UNIVERSIDADE FEDERAL DE SANTA MARIA CENTRO DE CIÊNCIAS NATURAIS E EXATAS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA

Suzan Gonçalves Rosa

O SISTEMA OPIOIDE CONTRIBUI PARA O EFEITO DO TIPO ANTIDEPRESSIVO DO DISSELENETO DE *m*-TRIFLUORMETIL-DIFENILA EM CAMUNDONGOS

Santa Maria, RS
2018

O SISTEMA OPIOIDE CONTRIBUI PARA O EFEITO DO TIPO ANTIDEPRESSIVO DO DISSELENETO DE *m*-TRIFLUORMETIL-DIFENILA EM CAMUNDONGOS

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito para obtenção do título de **Doutor em Bioquímica Toxicológica**.

Orientadora: Prof.ª Dr.ª Cristina Wayne Nogueira

Santa Maria, RS
2018

Suzan Gonçalves Rosa

O SISTEMA OPIOIDE CONTRIBUI PARA O EFEITO DO TIPO ANTIDEPRESSIVO DO DISSELENETO DE *m*-TRIFLUORMETIL-DIFENILA EM CAMUNDONGOS

Tese apresentada ao Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito para obtenção do título de **Doutor em Bioquímica Toxicológica**.

Aprovado em 20 de julho de 2018:

_____

Cristina Wayne Nogueira, Dr.ª (UFSM)
(Presidente/Orientadora)

_____

Iraci Lucena da Silva Torres, Dr.ª (UFRGS)

_____

Jeferson Luis Franco, Dr. (UNIPAMPA)

_____

Marina Prigol, Dr.ª (UNIPAMPA)

_____

Mauro Schneider Oliveira, Dr. (UFSM)

Santa Maria, RS

2018

À minha família

AGRADECIMENTOS

Agradeço a Deus pela vida, por iluminar meu caminho e me proteger sempre.

À minha família, meu pai Geraldo, minha mãe Ana e meus irmãos Sandrigo e Ricardo, por estarem sempre ao meu lado, acreditarem em mim e nunca medirem esforços para me ajudar. Obrigada por todo apoio, cuidado e amor. Vocês são meus maiores incentivadores. Amo vocês!

Agradeço imensamente à minha orientadora Cristina, por toda dedicação, compreensão, paciência, ensinamentos que foram além do seu papel de orientadora e pelo carinho e amizade!
Ao GZ, pela dedicação, exemplo profissional, carinho e amizade.

Ao meu namorado, Bernardo, obrigada por acreditar em mim, pela paciência, amor, cuidado e pelo apoio em todos os momentos!

Agradeço à minhas amigas de infância, Jordana, Liliane, Thamise, Tienny e Uly por compreenderem minhas faltas e torcerem por mim independentemente do tempo ou distância.

Aos colegas de Lab Cris: Bruna, Carla, Caroline, César, Daniela, Isabella, Juliano, Lina, Marcel, Natalia, Nícolas, Paulo César, Pietro, Renata, Sabrina, Suelen, Vanessa e Vinicius, pela ajuda, paciencia, compreensão e amizade. Em especial às minhas coautoras e amigas Ana Paula e Franciele.

A todos antigos colegas do Lab Cris, especialmente à Juliana, Elusa e Crisinha pelos incentivos, conselhos, amizade e ensinamentos profissionais.
Aos colegas do Lab Gz pela amizade.
Ao Rinaldo pelo cuidado com os animais e amizade.
À banca examinadora por participarem da avaliação dessa tese.

À Universidade Federal de Santa Maria e ao Programa de Pós-Graduação em Bioquímica Toxicológica pela possibilidade de realização desse curso.
À CAPES, pelo auxílio financeiro.

Enfim, agradeço a todos que de alguma forma contribuíram para a realização deste trabalho.

"A menos que modifiquemos a nossa maneira de pensar, não seremos capazes de resolver os problemas causados pela forma como nos acostumamos a ver o mundo."

(Albert Einstein)

RESUMO

O SISTEMA OPIOIDE CONTRIBUI PARA O EFEITO DO TIPO ANTIDEPRESSIVO DO DISSELENETO DE *m*-TRIFLUORMETIL-DIFENILA EM CAMUNDONGOS

Autora: Suzan Gonçalves Rosa
Orientadora: Dr.ª Cristina Wayne Nogueira

A depressão é um transtorno mental de patofisiologia complexa e caracterizada por múltiplos sintomas, o que dificulta o tratamento desta doença. Nas últimas décadas, o sistema opioide tornou-se foco de pesquisas sobre a depressão, uma vez que este sistema exerce um importante efeito regulatório sobre o humor. No entanto, o uso de opioides na clínica é limitado pelo desenvolvimento de tolerância e dependência após a administração repetida. O composto orgânico de selênio disseleneto de *m*-trifluormetil-difenila (*m*-CF_3-PhSe)$_2$ apresenta efeito do tipo antidepressivo em modelos animais por meio de diferentes alvos de ação, que incluem o sistema opioide. Assim, o principal objetivo desta tese foi avaliar a contribuição do sistema opioide para o efeito farmacológico do composto orgânico de selênio (*m*-CF_3-PhSe)$_2$ em modelos de depressão em camundongos Swiss machos. Os resultados do **artigo 1** demostraram o efeito do tipo antidepressivo do (*m*- CF_3-PhSe)$_2$ no teste do nado forçado (TNF) e a contribuição de cada receptor opioide para este efeito, por meio do uso de antagonistas seletivos destes receptores, sugerindo que a ativação de receptores μ e δ e o bloqueio dos receptores κ estão envolvidos na ação do tipo antidepressiva do composto. Além disso, o **artigo 1** revelou o efeito do tipo antidepressivo da administração aguda ou repetida do (*m*-CF_3-PhSe) no TNF e teste de suspensão da cauda (TSC) modificado. Neste último, o envolvimento do sistema opioide na ação do tipo antidepressiva do composto foi evidenciado por meio do aumento de comportamentos característicos da modulação do sistema opioide. O a**rtigo 1** também demonstrou que a administração repetida do (*m*-CF_3-PhSe)$_2$ não induz tolerância no TNF e sinais físicos de abstinência induzidos por naloxona e não altera parâmetros de toxicidade sistêmica. Com base nestes resultados, buscou-se investigar mecanismos moleculares pelos quais o sistema opioide contribui para os efeitos do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$. Para isso, modelos de depressão induzidas por estresse foram utilizados, uma vez que o sistema opioide está envolvido nas diferentes respostas ao estresse. No **artigo 2,** o efeito de uma ou repetidas exposições ao estresse de natação forçada (ENF) sobre o comportamento do tipo depressivo e nos níveis de receptores opioides em córtex pré-frontal de camundongos foi demonstrada. Neste estudo, o (*m*-CF_3-PhSe)$_2$ foi efetivo contra os sintomas do tipo depressivos no TNF, TSC e *splash* teste induzidos pelo ENF repetido por meio da regulação dos níveis de receptores μ e κ. Portanto, o efeito do (*m*-CF_3-PhSe)$_2$ foi investigado em um modelo de estresse mais severo e prolongado no **artigo 3**. Neste estudo, o estresse de derrota social (EDS) induziu aversão social e alterou os níveis dos três tipos de receptores opioides em córtex pré-frontal de camundongos suscetíveis. Além disso, o (*m*-CF_3-PhSe)$_2$ foi efetivo contra estas alterações promovendo resiliência ao EDS, além de aumentar a sociabilidade natural entre os camundongos. Em conjunto, os resultados desta tese contribuem para a compreensão dos mecanismos opioides envolvidos no efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ e indicam que este composto pode ser uma alternativa terapêutica para o tratamento da depressão.

Palavras-chave: Depressão. Selênio. Sistema opioide. Estresse.

ABSTRACT

OPIOID SYSTEM CONTRIBUTES TO *m*-TRIFLUOROMETHYL-DIPHENYL DISELENIDE ANTIDEPRESSANT-LIKE EFECT IN MICE

Author: Suzan Gonçalves Rosa
Advisor: Cristina Wayne Nogueira, PhD

Depression is a mental disorder with complex pathophysiology and characterized by multiple symptoms, which makes it difficult to treat this disease. In the last decades, the opioid system has become the focus of depression researches, given that this system has an important regulatory effect on mood. However, the opioids use in the clinic is limited by the development of tolerance and dependence after repeated administration. The organoselenium compound *m*-trifluoromethyl-diphenyl diselenide **(***m*-CF_3-PhSe)$_2$ elicits antidepressant-like effect in animal models through different action targets, including the opioid system. Thus, the main objective of this thesis was to evaluate the opioid system contribution to the pharmacological effect of the organic compound of selenium (*m*-CF_3-PhSe)$_2$ in models of depression in swiss male mice. The results of **article 1** demonstrated the (*m*-CF_3-PhSe)$_2$ antidepressant-like effect on the forced swimming test (FST) and the contribution of each opioid receptor to this effect through the use of selective antagonists of these receptors, suggesting that μ and δ receptors activation and κ-receptor blockade are involved in the antidepressant-like action of the compound. In addition, **article 1** revealed the antidepressant-like effect of acute or repeated administration of (*m*-CF_3-PhSe)$_2$ on the FST and the modified tail suspension test (TST). In the modified TST, the involvement of the opioid system in the antidepressant-like action of the compound was demonstrated by the increase of behaviors characteristic of opioid system modulation. The **article 1** also demonstrated that repeated administration of (*m*-CF_3-PhSe)$_2$ neither induce tolerance in the FST nor physical signs of naloxone-induced withdrawal and did not alter systemic toxicity parameters. Based on these results, it was investigated the molecular mechanisms by which the opioid system contributes to the antidepressant-like effect of (*m*-CF_3-PhSe)$_2$. For this, stress-induced depression models were carried out, given that the opioid system is involved in the different responses to stress. The **article 2** revealed that one or repeated forced swimming stress (FSS) exposures induced depressant-like behavior and affected the prefrontal cortical opioid receptor levels of mice. In this study, (*m*-CF_3-PhSe)$_2$ was effective against depressant-like symptoms induced by repeated FSS in the FST, the TST and the splash test through of μ and κ receptor levels regulation. Therefore, the (*m*-CF_3- PhSe)$_2$ effect was investigated in a more severe and prolonged stress model in the **article 3**. In this study, social defeat stress (SDS) induced social aversion and altered the levels of the three opioid receptors types in the prefrontal cortex of susceptible mice. (*m*-CF_3-PhSe)$_2$ was effective against these changes, promoting resilience to SDS and increasing the natural sociability among mice. Together, the results of this thesis contributes to the understanding of the opioid mechanisms involved in the antidepressant-like effect of (*m*-CF_3-PhSe)$_2$ and indicates that this compound may be an therapeutic alternative for the treatment of depression.

Keywords: Depression. Selenium. Opioid System. Stress.

LISTA DE FIGURAS

LISTA DE ABREVIATURAS

(*m*-CF_3-PhSe)$_2$	Disseleneto de *m*-trifluormetil-difenila
$(PhSe)_2$	Disseleneto de difenila
5-HT	5-Hidroxitriptamina, serotonina
ACTH	hormônio adrenocorticotrófico
ADT	Antidepressivos tricíclicos
Akt	Proteína quinase B
ALT	Alanina aminotransferase
AMPc	Adenosina 3',5'-monofosfato cíclico
HPA	Hipotálamo-pituitária-adrenal
AST	Aspartato aminotransferase
CK	Creatina quinase
CRH	Hormônio liberador de corticotrofina
DL50	Dose letal em 50% dos animais
EDS	Estresse de derrota social
ENF	Estresse de natação forçada
ERK	Quinase regulada por sinal extracelular
GRK	Proteína quinase do receptor acoplado à proteína G
Imao	Inibidores da monoaminoxidase
ISRN	Inibidores seletivos da recaptação da noradrenalina
ISRS	Inibidores seletivos da recaptação da serotonina
JNK	Quinase c-Jun N-terminal
MAPKs	Proteínas quinases ativadas por mitógeno
MAO	Monoamina oxidase
NMDA	N-metil-D-aspartato
PKA	Proteína quinase dependente de cAMP
p38 MAPK	Proteína quinase ativada por mitógeno p38
SNC	Sistema Nervoso Central
TNF-α	Fator de necrose tumoral alfa
TNF	Teste do nado forçado
TSC	Teste da suspensão da cauda

LISTA DE ANEXOS

SUMÁRIO

1 INTRODUÇÃO

1.1 DEPRESSÃO

A depressão é um transtorno mental grave e altamente recorrente, associado à diminuição da qualidade de vida e aumento da mortalidade em todo o mundo (MARCUS et al., 2012). Estudos epidemiológicos estimam que, globalmente, mais de 322 milhões de pessoas de todas as idades sofrem de depressão (WHO, 2017). A Organização Mundial de Saúde classificou a depressão como a 4ª causa de incapacidade mundial e estimou que, até 2020, será a segunda causa principal, podendo ser o mal mais prevalente do planeta até 2030 (WHO, 2012). No Brasil, cerca de 11,5 milhões de pessoas, 5,8% da população, apresentam sintomas depressivos, o que classifica o país como o primeiro em maior prevalência de depressão da América Latina e o segundo nas Américas, ficando atrás somente dos Estados Unidos, com 5,9% da população depressiva (WHO, 2017). Entre os estados brasileiros, os que concentram o maior número de adultos deprimidos encontram-se no sul do país, sendo o Rio Grande do Sul o estado com maior número de pessoas diagnosticadas com depressão, cerca de 13,2% da população (IBGE, 2014).

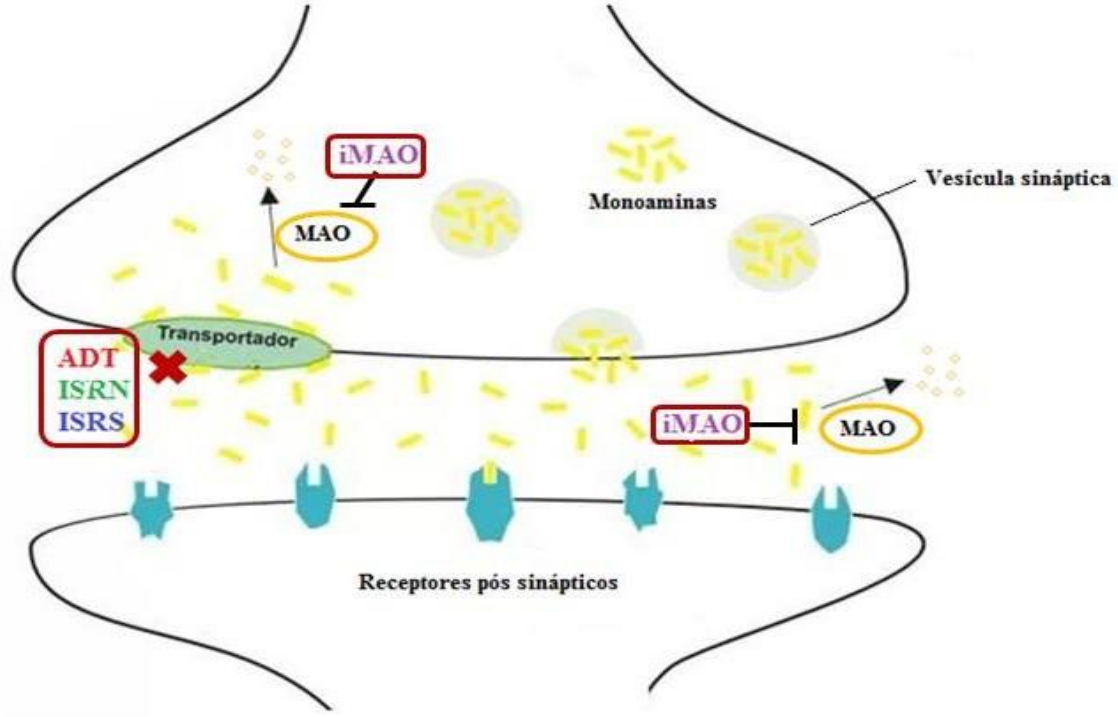
O Manual de Diagnóstico e Estatística dos Transtornos Mentais descreve a depressão como um conjunto de sintomas que duram pelo menos duas semanas e incluem humor deprimido, perda de interesse ou prazer e pelo menos cinco das seguintes ocorrências: mudança significativa de peso ou apetite, distúrbios do sono, perda de autoestima, dificuldade de concentração, fadiga, sentimento de culpa ou fracasso, e planos ou tentativas de suicídio (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). Coletivamente, esses sintomas causam substanciais prejuízos na qualidade de vida dos indivíduos, interferindo na capacidade de autocuidado e na execução de suas responsabilidades diárias (WHO, 2012).

A complexidade da depressão reflete-se na variedade de mecanismos genéticos, neurológicos, hormonais, imunológicos e neuroendócrinos envolvidos na gênese da doença, os quais caracterizam a depressão como uma doença heterogenia e multifatorial e dificultam a total compreensão da sua patofisiologia (SAVEANU e NEMEROFF, 2012).

Durante décadas, a teoria monoaminérgica esteve no centro das pesquisas clínicas e pré-clínicas sobre a neurobiologia e tratamento da depressão. Esta teoria postula que a base patofisiológica da depressão envolve a depleção dos neurotransmissores serotonina, norepinefrina ou dopamina no sistema nervoso central (SNC), seja por aumento da degradação de monoaminas pela enzima monoaminoxidase (MAO) e/ou pelo aumento da recaptação desses neurotransmissores para o neurônio pré-sináptico e consequente diminuição dos mesmos

na fenda sináptica (HIRSCHFELD, 2000; SCHILDKRAUT, 1965). Esta patofisiologia hipotética é apoiada pelo mecanismo de ação de antidepressivos, agentes que elevam os níveis de serotonina, norepinefrina ou dopamina no SNC mostraram-se eficazes no alívio dos sintomas depressivos (WILLNER et al., 2013). Desta forma, as principais classes de antidepressivos utilizados na clínica incluem os inibidores seletivos da recaptação da serotonina (ISRS), inibidores seletivos da recaptação da noradrenalina (ISRN), inibidores da monoamina oxidase (iMAO) e antidepressivos tricíclicos (ADT) (CARVALHO et al., 2016) (Figura 1).

Figura 1- Representação dos mecanismos de ação de antidepressivos monoaminérgicos



Fonte: Adaptado de YOUDIM et al. (2006).

No entanto, apesar da importância da hipótese monoaminérgica para a investigação e o tratamento da depressão, muitos pacientes, cerca de 30-40%, não respondem ou respondem parcialmente a este tipo de tratamento. Somado a isso, o longo prazo para a resposta terapêutica e os diversos efeitos indesejáveis destes fármacos, tem impulsionado as buscas por outros possíveis mecanismos e terapias antidepressivas que vão além da hipótese das monoaminas (BENTLEY et al., 2014; CARVALHO et al., 2016).

Neste contexto, o sistema opioide tornou-se foco de pesquisas sobre a patofisiologia e o

tratamento da depressão nos últimos anos. Isso se deu principalmente a partir das diferenças na distribuição e densidade de receptores opioides entre pessoas saudáveis e deprimidas e dos potentes efeitos antidepressivos de fármacos opioides em pacientes e em modelos animais (BERSHAD et al., 2018; CALLAGHAN et al., 2018; SCARONE et al., 1990; ZALSMAN et al., 2005). Além disso, o envolvimento do sistema opioide em respostas emocionais ao estresse tem sido demonstrado por estudos que indicam que a variabilidade das respostas ao estresse pode ser regulada por opioides, tanto endógenos como as encefalinas, quanto exógenos como antagonistas de receptores κ (BALI et al., 2015; BERUBE et al., 2014; DONAHUE et al., 2015; WILLIAMS et al., 2018).

Nas últimas décadas, a depressão induzida por estresse tem sido amplamente estudada, uma vez que o estresse tornou-se um dos fatores de risco mais comuns para o desenvolvimento da doença (TAFET e NEMEROFF, 2016) . Estudos indicam que a maior parte de episódios depressivos, sejam eles iniciais ou reincidentes, são consequências prováveis de uma experiência estressante grave ou de episódios estressantes recorrentes (SALAVECZ et al., 2014; TAFET e NEMEROFF, 2016). Além disso, estudos clínicos demonstram que o estresse causa alterações em diferentes regiões do encéfalo, incluindo o hipocampo, a amígdala e o córtex pré-frontal, as quais são fundamentais para o processamento emocional (FRODL e O'KEANE, 2013; TREADWAY et al., 2015). A etiologia dos transtornos depressivos induzidos por estresse envolve alterações na funcionalidade de diferentes sistemas e vias de sinalização neuronais, as quais modificam a capacidade dos indivíduos em lidar com um estressor e consequentemente definem se as respostas ao estresse serão adaptativas ou patológicas (BALE, 2006). Além disso, o desenvolvimento e a gravidade dos sintomas depressivos induzidos por estresse dependem além de fatores interpessoais e ambientais, da duração, intensidade e natureza do estressor (TAFET e NEMEROFF, 2016).

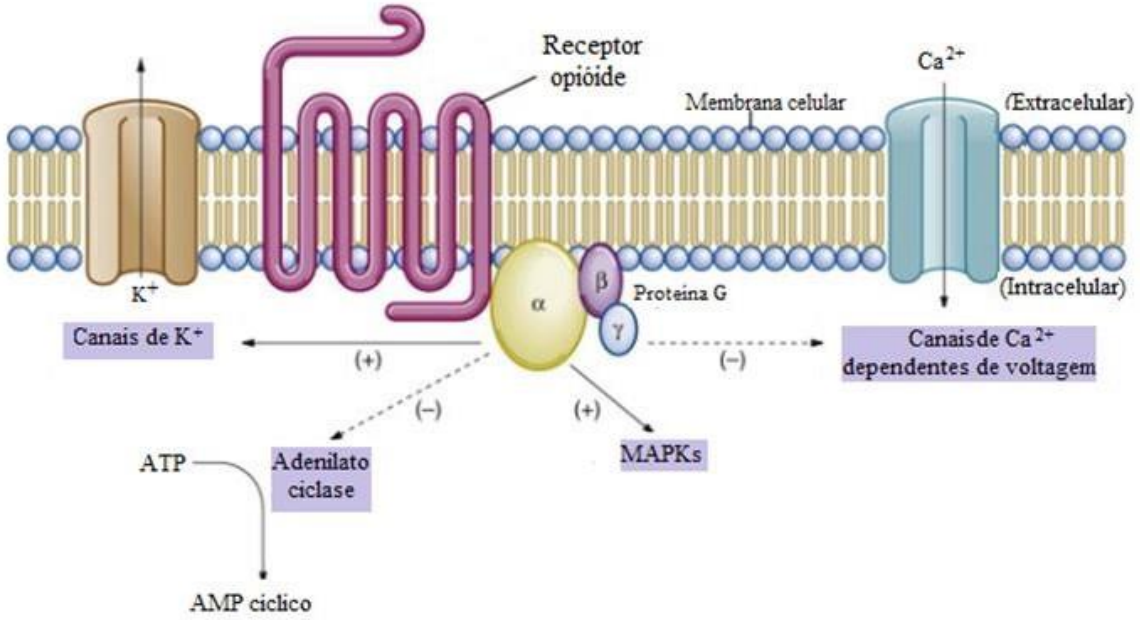
1.2 MECANISMOS DE AÇÃO OPIOIDE NA DEPRESSÃO E NO ESTRESSE

Embora os receptores opioides sejam amplamente reconhecidos por seus efeitos analgésicos, o envolvimento destes receptores na regulação do humor e de respostas ao

estresse tem sido cada vez mais estudado e ratificado (BALI et al., 2015; BERSHAD et al., 2018; CALLAGHAN et al., 2018; LUTZ e KIEFFER, 2013).

