Dale's Pharmacology**. 8th edition. – Rio de Janeiro: Elsevier, 2016.
- RAZAVI, Y. et al., **Neuroprotective effect of chronic administration of cannabidiol during the abstinence period on methamphetamine-induced impairment of recognition memory in the rats**. Behavioural pharmacology, v. 31(4), p. 385–396, 2020.
- REN, Y. et al. Cannabidiol, a Nonpsychotropic Component of Cannabis, Inhibits Cue-Induced Heroin Seeking and Normalizes Discrete Mesolimbic Neuronal Disturbances. **J Neurosci**, v. 29 (47), p. 14764 –14769, 2009.
- RENARD, J. et al. **Cannabidiol Counteracts Amphetamine-Induced Neuronal and Behavioral Sensitization of the Mesolimbic Dopamine Pathway through a Novel mTOR/p70S6 Kinase Signaling Pathway**. *J. Neurosci.* v. 36(18), p. 5160–5169, 2016.
- ROBBE, D. et al. **Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens**. *Proc. Natl. Acad. Sci. USA* v. 99(12), p. 8384-8, 2002.
- ROBINSON, T.E.; BERRIDGE, K.C. The neural basis of drug craving: an incentive-sensitization theory of addiction. **Brain Res. Rev.**, v. 18, p. 247-291, Sep. 1993.
- ROITMAN, M.F.; WHEELER, R.A.; WIGHMAN, R.M.; CARELLI, R.M. Real-time chemical responses in the nucleus accumbens differentiate rewarding and aversive stimuli.

Nature Publishing Group, v. 1376–1377, 2008.

RUSSO, E.B. Cannabidiol claims and misconceptions. **Trends Pharmacol Sci.**v. 38, p. 198–201, 2017.

RYBERG, E. et al. The orphan receptor GPR55 is a novel cannabinoid receptor. **Br J Pharmacol.**, v. 152(7), p. 1092–1101, 2007.

SADEGHZADEH, F.; BABAPOUR, V.; HAGHPARAST, A. Role of dopamine D1-like receptor within the nucleus accumbens in acute food deprivation- and drug priming-induced reinstatement of morphine seeking in rats. **Behav Brain Res**, v. 287, p. 172–181, 2015.

SAMSHA - Substance Abuse and Mental Health Services Administration, Results from the 2013 National Survey on Drug Use and Health: Summary of National Findings, NSDUH Series H-48, **HHS Publication No.** (SMA) 14-4863. Rockville, MD: Substance Abuse and Mental Health Services Administration, 2014.

SANCHIS-SEGURA, C.; SPANAGEL, R. Behavioural assessment of drug reinforcement and addictive features in rodents: an overview. **Addict. Biol.**, v. 11, p. 2–38, 2006.

SARTIM, A.G.; GUIMARAES, F.S.; JOCA, S.R. Antidepressant-like effect of cannabidiol injection into the ventral medial prefrontal cortex-possible involvement of 5-HT_{1A} and CB₁ receptors. **Behav Brain Res.**, v. 303, p. 218–227, 2016.

SCHEFFER, D.L. et al. Moderate running exercise prevents excessive immune system activation. **Physiol Behav.**, v. 204, p. 248–255, 2019.

SCHIAVON, A.P. et al. Influence of single and repeated cannabidiol administration on emotional behavior and markers of cell proliferation and neurogenesis in non-stressed mice. **Prog Neuropsychopharmacol Biol Psychiatry.**, v. 64, p. 27–34, 2016.

SCHMEICHEL, B.E.; BERRIDGE, C.W. Amphetamine acts within the lateral hypothalamic area to elicit affectively neutral arousal and reinstate drug-seeking. **Int. J. Neuropsychopharmacol.**, v. 17, p. 63–75, 2014.

SEDKI, F.; EIGENMANN, K.; GELINAS, J.; SCHOUELA, N.; COURCHESNE, S.; SHALEV, U. A role for kappa-, but not mu-opioid, receptor activation in acute food deprivation induced reinstatement of heroin seeking in rats. **Addict. Biol.**, v. 20, p. 423–432, 2015.

SEGAT, H. J. et al. Exercise modifies amphetamine relapse: Behavioral and oxidative markers in rats. **Behav Brain Res**,v. 262, p. 94–100, 18 Jan. 2014.

SEGAT, H. J. et al. m-Trifluoromethyl-diphenyldiselenide as a pharmacological tool to treat preference symptoms related to AMPH-induced dependence in rats. **Prog Neuro Psycho**, v. 66, p. 1–7, Apr. 2016.