O sistema opioide abrange os diferentes peptídeos endógenos, as endorfinas, encefalinas e dinorfinas, que atuam sobre três principais receptores, mu (μ), delta (δ) e kappa (κ), respectivamente (OGURA e EGAN, 2013). Os três receptores opioides possuem todos os sete domínios transmembrana característicos da superfamília de receptores acoplados a proteína G e utilizam as proteínas heterotriméricas Gi e Go para transdução de sinal. Após a ativação destes receptores, as subunidades Gα e Gβy dissociam-se e subsequentemente atuam sobre várias vias efetoras intracelulares, incluindo a inibição da adenilato ciclase, enzima que catalisa síntese de adenosina 3',5'-monofosfato cíclico (AMPc), a diminuição da atividade de canais de cálcio e a estimulação de canais de potássio, diminuindo, desta forma, a excitabilidade neuronal e a liberação de neurotransmissores (AL-HASANI e BRUCHAS, 2011; LAW et al., 2000). Além disso, os receptores opioides também regulam cascatas de sinalização de proteínas quinases, como as da família das proteínas quinases ativadas por mitógenos (MAPKs) (OGURA e EGAN, 2013) (Figura 2).

Figura 2- Representação dos receptores opioides e suas principais vias de sinalização.



Fonte: Adaptado de OGURA e EGAN (2013)

O sistema endógeno de opioides está envolvido na resposta neuroendócrina ao estresse. O estresse ativa o sistema nervoso simpático e o eixo hipotálamo-pituitária- adrenal (HPA). O primeiro resulta no aumento da frequência cardíaca e pressão arterial. Já a estimulação do eixo HPA leva a liberação do hormônio liberador de corticotrofina (CRH) e consequente produção do hormônio adrenocorticotrófico (ACTH) a partir da hipófise anterior, levando à liberação de cortisol pelas glândulas supra-renais. A endorfina endógena compartilha um precursor comum com o ACTH e, quando a liberação de cortisol é estimulada, também são produzidas endorfinas (BODNAR, 2013; KJÆR et al., 1992). Desta forma, o sistema opioide e o eixo HPA estão fisiologicamente interligados.

Além disso, os peptídeos opioides endógenos e seus receptores estão densamente distribuídos em regiões corticais implicadas na regulação do humor, estímulos emocionais e de resposta a estressores, incluindo o córtex pré-frontal (MANSOUR et al., 1995; SHARP, 2004).

Neste contexto, estudos têm demonstrado que pacientes com depressão podem apresentar deficiência na atividade opioide endógena e que, por outro lado, opioides como a β-endorfina, inibidores da degradação da encefalina e a buprenorfina, um agonista parcial μ e antagonista do receptor opioide κ, apresentam efeitos antidepressivos na clínica (DARKO et al., 1992; JUTKIEWICZ et al., 2006; KARP et al., 2014).

Em modelos pré-clínicos, o fenótipo pró-depressivo produzido por estresse, pode estar relacionado à modulação de receptores opioides e seus subsequentes eventos de sinalização (BALI et al., 2015; BERUBE et al., 2013). O estresse altera a liberação de peptídeos opioides endógenos em regiões límbicas, os quais exercem efeitos particulares sobre os comportamentos depressivos e em respostas adaptativas ou patológicas ao estresse de acordo com cada tipo de receptor opioide (BODNAR, 2017; DROLET et al., 2001; NABESHIMA et al., 1992; SMITH et al., 2012).

O envolvimento do receptor opioide μ na resposta ao estresse e transtornos depressivos tem sido demonstrado por meio de pesquisas em humanos e animais. Estudos clínicos demonstraram uma diminuição na ativação e na densidade do receptor opioide μ e seu ligante endógeno em pacientes com depressão e suicidas (HU et al., 2015; ZALSMAN et al., 2005). Além disso, (DARKO et al., 1992) reportaram que a administração de β-endorfina produz rapidamente um efeito antidepressivo em pacientes deprimidos. Em roedores, a ativação farmacológica dos receptores opioides μ reduz comportamentos depressivos e o bloqueio destes receptores previne os efeitos de antidepressivos tricíclicos (ONALI et al., 2010; ROBINSON, S. A. et al., 2017). Por outro lado, estudos demonstram uma diminuição de

comportamentos depressivos e de respostas negativas ao estresse em camundongos com deleção genética de receptores opioides μ e uma elevação da expressão e da funcionalidade desses receptores na depressão induzida por estresse em roedores (HAJ-MIRZAIAN et al., 2016; KOMATSU et al., 2011; NIKULINA et al., 2005). Desta forma, acredita-se que os receptores opioides μ são regulados de acordo com os estímulos, e que tanto uma diminuição/bloqueio ou um aumento/ativação destes receptores pode ter consequências prejudiciais sobre o humor (LUTZ e KIEFFER, 2013).

Diferentemente dos receptores opioides μ, os efeitos dos receptores opioides δ e κ são bem estabelecidos em transtornos depressivos e no estresse (LUTZ e KIEFFER, 2013). Em humanos, a diminuição de encefalina cerebral está relacionada com a vulnerabilidade ao estresse, indicando o envolvimento do receptor opioide δ em transtornos psiquiátricos relacionados a este evento (SCHWARTZ et al., 1988). No mesmo sentido, estudos demonstram que as encefalinas facilitam a adaptação comportamental ao estresse em roedores (BERUBE et al., 2014; HENRY et al., 2017). Além disso, corroborando estes dados, o efeito do tipo antidepressivo de agonistas de receptores opioides δ em diferentes testes comportamentais tem sido amplamente reportado (DRIPPS, I. J. et al., 2018; DRIPPS, ISAAC J e JUTKIEWICZ, 2017; POULIN et al., 2014; SAITOH et al., 2011).

As dinorfinas e seus receptores opioides κ têm sido apontados como fatores-chave na regulação do humor e respostas ao estresse (KNOLL e CARLEZON, 2010). A administração de agonistas e antagonistas destes receptores produz efeitos caracterizados como pró-depressivos ou antidepressivos, respectivamente, em diferentes testes comportamentais (CARLEZON et al., 2006; FALCON et al., 2016; WILLIAMS et al., 2018). Evidências clínicas também já demonstraram os efeitos disfóricos (estado desagradável ou aversivo) da ativação de receptores opioides κ em humanos (PFEIFFER et al., 1986). Além disso, sabe-se que a exposição ao estresse sensibiliza o sistema dinorfina-receptores κ, uma vez que há um aumento na liberação de dinorfinas e expressão de receptores opioides κ durante o estresse (DONAHUE et al., 2015; SMITH et al., 2012). Por outro lado, a deleção genética de pró- dinorfina ou o tratamento com antagonistas destes receptores bloqueia respostas depressivas dependentes do estresse (FALCON et al., 2016; MCLAUGHLIN et al., 2006a; WILLIAMS et al., 2018).

Os receptores opioides, tem como parte da sua via de sinalização a ativação de MAPKs, as quais desempenham um importante papel na regulação de respostas ao estresse e em doenças psiquiátricas relacionadas a ele, em especial a depressão (OGURA e EGAN, 2013; YANG et al., 2017). Evidências sugerem que, assim como a modulação de receptores opioides produz efeitos comportamentais e neurológicos particulares a cada tipo de receptor, a ativação destes

receptores pode variar na sua capacidade de regular a ativação das MAPKs (CHEN et al., 2016; GUTSTEIN et al., 1997; LOVELL et al., 2015). Além de contribuir para o efeito antidepressivo de opioides, as MAPKs podem regular tanto efeitos agudos do estresse quanto pode alterar respostas às experiências estressantes prolongadas (QI, XIAOLI et al., 2006b; THOMAS e HUGANIR, 2004).

1.3 MECANISMOS DE TOLERÂNCIA E ABSTINÊNCIA AOS OPIOIDES

Conforme mencionado anteriormente, muitos estudos clínicos e pré-clínicos comprovam a eficácia de fármacos opioides na melhora do humor, nos sintomas depressivos e na adaptação ao (BALI et al., 2015; BERROCOSO et al., 2009; BERSHAD et al., 2018) estresse . No entanto, a aplicação clínica de opioides para esse fim é bastante limitada, uma vez que o uso frequente destes fármacos leva a um rápido desenvolvimento de tolerância e dependência (FARRAR et al., 2010; LIN et al., 2015).

A tolerância é definida pela necessidade de doses cada vez maiores para se manter os efeitos de uma fármaco, enquanto a dependência é caracterizada por fortes sintomas de abstinência após a descontinuação do tratamento. Os sintomas de abstinência são muitas vezes contrários aos efeitos da fármaco e vão desde sinais físicos desagradáveis até o desenvolvimento de transtornos psicológicos como a ansiedade e a depressão (BISAGA et al., 2001; WAKIM, 2012).

Os mecanismos implicados na tolerância e abstinência a opioides são complexos e envolvem vias de sinalização, adaptações a níveis de receptores e interações entre sistemas opioide e não opioides. Neste contexto, estudos têm demonstrado que a tolerância e dependência aos opioides resulta principalmente da dessensibilização dos receptores opioides e da regulação positiva da via do cAMP (ALLOUCHE et al., 2014; CHAN e LUTFY, 2016).

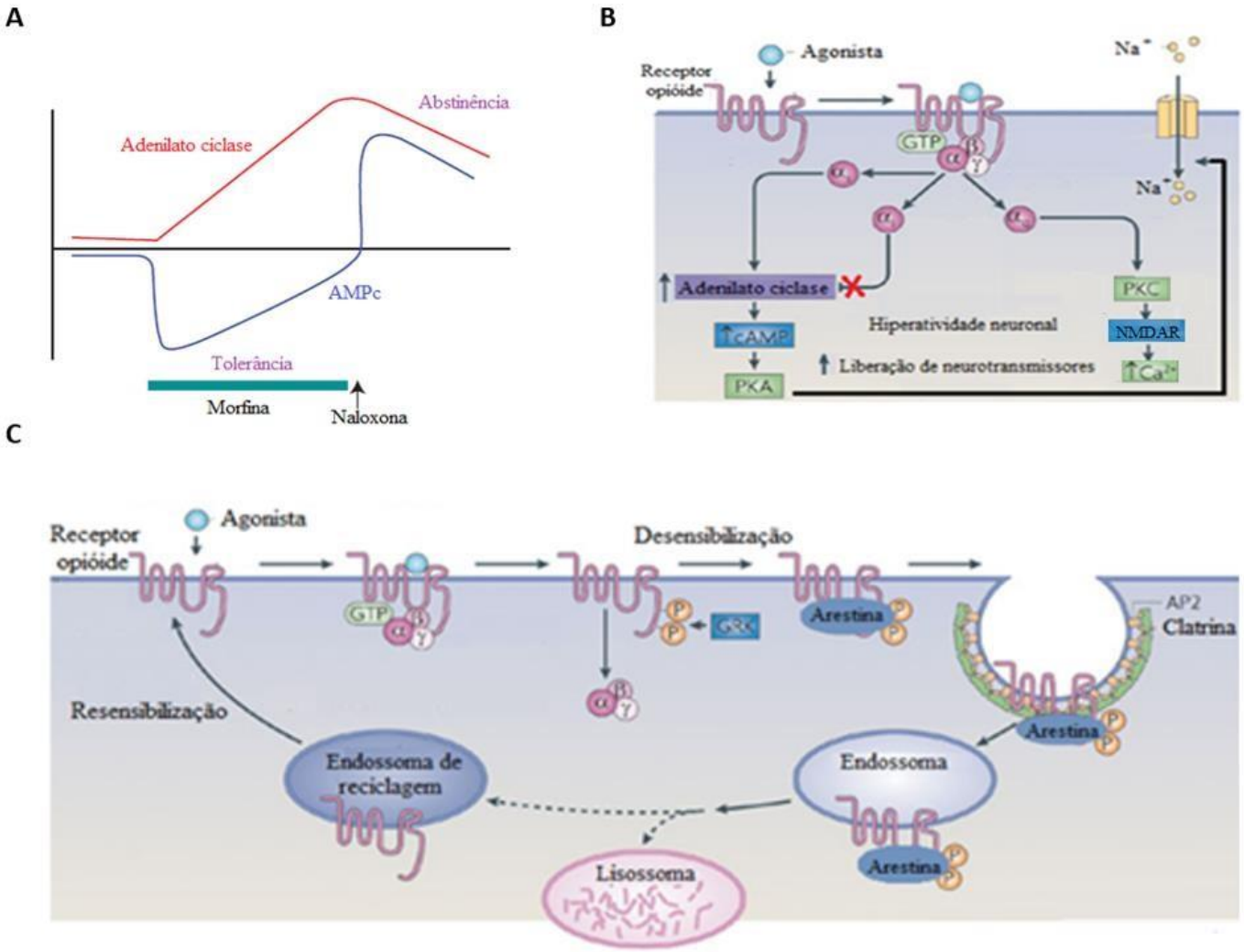
A dessensibilização dos receptores opioides pode ser mediada pela fosforilação destes receptores pela proteína quinase do receptor acoplado à proteína G (GRK). A fosforilação dos receptores aumenta a ligação com β-arrestinas levando ao desacoplamento de receptores opioides das proteínas G inibitórias. Após a dessensibilização, as β-arrestinas ligam-se à clatrina iniciando a internalização dos receptores, os quais podem ser reciclados para a membrana (resensibilização) ou direcionados para a degradação (ALLOUCHE et al., 2014). Esses processos podem contribuir diretamente para a tolerância aos efeitos dos opioides ao diminuir o número de receptores funcionais na superfície celular (Figura 3A).

De forma aguda, os opioides inibem a adenilato ciclase, diminuindo a ativação da via do AMPc, como mencionado anteriormente (LAW et al., 2000). No entanto, durante a exposição repetida, ocorre uma resposta compensatória homeostática, a qual leva a normalização desta via gradualmente, desencadeando o fenômeno da tolerância farmacológica. Além disso, após a remoção da fármaco opioide (retirada espontânea abrupta ou pela administração de um antagonista opioide) a atividade da adenilato ciclase e os níveis de AMPc aumentam significativamente, podendo ser considerados um sinal de abstinência (Figura 3B) (CHAN e LUTFY, 2016). Resumidamente, a superativação da adenilato ciclase e o consequente aumento dos níveis de AMPc ativam a proteína quinase dependente de cAMP (PKA), a qual aumenta as correntes de Na^+, aumentando a excitabilidade neuronal. A ativação desta corrente pode explicar por que as taxas de disparo neuronais retornam ao normal, apesar da presença contínua de um opioide (tolerância) (KOPNISKY e HYMAN, 2002). Além disso, a exposição crônica à agonistas opioides, ativa a proteína quinase Cγ (PKCγ), a qual também contribui para a tolerância opioide, ativando receptores glutamatérgicos do tipo N-metil-D-aspartato (NMDA) e levando a um consequente aumento no influxo de Ca^{2+}. A ativação prolongada de vias de sinalização dependentes de Ca^{2+} e o aumento da liberação de neurotransmissores, incluindo o glutamato, têm sido associados ao desenvolvimento de tolerância e sinais de abstinência à opioides, como a morfina (LU et al., 1999; SIGGINS et al., 2003) (Figura 3C). Além disso, os níveis aumentados de AMPc também estão relacionados com o aumento da liberação de noradrenalina, dependente de cálcio, em neurônios presentes no locus coeruleus. A transmissão de impulsos noradrenérgicos a várias partes do encéfalo estimula a vigília, a respiração, a pressão arterial, o nervosismo e a ansiedade, entre outros sintomas que caracterizam a síndrome de abstinência (CHAIJALE et al., 2013; PARLATO et al., 2010)

Confirmando o envolvimento do sistema glutamatérgico na tolerância e abstinência aos opioides, estudos têm demonstrado que a administração de naloxona a camundongos dependentes de morfina leva ao aumento da liberação e dos níveis de glutamato, altera a expressão de receptores glutamatérgicos e induz uma diminuição dos transportadores de glutamato em diferentes regiões cerebrais (MAO et al., 2002; SIGGINS et al., 2003; WANG, X. F. et al., 2011). Além disso, os efeitos de antagonistas dos receptores NMDA e da ativação de transportadores de glutamato em atenuar o desenvolvimento de tolerância e dependência à morfina também tem sido reportados em pesquisas pré-clínicas e clínicas (BISAGA et al., 2001; HARRIS et al., 2008; MENDEZ e TRUJILLO, 2008). Desta forma, a transmissão glutamatérgica parece estar envolvida com a dependência à opioides, contribuindo para o surgimento da tolerância e dos sinais físicos de abstinência.

Levando em conta a eficácia de fármacos opioides para o tratamento de transtornos depressivos e doenças relacionadas ao estresse, a busca de fármacos que possam agir sobre receptores opioides, porém desencadeando menores ou nenhum efeito de tolerância e abstinência, é de grande importância e têm crescido ao longo dos últimos anos.

Figura 3- Mecanismos de tolerância e abstinência aos opioides.



Fonte: Adaptado de (MAZEI-ROBISON e NESTLER, 2012) e (RITTER e HALL, 2009).

1.4 MODELOS ANIMAIS PARA ESTUDO DA DEPRESSÃO E ESTRESSE

A depressão, como anteriormente mencionado, é considerada uma doença heterogênea que frequentemente manifesta-se com sintomas nos níveis psicológico, comportamental e fisiológico (BENTLEY et al., 2014). Dessa forma, o estudo da depressão *in vivo* permite que a natureza multidimensional desta doença seja examinada. Neste sentido, os modelos animais para estudo da depressão e estresse são ferramentais importantes para a compreensão da patofisiologia da depressão e da variabilidade de respostas ao estresse, permitindo o estudo de diferentes sintomas da doença e a descoberta de novos alvos terapêuticos para o seu tratamento.

Embora muitos dos sinais da depressão, como perda de peso, falta de interesse ou prazer (anedonia), diminuição da habilidade de enfrentar situações adversas e aversão social, possam ser mimetizados em modelos animais, alguns dos sintomas que caracterizam a depressão em humanos, como sentimento de culpa e fracasso, humor deprimido e tendências suicidas, não podem ser fidedignamente reproduzidos experimentalmente (ABELAIRA et al., 2013; DUMAN, 2010). No entanto, de uma forma geral, os modelos de depressão reproduzem comportamentos que se assemelham aos sintomas depressivos humanos e que, portanto, podem ser chamados de comportamentos do tipo depressivos (do inglês, *depressive- like*) (DUMAN, 2010; STEWART e KALUEFF, 2015).

Além da importância de reproduzir os sintomas clínicos da doença, que caracterizam a validade aparente dos modelos experimentais, outros dois critérios são úteis para confiabilidade e reprodutibilidade de modelos experimentais: a validade preditiva, a qual diz respeito à capacidade do modelo em detectar os tratamentos clinicamente úteis e a validade construtiva, referente à semelhança de fatores causais da doença no modelo experimental e em pacientes (WILLNER e MITCHELL, 2002). Baseado nestes critérios, diversos modelos experimentais têm sido desenvolvidos para o estudo da depressão e do estresse em roedores de laboratório.

1.4.1 Modelos preditivos de atividade antidepressiva

Entre os testes mais comumente utilizados pelos pesquisadores para investigar novos fármacos antidepressivos estão o teste do nado forçado (TNF) e o teste de suspensão da cauda (TSC). Embora, estes testes não possam ser considerados modelos animais de depressão por não cumprir todos os critérios de validade experimental, ambos são importantes ferramentas

para a triagem de fármacos antidepressivos em ratos e camundongos, devido a sua alta validade preditiva, facilidade de desenvolvimento e baixo custo, aliados à boa reprodutividade (YIN et al., 2016). No TNF, descrito por PORSOLT et al. (1977), o animal é colocado em um cilindro cheio de água de onde é incapaz de sair, gerando um estresse agudo inevitável e incontrolável. Inicialmente, o animal tenta escapar, mas eventualmente adota uma postura de imobilidade que é interpretada como comportamento do tipo depressivo. Essa mesma abordagem também é utilizada no TSC descrito por STERU et al. (1985). No entanto, neste teste, o estresse inevitável e incontrolável é gerado pela suspensão dos camundongos pela cauda por meio de uma fita adesiva. A validade preditiva de atividade antidepressiva do TNF e TSC deve-se ao efeito de um amplo espectro de agentes antidepressivos em diminuir a imobilidade dos animais nestes testes (YIN et al., 2016).

Modificações no TNF e TSC propostas por DETKE et al. (1995) e BERROCOSO et al. (2013), respectivamente, trouxeram a possibilidade de predizer o mecanismo de ação de fármacos antidepressivos nestes testes. No TNF modificado, fármacos que agem por vias noradrenérgicas ou serotoninérgicas induzem diferentes comportamentos ativos, de forma que, além de diminuir a imobilidade dos animais, antidepressivos noradrenérgicos aumentam o comportamento de escalada, enquanto que os fármacos serotoninérgicos aumentam o tempo de natação dos animais neste teste. Por outro lado, no TSC modificado, é possível a avaliação de mecanismo opioide ou monoaminérgico dos antidepressivos. Neste teste, antidepressivos opioides diminuem a imobilidade dos animais e aumentam o comportamento ondulatório, enquanto antidepressivos monoaminérgicos reduzem a imobilidade e aumentam o comportamento oscilatório dos animais. Desta forma, o uso do TNF e TSC nas suas formas modificadas facilita o direcionamento do estudo dos mecanismos de ação de fármacos antidepressivos.

Embora o TNF e TSC sejam tradicionalmente empregados para avaliação de efeitos de antidepressivos, estudos atuais têm demonstrado a utilidade destes testes na avaliação do comportamento do tipo depressivo, induzido por exemplo, pela exposição prolongada a estressores, onde se observa um aumento da imobilidade dos animais e alterações neuroquímicas que reproduzem os efeitos do estresse no SNC (BONDAR et al., 2017; MUL et al., 2016).

1.4.2 Modelo de depressão induzida por nado forçado repetido

Embora, como descrito acima, o TNF seja um tipo de estressor inescapável utilizado principalmente como modelo preditivo de atividade antidepressiva, a exposição repetida à natação forçada pode ser considerada um modelo de depressão induzida por estresse, uma vez que a reincidência de experiências estressantes, como o estresse inevitável e incontrolável da natação, gera sintomas semelhantes a depressão em diferentes testes comportamentais, os quais são revertidos por fármacos antidepressivos (QI, X. et al., 2008). Neste modelo, os animais são expostos a repetidos episódios de natação forçada, de forma semelhante ao anteriormente descrito para o TNF, a quantidade de exposições bem como o tempo de intervalo entre elas pode variar de minutos a dias dependendo do tipo de situação estressante pretende-se mimetizar, por exemplo, pequenos e recorrentes episódios de estresse, estresse moderado e intermitente ou ainda estresse prolongado (DAL-ZOTTO et al., 2000). Diversos estudos têm relacionado o tipo de exposição ao estresse de natação repetida às respostas adaptativas ou patológicas ao estresse e muitas alterações cerebrais comumente encontradas em pacientes depressivos já foram descritas em animais submetidos ao estresse do nado forçado repetido, como modificações no sistema serotoninérgico, indicando que este modelo de depressão induzida por estresse reproduz de forma eficaz esta doença em humanos (BRUCHAS et al., 2008; QI, XIAOLI et al., 2006b; SHISHKINA et al., 2008).

1.4.3 Modelo de depressão induzida por estresse de derrota social

O conflito social é um dos estressores mais predominantes nos seres humanos e está associado a um alto risco de desenvolvimento de doenças psiquiátricas como depressão e ansiedade (BJORKQVIST, 2001; SANTINI et al., 2015). Desta forma, modelos que envolvam o estresse social podem mimetizar os sintomas e reproduzir os mecanismos da depressão em humanos, tornando-se bastante úteis para o estudo da depressão induzida por estresse.

O modelo do estresse da derrota social tem sido bastante utilizado para o estudo da depressão e na busca por novas terapias para essa doença, uma vez que, induz um fenótipo depressivo robusto marcado por anedonia, ansiedade e aversão social (BONDAR et al., 2017; HENRIQUES-ALVES e QUEIROZ, 2015). Além disso, este modelo permite avaliar a variação de respostas comportamentais e cerebrais ao estresse, classificando os animais em suscetíveis ou resilientes, fato que aumenta a validade preditiva deste modelo para o estudo de tratamentos

antidepressivos efetivos, uma vez que, traduz respostas diferenciadas de indivíduos expostos a estressores (BAGOT et al., 2017; KRISHNAN et al., 2007). O estresse de derrota social é um modelo baseado em uma ou mais exposições a um tipo de estresse físico e emocional, onde camundongos são submetidos a derrotas sociais físicas por diferentes camundongos agressores (um a cada dia) e a fases de contato sensorial durante a qual o animal derrotado tem contato visual, auditivo e olfativo com o agressor a fim de manter o estresse psicológico. Ao fim do protocolo, a diminuição da interação social entre os animais, assim como outros comportamentos do tipo depressivo e ansioso e as inúmeras alterações cerebrais encontradas neste modelo caracterizam a depressão induzida por estresse (BAGOT et al., 2017; GOLDEN et al., 2011; LIU, Y. Y. et al., 2017b).

1.5 A BUSCA POR NOVOS TRATAMENTOS ANTIDEPRESSIVOS E OS COMPOSTOS ORGÂNICOS DE SELÊNIO

Ao longo de muitas décadas, inúmeras fármacos antidepressivos foram descobertas e estudadas. No entanto, devido aos efeitos adversos, como a cardiotoxicidade no caso dos ADT, bem como a interação com outros medicamentos e alimentos induzida pelo uso de iMAOs, estimulou a busca por antidrepressivos mais seletivos e possivelmente melhor tolerados. Os IRSNs foram então introduzidos no mercado entre os anos de 1980 e 1990 e continuam entre as fármacos de maior sucesso para o tratamento das doenças psiquiátricas. Porém, como mencionado anteriormente, a eficácia destes medicamentos ainda é limitada devido ao elevado tempo necessário para o início dos efeitos terapêuticos, a alta taxa de pacientes que não respondem ao tratamento e os inúmeros efeitos indesejáveis, os quais dificultam a adesão dos pacientes ao tratamento (CARVALHO et al., 2016). Desta forma, pesquisas vêm sendo desenvolvidas na busca de novos fármacos antidepressivos que atuem por diferentes mecanismos e que possam ser mais eficazes e com menores efeitos colaterais (MANJI e ZARATE, 2002).

Neste sentido, o selênio, um elemento traço nutricionalmente essencial, tem sido alvo de muitos estudos devido aos seus importantes papéis fisiológicos tais como, componente estrutural de enzimas antioxidantes, imunomodulador, anti-inflamatório e antiviral (DUNTAS e BENVENGA, 2015; RAYMAN, 2000).

Segundo a Agência Nacional de Vigilância Sanitária (ANVISA) a ingesta diária de selênio recomendada é de 70 µg para adultos, seja por meio de alimentos que contenham este elemento, como castanha-do-pará, alho, cebola, brócolis, cogumelos, cereais, ovos e carnes, ou

por suplementação (DUMONT et al., 2006). Outros estudos têm indicado que a ingesta diária de selênio ideal para adultos fica entre 47-105 μg (FAIRWEATHER-TAIT et al., 2011; HURST et al., 2010), sendo que uma ingestão maior que 400 μg por dia, excede a capacidade corporal de eliminação do selênio, o que pode provocar efeitos tóxicos, denominado selenoses (DUMONT et al., 2006).

Por outro lado, estudos clínicos e pré-clínicos têm indicado uma possível ligação entre a deficiência de selênio e a predisposição ao desenvolvimento de doenças como a depressão. Um estudo caso-controle revelou que a baixa ingestão de selênio está associada ao alto risco de recaída à depressão (PASCO et al., 2012). No mesmo sentido, concentrações séricas de selênio tem sido inversamente correlacionadas com a gravidade da depressão (IBARRA et al., 2014), enquanto altos níveis de selênio estão relacionados com a redução de sintomas depressivos em adultos jovens (CONNER et al., 2015), com menores índices de depressão em pacientes idosos (GAO et al., 2012) e com a prevenção de depressão pós-parto (GAVIN et al., 2005).

O selênio é raramente encontrado na natureza em sua forma livre, podendo combinar-se com metais ou não metais para formar compostos inorgânicos (selenito e selenato) ou apresentar-se na forma orgânica (selenocisteína, selenocistina e selenometionina), a qual possui menor toxicidade e maior biodisponibilidade quando comparada às formas inorgânicas (NAKAMURO et al., 2000; NARAJJI et al., 2007). Ao longo das últimas décadas, o interesse por compostos orgânicos de selênio sintéticos têm aumentado devido às diversas propriedades farmacológicas que estas moléculas apresentam em uma vasta gama de modelos de doenças humanas (NOGUEIRA e ROCHA, 2011; NOGUEIRA et al., 2004) Dentre os compostos orgânicos de selênio estudados por nosso grupo de pesquisa, o disseleneto de difenila ($(PhSe)_2$), protótipo da classe dos disselenetos, apresenta importantes efeitos antioxidantes, antimicrobiano, antiviral, antinociceptivo, anti-inflamatório, neuroprotetor, ansiolítico e do tipo antidepressivo em roedores (BRUNING et al., 2012; QUINES et al., 2016b; ROSA et al., 2015; ROSA et al., 2016; SARTORI et al., 2016). Além disso, a substituição de grupos funcionais na estrutura do diaril disseleneto têm gerado compostos com diversas atividades farmacológicas, como antinociceptiva, do tipo antidepressiva, nootrópica e anti- hiperglicemiante (OLIVEIRA et al., 2016; QUINES et al., 2016a; ZBOROWSKI et al., 2016). Nesse contexto, a inserção de grupamentos CF_3 na posição *meta* na molécula do diaril disseleneto gerou o disseleneto de *mc*-trifluormetil-difenila (*m*-CF_3-$PhSe)_2$ (Figura 4), um composto com inúmeras propriedades farmacológicas já descritas, como do tipo antidepressiva, ansiolítica, anticonvulsivante, antinociceptiva (BRUNING et al., 2014; BRUNING et al., 2015a; BRUNING et al., 2009;

MAGNI et al., 2012).

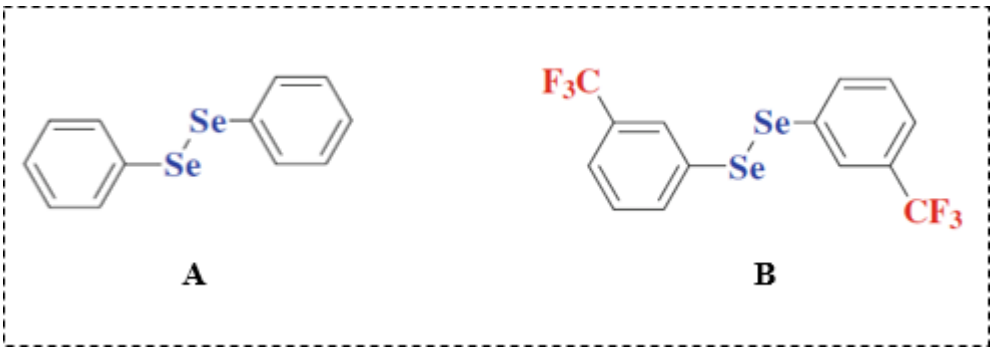
A inserção de átomos de flúor na estrutura de fármacos é comumente utilizada na química farmacêutica para melhorar a estabilidade metabólica, e a biodisponibilidade de moléculas, uma vez que as torna mais resistentes ao ataque oxidativo (PURSER et al., 2008). A inserção estratégica de flúor em fármacos já culminou na produção de alguns medicamentos disponíveis no mercado, como é o caso do antidepressivo fluoxetina. Estudos de relação estrutura-atividade mostraram que a inclusão de um grupo trifluorometila no anel fenólico aumentou a potência da fluoxetina em inibir a captação de 5-HT, comparada com o composto não fluorado (ROMAN et al., 2003). No mesmo sentido a inserção do substituinte difluormetileno em um análogo da vitamina D, além de evitar que ela seja degradada rapidamente, aumentou sua atividade biológica (FUJISHIMA et al., 2010). Embora, não existam estudos demonstrando a relação estrutura atividade do composto (*m*-CF_3-PhSe)$_2$, a presença do grupamento trifluorometila possivelmente contribua para seus efeitos farmacológicos duradouros e ampla distribuição.

Pouco se saibe sobre as propriedades farmacocinéticas do (*m*-CF_3-PhSe)$_2$, no entanto, de acordo com programa computacional Molinspiration, o composto apresenta propriedades compatíveis com a regra dos 5 de Lipinski (LIPINSKI et al., 1997), a qual permite inferir se os compostos analisados terão problemas com a absorção e permeabilidade, por meio da identificação de parâmetros físico-químicos. Segundo esta regra, a maioria das moléculas com boa biodisponibilidade oral apresenta logP $\leq$ 5 (6,71), peso molecular $\leq$ 500 (448), número de aceitadores de ligações de hidrogênio $\leq$ 10 (0) e número de doadores de pontes de hidrogênio $\leq$ 5 (0). Dessa forma, moléculas que violem mais de um destes parâmetros podem ter, pela via oral, redução da biodisponibilidade. Dentre as propriedades citadas, o composto apresenta somente uma propriedade incompatível, logP>5 e, portanto, possivelmente apresenta uma boa absorção e biodisponibilidade após administração oral.

Neste contexto, um perfil de distribuição e excreção de (*m*-CF_3-PhSe)$_2$ foi sugerido por BRUNING et al. (2014) por meio da determinação dos níveis de Se em diferentes tecidos. Embora, o Se quantificado possa ser derivado do (*m*-CF_3-PhSe)$_2$ não-biotransformado ou dos seus possíveis metabólitos, este estudo indicou uma ampla distribuição de Se em diferentes tecidos, incluindo fígado, rins, pulmão, gordura e encéfalo. Quanto aos padrões de excreção, os resultados mostraram que a urina é uma importante rota de eliminação de (*m*-CF_3-PhSe)$_2$ ou seus metabólitos. Além disso, níveis elevados de Se foram observados nas fezes, o que pode indicar a eliminação intestinal hepática ou mesmo não biliar do composto devido à natureza lipofílica do (*m*-CF_3-PhSe)$_2$.

Os efeitos farmacológicos do (*m*-CF_3-PhSe)$_2$ têm sido estudados em diferentes modelos de doenças humanas e seus diferentes mecanismos de ação já descritos caracterizam o (*m*-CF_3-PhSe)$_2$ como um composto multi-alvo (BRUNING et al., 2014; BRUNING et al., 2015a; BRUNING et al., 2015b; BRUNING et al., 2011). Os primeiros estudos sobre a ação farmacológica do (*m*-CF_3-PhSe)$_2$ demonstraram a eficácia deste composto em atenuar a estereotipia induzida pela apomorfina (MACHADO et al., 2006) e inibir a recaptação de 5-HT em sinaptossomas de ratos *in vitro*, e revelaram (BORGES et al., 2009) suas propriedades antimutagênica, antigenotóxica (MACHADO MDA et al., 2009), anticonvulsivante (PRIGOL et al., 2009) e ansiolítica (BRUNING et al., 2009).

Figura 4- Estrutura química do disseleneto de difenila (PhSe)$_2$ (A) e do disseleneto de *m*-trifluormetil-difenila (*m*–CF_3–PhSe)$_2$ (B).



Fonte: Adaptado de NOGUEIRA e ROCHA (2011)

Além disso, os efeitos do (*m*-CF_3-PhSe)$_2$ foram amplamente explorados em diversos modelos de dor e de depressão. Sobre o mecanismo antinociceptivo deste composto, evidências farmacológicas demonstraram o envolvimento do sistema serotoninérgico, mais especificamente dos receptores 5-HT_{1A} e 5-HT_{2A}, neste efeito que também parece estar relacionado ao fato do (*m*-CF_3-PhSe)$_2$ inibir a captação e a ligação específica de serotonina em encéfalo de camundongos *ex vivo* e *in vitro*, respectivamente (BRUNING et al., 2014). De forma semelhante, o sistema serotoninérgico também contribui para o efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$, o qual é mediado pelos receptores 5-HT_{1A} e 5-$HT_{2A/2C}$ e 5-HT $_3$. Além disso, um estudo recente revelou a eficácia do (*m*-CF_3-PhSe)$_2$ em um modelo de

depressão induzida pelo fator de necrose tumoral alfa (TNF-α), indicando que o mecanismo anti-inflamatório do composto também está envolvido em seu efeito do tipo antidepressivo. Ainda, o estudo do (*m*-CF_3-PhSe)$_2$ em um modelo de díade dor-depressão reforçou seus efeitos antinociceptivo e do tipo antidepressivo e seus diferentes mecanismos de ação (BRUNING et al., 2015a; BRUNING et al., 2015b).

Interessantemente, o (*m*-CF_3-PhSe)$_2$ apresenta uma particularidade em relação ao protótipo (PhSe)$_2$ e aos demais compostos orgânicos de selênio estudados por nosso grupo de pesquisa, qual seja, o envolvimento do sistema opioide em seu mecanismo de ação antinociceptivo e do tipo antidepressivo. Um estudo de BRUNING et al. (2010) evidenciou, por meio do uso de antagonistas opioides seletivos, a contribuição de cada receptor opioide para o efeito antinociceptivo do (*m*-CF_3-PhSe)$_2$, revelando que a ativação dos receptores μ e δ contribui para este efeito do composto, no qual o receptor κ parece não estar envolvido. Ainda, a administração de naloxona, um antagonista não seletivo de receptores opioides, bloqueia a ação do tipo antidepressiva do (*m*-CF_3-PhSe)$_2$ indicando que o sistema opioide está envolvido em ambos os efeitos, antinociceptivo e do tipo antidepressivo, deste composto (BRUNING et al., 2011). No entanto, o envolvimento de cada receptor opioide no efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ permanece totalmente inexplorado. Desta forma, a investigação dos receptores opioides envolvidos na ação do tipo antidepressiva do (*m*-CF_3- PhSe)$_2$ e de que forma cada um destes receptores poderia contribuir para esse efeito é de grande importância para a melhor compreensão dos mecanismos farmacológicos do composto, principalmente, no que diz respeito ao envolvimento do sistema opioide, o qual exerce papel importante na regulação de transtornos psiquiátricos (LUTZ e KIEFFER, 2013).

2 OBJETIVOS

2.1 OBJETIVOS GERAIS

Considerando os aspectos mencionados anteriormente, o principal objetivo desta tese foi avaliar a contribuição do sistema opioide para o efeito farmacológico do composto orgânico de selênio (*m*-CF_3-PhSe)$_2$ em modelos de depressão em camundongos.

2.2 OBJETIVOS ESPECÍFICOS

- Investigar o envolvimento do sistema opioide na ação do tipo antidepressiva do tratamento agudo e repetido com (*m*-CF_3-PhSe)$_2$;
- Determinar se o tratamento repetido com o (*m*-CF_3-PhSe)$_2$ induz tolerância;
- Investigar se a retirada do (*m*-CF_3-PhSe)$_2$ leva a sinais físicos de abstinência e a alterações neuroquímicas em córtex e hipocampo de camundongos.
- Avaliar parâmetros toxicológicos após a administração repetida do composto em diferentes doses;
- Avaliar se o tratamento com o (*m*-CF_3-PhSe)$_2$ é eficaz em reverter o comportamento do tipo depressivo induzido por estresse repetido de natação forçada;
- Investigar o efeito do (*m*-CF_3-PhSe)$_2$ nos níveis de receptores opioides após única ou repetida exposições ao estresse de natação forçada;
- Determinar se o tratamento com o (*m*-CF_3-PhSe)$_2$ induz resiliência ao estresse de derrota social;
- Avaliar o efeito do tratamento com o (*m*-CF_3-PhSe)$_2$ nos níveis de receptores opioides e MAPKs em camundongos expostos ao estresse de derrota social;

3 DESENVOLVIMENTO

O desenvolvimento desta tese está apresentado sob a forma de três artigos científicos. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se nos próprios artigos, os quais estão estruturados de acordo com as normas de cada revista onde foram publicados. Em anexo a esta tese encontram-se a carta de aprovação do projeto de pesquisa pela Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal de Santa Maria e as autorizações das editoras para reprodução dos artigos científicos.

3.1 ARTIGO 1

Contribuição do sistema opioide para o efeito do tipo antidepressivo do disseleneto de *m*-trifluormetil-difenila em camundongos: Um composto que não induz tolerância e síndrome de retirada

Opioid system contribution to the antidepressant-like action of *m*-trifluoromethyl-diphenyl diselenide in mice: A compound devoid of tolerance and withdrawal syndrome

Suzan Gonçalves Rosa , Ana Paula Pesarico , Carolina Fagan Tagliapietra, Sônia C. Almeida da Luz, Cristina Wayne Nogueira

Journal of Psychopharmacology 2017, 31(9) 1250– 1262 DOI:10.1177/0269881117724353

Original Paper

Psychopharm

Journal of Psychopharmacology
2017, Vol. 31(9) 1250–1262

DOI: 10.1177/0269881117724353
journals.sagepub.com/home/jop
SAGE

Opioid system contribution to the antidepressant-like action of *m*-trifluoromethyl-diphenyl diselenide in mice: A compound devoid of tolerance and withdrawal syndrome

Suzan G Rosa[1], Ana P Pesarico[1], Carolina F Tagliapietra[1], Sônia CA da Luz[2] and Cristina W Nogueira[1]

Abstract

Animal and clinical researches indicate that the opioid system exerts a crucial role in the etiology of mood disorders and is a target for intervention in depression treatment. This study investigated the contribution of the opioid system to the antidepressant-like action of acute or repeated *m*-trifluoromethyl-diphenyl diselenide administration to Swiss mice. *m*-Trifluoromethyl-diphenyl diselenide (50 mg/kg, intragastric) produced an antidepressant-like action in the forced swimming test from 30 min to 24 h after treatment. This effect was blocked by the μ and δ-opioid receptor antagonists, naloxonazine (10 mg/kg, intraperitoneally) and naltrindole (3 mg/kg, intraperitoneally), and it was potentiated by a κ-opioid receptor antagonist, *norbinaltrophimine (*1 mg/kg, subcutaneously*). Combined treatment with subeffective doses of m*-trifluoromethyl-diphenyl diselenide (10 mg/kg, intragastric) and morphine (1 mg/kg, subcutaneously) resulted in a synergistic antidepressant-like effect. The opioid system contribution to the *m*-trifluoromethyl-diphenyl diselenide antidepressant-like action was also demonstrated in the modified tail suspension test, decreasing mouse immobility and swinging time and increasing curling time, results similar to those observed using morphine, a positive control. Treatment with *m*-trifluoromethyl-diphenyl diselenide induced neither tolerance to the antidepressant-like action nor physical signs of withdrawal, which could be associated with the fact that *m*-trifluoromethyl-diphenyl diselenide did not change the mouse cortical and hippocampal glutamate uptake and release. *m*-Trifluoromethyl-diphenyl diselenide treatments altered neither locomotor nor toxicological parameters in mice. These findings demonstrate that *m*-trifluoromethyl-diphenyl diselenide elicited an antidepressant-like action by direct or indirect μ and δ-opioid receptor activation and the κ-opioid receptor blockade, without inducing tolerance, physical signs of withdrawal and toxicity.

Keywords

Antidepressant-like, opioid system, tolerance, withdrawal signs, selenium

Introduction

Depression is a debilitating psychiatric disorder characterized by a wide range of emotional and physical symptoms (Berrocoso and Mico, 2009) and estimated to affect 350m people (WHO, 2012). Current pharmacological treatments for depression are based on the use of drugs that act mainly by enhancing the brain monoaminergic neurotransmission. However, a large number of patients present partial or lack of response to antidepressant treatments (Ruhé et al., 2012), which indicates the need for the research and discovery of novel drugs, with alternative mechanisms of action, to find more effective interventions for this disease.

Substantial pharmacological, neurochemical and behavioral evidence supports a role for endogenous opioids in the regulation of mood and depression, suggesting that compounds that enhance the opioid neurotransmission may exert genuine antidepressant effects (Bodnar, 2017; Lutz and Kieffer, 2013; Robinson et al., 2017). Besides, it has been identified that there is a high density of endogenous opioid peptides and receptors, mu (μ), delta (δ) and kappa (κ), in the limbic brain areas (Mansour et al., 1988) and a distinct control of each of the opioid receptors over mood-related processes (Lutz and Kieffer, 2013). Studies with rodents have demonstrated that μ or δ-opioid receptor agonists and κ-opioid receptor antagonists elicit an antidepressant-like effect (Filho et al., 2013; Saitoh et al., 2011; Wang et al., 2015). In addition, clinical trials have indicated that opioid drugs, such as oxycodone and buprenorphine, are effective in patients with refractory depression (Cowan, 2007; Stoll and Rueter, 1999).