SEGAT, H.J. et al. Influence of physical activity on addiction parameters of rats exposed to amphetamine which were previously supplemented with hydrogenated vegetable fat. **Brain Res. Bul.** v. 135, p. 69–76, Oct. 2017.

SEGAT, H.J. et al. Substitution therapy with amphetamine-isotherapeutic attenuates amphetamine toxicological aspects of addiction. **Neurosci. Lett.**, v. 690, p. 138–144, 2019.

SHAHAM, Y. et al. The reinstatement model of drug relapse: History, methodology and major findings. **Psychopharmacol**, v. 168, p.3–20, 2003.

SHAHAM, Y.; HOPE B. T. The role of neuroadaptations in relapse to drug seeking. **Nat. neurosci.** v. 8, n. 11, p. 1437 - 1439, Nov. 2005.

SHARIFI, A. et al., **Cannabidiol impairs the rewarding effects of methamphetamine: Involvement of dopaminergic receptors in the nucleus accumbens.** Progress in neuro-psychopharmacology & biological psychiatry, v.113, p.110458, 2022.

SHEN, Y.L.; CHANG, T.Y.; CHANG, I.C.; TIEN, H.H.; YANG, F.C.; WANG, P.Y.; LIAO, R.M. Elevated BDNF mRNA expression in the medial prefrontal cortex after d-amphetamine reinstated conditioned place preference in rats. **Neurosci.**, v. 263, p. 88–95, 2014.

SIDHPURA, N.; PARSONS, L.H. Endocannabinoid-mediated synaptic plasticity and addiction-related behavior. **Neuropharmacol** v. 61, p. 1070–87, 2011.

SITTE, H.H., FREISSMUTH, M. Amphetamines, new psychoactive drugs and the monoamine transporter cycle. **Trends Pharmacol Sci.**, v. 36(1), p. 41-50, 2015

SMAGA, I. et al. **Changes in the Brain Endocannabinoid System in Rat Models of Depression.** Neurotox. Res. v. 31, p. 421–435, 2017.

SOLINAS, M.; JUSTINOVA, Z.; GOLDBERG, S.R.; TANDA, G. Anandamide administration alone and after inhibition of fatty acid amide hydrolase (FAAH) increases dopamine levels in the nucleus accumbens shell in rats. **J. Neurochem.**, v. 98, p. 408–419, 2006.

SORG, B. A. et al. Repeated daily cocaine alters subsequent cocaine-induced increase of extracellular dopamine in the medial prefrontal cortex. **J. Pharmacol. Exp. Ther.**, v. 281, p. 54-61, 1997.

STANDAERT, DAVID G.; GALANTER, JOSHUA M. **Farmacologia da Neurotransmissão Dopaminérgica.** 4ª edição. Ano 2008. Cap.12. Pg 166 a 185.

STHAL, S.M. **Psicofarmacologia – Bases Neurocientíficas e Aplicações Práticas,** 4ª edição. Guanabara Koogan, 09 /2014.

STRIPLIN, C. D., KALIVAS, P. W. Robustness of G protein changes in cocaine sensitization shown with immunoblotting. **Synapse**, v. 14, p. 10-15, 1993.

SULZER, D. How addictive drugs disrupt presynaptic dopamine neurotransmission. **Neuron.** v. 69, p. 628-649, Feb. 2011.

SZABO, B; SIEMES, S.; WALLMICHATH, I. Inhibition of GABAergic neurotransmission in the ventral tegmental area by cannabinoids. **Eur. J. Neurosci.**, v. 15, p. 2057–2061, 2002.

TANIMURA, A. et al. The endocannabinoid 2- arachidonoylglycerol produced by diacylglycerol lipase alpha mediates retrograde suppression of synaptic transmission. **Neuron**, v. 65, p. 320–7, 2010.

TIEGES, Z. et al. Caffeine does not modulate inhibitory control. **Brain and Cognition**. v. 69, p. 316-327, Mar. 2009.

TZCHENTKE, T.M. Measuring reward with the conditioned place preference paradigm: a comprehensive review of drug effects, recent progress and new issues. **Prog. Neurobiol.** v. 56, n. 6, p. 613–672, Dec. 1998.

TZCHENTKE, T.M. Measuring reward with the conditioned place preference (CPP) paradigm: update of the last decade. **Addict. Biol.** v. 12, p. 227- 462, Sep. 2007.

ULUGÖL, A. The Endocannabinoid System as a Potential Therapeutic Target for Pain Modulation. **Balkan Medical Journal**, v. 31(2), p. 115-120, 2014.

UNODC – United Nations Office for Drug Control and Crime Prevention. World Drug Report 2021, 2021.