[1]Departamento de Bioquímica e Biologia Molecular, Universidade Federal de Santa Maria, Santa Maria, Brasil
[2]Departamento de Patologia, Universidade Federal de Santa Maria, Santa Maria, Brazil

Corresponding author:
Cristina W Nogueira, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, CEP 97105-900, RS, Brazil.
Email: criswn@ufsm.br

Although there has been substantial scientific progress on understanding the role of endogenous opioid system dysregulation in the context of depression (Berrocoso et al., 2009), the clinical use of opioids as antidepressants remains greatly limited, owing to the inherent risk of development of tolerance and withdrawal syndrome (Christie, 2008). The mechanisms behind opioid tolerance and withdrawal are complex and involve adaptations in the expression of receptors, modulation of downstream signaling cascades, and interactions between opioid and nonopioid systems. Moreover, downregulation of glutamate transporters expression, activation of N-methyl-D-aspartate (NMDA) receptors, and a consequent progression of neuronal excitability have been reported as contributing factors to the development of opioid dependence and tolerance (Mao et al., 2002; Murray et al., 2007).

Organoselenium compounds are effective in different models of depression (Bruning et al., 2015a; Gai et al., 2014; Quines et al., 2016), among them *m*-trifluoromethyl diphenyl diselenide ((*m*-CF_3-PhSe)$_2$) has been reported to have pharmacological properties mediated by the opioid system (Bruning et al., 2010; Bruning et al., 2011b). Furthermore, (*m*-CF_3-PhSe)$_2$ is a small molecule with multiple biological targets, which elicits antidepressant-like action through its anti-inflammatory property and modulation of the serotonergic system (Bruning et al., 2011b; Bruning et al., 2015b). Although the antidepressant-like action of (*m*-CF_3-PhSe)$_2$ has been documented (Bruning et al., 2011b, 2015a,b), some important points such as duration of this effect, dose-effect relationship in different behavioral tests, and toxicity of repeated administration of this compound remain largely unknown. Moreover, despite the fact that we reported (Bruning et al., 2011a) that naloxone blocks the antidepressant-like action of (*m*-CF_3-PhSe)$_2$, there is no evidence for the contribution of opioid receptors to this effect.

The aim of this study was to evaluate: (a) the antidepressant-like action of acute or repeated administration of (*m*-CF_3-PhSe)$_2$; (b) the contribution of opioid system in this action; and (c) whether (*m*-CF_3-PhSe)$_2$ administration induces tolerance to the antidepressant-like action, signs of withdrawal syndrome, and systemic toxicity in mice.

Materials and methods

Animals

The experiments were carried out using male adult (two-months-old) Swiss mice (25–30 g) from our breeding colony. The animals were housed in cages (five mice per cage) with free access to food and water. They were kept in a separate animal room, on a 12-hour light/12-hour dark cycle; the lights were turned on every day at 07:00, in a controlled temperature environment (22±2ºC). A commercial diet (Guaiba, Rio Grande de Sul, Brazil) and tap water were supplied ad libitum. The experiments were performed according to a randomized schedule and each animal was used only once in each test. The present experimental study was approved by the Institutional Ethics Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Rio Grande de Sul, Brazil and registered under the number 7770060215. The procedures in this study were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

Drugs

The drug (*m*–CF_3–PhSe)$_2$ was prepared and characterized in our laboratory by the method previously described (Paulmier, 1986). Analysis of the ^{1}H NMR and ^{13}C NMR (nuclear magnetic resonance) spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of (*m*–CF_3–PhSe)$_2$ (99.9%) was determined by gas chromatography-mass spectrometry (GC-MS; Shimadzu QP2010PLUS GC/MS combination). Naltrindole hydrochloride, norbinaltrophimine (nor-BNI) dihydrochloride and naloxone hydrochloride were obtained from Sigma (St Louis, Missouri, USA). Morphine was purchased from Merck (Darmstdat, Germany). All drugs were dissolved in saline except (*m*–CF_3–PhSe)$_2$ which was dissolved in canola oil. The mice received all drugs at a constant volume of 10 mL/kg body weight. Appropriate vehicle-treated groups were also assessed simultaneously.

Experimental design

Time-course curve of antidepressant-like effect of (m–CF_3–PhSe)$_2$ in the forced swimming test (FST). This experimental protocol was performed in order to determine the time-course curve of (*m*–CF_3–PhSe)$_2$ antidepressant-like action in the FST. To this end, different groups of mice received a single administration of vehicle (canola oil, 10 mL/kg) or (*m*–CF_3–PhSe)$_2$ at a dose of 50 mg/kg (Bruning et al., 2011b) by the intragastric (i.g.) route.

At different times after treatment with vehicle or (*m*–CF_3–PhSe)$_2$ (30 min, 1, 2, 4, 8, 24, and 48 h) animals performed the FST (eight animals/group). The vehicle group represents the mean of immobility time of mice treated with vehicle at different times. The dose and protocol regimen for (*m*–CF_3–PhSe)$_2$ administration was chosen based on our previous study (Bruning et al., 2011).

Contribution of opioid system to the antidepressant-like action of (m–CF_3–PhSe)$_2$. A pharmacological protocol was delineated to test the hypothesis that μ, δ, and κ-opioid receptors contribute to the antidepressant-like action of (*m*–CF_3–PhSe)$_2$. In this protocol, separate groups of mice were treated with naloxonazine (10 mg/kg, intraperitoneally (i.p.), a selective μ-opioid receptor antagonist) (nine animals/group) or naltrindole (3 mg/kg, i.p., a selective δ-opioid receptor antagonist) (eight animals/group) or nor-BNI (10 mg/kg, subcutaneously (s.c.), a selective κ-opioid receptor antagonist) (nine animals/group) (Carr et al., 2010; Zomkowski et al., 2005). Thirty minutes after naltrindole and nor-BNI or 24 h after naloxonazine administration, the mice received vehicle or (*m*–CF_3–PhSe)$_2$ (50 mg/kg, i.g.) and 30 min afterwards the mice performed the FST.

In order to investigate the possible synergistic effect of (*m*–CF_3–PhSe)$_2$ and the κ-opioid receptor antagonist, the mice were treated with a subeffective dose of nor-BNI (1 mg/kg, s.c) and after 30 min they received a subeffective dose of (*m*–CF_3–PhSe)$_2$ (10 mg/kg, i.g.) (Bruning et al., 2011; Carr et al., 2010). Thirty minutes later, the mice performed the FST.

In another set of experiments, a subeffective dose of morphine (1 mg/kg, s.c.) (Zomkowski et al., 2005) was injected in mice 20 min before the administration of a subeffective dose of $(m\text{–}CF_3\text{–}PhSe)_2$ (10 mg/kg, i.g.) (Bruning et al., 2011). Thirty minutes later, the mice performed the FST (10 animals/group).

The modified tail suspension test (mTST) was carried out to further support our hypothesis that the opioid system contributes to the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ in mice. This test allows to differentiate behaviors involving the opioid and monoaminergic systems (Berrocoso et al., 2013). The dose-response curve of the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ in the mTST was carried out 30 min after the administration of different doses (5, 25, or 50 mg/kg, i.g.) of compound or one hour after the morphine injection (a positive control, 5 mg/kg, s.c.) (Zomkowski et al., 2005) (eight animals/group).

All doses and time intervals of treatments with opioid receptor antagonists or $(m\text{–}CF_3\text{–}PhSe)_2$ used in this protocol were chosen based on previous reports (Bruning et al., 2011; Carr et al., 2010; Zomkowski et al., 2005).

Antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ repeated administration. A dose-response curve of $(m\text{–}CF_3\text{–}PhSe)_2$ was carried out in order to investigate the antidepressant-like action of repeated administration with this compound in the mouse FST and the mTST. Separate groups of mice received once a day vehicle or $(m\text{–}CF_3\text{–}PhSe)_2$ at different doses (5, 25, or 50 mg/kg) for eight days. Thirty minutes after the last administration (Bruning et al., 2011), the mice performed the mTST (seven animals/group) and subsequently the FST (eight animals/group). The $(m\text{–}CF_3\text{–}PhSe)_2$ treatment time was defined based on a pilot study performed in our research laboratory. The doses of $(m\text{–}CF_3\text{–}PhSe)_2$ tested in this protocol were chosen based on the effect of a single administration of this compound in the mouse FST. Morphine (5 mg/kg, s.c.), a positive control, was injected once a day for eight days and one hour after its last administration the mice performed the FST (eight animals/group) and the mTST (seven animals/group) (Zomkowski et al., 2005).

Signs of tolerance and withdrawal syndrome. The drug $(m\text{–}CF_3\text{–}PhSe)_2$ or morphine was administered at the same treatment regimen to mice to investigate whether the administration of $(m\text{–}CF_3\text{–}PhSe)_2$ induces tolerance and withdrawal syndrome as do classical opioid drugs (Christie, 2008), Different groups of mice (nine animals/group) received vehicle, $(m\text{–}CF_3\text{–}PhSe)_2$ (i.g.), or morphine (s.c) at a dose of 5 mg/kg, twice daily with 12 h intervals for seven days (Abdel-Zaher et al., 2010). With the purpose of evaluating the development of tolerance to the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$, the mice performed the FST 30 min or one hour after administration of $(m\text{–}CF_3\text{–}PhSe)_2$ or morphine, respectively, at days 1, 3, 5, and 7 of treatment. The FST was performed by separate groups of mice for each day of treatment (1, 3, 5, and 7) with the aim of discarding the interference of stress generated by repeated exposure to the FST (Bruchas et al., 2007; Mul et al., 2016).

On the eighth day of treatment, the mice received a single administration of vehicle, $(m\text{–}CF_3\text{–}PhSe)_2$ at a dose of 5 mg/kg (i.g.), or morphine at the same dose (s.c.) and after 30 min and one hour, respectively, the animals were treated with naloxone at a dose of 5 mg/kg (i.p.) to induce the opiate withdrawal syndrome (Abdel-Zaher et al., 2010). Immediately after naloxone administration, each mouse was placed individually in a transparent acryl cylinder (20 cm in diameter, 35 cm in height) to record signs of withdrawal syndrome (rearing, jumping, teeth chattering, paw tremor, and diarrhea occurrence) for 30 min (Abdel-Zaher et al., 2010). In this experimental protocol we did not include the vehicle/saline group because there were no physical withdrawal signs in mice that did not receive opioid treatment (Gao et al., 2016; Rehni et al., 2008).

Immediately afterwards, the mice were killed by cervical dislocation and the prefrontal cortices and hippocampi were excised from the brains of mice (five animals/group) to determine [^{3}H] glutamate release and [^{3}H]glutamate uptake assays.

Spontaneous locomotor activity. With the purpose of excluding sedative or motor abnormality induced by treatments, the mice performed the open-field test 4 min before the FST and the mTST in all experimental protocols carried out in this study.

Behavioral tests

FST. The FST, as originally described by Porsolt et al. (1977a), is the most widely used and recognized pharmacological behavioral test to evaluate the antidepressant efficacy of new compounds. In this test, mice were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm), containing 19 cm of water at 25±1°C. The total duration of immobility was recorded during a six-minute period. Each mouse was judged to be immobile when it ceased struggling and remained floating motionless in the water, making only those movements necessary to keep its head above water. The decrease in the duration of immobility time is indicative of an antidepressant-like effect (Porsolt et al., 1977a,b).

mTST. The mTST allows for differentiating between two active behaviors, swinging and curling (Berrocoso et al., 2013). By using this modified behavioral test, it is possible to evaluate the antidepressant-like action of opioid drugs. In this test, monoaminergic antidepressants specifically increase swinging behavior, whereas opioids increase curling behavior. Importantly, both classic antidepressants and opioids diminish the immobility of the mice, the traditional measure of antidepressant-like activity in the TST. Mice were individually secured to the bar by adhesive tape placed 1 cm from the tip of the tail and the behavioral response was recorded during 6 min. The mouse was considered immobile when it hung passively and developed no active behavior. The times of swinging and curling were recorded, respectively, when the mouse continuously moved its paws in the vertical position while keeping its body straight and/or it moved its body from side to side or when the mouse performed twisting movements of the entire body (Berrocoso et al., 2013).

Open-field test. Mice performed the open-field test in a box of plywood surrounded by walls 30 cm in height. The floor of the open-field (45 cm in length and 45 cm in width) was divided by masking tape markers into nine squares (three rows of three). Each animal was placed individually at the center of the apparatus and observed for 4 min to record the locomotor activity (number of segments crossed with the four paws) (Walsh and Cummins, 1976).

Ex vivo analysis

The ex vivo analyses were performed in triplicate to generate an individual data point in each of the independent experiments.

Parameters of toxicity. A single administration of different doses of (m–CF_3–PhSe)$_2$ has no systemic toxicity in mice (Savegnago et al., 2009). In order to investigate possible toxic effects caused by repeated administration of (m–CF_3–PhSe)$_2$, the mice (seven animals/group) received once a day (m–CF_3–PhSe)$_2$ (50 mg/kg, i.g., the highest dose of the compound used in this study), or vehicle for eight days. Thirty minutes after the last administration, the mice were slightly anesthetized with ketamine/xylazine (150 and 10 mg/kg, respectively) to enable blood collection by heart puncture and samples of brain, liver, and kidney were removed. The activities of plasma aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were determined as biochemical markers of hepatic damage. The levels of urea and the activity of creatine kinase (CK), markers of renal and heart functions, were determined in plasma. The AST, ALT, and CK activities and urea levels were determined by enzymatic colorimetric methods using commercial kits (Labtest Diagnostica, Mina Gerais, Brazil). In addition, the samples of liver and kidney were used to provide histological qualitative evaluation. The samples were placed in 10% buffered formalin solution during 24 h. In sequence, they were dehydrated in ethanol, cleared in xylene, and then embedded in paraffin. Serial sections 20 of 4 μm in thickness were cut and stained with hematoxylin and eosin (H&E), and evaluated under light microscopy.

[^{3}H]glutamate release assay. The determination of [^{3}H] glutamate release was accomplished according to the method previously described (Migues et al., 1999). The prefrontal cortical and hippocampal synaptosomal suspensions were loaded with 0.25 μCi [^{3}H] glutamate (Amersham, specific activity 53 mCi/mmol, final concentration 5 μM) and pre-incubated in Tris/HCl-buffered salt solution (composition in mM Tris/HCl 27, NaCl 133, KCl 2.4, $MgSO_4$ 1.2, KH_2PO_4 1.2, Glucose 12, CaCl2 1.0) pH 7.4 at 37°C for 15 min. Aliquots of labeled synaptosomes (1.4 mg protein) were centrifuged at 16,000×g for 1 min. Supernatants were discarded, and the pellets were washed four times in Tris/HCl buffer by centrifugation at 16,000×g for 1 min (at 4°C). To assess the basal release of [^{3}H] glutamate, the final pellet was resuspended in Tris/HCl buffer and incubated for 1 min in this medium, at 37°C. Incubation was terminated by immediate centrifugation (16,000×g, 1 min, 4°C). Radioactivity present in supernatants and pellets was separately determined in a scintillation counter. The released [^{3}H] glutamate was calculated as a percentage of the total amount of radiolabel present in the synaptosomes at the start of the incubation.

[^{3}H]glutamate uptake assay. The glutamate uptake of prefrontal cortex and hippocampus slices was determined according to a previous study (Thomazi et al., 2004). The slices were washed with 1.0 mL Hank's buffered salt solution (HBSS). After 10 min of pre-incubation, the uptake assay was performed by adding 13.3 μM [^{3}H] glutamate in 300 μL HBSS at 37ºC. Incubation was terminated after 5 min by three ice-cold washes with milli-Q water immediately followed by the addition of 0.5M NaOH, which was kept overnight. Unspecific uptake was measured using the same protocol described above, with differences in the temperature (4ºC) and medium composition (choline chloride stead of sodium chloride). Na^+-dependent uptake was considered as the difference between the total uptake and the unspecific uptake. Incorporated radioactivity was measured using a liquid scintillation counter.

Statistical analysis. The normality of data was analyzed using a D'Agostino and Pearson omnibus normality test. The results are presented as mean±standard error of the mean (SEM). Effects of (m–CF_3–PhSe)$_2$ and morphine in the behavioral tests and ex vivo analyses were performed by one-way analysis of variance (ANOVA) followed by the Newman-Keuls test.

The two-way ANOVA of variance followed by the Newman-Keuls test was used to analyze the effects of (m–CF_3–PhSe)$_2$×opioid antagonists and (m–CF_3–PhSe)$_2$×morphine in the FST.

All analyses were performed by using the STATISTICA for Windows software Version 7 (StatSoft, Oklahoma, USA) by a blinded investigator, who did not know which samples/animals represented treatments or controls. Probability values less than 0.05 (p<0.05) were considered as statistically significant.

Results

Time-course curve of the (m–CF_3–PhSe)$_2$ antidepressant-like action in the FST

The time-course curve (scheme of the experimental design, Figure 1(a)) of the (m–CF_3–PhSe)$_2$ effect at a dose of 50 mg/kg on the immobility time in the mouse FST is shown in Figure 1(b). The (m–CF_3–PhSe)$_2$ administered to mice 30 min to 24 h before the FST decreased the immobility time when compared to that of the control group (F(7,63)=4.28, p<0.001). The antidepressant-like action of this compound in the FST was lost 48 h after (m–CF_3–PhSe)$_2$ administration to the mice.

The opioid system contributes to the (m–CF_3–PhSe)$_2$ antidepressant-like action

Figure 2 shows the effect of naloxonazine, naltrindole or nor-BNI (scheme of the experimental design, Figure 2(a)) on the antidepressant-like action of (m–CF_3–PhSe)$_2$ in the FST. The two-way ANOVA of immobility time demonstrated significant (m-CF_3-PhSe)$_2$×naloxonazine (F(1,32)=6.006, p<0.05), (m-CF_3-PhSe)$_2$×naltrindole interactions (F(1,28)=5.979, p<0.05) and (m-CF_3-PhSe)$_2$ (F(1,28)=29.07, p<0.05) and nor-BNI (F(1,28)=37.21, p<0.05) main effects.

Naloxonazine (Figure 2(b)) and naltrindole (Figure 2(c)) were effective against the antidepressant-like action of (m-CF_3-PhSe)$_2$ in the FST, whereas treatment with nor-BNI (Figure 2(d)) potentiated the action of this compound.

The two-way ANOVA of immobility time data demonstrated a significant (m-CF_3-PhSe)$_2$×nor-BNI interaction when administered to mice (F(1,32)=4.225, p<0.05). The Supplementary Material, Table 1S shows that combination of (m-CF_3-PhSe)$_2$ and nor-BNI at subeffective doses elicited an antidepressant-like action in the mouse FST.

Figure 3 shows the effect of (m-CF_3-PhSe)$_2$ and morphine at subeffective doses in in the mouse FST (scheme of the experimental design, Figure 3(a)). The two-way ANOVA of immobility

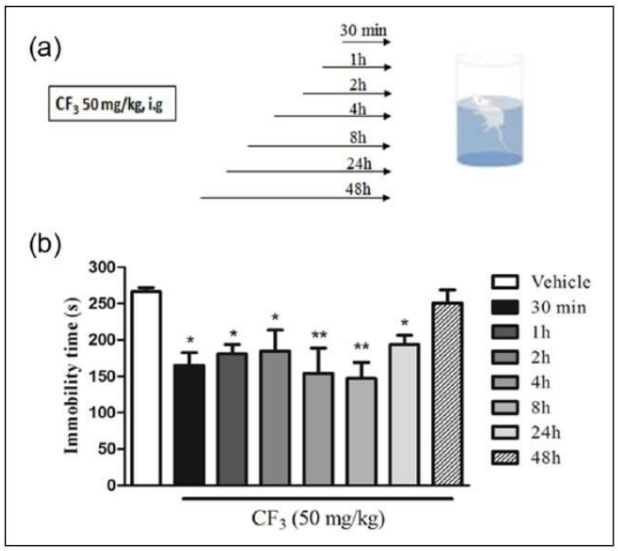


Figure 1. Time-course curve of $(m\text{–}CF_3\text{–}PhSe)_2$ (CF_3) antidepressant-like action in the mouse forced swimming test (FST) (b). The scheme of the experimental design is given in (a) and $(m\text{–}CF_3\text{–}PhSe)_2$ (50 mg/kg, intragastric (i.g.)) was administered 30 min to 48 h before test. Values are expressed as mean±standard error of the mean (SEM) of eight animals. Asterisks denote the significance levels when compared to the control group: *p<0.05 and **p<0.01 (one-way analysis of variance (ANOVA) followed by the Newman-Keuls test).

time demonstrated a significant $(m\text{-}CF_3\text{-}PhSe)_2$×morphine interaction ($F$(1,36)=4.942, p<0.05). The combination of morphine and $(m\text{-}CF_3\text{-}PhSe)_2$ at subeffective doses decreased the immobility time in the mouse FST (Figure 3(b)).

The effect of $(m\text{–}CF_3\text{–}PhSe)_2$ in the mouse mTST is shown in Figure 4 (scheme of the experimental design, Figure 4(a)). The administration of $(m\text{–}CF_3\text{–}PhSe)_2$ at all doses tested decreased the time of immobility (F(4,39)=3.495, p<0.05) and swinging (F(4,39)=9.501, p<0.001), but it increased the curling time (F(4,39)=3.751, p<0.05) in the mouse mTST when compared to those of the control group (Figure 4(b)). These results were similar to those observed using morphine.

Repeated administration of $(m\text{-}CF3\text{-}PhSe)_2$ elicited an antidepressant-like action in the FST and mTST

The effects of repeated administration of $(m\text{-}CF3\text{-}PhSe)_2$ in the FST and mTST are illustrated in Figure 5 (scheme of the experimental design, Figure 5(a)). Repeated administration of $(m\text{-}CF3\text{-}PhSe)_2$ at all doses tested or morphine decreased the immobility time of mice in the FST (F(1,39)=9.523, p<0.001) (Figure 5(b)). Similarly to the behavior of mice treated with morphine, repeated administration of $(m\text{-}CF3\text{-}PhSe)_2$ at all doses tested decreased the immobility time (F(4,34)=3.896, p<0.05) and swinging time (F(4,34)=6.171, p<0.001) but increased the curling time (F(4,34)=5.127, p<0.01) of mice in the mTST (Figure 5(c)).

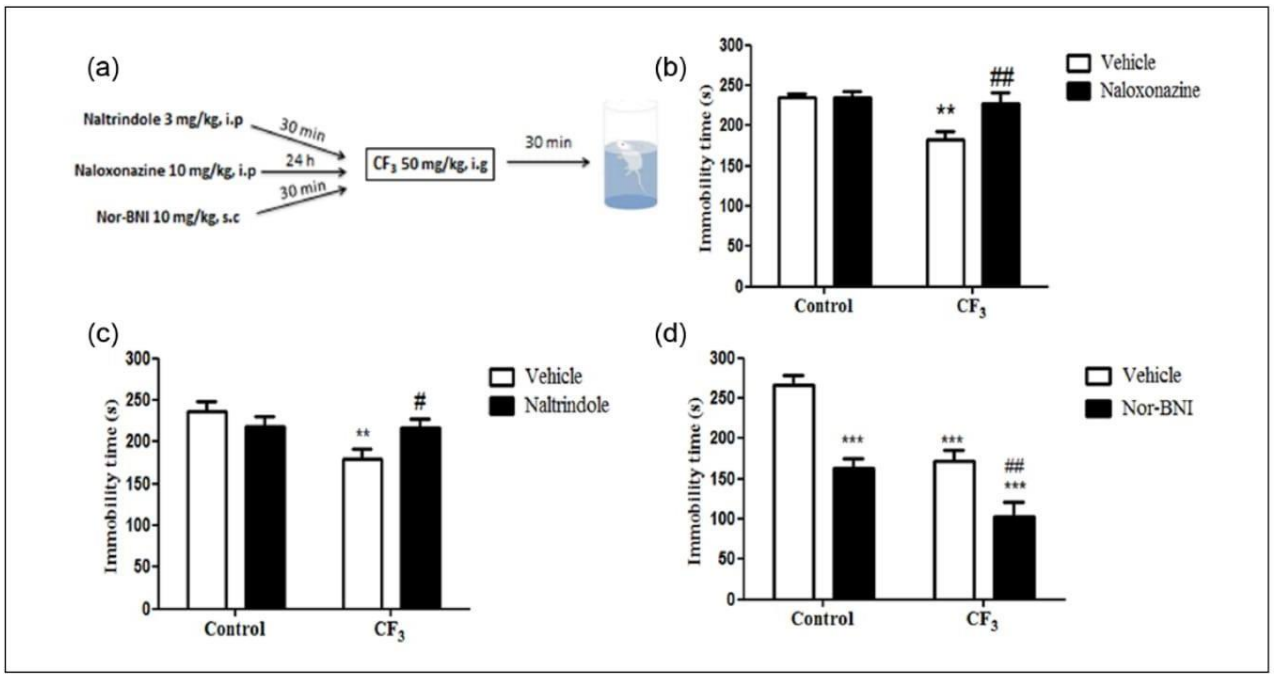


Figure 2. Effect of naloxonazine (10 mg/kg, intraperitoneally (i.p.)) (b), naltrindole (3 mg/kg, i.p.) (c), or *norbinaltrophimine* (nor-BNI) (10 mg/kg, subcutaneously (s.c.)) (d) administration to mice on the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ (CF_3; 50 mg/kg, intragastric (i.g.)) in the forced swimming test (FST). The scheme of the experimental design is given in (a) and the administration of antagonists was 30 min (naltrindole/nor-BNI) or 24 h (naloxonazine) before $(m\text{–}CF_3\text{–}PhSe)_2$. Mice performed the FST 30 min after $(m\text{–}CF_3\text{–}PhSe)_2$ administration. Values are expressed as mean±standard error of the mean (SEM) of nine ((b) and (d)) and eight (c) animals. Asterisks denote the significance levels when compared to the control group: **p<0.01 and ***p<0.001. Hashtags denote the significance levels when compared to the CF_3 group: #p<0.05 and ##p<0.01 (two-way analysis of variance (ANOVA) followed by the Newman-Keuls test).

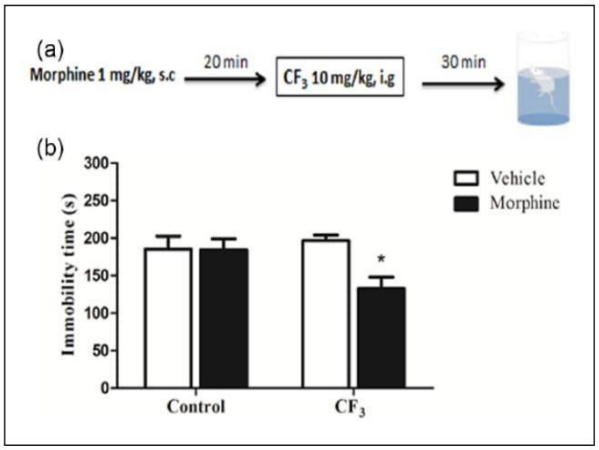


Figure 3. Effect of morphine (1 mg/kg, intraperitoneally (i.p.)) and (m–CF_3–PhSe)$_2$ (CF_3; 10 mg/kg, intragastric (i.g.)) subeffective doses on the mouse forced swimming test (FST) (b). The scheme of the experimental design is given in (a) and CF_3 was administered 20 min after morphine and mice performed the FST 30 min after CF_3 administration. Values are expressed as mean±standard error of the mean (SEM) of 10 animals. An asterisk denotes the significance levels when compared to the control group: *p<0.05 (two-way analysis of variance (ANOVA) followed by the Newman-Keuls test).

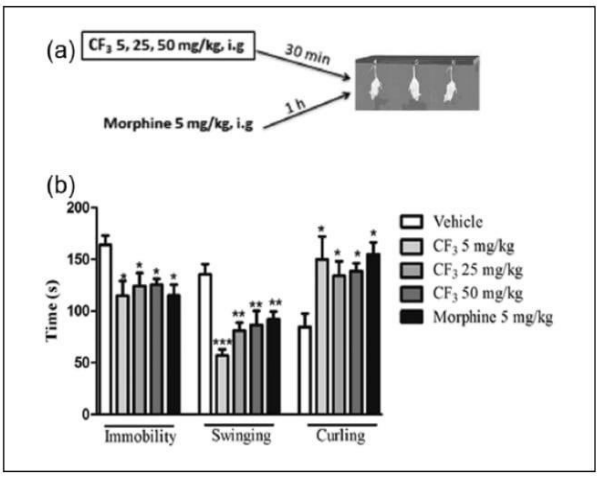


Figure 4. Effect of (m–CF_3–PhSe)$_2$ (CF_3) administration in the mouse modified tail suspension test (mTST) (b). The scheme of the experimental design is given in (a) and CF_3 (5–50 mg/kg, intragastric (i.g.)) or morphine (5 mg/kg, subcutaneously (s.c.)) was administered 30 min or 1 h, respectively, before test. Values are expressed as mean±standard error of the mean (SEM) of eight animals. Asterisks denote the significance levels when compared to the control group: *p<0.05, **p<0.01 and ***p<0.001 (one-way analysis of variance (ANOVA) followed by the Newman-Keuls test).

Repeated administration of (m-CF3-PhSe)$_2$ induced neither tolerance to its antidepressant-like action nor signs of withdrawal syndrome

The effect of the administration of (m-CF_3-PhSe)$_2$ or morphine at a dose of 5 mg/kg, twice daily during seven days, to mice in the FST is illustrated in Figure 6 (scheme of the experimental design, Figure 6(a)). Post-hoc comparisons revealed a decrease of the immobility time of mice at the third (F(2,26)=7.272, p<0.01), at the fifth (F(2,26)=9.429, p<0.001) and at the seventh day (F(2,26)=8.507, p<0.01) of (m-CF3-PhSe)$_2$ treatment and a decrease of immobility time of mice in the first (F(2,26)=3.488, p<0.05) and the third (F(2,26)=7.272, p<0.01) day of morphine treatment (Figure 6(b)). However, the morphine effect on immobility time of mice in the FST was lost on the fifth day of treatment.

The administration of naloxone in (m-CF_3-PhSe)$_2$-treated mice (scheme of the experimental design, Figure 7(a)) did not induce signs of withdrawal syndrome (Figure 7(b)–(f)). There was no significant effect of naloxone in (m-CF_3-PhSe)$_2$-treated mice in rearing (F(2,26)=24.11), jumping (F(2,26)=7.311), teeth chattering (F(2,26)=16.93) and paw tremor (F(2,26)=21.78, p>0.05). By contrast, naloxone induced an increase in rearing (F(2,26)=24.11, p<0.001), jumping (F(2,26)=7.311, p<0.01), teeth chattering (F(2,26)=16.93, p<0.001) and paw tremor (F(2,26)=21.78, p<0.001) in morphine-treated mice (Figure 7(b)–(e)).

In addition, Fisher's exact test revealed an increase in diarrhea occurrence induced by naloxone in morphine-treated mice (p<0.01). The naloxone injection increased diarrhea occurrence in mice treated with morphine (Figure 7(f)).

(m–CF_3–PhSe)$_2$ withdrawal induced by naloxone administration did not alter the glutamate uptake and release in the prefrontal cortex and hippocampus of mice

Figure 8 shows the results on the glutamate uptake and release in the prefrontal cortex (Figure 8(a) and (b), respectively) and hippocampus (Figure 8(c) and (d), respectively) of mice treated with (m–CF_3–PhSe)$_2$ or morphine during withdrawal induced by naloxone. The naloxone injection did not alter the [^{3}H] glutamate uptake and [^{3}H] release in the prefrontal cortex and hippocampus of mice pretreated with (m–CF_3–PhSe)$_2$, p>0.05.

The mice treated with morphine showed a decrease in the [^{3}H] glutamate uptake in the prefrontal cortex (F(2,14)=4.747, p<0.05) and hippocampus (F(2,14)=6.671, p<0.05) but an increase in the [^{3}H] glutamate release in the prefrontal cortex (F(2,14)=6.749, p<0.01) and hippocampus (F(2,14)=5.252, p<0.05).

(m-CF_3-PhSe)$_2$ repeated administration did not induce systemic toxicity

The repeated administration of (m-CF3-PhSe)$_2$ at different doses did not change parameters of hepatic, cardiac, and renal toxicity, such as plasma AST, ALT, and CK activities and urea levels, p>0.05 (Table 1).

In addition, histopathological analyses are illustrated in the Supplementary Material, Figure 1S. The mice pretreated either with vehicle or (m-CF3-PhSe)$_2$ showed: normal cellular architecture of hepatic tissue; with central vein at the center of lobule and cords of hepatocytes radiating from central vein towards portal triads (Supplementary Material, Figure 1S (a) and (c)), and normal cellular architecture of renal cortex tissue, showing tubular

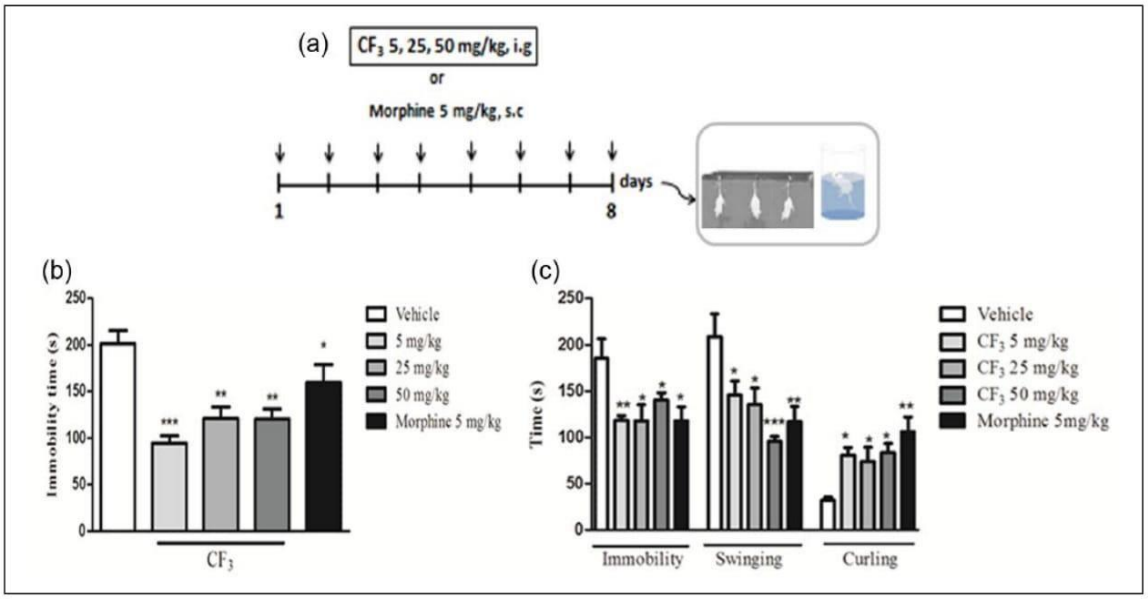


Figure 5. Effect of (m–CF_3–PhSe)$_2$ (CF_3) repeated administration in the mouse forced swimming test (FST) (b) and mTST (c). The scheme of the experimental design is given in (a) and CF_3 (10–50 mg/kg, intragastric (i.g.)) or morphine (5 mg/kg, subcutaneously (s.c.)) was administered for eight days, 30 min or 1 h, respectively, and after the last administration mice performed the behavioral tests. Values are expressed as mean±standard error of the mean (SEM) of eight (a) or seven (b) animals. Asterisks denote the significance levels when compared to the control group: *p<0.05, **p<0.01 and ***p<0.001 (one-way analysis of variance (ANOVA) followed by the Newman-Keuls test).

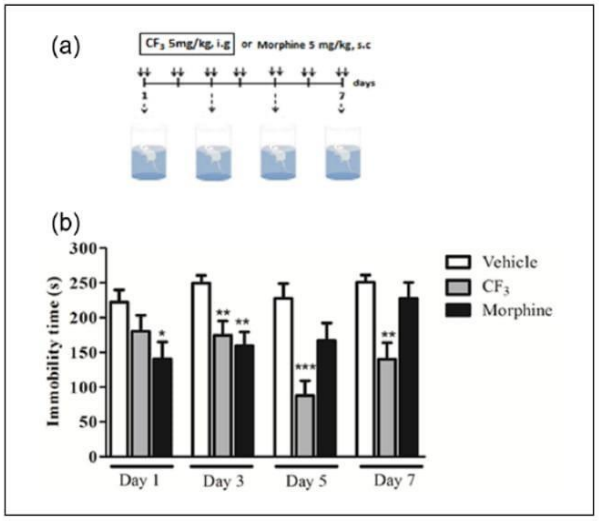


Figure 6. Effect of (m–CF_3–PhSe)$_2$ (CF_3) on the development of tolerance in the mouse forced swimming test (FST) (b). The scheme of the experimental design is given in (a) and a dose of 5 mg/kg of CF_3 or morphine, a positive control, was administered twice daily to mice for seven days. At days 1, 3, 5, and 7 of treatment, the mice performed the FST 30 min or 1 h after administration of CF_3 or morphine, respectively. Values are expressed as mean±standard error of the mean (SEM) of nine animals. Asterisks denote the significance levels when compared to the control group: *p<0.05, **p<0.01 and ***p<0.001 (one-way analysis of variance (ANOVA) followed by the Newman-Keuls test).

cells with normal nuclei, and regular glomeruli (Supplementary Material, Figure 1S (b) and (d)).

The drug treatments did not induce alterations in the locomotor activity of mice on the open-field test

There were no significant differences (± >0.05) among groups in the number of crossings of mice assessed in the open-field test after any treatment carried out in this study (Tables 2 and 3).

Discussion

Clinical and animal research data support the notion that endogenous opioids have a crucial role in the etiology of mood disorders and can be targets for intervention in depressive disorders (Berrocoso et al., 2009; Lutz and Kieffer, 2013). In the present study, we investigated the contribution of the opioid system to the antidepressant-like action of (m-CF_3-PhSe)$_2$ in mice. Acute and repeated administrations of this compound abolished the depressant-like behavior of mice in the FST and the mTST. The pharmacological findings provided evidence that μ- and δ- receptor activation and the κ-opioid receptor blockade contributed to the antidepressant-like action of (m-CF_3-PhSe)$_2$. Importantly, this study also revealed that (m-CF_3-PhSe)$_2$ induced neither tolerance to the antidepressant-like action nor physical signs of withdrawal, as classical opioid drugs do, nor systemic toxicity, indicating the safe use of this compound.

Previous studies from our research group have reported that (m-CF_3-PhSe)$_2$ elicits an antidepressant-like action in the mouse FST and TST (Bruning et al., 2011, 2015a,b). The findings of the present study revealed that a single administration of (m-CF_3-PhSe)$_2$ decreased immobility time of mice in the FST for a period of 30 min to 24 h after treatment. Interestingly, this time profile of antidepressant-like action of (m-CF_3-PhSe)$_2$ is

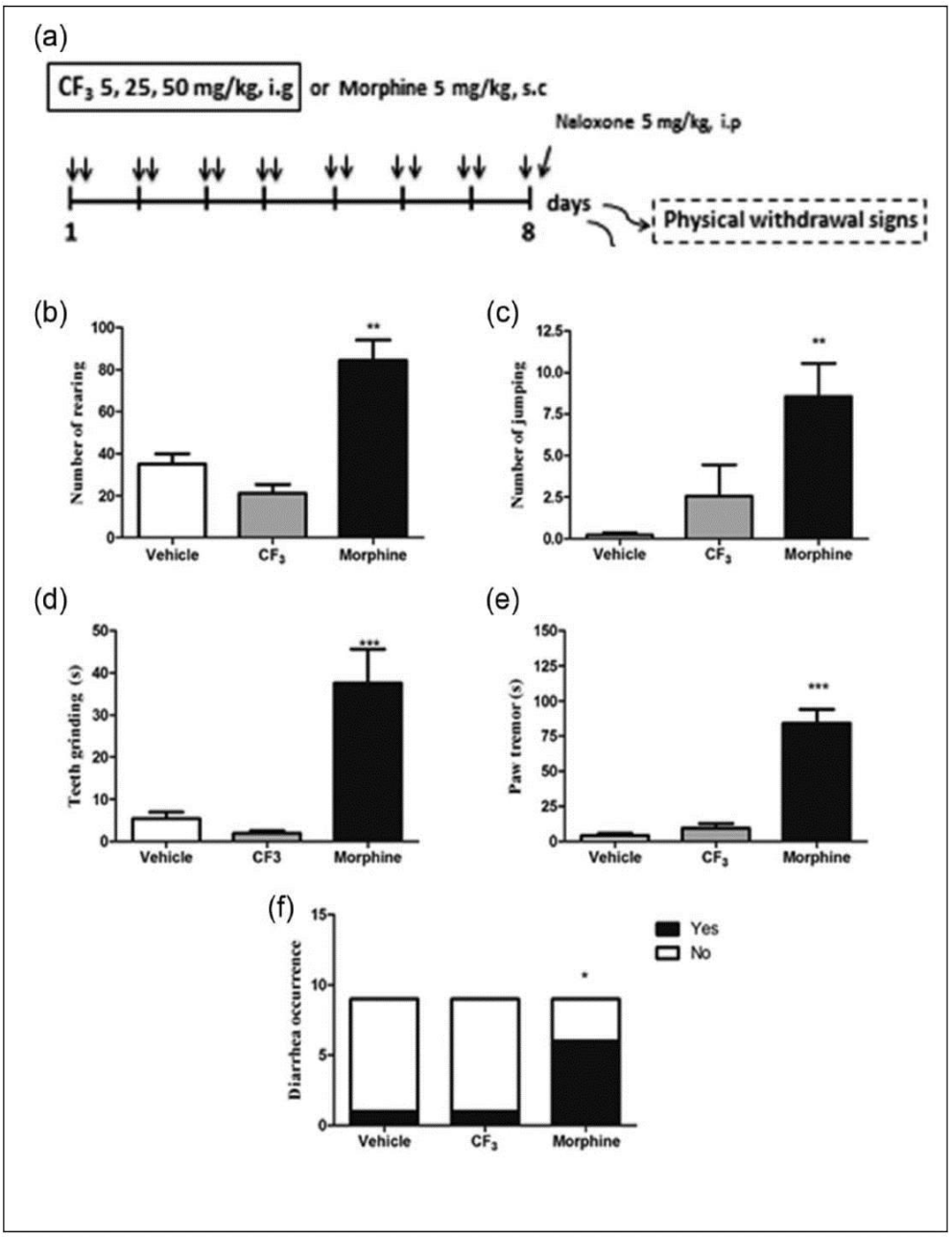


Figure 7. Effect of (m-CF_3-PhSe)$_2$ (CF_3) on signs of withdrawal syndrome after naloxone administration in mice. The scheme of the experimental design (a) CF_3 or morphine (5 mg/kg, intragastric (i.g.)) was administrated to mice twice a day for seven days. On the eighth day mice received a dose of CF_3 or morphine 30 min before naloxone (5 mg/kg, i.g.) injection. Signs of withdrawal syndrome, the number of rearings (b) and jumping (c), teeth grinding (d), paw tremor (e), and diarrhea occurrence (f) were recorded immediately after the naloxone administration. Values are expressed as mean±standard error of the mean (SEM) of nine animals. Asterisks denote the significance levels when compared to the control group: *p<0.05, **p<0.01 and ***p<0.001 (one-way analysis of variance (ANOVA) followed by the Newman-Keuls test (b), (c), (d) and (e), Fisher's exact test (f)).

similar to that of ketamine and buprenorphine, antidepressants known for rapid and sustained effect (Falcon et al., 2015; Murrough et al., 2013).

The antidepressant-like action of (m-CF_3-PhSe)$_2$ has been associated with the serotonergic modulation and anti-inflammatory action of this compound (Bruning et al., 2011, 2015b). In addition, experimental data demonstrated that naloxone, a non-selective antagonist of opioid receptors, blocks the decrease of immobility time induced by (m–CF_3–PhSe)$_2$ in the FST, suggesting that the opioid system can also contribute to the antidepressant-like action of this compound (Bruning et al., 2011). Research findings demonstrate that as opposed to the

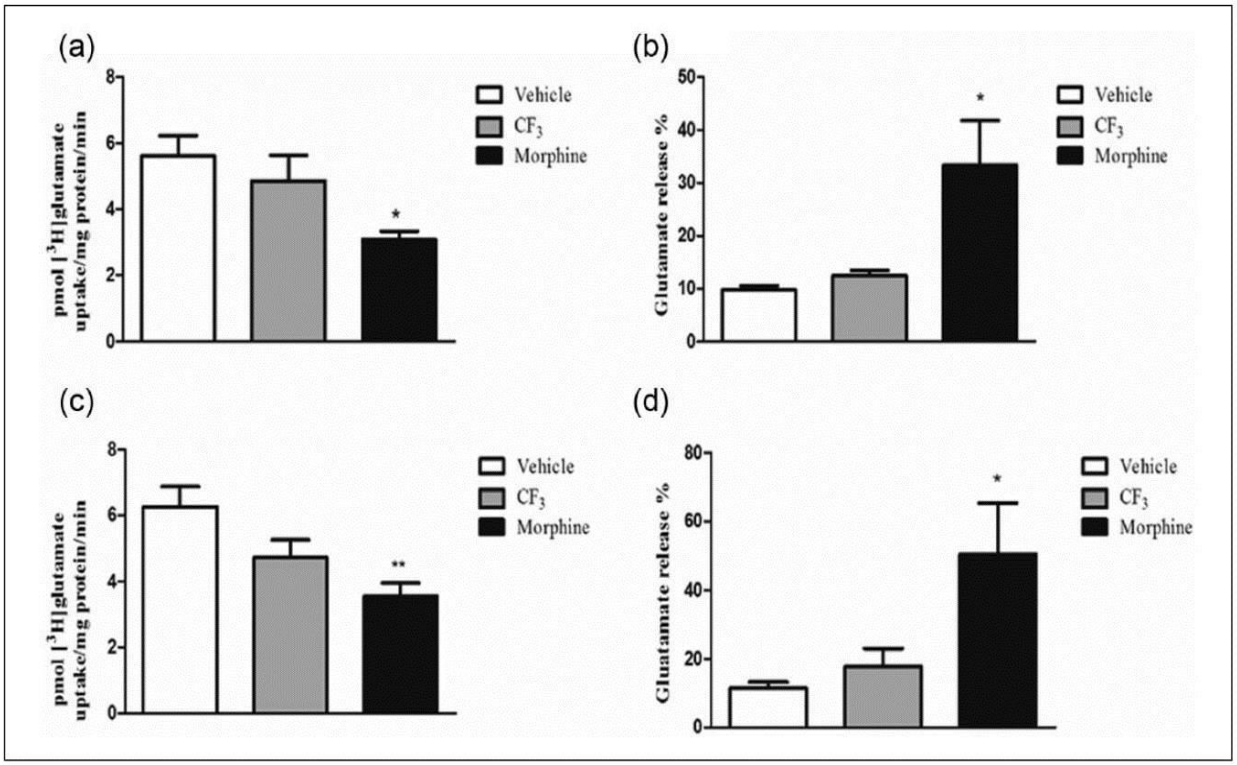


Figure 8. Effect of $(m\text{-}CF_3\text{-}PhSe)_2$ (CF_3) on [^{3}H] glutamate uptake and release in mice administered with naloxone, CF_3 or morphine (5 mg/kg, intragastric (i.g.)) was administrated twice a day for seven days. On the eighth day, mice received a dose of CF_3 30 min before naloxone injection. [^{3}H] glutamate uptake ((a) and (c)) and [^{3}H] glutamate release ((b) and (d)) were determined in the prefrontal cortex ((a) and (b)) hippocampus ((c) and (d)) 30 min after naloxone injection. Values are expressed as mean±standard error of the mean (SEM) of five animals. Asterisks denote the significance levels when compared to the control group: *p<0.05 and **p<0.01 (one-way analysis of variance (ANOVA) followed by the Newman-Keuls test).