VALLOF, D et al. The Anorexigenic Peptide Neuromedin U (NMU) Attenuates Amphetamine-Induced Locomotor Stimulation, Accumbal Dopamine Release and Expression of Conditioned Place Preference in Mice. **PLoS ONE** v. 11, p. 5, 2016.

VAN DER MEER, F.J.; VELTHORST, E. Genet. Risk Outcome Psychos. (GROUP) Investig. Course of cannabis use and clinical outcome in patients with non-affective psychosis: a 3-year follow-up study. **Psychol. Med.**, v. 45, p. 1977–88, 2015.

VAZQUEZ, V. et al. Brief early handling increases morphine dependence in adult rats. **Behav. Brain Res.**, v. 170, p. 211-218, 30 Jun. 2006.

VIUDEZ-MARTÍNEZ, A. et al. Cannabidiol does not display drug abuse potential in mice behavior. **Acta Pharmacologica Sinica**, v. 0, p. 1–7, 2018.

VIUDEZ-MARTÍNEZ, A., et al. Gender differences in the effects of cannabidiol on ethanol binge drinking in mice. **Addict Biol.**, v. 25, p. 12765, 2020.

VOLKOW, N.D. et al. Effects of modafinil on dopamine and dopamine transporters in the male human brain: clinical implications. **JAMA**, 18 Mar. 2009.

VOLKOW, N.D.; HAMPSON, A.J.; BALER, R.D. Don't Worry, Be Happy: Endocannabinoids and Cannabis at the Intersection of Stress and Reward. **Annu. Rev. Pharmacol. Toxicol.**, v. 57, p. 285–308, 2017.

- WANG, H.; LUPICA, C.R. Release of endogenous cannabinoids from ventral tegmental area dopamine neurons and the modulation of synaptic processes. **Prog. Neuro Psychopharmacol. Biol. Psychiatry**, v. 52, p. 24–27, 2014.
- WEISS, F.; MARTIN-FARDON, R.; CICCOCIOPPO, R.; KERR, T.M.; SMITH, D.L.; BEN-SHAHAR, O. Enduring resistance to extinction of cocaine-seeking behavior induced by drug-related cues *Neuropsychopharmacol.*, v. 25, p. 361-372, 2001.
- WESTOVER, A.N.; NAKONEZKY, P.A.; HALEY, R.W. Acute myocardial infarction in young adults who abuse amphetamines. **Drug Alcohol Depen.**, v. 96, p. 49–56, 1 Jul. 2008.
- WHITE, F. J., WANG, R. Y. Electrophysiological evidence for A10 dopamine autoreceptor subsensitivity following chronic d-amphetamine treatment. **Brain Res.**, v. 309, p. 283-292, 1984.
- WHITE, F. J. et al. Repeated administration of cocaine or amphetamine alters neuronal responses to glutamate in the mesoaccumbens dopamine system. **J. Pharmacol. Exp. Ther.** v. 273, p. 445-454, 1995.
- WILSON, R.I.; KUNOS, G.; NICOLL, R.A. Presynaptic specificity of endocannabinoid signaling in the hippocampus. **Neuron**, v. 31, p. 453–62, 2001.
- WILSON, R.I.; NICOLL, R.A. Endogenous cannabinoids mediate retrograde signalling at hippocampal synapses. **Nature**, v. 410, p. 588–92, 2001.
- WISE, R.A. Dopamine, learning, and motivation. **Nature Reviews Neuroscience**. v. 5, n. 6, p. 483-494, Jun. 2004.
- YAHYAVI-FIROUZ-ABADI, N.; SEE, R.E. Anti-relapse medications: preclinical models for drug addiction treatment. **Pharmacology & therapeutics**, v. 124(2), p. 235–247, 2009.
- YANG, G. et al. **Cannabidiol attenuates methamphetamine-induced conditioned place preference via the Sigma1R/AKT/GSK-3 β /CREB signaling pathway in rats.** *Toxicol Res (Camb)*. v. 9 (3), p. 202-211, 2020.
- ZANELATTI, T.V. et al. Antidepressant-like effects of cannabidiol in mice: possible involvement of 5-HT1A receptors. **Brit J Pharmacol** v.159, p. 122-128, 2010.
- ZHANG, X. F. et al. Increased responsiveness of ventral tegmental area dopamine neurons to glutamate after repeated administration of cocaine or amphetamine is transient and selectively involves AMPA receptors. **J. Pharmacol. Exp. Ther.**, v. 281, p. 699-706, 1997.
- ZUARDI, A.W. et al. Cannabidiol for the treatment of psychosis in Parkinson's disease. **J Psychopharmacol.**, v. 23, p. 979–83, 2009.