Table 1. Effect of repeated treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ (CF_3) on serum toxicity parameters.

Group	AST U/L	ALT U/L	CK U/L	Urea mg/dL
Control	178.6 ± 22.04	45.71 ± 6.75	61.29 ± 8.22	27.00 ± 1.49
CF_3 10 mg/kg	158.6 ± 14.23	55.14 ± 9.04	41.86 ± 3.95	22.86 ± 1.99
CF_3 25 mg/kg	170.3 ± 14.05	48.14 ± 3.33	56.14 ± 6.12	22.14 ± 1.83
CF_3 50 mg/kg	142.3 ± 28.56	58.00 ± 3.57	44.29 ± 7.29	25.29 ± 1.89

ALT: alanine aminotransferase; AST: aspartate aminotransferase; CK: creatine kinase.
Values are reported as means±standard error of the mean (SEM). for seven animals per group. Data were analyzed by using a one-way analysis of variance (ANOVA).

Table 2. Spontaneous locomotor activity of mice treated with $(m\text{-}CF_3\text{-}PhSe)_2$ (CF_3) and/or opioid receptor antagonists on open-field test.

Groups	Number of crossing
Control	46.69 ± 8.08
CF_3	40.55 ± 3.41
Naloxonazine	41.29 ± 4.71
CF_3 + naloxonazine	31.62 ± 5.59
Control	56.14 ± 4. 90
CF_3	51.85 ± 6.18
Naltrindole	58.71 ± 4.59
CF_3 + naltrindole	49.00 ± 5.59
Control	59.20 ± 8.84
CF_3	48.60 ± 4.53
Nor-BNI	59.20 ± 3.92
CF_3 + nor-BNI	54.11 ± 4.92

nor-BNI: *norbinaltrophimine*.
Values are reported as means±standard error of the mean (SEM). for 8–10 animals per group. Data were analyzed by using a two-way analysis of variance (ANOVA). CF_3: $(m\text{-}CF_3\text{-}PhSe)_2$. CF_3 was administered at a dose of 50 mg/kg, naloxonazine at 10 mg/kg, naltrindole at 3 mg/kg and nor-BNI at 10 mg/kg.

antidepressant-like action induced by the μ- and δ-receptor agonists (Gassaway et al., 2014; Saitoh et al., 2011), the κ-receptor agonist induces depressive behaviors (Kanneti et al., 2011), while the κ-opioid receptor antagonist exerts antidepressant-like effect in different behavioral models of depression (Carr et al., 2010; Shirayama et al., 2004). Accordingly, opioid agonists, such as oxycodone and oxymorphone, and the partial μ-agonist with κ-antagonist activity, buprenorphine, were effective in patients with refractory major depression (Bodkin et al., 1995; Stoll and Rueter, 1999).

In the present study, evidence was found to suggest that direct or indirect activation of δ and μ-opioid receptors contributes to

Table 3. Spontaneous locomotor activity of mice after repeated treated with $(m\text{–}CF_3\text{–}PhSe)_2$ (CF_3).

Group	Number of crossing
Control	52.62 ± 3.47
CF_3 10 mg/kg	43.88 ± 3.10
CF_3 25 mg/kg	41.63 ± 7.34
CF_3 50 mg/kg	46.00 ± 5.55
Morphine 5 mg/kg	62.53 ± 6.67

Values are reported as means±standard error of the mean (SEM). for eight animals per group. Data were analyzed by using a one-way analysis of variance (ANOVA).

the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$. This idea is further supported by the fact that the selective μ- and δ-opioid receptor antagonists, naloxonazine and naltrindole, blockaded the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ in the mouse FST. Moreover, the administration of a selective κ-opioid receptor antagonist, nor-BNI, and $(m\text{–}CF_3\text{–}PhSe)_2$, either at effective or subeffective doses elicited an antidepressant-like action in the FST, suggesting that nor-BNI and $(m\text{–}CF_3\text{–}PhSe)_2$ act through a common pathway to produce this action. Therefore, it is possible that the antidepressant-like action elicited by $(m\text{–}CF_3\text{–}PhSe)_2$ is mediated, at least in part, by the direct or indirect antagonism of the κ-opioid receptors.

Furthermore, it is known that morphine, an alkaloid opiate with substantial affinity to the μ-opioid receptors, elicits an antidepressant-like effect in the FST (Kaster et al., 2007). In the current study, sub-effective doses of $(m\text{–}CF_3\text{–}PhSe)_2$ and morphine demonstrated a synergistic antidepressant-like action in the mouse FST, reinforcing the assumption that μ-opioid receptor activation contributes to the antidepressant-like effect of $(m\text{–}CF_3\text{–}PhSe)_2$.

The relevance of these pharmacological results comes from the fact that each opioid receptor subtype could contribute to the $(m\text{–}CF_3\text{–}PhSe)_2$ antidepressant-like action, which corroborates with the effectiveness of the opioid μ- and δ-agonists and the κ-antagonists in rodent models of depression (Carr et al., 2010; Gassaway et al., 2014; Saitoh et al., 2011).

The involvement of opioid system, direct or indirect, has been reported in the antidepressant effect of drugs (Haj-Mirzaian et al., 2016; Gassaway et al., 2014; Zomkowski et al., 2005). Zomkowski et al. (2005) demonstrated pharmacologically the involvement of opioid system in the antidepressant-like effect of agmatine but this drug does not bind to opioid receptors (Jin et al., 1999), which suggests an indirect effect of agmatine on the opioid system. Moreover, naloxone reverses the antidepressant-like effect of venlafaxine and fluoxetine, selective serotonin reuptake inhibitors, and of imipramine, a mixed serotonin and noradrenaline reuptake inhibitor (Haj-Mirzaian et al., 2016; Tejedor-Real et al., 1995), suggesting an indirect involvement of the opioid system in the effect of these drugs. On the other hand, drugs that have antidepressant effect, such as tianeptine and buprenorphine, directly bind to opioid receptors (Cowan, 2007; Falcon et al., 2015; Gassaway et al., 2014). In the present study, the pharmacological results, although contributing to the understanding of $(m\text{–}CF_3\text{–}PhSe)_2$ antidepressant-like effects, do not allow us to affirm if this compound acts directly or indirectly on the opioid receptors. The possibility that the involvement of opioid system in the antidepressant-like action of compound is indirectly mediated by modulation of other neurotransmitter systems or by regulation of endogenous opioid peptide release cannot be ruled out because $(m\text{–}CF_3\text{–}PhSe)_2$ is a multi-target compound. Therefore, we acknowledge the lack of the opioid-binding assay as a limitation of the present study.

To further investigate the contribution of opioid system to the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$, we used the modified form of the TST, a predictive behavioral test of antidepressant activity (Steru et al., 1985). This behavioral test evaluates active behaviors, allowing us to distinguish between antidepressants with different mechanisms of action (Berrocoso et al., 2013). Different antidepressant classes reduce the immobility time of animals in the mTST (Berrocoso et al., 2013). Moreover, the antagonism or genetic deletion of opioid receptors decreases curling behavior (Berrocoso et al., 2013). Similar to morphine, $(m\text{–}CF_3\text{–}PhSe)_2$ decreased immobility and swinging times, whereas it increased the curling time of animals in the mTST, a behavior profile characteristic of opioid drugs, strengthening the notion that the opioid system contributes to the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ in both FST and mTST tests for screening antidepressant drugs.

Considering that acute administration of $(m\text{–}CF_3\text{–}PhSe)_2$ elicited an antidepressant-like action in the FST and mTST and that the opioid system contributed to this effect as well as the fact that antidepressants are commonly used in long-lasting treatments, we investigated the antidepressant-like action of repeated doses of $(m\text{–}CF_3\text{–}PhSe)_2$. Similar to morphine, repeated administration of this compound at all tested doses decreased the immobility time of mice in the FST and induced the same behavior profile as mice administered acutely with $(m\text{–}CF_3\text{–}PhSe)_2$ in the mTST, suggesting the contribution of opioid system in the antidepressant-like action of $(m\text{–}CF_3\text{–}PhSe)_2$ when administered repeatedly. These results further indicate that subchronic administration of $(m\text{-}CF_3\text{-}PhSe)_2$ could remain clinically effective for treating depressive disorders.

In the present study, the mice performed the open-field test because the FST and mTST may yield false positive results with drugs that increase locomotor activity, and correspondingly, decrease immobility. The findings indicate that the antidepressant-like effect of $(m\text{–}CF_3\text{–}PhSe)_2$ or morphine was not accompanied by changes in the locomotor activity of animals, which means that we can discard interference in the FST and mTST caused by sedative or stimulant effect. Regarding the well-known psychostimulant effect of morphine, it has been reported that antidepressant-like and psychostimulant effects are dissociated (Haj-Mirzaian et al., 2016; Zomkowski et al., 2005) which is in agreement with the findings of this study.

Although opioid compounds have been documented as potential drugs to treat depressive disorders (Almatroudi et al., 2015; Brocardo et al., 2009; Wang et al., 2015), the clinical utility of opioids for this purpose is quite limited by the development of tolerance and dependence during prolonged treatment and by the emergence of physical signs of withdrawal after discontinuation of use (Hu et al., 2015; Lin et al., 2015). Chronic administration of morphine is associated with a decrease of drug effect and a consequent need for progressively increasing doses, known as tolerance, which often lead to drug-seeking behaviors, dependency, and abstinence signs (Mansouri et al., 2014). In animals, the appearance of characteristic behaviors such as teeth chattering, paw tremor, diarrhea, rearing, and jumping induced by

naloxone administration to morphine-treated animals has been largely associated with the opiate withdrawal syndrome and indicates the establishment of morphine dependency (Abdel-Zaher et al., 2010; Wei et al., 1973). The current results demonstrated that repeated administration of $(m\text{-}CF_3\text{-}PhSe)_2$ elicited an antidepressant-like action from the second to the last day of treatment. Furthermore, the naloxone administration did not induce the emergence of physical signs of opiate withdrawal syndrome in the $(m\text{-}CF_3\text{-}PhSe)_2$-treated mice, indicating that although the opioid system could contribute to the antidepressant-like action of this compound, repeated administration did not induce tolerance and withdrawal physical signs, or adverse effects associated with prolonged use of opiates (Heishman et al., 1989; Song et al., 2015). By contrast, the loss of antidepressant-like action of morphine by the fifth day of treatment indicated tolerance induced by this opioid drug. Furthermore, naloxone induced an increase of physical signs of opiate withdrawal syndrome only in mice treated with morphine, confirming the establishment of morphine tolerance and dependency, which is widely documented in animal models and human beings (Mansouri et al., 2014).

In the context of opiate tolerance, dependence, and withdrawal, several key neurotransmitters have been documented, in particular a critical role of glutamatergic neurotransmission dysfunctions (Mao et al., 2002; Murray et al., 2007). It has been proven that naloxone administration to morphine-tolerant mice leads to increases in glutamate levels, glutamate release, and alters the expression of glutamate receptors, especially NMDA receptors in different brain regions (Glass et al., 2004; Siggins et al., 2003; Wang et al., 2011). In agreement with these studies, the present findings demonstrate that the naloxone administration altered the glutamatergic neurotransmission in the prefrontal cortex and hippocampus of mice treated with morphine. However, naloxone did not cause any change of glutamate uptake and release in the prefrontal cortex and hippocampus of mice treated with $(m\text{-}CF_3\text{-}PhSe)_2$. The fact that the opioid system contributes to the $(m\text{-}CF_3\text{-}PhSe)_2$ antidepressant-like action although this compound does not induce withdrawal syndrome could be explained by effects on downstream signaling cascades of opioid receptors, for example the absence of withdrawal syndrome could be attributed to unaltered glutamate release and uptake observed in mice treated with $(m\text{-}CF_3\text{-}PhSe)_2$ whereas mice treated with morphine showed reduced glutamate uptake and increased glutamate release. However, this hypothesis and other possible reasons why $(m\text{-}CF_3\text{-}PhSe)_2$ did not induce withdrawal syndrome should be investigated in the near future. In addition, studies have demonstrated that some drugs that show an opioid mechanism, including direct interaction with opioid receptors, such as buprenorphine and tianeptine, induce very few or no signs of withdrawal (Jasinski et al., 1978; Samuels et al., 2017; Tompkins et al., 2014).

Considering the pharmacological effects of $(m\text{-}CF_3\text{-}PhSe)_2$ demonstrated in this study and the fact that organoselenium compounds can induce toxicity at high doses in rodents (Nogueira et al., 2004; Nogueira and Rocha, 2011), we delineated studies to evaluate the possible toxicological effects of this compound. Regarding parameters of hepatic, renal, and cardiac functions, $(m\text{-}CF_3\text{-}PhSe)_2$ did not alter these factors in the plasma of mice. The histology of liver and kidney tissues indicated that the morphology of these tissues was preserved after $(m\text{-}CF_3\text{-}PhSe)_2$ treatment. In addition, all treatments carried out in this study were devoid of any significant alteration in the spontaneous locomotor activity of the animals. Together these results indicate a relatively safe treatment with $(m\text{-}CF_3\text{-}PhSe)_2$ and exclude possible experimental bias in the behavioral tests (Cryan et al., 2005).

In conclusion, the results found in this study demonstrate that acute and repeated administration of $(m\text{-}CF_3\text{-}PhSe)_2$ elicited an antidepressant-like action in the FST and mTST and that the opioid system, by direct or indirect μ- and δ-opioid receptors activation and κ-opioid receptor antagonism, contributed to this action. In addition, the current study revealed that repeated treatment did not develop tolerance to $(m\text{-}CF_3\text{-}PhSe)_2$ and opiate withdrawal syndrome in mice. These results suggest that $(m\text{-}CF_3\text{-}PhSe)_2$ is a candidate for future studies as a new therapeutic tool for treatment of depression because it unites the potent antidepressant-like action of opioids with a relatively safe use of this compound. Nevertheless, further studies are needed to understand the molecular mechanisms whereby this compound leads to modulation of opioid receptors exerting antidepressant-like action.

Declaration of conflicting interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This work was supported by Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) (2239-2551/14) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (441405/2014-2) for the financial support. CWN is the recipient of a CNPq fellowship.

References

Abdel-Zaher AO, Abdel-Rahman MS and ELwasei FM (2010) Blockade of nitric oxide overproduction and oxidative stress by Nigella sativa oil attenuates morphine-induced tolerance and dependence in mice. *Neurochem Res* 35: 1557–1565.

Almatroudi A, Husbands SM, Bailey CP, et al. (2015) Combined administration of buprenorphine and naltrexone produces antidepressant-like effects in mice. *J Psychopharmacol* 29: 812–821.

Berrocoso E, Ikeda K, Sora I, et al. (2013) Active behaviours produced by antidepressants and opioids in the mouse tail suspension test. *Int J Neuropsychopharmacol* 16: 151–162.

Berrocoso E and Mico JA (2009) Cooperative opioid and serotonergic mechanisms generate superior antidepressant-like effects in a mice model of depression. *Int J Neuropsychopharmacol* 12: 1033–1044.

Berrocoso E, Sanchez-Blazquez P, Garzon J, et al. (2009) Opiates as antidepressants. *Curr Pharm Des* 15: 1612–1622.

Bodkin JA, Zornberg GL, Lukas SE, et al. (1995) Buprenorphine treatment of refractory depression. *J Clin Psychopharmacol* 15: 49–57.

Bodnar RJ (2017) Endogenous opiates and behavior: 2015. *Peptides* 88: 126–188.

Brocardo PS, Budni J, Lobato KR, et al. (2009) Evidence for the involvement of the opioid system in the antidepressant-like effect of folic acid in the mouse forced swimming test. *Behav Brain Res* 200: 122–127.

Bruchas MR, Land BB, Aita M, et al. (2007) Stress-induced p38 mitogen-activated protein kinase activation mediates kappa-opioid-dependent dysphoria. *J Neurosci* 27: 11614–11623.

Bruning CA, Martini F, Soares SM, et al. (2015a) m-Trifluoromethyldiphenyl diselenide, a multi-target selenium compound, prevented mechanical allodynia and depressive-like behavior in a mouse comorbid pain and depression model. *Prog Neuropsychopharmacol Biol Psychiatry* 63: 35–46.

Bruning CA, Martini F, Soares SM, et al. (2015b) Depressive-like behavior induced by tumor necrosis factor-alpha is attenuated by m-trifluoromethyl-diphenyl diselenide in mice. *J Psychiatr Res* 66–67: 75–83.

Bruning CA, Prigol M, Roehrs JA, et al. (2010) Evidence for the involvement of mu-opioid and delta-opioid receptors in the antinociceptive effect caused by oral administration of m-trifluoromethyl-diphenyl diselenide in mice. *Behav Pharmacol* 21: 621–626.

Bruning CA, Souza AC, Gai BM, et al. (2011) Antidepressant-like effect of m-trifluoromethyl-diphenyl diselenide in the mouse forced swimming test involves opioid and serotonergic systems. *Eur J Pharmacol* 658: 145–149.

Carr GV, Bangasser DA, Bethea T, et al. (2010) Antidepressant-like effects of kappa-opioid receptor antagonists in Wistar Kyoto rats. *Neuropsychopharmacology* 35: 752–763.

Christie M (2008) Cellular neuroadaptations to chronic opioids: Tolerance, withdrawal and addiction. *Br J Pharmacol* 154: 384–396.

Cowan A (2007) Buprenorphine: The basic pharmacology revisited. *J Addict Med* 1: 68–72.

Cryan JF, Valentino RJ and Lucki I (2005) Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. *Neurosci Biobehav Rev* 29: 547–569.

Falcon E, Maier K, Robinson SA, et al. (2015) Effects of buprenorphine on behavioral tests for antidepressant and anxiolytic drugs in mice. *Psychopharmacology* 232: 907–915.

Filho CB, Del Fabbro L, de Gomes MG, et al. (2013) Kappa-opioid receptors mediate the antidepressant-like activity of hesperidin in the mouse forced swimming test. *Eur J Pharmacol* 698: 286–291.

Gai BM, Bortolatto CF, Bruning CA, et al. (2014) Depression-related behavior and mechanical allodynia are blocked by 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene in a mouse model of neuropathic pain induced by partial sciatic nerve ligation. *Neuropharmacology* 79: 580–589.

Gao S, Gao H, Fan Y, et al. (2016) Yiguanjian cataplasm attenuates opioid dependence in a mouse model of naloxone-induced opioid withdrawal syndrome. *J Tradit Chin Med* 36: 464–470.

Gassaway MM, Rives ML, Kruegel AC, et al. (2014) The atypical antidepressant and neurorestorative agent tianeptine is a mu-opioid receptor agonist. *Transl Psychiatry* 4: e411.

Glass MJ, Kruzich PJ, Kreek MJ, et al. (2004) Decreased plasma membrane targeting of NMDA-NR1 receptor subunit in dendrites of medial nucleus tractus solitarius neurons in rats self-administering morphine. *Synapse* 53: 191–201.

Haj-Mirzaian A, Kordjazy N, Ostadhadi S, et al. (2016) Fluoxetine reverses the behavioral despair induced by neurogenic stress in mice: Role of N-methyl-d-aspartate and opioid receptors. *Can J Physiol Pharmacol* 94: 599–612.

Heishman SJ, Stitzer ML, Bigelow GE, et al. (1989) Acute opioid physical dependence in postaddict humans: Naloxone dose effects after brief morphine exposure. *J Pharmacol Exp Ther* 248: 127–134.

Hu X, Huang F, Szymusiak M, et al. (2015) Curcumin attenuates opioid tolerance and dependence by inhibiting Ca2+/calmodulin-dependent protein kinase II α activity. *J Pharmacol Exp Ther* 352: 420–428.

Jasinski DR, Pevnick JS and Griffith JD (1978) Human pharmacology and abuse potential of the analgesic buprenorphine: A potential agent for treating narcotic addiction. *Arch Gen Psychiatry* 35: 501–516.

Jin L, Xin L, Gang P, et al. (1999) Relationship between inhibition of agmatine on rat morphine physical dependence and opiate receptors. *Chin J Drug Depend* 3: 178–181.

Kanneti R, Bhavesh D, Paramar D, et al. (2011) Development and validation of a high-throughput and robust LC-MS/MS with electrospray ionization method for simultaneous quantitation of oseltamivir phosphate and its oseltamivir carboxylate metabolite in human plasma for pharmacokinetic studies. *Biomed Chromatogr* 25: 727–733.

Kaster MP, Budni J, Santos AR, et al. (2007) Pharmacological evidence for the involvement of the opioid system in the antidepressant-like effect of adenosine in the mouse forced swimming test. *Eur J Pharmacol* 576: 91–98.

Lin C-P, Kang K-H, Lin T-H, et al. (2015) Role of spinal CXCL1 (GROα) in opioid tolerance: A human-to-rodent translational study. *Anesthesiology* 122: 666.

Lutz PE and Kieffer BL (2013) Opioid receptors: Distinct roles in mood disorders. *Trends Neurosci* 36: 195–206.

Mansour A, Khachaturian H, Lewis ME, et al. (1988) Anatomy of CNS opioid receptors. *Trends Neurosci* 11: 308–314.

Mansouri MT, Naghizadeh B and Ghorbanzadeh B (2014) Ellagic acid enhances morphine analgesia and attenuates the development of morphine tolerance and dependence in mice. *Eur J Pharmacol* 741: 272–280.

Mao J, Sung B, Ji RR, et al. (2002) Chronic morphine induces downregulation of spinal glutamate transporters: implications in morphine tolerance and abnormal pain sensitivity. *J Neurosci* 22: 8312–8323.

Migues PV, Leal RB, Mantovani M, et al. (1999) Synaptosomal glutamate release induced by the fraction Bc2 from the venom of the sea anemone Bunodosoma caissarum. *Neuroreport* 10: 67–70.

Mul JD, Zheng J and Goodyear LJ (2016) Validity assessment of 5 day repeated forced-swim stress to model human depression in young-adult C57BL/6J and BALB/cJ mice. *eNeuro* 3: ENEURO. 0201–0216.2016.

Murray F, Harrison NJ, Grimwood S, et al. (2007) Nucleus accumbens NMDA receptor subunit expression and function is enhanced in morphine-dependent rats. *Eur J Pharmacol* 562: 191–197.

Murrough JW, Perez AM, Pillemer S, et al. (2013) Rapid and longer-term antidepressant effects of repeated ketamine infusions in treatment-resistant major depression. *Biol Psychiatry* 74: 250–256.

Nogueira CW and Rocha JB (2011) Toxicology and pharmacology of selenium: Emphasis on synthetic organoselenium compounds. *Arch Toxicol* 85: 1313–1359.

Nogueira CW, Zeni G and Rocha JBT (2004) Organoselenium and organotellurium compounds: Toxicology and pharmacology. *Chem Rev* 104: 6255–6285.

Paulmier C (1986) Selenoorganic functional groups. In: Paulmier C (ed.) *Selenium Reagents and Intermediates in Organic Synthesis*. 1st ed. Oxford: Pergamon Press, pp. 25–51.

Porsolt RD, Bertin A and Jalfre M (1977a) Behavioral despair in mice: Primary screening-test for antidepressants. *Arch Int Pharmacodyn Ther* 229: 327–336.

Porsolt RD, Lepichon M and Jalfre M (1977b) Depression: New animal-model sensitive to antidepressant treatments. *Nature* 266: 730–732.

Quines CB, Rosa SG, Velasquez D, et al. (2016) Diphenyl diselenide elicits antidepressant-like activity in rats exposed to monosodium glutamate: A contribution of serotonin uptake and Na(+), K(+)-ATPase activity. *Behav Brain Res* 301: 161–167.

Rehni AK, Bhateja P, Singh TG, et al. (2008) Nuclear factor-kappa-B inhibitor modulates the development of opioid dependence in a mouse model of naloxone-induced opioid withdrawal syndrome. *Behav Pharmacol* 19: 265–269.

Robinson SA, Erickson RL, Browne CA, et al. (2017) A role for the mu opioid receptor in the antidepressant effects of buprenorphine. *Behav Brain Res* 319: 96–103.

Ruhé HG, van Rooijen G, Spijker J, et al. (2012) Staging methods for treatment resistant depression. A systematic review. *J Affect Disord* 137: 35–45.

Saitoh A, Sugiyama A, Nemoto T, et al. (2011) The novel delta opioid receptor agonist KNT-127 produces antidepressant-like and antinociceptive effects in mice without producing convulsions. *Behav Brain Res* 223: 271–279.

Samuels BA, Nautiyal KM, Kruegel AC, et al. (2017) The Behavioral Effects of the Antidepressant Tianeptine Require the Mu-Opioid Receptor. *Neuropsychopharmacology*. Epub ahead of print 19 April 2017. doi: 10.1038/npp.2017.60.

Savegnago L, Jesse CR and Nogueira CW (2009) Structural modifications into diphenyl diselenide molecule do not cause toxicity in mice. *Environ Toxicol Pharmacol* 27: 271–276.

Shirayama Y, Ishida H, Iwata M, et al. (2004) Stress increases dynorphin immunoreactivity in limbic brain regions and dynorphin antagonism produces antidepressant-like effects. *J Neurochem* 90: 1258–1268.

Siggins GR, Martin G, Roberto M, et al. (2003) Glutamatergic transmission in opiate and alcohol dependence. *Ann NY Acad Sci* 1003: 196–211.

Song L, Wu C and Zuo Y (2015) Melatonin prevents morphine-induced hyperalgesia and tolerance in rats: role of protein kinase C and N-methyl-D-aspartate receptors. *BMC Anesthesiol* 15: 12.

Steru L, Chermat R, Thierry B, et al. (1985) The tail suspension test: A new method for screening antidepressants in mice. *Psychopharmacology* 85: 367–370.

Stoll AL and Rueter S (1999) Treatment augmentation with opiates in severe and refractory major depression. *Am J Psychiatry* 156: 2017.

Tejedor-Real P, Mico JA, Maldonado R, et al. (1995) Implication of endogenous opioid system in the learned helplessness model of depression. *Pharmacol Biochem Behav* 52: 145–152.

Thomazi AP, Godinho GFRS, Rodrigues JM, et al. (2004) Ontogenetic profile of glutamate uptake in brain structures slices from rats: Sensitivity to guanosine. *Mech Ageing Dev* 125: 475–481.

Tompkins DA, Smith MT, Mintzer MZ, et al. (2014) A double blind, within subject comparison of spontaneous opioid withdrawal from buprenorphine versus morphine. *J Pharmacol Exp Ther* 348: 217–226.

Walsh RN and Cummins RA (1976) The open-field test: A critical review. *Psychol Bull* 83: 482–504.

Wang FR, Qiao MQ, Xue L, et al. (2015) Possible involvement of mu opioid receptor in the antidepressant-like effect of shuyu formula in restraint stress-induced depression-like rats. *Evid Based Complement Alternat Med* 2015: 452412.

Wang XF, Wu N, Su RB, et al. (2011) Agmatine modulates neuroadaptations of glutamate transmission in the nucleus accumbens of repeated morphine-treated rats. *Eur J Pharmacol* 650: 200–205.

Wei E, Loh HH and Way EL (1973) Quantitative aspects of precipitated abstinence in morphine-dependent rats. *J Pharmacol Exp Ther* 184: 398–403.

WHO (2012) *Depression: A Global Crisis*. Available at: http://www.who.int/mental_health/management/depression/wfmh_paper_depression_wmhd_2012.pdf (accessed 15 May 2016).

Zomkowski ADE, Santos ARS and Rodrigues ALS (2005) Evidence for the involvement of the opioid system in the agmatine antidepressant-like effect in the forced swimming test. *Neuroscience Letters* 381: 279–283.

Supplementary Material

Figure 1S.

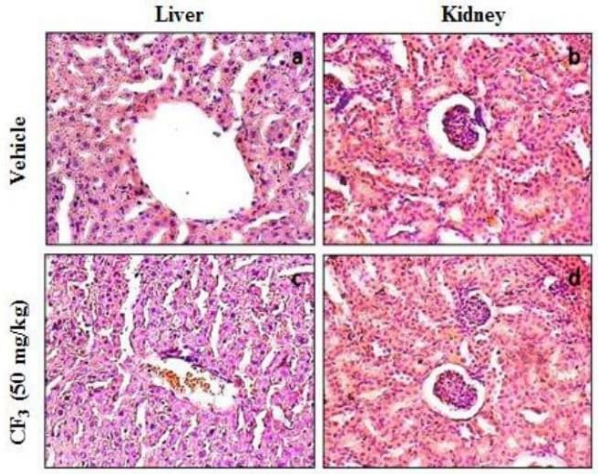


Fig.1S. Representative histological photomicrographs of mice liver and kidney after (*m*–CF_3–PhSe)$_2$ treatment. (*m*–CF_3–PhSe)$_2$ (50 mg/kg, i.g.) or vehicle was administered once a day for eight days. Thirty min after the last administration, samples of liver and kidney were removed to histopathology analysis. A and C: are shown of the vehicle and CF_3 groups showed normal cellular architecture of hepatic tissue; with central vein at the center of lobule and cords of hepatocytes radiating from central vein towards portal triads. B and D: vehicle and CF_3 groups showed normal cellular architecture of renal cortex tissue showing tubular cells with normal nuclei, and regular glomeruli (H&E 20X). CF_3: (*m*-CF_3-PhSe)$_2$.

Table 1S. Effect of (*m*–CF_3–PhSe)$_2$ and κ opioid receptor antagonist subeffective doses in the mouse FST.

Groups	**Immobility time (s)**
Control	259.00 ± 9.32
CF_3	259.33± 6.82
Nor-BNI	253.22 ± 13.55
CF_3+ Nor-BNI	212.11 ± 9.45**

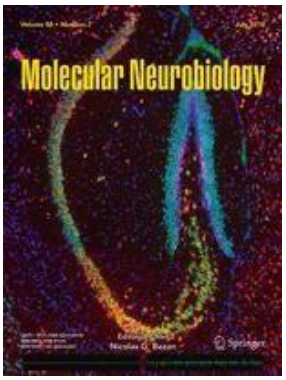
Values are reported as means ± S.E.M. for ten animals per group. Data were analyzed by using a two-way analysis of variance (ANOVA). $^{(**)}$P>0.01 when compared to the control group.CF_3: (*m*-CF_3-PhSe)$_2$. CF_3 was administered at a dose of 10 mg/kg to mice. Nor-BNI was administered at a dose of 1 mg/kg to mice.

3.2 ARTIGO 2

Disseleneto de *m*-Trifluormetil-Difenila regula os níveis das proteínas MOR e KOR em córtex pré-frontal e bloqueia o fenótipo induzido pelo estresse do nado forçado repetido em camundongos

m-Trifluoromethyl-diphenyl Diselenide Regulates Prefrontal Cortical MOR and KOR Protein Levels and Abolishes the Phenotype Induced by Repeated Forced Swim Stress in Mice

Suzan Gonçalves Rosa, Ana Paula Pesarico, Franciele Martini, Cristina Wayne Nogueira



Molecular Neurobiology, 2018 (In Press)
DOI: 10.1007/s12035-018-1024-x

Molecular Neurobiology
https://doi.org/10.1007/s12035-018-1024-x

m-Trifluoromethyl-diphenyl Diselenide Regulates Prefrontal Cortical MOR and KOR Protein Levels and Abolishes the Phenotype Induced by Repeated Forced Swim Stress in Mice

Suzan Gonçalves Rosa[1] · Ana Paula Pesarico[1] · Franciele Martini[1] · Cristina Wayne Nogueira[1]

Received: 3 November 2017 / Accepted: 20 March 2018

Abstract
The present study aimed to investigate the *m*-trifluoromethyl-diphenyl diselenide [(*m*-CF_3-PhSe)$_2$] effects on prefrontal cortical MOR and KOR protein levels and phenotype induced by repeated forced swim stress (FSS) in mice. Adult Swiss mice were subjected to repeated FSS sessions, and after that, they performed the spontaneous locomotor/exploratory activity, tail suspension, and splash tests. (*m*-CF_3-PhSe)$_2$ (0.1 to 5 mg/kg) was administered to mice 30 min before the first FSS session and 30 min before the subsequent repeated FSS. (*m*-CF_3-PhSe)$_2$ abolished the phenotype induced by repeated FSS in mice. In addition, a single FSS session increased μ but reduced δ-opioid receptor contents, without changing the κ content. Mice subjected to repeated FSS had an increase in the μ content when compared to those of naïve group or subjected to single FSS. Repeated FSS induced an increase of δ-opioid receptor content compared to those mice subjected to single FSS. However, the δ-opioid receptor contents were lower than those found in the naïve group. The mice subjected to repeated FSS showed an increase in the κ-opioid receptor content when compared to that of the naïve mice. (*m*-CF_3-PhSe)$_2$ regulated the protein contents of μ and κ receptors in mice subjected to repeated FSS. These findings demonstrate that (*m*-CF_3-PhSe)$_2$ was effective to abolish the phenotype induced by FSS, which was accompanied by changes in the contents of cortical μ- and κ-opioid receptors.

Keywords Depression · Stress · Opioid receptors · Organoselenium

Introduction

Stress is a common experience of life, which a significant population is exposed daily [1]. The impact of stressful life events on physical and psychological well-being is highly variable and dependent of adaptive responses to stress [2]. Clinical studies have shown that stress can act as a precipitating factor of emotional disorders in human, such as depression [3], one of the most prevalent incapacitating psychiatric ailment in the world [4].

It is well-known that exposure to stressors leads to changes in the endogenous opioid system functionality [5], which is closely related to stressful stimuli processing and emotional regulation [5, 6]. Furthermore, the involvement of opioid system in depression disorders is demonstrated by opioid deficiency in post mortem studies with depressed and suicidal patients [7].

The organoselenium compounds have been studied as potential therapeutic agents due to their numerous pharmacological properties [8, 9], among them, a remarkable efficacy in different experimental models of depression [10–12]. Particularly, *m*-trifluoromethyl-diphenyl diselenide [(*m*-CF_3-PhSe)$_2$], a lipophilic compound, has been studied in our research group due to its lower toxicity in comparison to its structural analogues [13] and its effectiveness in various experimental models of mood disorders [12, 14]. Previous studies from our research group reported that the mechanism of antidepressant-like action of this compound involves its anti-inflammatory property and the modulation of serotonergic and opioid systems [15]. Besides, our recent study revealed that each opioid receptor can differently contribute to the antidepressant-like action of (*m*-CF_3-PhSe)$_2$ and that this compound does not induce tolerance to its antidepressant-like effect and physical signs of withdrawal as classical opioid treatments do [16]. However, the molecular mechanisms

✉ Cristina Wayne Nogueira
criswn@ufsm.br

[1] Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, CEP 97105-900, Santa Maria, Rio Grande do Sul RS, Brazil

Published online: 05 April 2018

involving the opioid system and the antidepressant-like action of (m-CF_3-PhSe)$_2$ remains largely unknown.

In view of the above considerations, the purpose of this study was to investigate the effect of (m-CF_3-PhSe)$_2$ on the phenotype induced by repeated forced swim stress in mice. Furthermore, it was investigated whether the (m-CF_3-PhSe)$_2$ effects on phenotype are accompanied by modulation of prefrontal cortical opioid receptor levels in stressed mice.

Materials and Methods

Animals

The experiments were carried out using male adult Swiss mice (25–35 g) obtained from our breeding colony and housed in cages (five mice per cage), with free access to food and water. The animals were kept in an air-conditioned room (22 ± 2 °C) under a 12:12 h light/dark cycle, with lights turned on at 7.00 a.m. The experimental procedures of this study were approved by the Institutional Ethics Committee on Care and Use of Experimental Animal Resources from the Federal University of Santa Maria - RS - Brazil (no. 7770060215). All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

Drug and Treatment

(m-CF_3-PhSe)$_2$ was prepared and characterized in our laboratory based on a previous study carried out by Paulmier [17]. Analysis of the ^{1}H NMR and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of (m-CF_3-PhSe)$_2$ (99.9%) was determined by gas chromatography mass spectrometry (Shimadzu QP2010PLUS GC/MS combination).

(m-CF_3-PhSe)$_2$ was dissolved in canola oil and administered to mice by the intragastric (i.g.) route in a constant volume of 10 ml/kg body weight. (m-CF_3-PhSe)$_2$ was administered at doses of 0.1, 1, and 5 mg/kg 30 min before the first forced swim stress (FSS) session (day 1). On day 2, the mice received again the same doses of (m-CF_3-PhSe)$_2$ 30 min before the subsequent repeated FSS sessions (Fig. 1). Moreover, an experimental naïve control group, which did not perform the behavioral tests, was used in ex vivo analyses.

Repeated FSS and Forced Swim Test

The modified Porsolt FSS [18] was used as a repeated and inescapable stress, which results in a progressive increase in the amount of time the animal remains immobile. Briefly, mice were placed in water to swim for a single trial, during a 15-min period. Twenty-four hours after the first FSS, animals were placed in water to swim through a series of four trials, each of them swam for 6 min long; between each trial, mice were towel dried and returned to their home cage for 6 min. The forced swim test (FST) was performed in the last fourth trial, when the time spent immobile was recorded during 6 min (Fig. 1). The control and (m-CF_3-PhSe)$_2$ per se groups were exposed only to the last trail of FSS in order to perform the FST. Immediately after the last FSS session, all experimental groups were subjected to the spontaneous locomotor/exploratory activity, tail suspension, and splash tests to evaluate the stress-induced depressive-like behaviors (n = 8 animals/group).

Tail Suspension Test

The tail suspension test (TST) was performed according to the study carried out by Steru et al. [19]. Each mouse both acoustically and visually isolated was suspended 50 cm above the floor by adhesive tape placed approximately 1 cm from the tip of the tail. The total immobility time was recorded during a 6-min period. Mice were considered immobile only when they hung passively and completely motionless.

Splash Test

The splash test consists of evaluating the grooming time of mouse as an index of self-care and motivational behavior [20]. A 10% sucrose solution was squirted on the dorsal coat of mouse in its home cage. Because of its viscosity, the sucrose solution dirties the mouse fur, and the animal initiate grooming behavior. The total grooming activity time of mouse (nose/face grooming, head washing, and body grooming) was recorded for 5 min after the sucrose spraying.

Spontaneous Locomotor/Exploratory Activity

With the proposal to discard sedative and motor abnormality, it evaluated the spontaneous locomotor/exploratory activity of mice. Testing took place in a clear Plexiglas cage (500 × 480 × 500 mm) equipped with 16 infrared sensors for the automatic recording of horizontal activity (Model EP149, Insight Instruments Ltda, São Paulo, Brazil). Each animal was initially placed in the center of the testing chamber and allowed to freely move while being tracked by an automated tracking system [21]. The data (crossings, distance, and speed) were recorded for 4 min. This time of test is sufficient to detect locomotor damage induced by drugs in rodents [10].

Ex Vivo Analyses

Immediately after the last behavioral test, the mice were killed by cervical dislocation and samples of prefrontal cortices were excised and stored at − 80 °C for Western blot analyses of μ-, δ-, and κ-opioid receptor contents (n = 5 animals/group). The

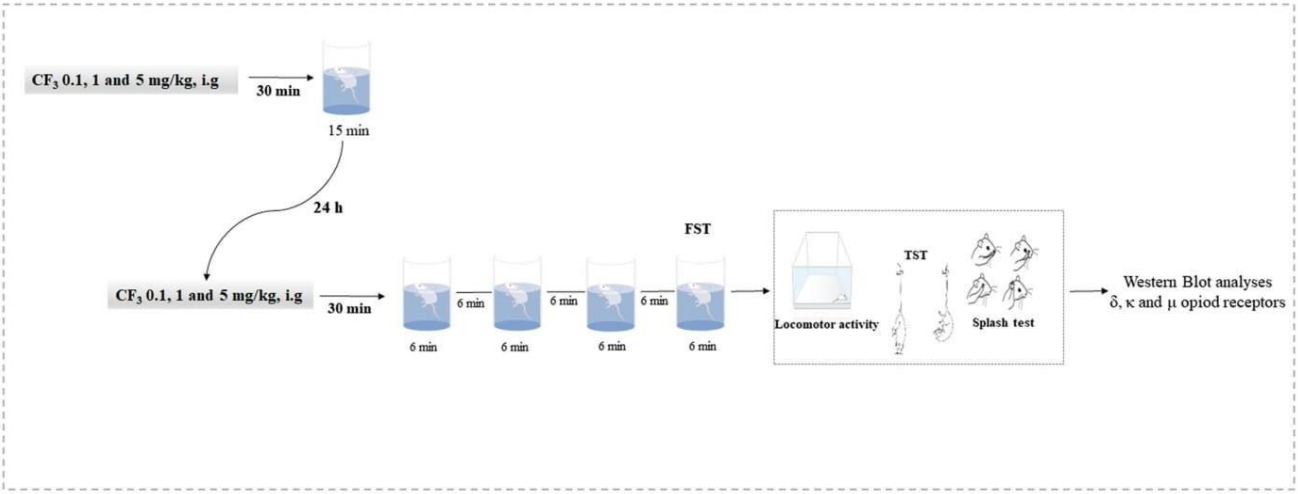


Fig. 1 Schematic representation of the experimental design of this study. FST (forced swim test), TST (tail suspension test)

(*m*-CF_3-PhSe)$_2$ dose of 5 mg/kg was chosen to be investigated in the ex vivo assays based on the results obtained in the behavioral tests. In addition, an experimental naïve control group, which did not perform the behavioral tests, was included in the Western blot analyzes to figure out the effect of behavioral tests exposure on the opioid receptor protein contents.

Western Blot Assay

Samples of prefrontal cortices ($n = 5$ animals/group) were homogenized in ice-cold 0.32 M sucrose buffer (pH 7.4) containing 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.1 mM ethylene glycol tetraacetic acid (EGTA), and 0.1 mM phenyl methyl sulfonyl fluoride in the presence of commercial protein inhibitor cocktail (Sigma-Aldrich Company, St. Louis, MO, USA). The protein concentration of samples was determined using the Bio-Rad DC Protein Asay kit (Bio-Rad Laboratories, Hercules, CA). Tissue extracts were diluted to a final protein concentration 2 μg/μl. The samples (40 μg of protein) and pre stained molecular weight standards (Sigma-Aldrich Company, St. Louis, MO, USA) were separated on 10% resolving with 4% concentrating SDS-PAGE electrophoresis gels. Proteins were transferred to nitrocellulose membrane using Transfer-Blot® Turbo™ Transfer System (1.0 mA; 45 min). After blocking with 3% bovine serum albumin solution, the blots were incubated overnight at 4 °C with rabbit anti-δ-opioid receptor (DOR) (1:1000; Santa Cruz Biotechnology), rabbit anti-κ-opioid receptor (KOR) (1:1000; Santa Cruz Biotechnology), or goat anti-μ-opioid receptor (MOR) (1:1000; Santa Cruz Biotechnology). Mouse anti-β-actin (1:5000, Abcam) was stained as additional control of the protein loading. After primary antibody incubation, membranes were washed and incubated with secondary antibodies conjugated with horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h at room temperature and developed with chemiluminescence kit (Amersham, São Paulo/Brazil). Optical density (OD) of the Western blotting bands was quantified using Image J (NIH, Bethesda, MD, USA) software for Windows. Each value was derived from the ratio between arbitrary units obtained by the protein band and the respective β-actin band. The results were shown as percent of control.

Statistical Analysis

All experimental results are presented as the mean ± S.E.M. Normality of data was analyzed using a D'Agostino and Pearson omnibus normality test. Statistical comparisons between experimental groups (control, CF_3, repeated FSS, and repeated FSS + CF_3) were performed using two-way analysis of variance (ANOVA) followed by the Newman–Keuls test. Western blot data of naïve control and repeated FSS groups were statistically analyzed by one-way ANOVA followed by the Newman–Keuls test. Main effects are presented only when the first order interaction was non-significant. All analyses were performed by using the STATISTICA for Windows software Version 7 (Stat Soft, OK, USA). Probability values less than 0.05 ($P < 0.05$) were considered to be significant.

Results

(*m*-CF_3-PhSe)$_2$ Treatment Abolishes the Phenotype Induced by Repeated FSS Sessions

Figure 2a, b shows the immobility time of mouse in the FST and TST. The two-way ANOVA analyses of immobility time revealed a significant (*m*-CF_3-PhSe)$_2$ × repeated FSS session interaction [$F_{(3,56)} = 3.89$, $P < 0.01$] in the FST and TST [$F_{(3,56)} = 2.77$, $P < 0.05$] (Table 2-statistical data).

Repeated FSS sessions increased the mouse depressive-like behavior represented by an increase in immobility time

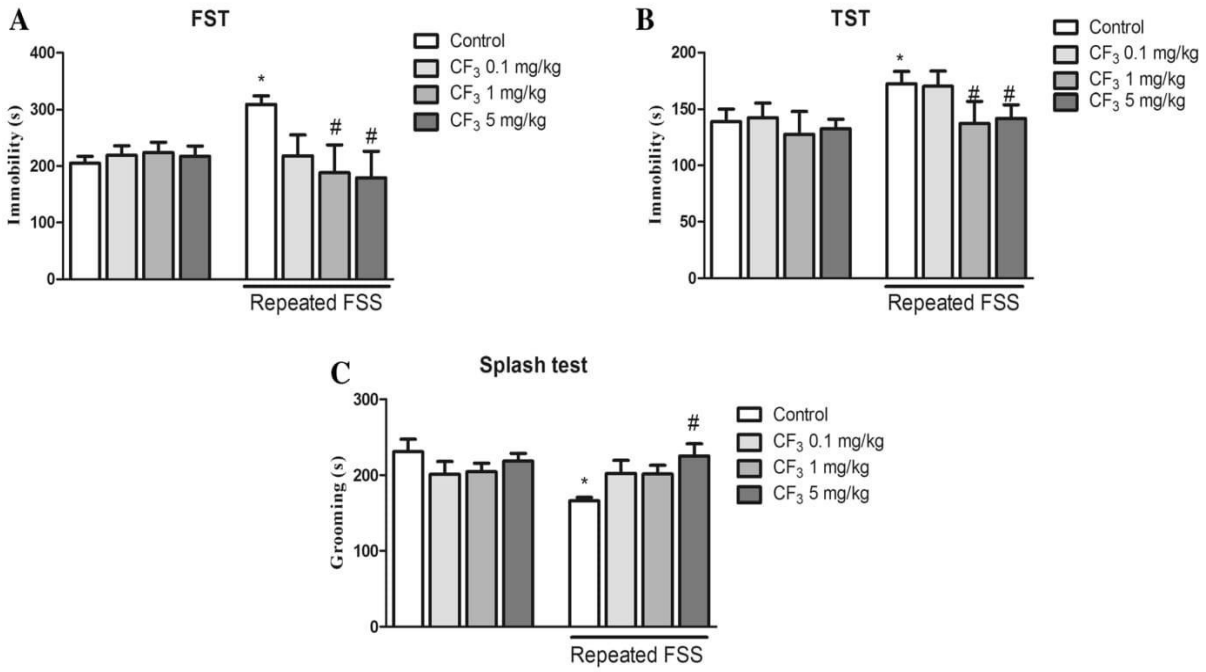


Fig. 2 (m-CF_3-PhSe)$_2$ treatment protects against depression-like behavior induced by repeated FSS sessions. Effects of (m-CF_3-PhSe)$_2$ (0.1, 1 and 5 mg/kg, i.g.) on depression-like behavior of mice in the FST (**a**), TST (**b**), and splash test (**c**). Values are expressed as mean ± S.E.M. of eight animals/groups. (*) $P < 0.05$ when compared to the control group. (#) $P < 0.05$ when compared to the repeated FSS control group (Two-way ANOVA followed by the Newman Keuls test). CF_3: (m-CF_3-PhSe)$_2$

in both behavioral tests when compared to that of the control group ($P < 0.05$), and (m-CF_3-PhSe)$_2$ at doses of 1 and 5 mg/kg was effective against this increase ($P < 0.05$).

(m-CF_3-PhSe)$_2$ at the dose of 0.1 mg/kg was ineffective against the increase in immobility time induced by repeated FSS sessions in the FST and TST (Fig. 2a, b).

The two-way ANOVA analyses of grooming time data revealed a significant (m-CF_3-PhSe)$_2$ × repeated FSS sessions interaction [$F_{(3,56)} = 4.19$, $P < 0.01$] (Table 2-Statistical Data).

Mice subjected to repeated FSS sessions spent less time in the grooming behavior when compared to those of the control group ($P < 0.01$). The time spent grooming was increased in mice subjected to repeated FSS sessions and treated with (m-CF_3-PhSe)$_2$ only at dose of 5 mg/kg ($P < 0.05$) (Fig. 2c).

(m-CF_3-PhSe)$_2$ Treatment and Repeated FSS Do Not Induce Alterations in the Mouse Spontaneous Locomotor/Exploratory Activity

Table 1 shows the effect of (m-CF_3-PhSe)$_2$ treatment and FFS sessions on the mouse spontaneous locomotor/exploratory activity. Neither repeated FSS sessions nor (m-CF_3-PhSe)$_2$ affected the numbers of crossings, distance traveled (mm), and speed (m/s) of mice ($P > 0.05$) (Table 2-statistical data).

Behavioral Exposure and Repeated FSS Sessions Induce Changes in the Prefrontal Cortical Contents of Opioid Receptors

Figure 3a–c shows the prefrontal cortical MOR (Fig. 3a), DOR (Fig. 3b), and KOR (Fig. 3c) protein contents of mice subjected to FSS (repeated FSS group), to behavioral tests (control group), or those not subjected to behavioral tests (naïve group).

The control mice had higher prefrontal cortical MOR protein content than those of the naïve group ($P < 0.05$). The mice that were exposed to repeated FSS sessions had higher prefrontal cortical MOR protein content when compared to the naïve group ($P < 0.001$) or control group ($P > 0.01$) (Fig. 3a) (one-way ANOVA [$F_{(2,14)} = 16.74$, $P < 0.001$]) (Table 3-statistical data).

The prefrontal cortical protein levels of DOR were reduced in the control mice when compared to those of the naïve group ($P < 0.001$). Similarly, mice subjected to repeated FSS showed a decrease of prefrontal cortical protein content of DOR when compared to those of the naïve group ($P < 0.01$). By contrast, the prefrontal cortical protein content of DOR in mice subjected to repeated FSS was higher than that found in the control mice (Fig. 3b) ($P < 0.05$) (one-way ANOVA [$F_{(2,14)} = 24.70$, $P < 0.001$]) (Table 3-statistical data).

The exposure to the behavioral paradigm (control mice) did not change the prefrontal cortical KOR protein

Table 1 Effects of treatment with (m-CF_3-PhSe)$_2$ on spontaneous locomotor activity of mice subjected to repeated FSS

Group	Number of crossing	Distance (mm)	Speed (mm/s)
Control	499.0 ± 44.2	8264.8 ± 648.8	38.3 ± 2.3
CF_3 0.1 mg/kg	527.0 ± 40.3	8625.9 ± 949.9	42.0 ± 1.9
CF_3 1 mg/kg	440.0 ± 42.7	8481.7 ± 1014.3	37.7 ± 4.3
CF_3 5 mg/kg	497.1 ± 36.0	8825.1 ± 861.7	37.9 ± 3.7
FSS	492.0 ± 25.3	7604.0 ± 453.7	36.2 ± 1.9
FSS + CF_3 0.1 mg/kg	482.4 ± 70.8	7676.4 ± 1167.7	42.9 ± 4.1
FSS + CF_3 1 mg/kg	440.0 ± 49.3	7017.0 ± 204.1	36.1 ± 2.0
FSS + CF_3 5 mg/kg	484.0 ± 29.6	9505.9 ± 1111.0	39.3 ± 5.7

Values are reported as means ± S.E.M. for seven animals per group. Data were analyzed by using one-way analysis of variance (ANOVA). *FSS* forced swimming stress; CF_3: (m-CF_3-PhSe)$_2$

content when compared to that of the naïve group ($P > 0.05$). Conversely, repeated exposure to FSS induced an increase in the prefrontal cortical KOR protein content when compared to those of the naïve group ($P < 0.05$) and the control group ($P < 0.05$) (Fig. 3c) (one-way ANOVA [$F_{(2,14)} = 4.27$, $P < 0.05$]) (Table 3-statistical data).

(m-CF_3-PhSe)$_2$ Treatment Protects Against the Increase in MOR and KOR Protein Contents Induced by Repeated FSS Sessions

The effect of (m-CF_3-PhSe)$_2$ treatment in the prefrontal cortical MOR, DOR, and KOR protein contents of stressed mice is shown in Fig. 4a–c.

The two-way ANOVA analyses of MOR protein content data revealed a significant (m-CF_3-PhSe)$_2$ × repeated FSS sessions interaction [$F_{(1,16)} = 4.56$, $P < 0.05$] (Table 4-statistical data). Repeated exposure to FSS induced an increase in the prefrontal cortical MOR protein content when compared to that of the control group ($P < 0.05$), which was protected by (m-CF_3-PhSe)$_2$ treatment ($P < 0.05$) (Fig. 4a).

The two-way ANOVA analyses of DOR protein content data showed a significant main effect of repeated FSS sessions [$F_{(1,16)} = 11.94$, $P < 0.01$] (Table 4-statistical data). The prefrontal cortical DOR protein content was increased in mice subjected to repeated FSS with or without (m-CF_3-PhSe)$_2$ treatment when compared to that of the control group ($P < 0.05$) (Fig. 4b).

Table 2 Effects of treatment with (m-CF_3-PhSe)$_2$ on the forced swim test (FST), tail suspension test (TST), splash test, and spontaneous locomotor activity of mice subjected to repeated FSS

Behavioral test	Variables two-way ANOVA	SS	DF	MS	F	P value
FST	FSS Factor	2783	1	2783	0.59	0.445
	CF_3 Factor	33,158	3	11,053	2.34	0.082
	FSS × CF_3	5571	3	18,357	3.89	0.013
TST	FSS Factor	6848	1	6848	6.25	0.015
	CF_3 Factor	20,391	3	6797	6.21	0.001
	FSS × CF_3	9093	3	3031	2.77	0.049
Splash test	FSS Factor	4953	1	4953	4.57	0.036
	CF_3 Factor	5421	3	1807	1.67	0.183
	FSS × CF_3	13,613	3	4538	4.19	0.009
Spontaneous locomotor activity						
Number of crossing	FSS Factor	7849	1	7849	0.57	0.453
	CF_3 Factor	19,902	3	6634	0.48	0.696
	FSS × CF_3	3030	3	1010	0.07	0.973
Distance	FSS Factor	5,014,957	1	5,014,957	0.96	0.330
	CF_3 Factor	16,773,190	3	5,591,063	1.07	0.368
	FSS × CF_3	8,798,537	3	2,932,846	0.56	0.641
Speed	FSS Factor	172.83	1	172.83	0.743	0.392
	CF_3 Factor	600.79	3	200.26	0.861	0.467
	FSS × CF_3	566.89	3	188.96	0.812	0.493

Two-way ANOVA table for Fig. 2. CF_3: (m-CF_3-PhSe)$_2$

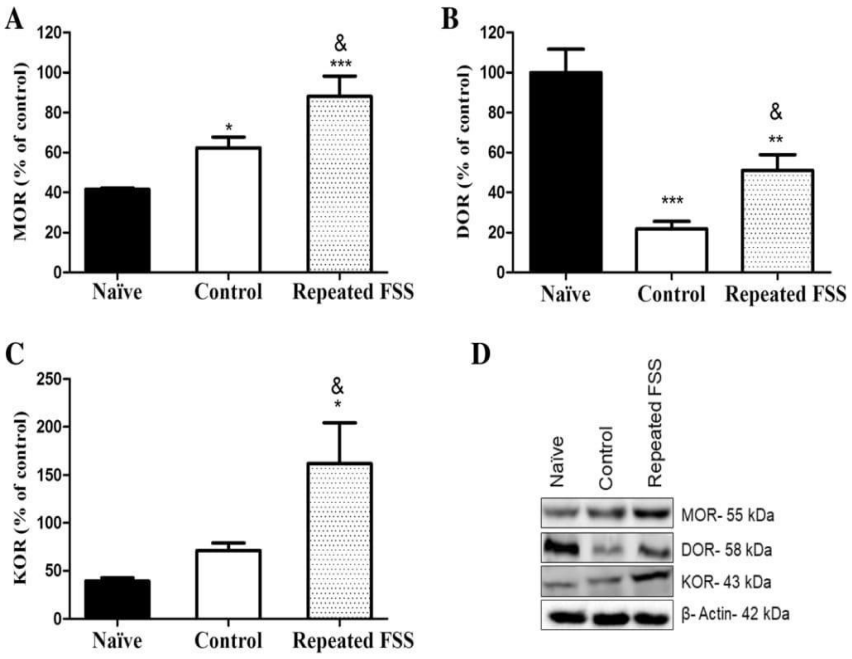


Fig. 3 Behavioral exposure and repeated FSS sessions induce changes in the opioid receptor contents. Effects of behavioral exposure and repeated FSS sessions on the prefrontal cortical MOR (**a**), DOR (**b**), and KOR (**c**) protein contents of mice. Values are expressed as mean ± S.E.M. of five animals/group. (*) $P < 0.05$, (**) $P < 0.01$, and (***) $P < 0.001$ when compared to the naïve group (&)$P < 0.05$ when compared to the control group (one-way ANOVA followed by the Newman Keuls test). Photographs are representation of qualitative Western blot analyses (**d**). CF_3: $(m\text{-}CF_3\text{-}PhSe)_2$

The two-way ANOVA of KOR protein content showed a significant $(m\text{-}CF_3\text{-}PhSe)_2$ × repeated FSS sessions interaction [$F_{(1,16)} = 9.092$, $P < 0.01$] (Table 4-statistical data). Repeated FSS increased the prefrontal cortical KOR levels when compared to those of the control group ($P < 0.05$), which was restored by $(m\text{-}CF_3\text{-}PhSe)_2$ treatment ($P < 0.05$) (Fig. 4c).

Discussion

The present study reveals the efficacy of organoselenium compound $(m\text{-}CF_3\text{-}PhSe)_2$ to abolish the phenotype induced by repeated FSS sessions in mice, without altering the locomotor activity. In addition, our results demonstrate that both the exposure to the behavioral paradigms and to repeated FSS sessions induced changes in the prefrontal cortical protein contents of opioid receptors in mice, which were specific to each type of opioid receptor. In this study, the antidepressant-like action of $(m\text{-}CF_3\text{-}PhSe)_2$ was accompanied by its effect in regulating the changes in the prefrontal cortical protein contents of μ- and κ-opioid receptors of mice subjected to FSS sessions, which can contribute to the improvement of adaptive responses to stress.

Forced swim is a type of inescapable stressor, which induces neuroadaptations that lead to the increase of animal immobility, and thus, it is used as an animal model of depression [22]. The increase of corticosterone concentrations in animals that exhibited depressive-like symptoms after being exposed to repeated forced swim sessions indicates that this is in fact a stressful experience, which is closely related to depression development [23]. Accordingly, in this study, the repeated FSS sessions induced a depressant-like phenotype in the FST, TST, and splash test. The efficacy of $(m\text{-}CF_3\text{-}PhSe)_2$ in improving active coping responses in the FST and TST and in self-care and motivational behaviors in the splash test indicates the potential antidepressant-like action of this compound in a depression model induced by stress. The antidepressant-like action of $(m\text{-}CF_3\text{-}PhSe)_2$ in different models of depression has been already shown in our previous studies [12, 14, 15, 24]. However, the current study

Table 3 Effects of a single or repeated forced swim stress (FSS) in cortical MOR, KOR, and DOR content

Western blot analyses	One-way ANOVA	SS	DF	MS	F	*P* value
MOR	Between groups	3398	2	1699	16.74	0.003
	Within groups	1217	12	101.5		
KOR	Between groups	43,120	2	21,560	4.27	0.039
	Within groups	60,520	12	5043		
DOR	Between groups	15,880	2	7941	24.70	0.000
	Within groups	3858	12	321.5		

One-way ANOVA for Fig. 3

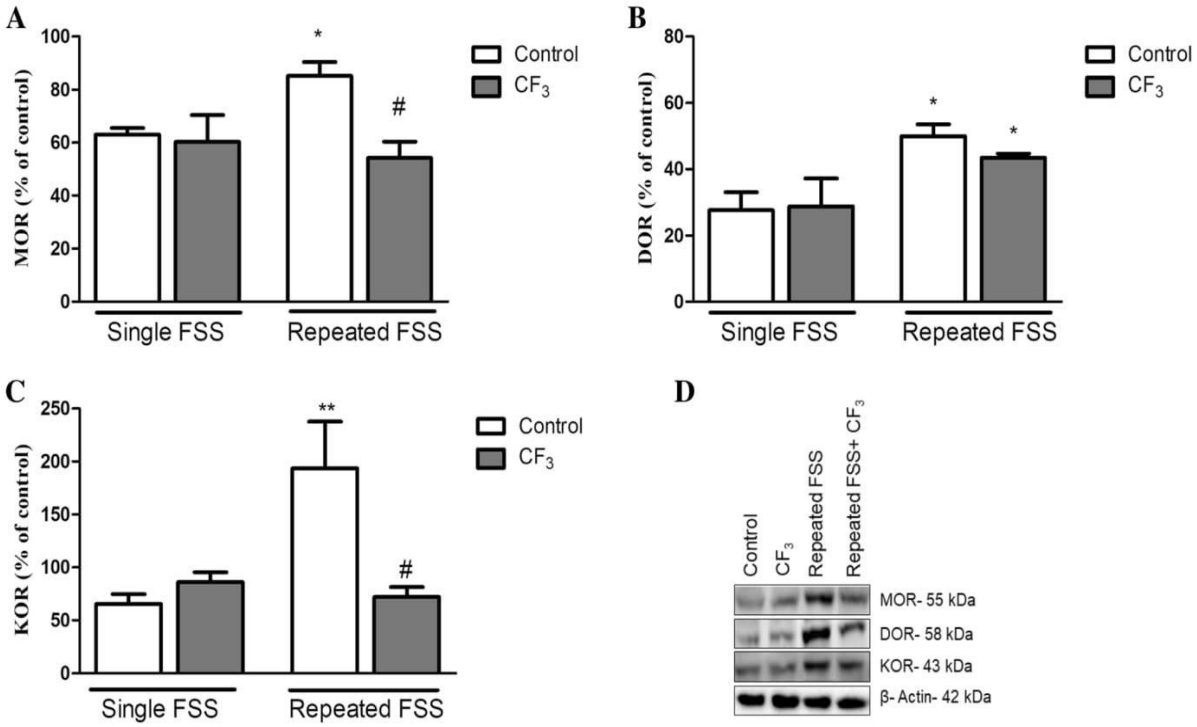


Fig. 4 (m-CF_3-PhSe)$_2$ treatment regulates the MOR and KOR contents altered by repeated FSS sessions. Effects of (m-CF_3-PhSe)$_2$ treatment (5 mg/kg, i.g.) on the prefrontal cortical MOR (**a**), DOR (**b**), and KOR (**c**) levels in mice subjected to repeated FSS sessions. Values are expressed as mean ± S.E.M. of five animals/group. (*) $P < 0.05$ and (**) $P < 0.01$ when compared to the control group. (#) $P < 0.05$ when compared to the repeated FSS group. (Two-way ANOVA followed by the Newman Keuls test). Photographs are representation of qualitative Western blotting analysis (**d**). CF_3: (m-CF_3-PhSe)$_2$

demonstrates, for the first time, the action of this compound in a depression model induced by acute stress, a common precipitating factor of emotional disorders in human beings [3].

The low doses of (m-CF_3-PhSe)$_2$ used in this study were based on the fact that previous studies of our research group have shown that (m-CF_3-PhSe)$_2$ and other organoselenium compounds, such as diphenyl diselenide, elicit a higher pharmacological potency in models of depression (stress, partial sciatic nerve ligation, tumor necrosis factor-alfa) than in the FST, a behavioral model for testing antidepressant efficacy, used originally in naïve animals [12, 14, 16]. Regarding the (m-CF_3-PhSe)$_2$ action demonstrated in this study, two factors are especially important and give more predictive validity to the antidepressant-like action of this compound: first, (m-CF_3-PhSe)$_2$ was administered concomitantly to the depressogenic stimulus and second, it showed a higher antidepressant-like potency in stressed mice, when a depressogenic stimulus was applied.

In addition, whereas (m-CF_3-PhSe)$_2$ elicited an antidepressant-like action at doses equal or higher than 1 mg/kg in the FST and TST, the compound was effective only at the dose of 5 mg/kg in the splash test. A possible explanation to this result is that mice were subjected to the sequential

Table 4 Effects of (m-CF_3-PhSe)$_2$ in cortical MOR, KOR, and DOR content of mice subjected to forced swim stress (FSS)

Western blot analyses	Variables two-way ANOVA	SS	DF	MS	F	P value
MOR	FSS Factor	321,39	1	321,39	1.47	0.242
	CF_3 Factor	1406.65	1	1406.65	6.45	0.021
	FSS × CF_3	994.1	1	994.1	4.56	0.048
KOR	FSS Factor	16,244.8	1	16,244.8	5.85	0.027
	CF_3 Factor	12,709.2	1	12,709.2	4.57	0.048
	FSS × CF_3	25,240.6	1	25,240.6	9.09	0.008
DOR	FSS Factor	1716.31	1	1716.31	11.94	0.003
	CF_3 Factor	34.34	1	34.34	0.2391	0.631
	FSS × CF_3	74.26	1	76.26	0.516	0.482

Two-way ANOVA for Fig. 4. CF_3: (m-CF_3-PhSe)$_2$

behavioral tests, which can produce stress in the animals and modify the results obtained in the splash test, the last behavioral test performed by mice. Thus, we assume that the continuous exposure to different behavioral tests could be a confounding factor of present experimental protocol.

The mechanisms already described for the antidepressant-like action of $(m\text{-}CF_3\text{-}PhSe)_2$ include the anti-inflammatory property and the modulation of serotoninergic, monoaminergic and opioid systems, which characterize this compound as a multi-target drug [12, 14–16]. Of particular importance, the opioid system has been studied as a component of multiple stress-induced behavioral responses, given that this system has a prominent regulatory role in the processing of stressful stimuli [5]. Furthermore, it has been demonstrated that each subtype of opioid receptor contributes in a particular way in behavioral and cellular stress responses [25].

The molecular changes in the central nervous system are essential to adaptive or unhealthy responses to stress [26]. Considering that the exposure of mice to the behavioral tests can generate some amount of stress, the current study investigated whether different levels of stress (exposure only to the behavioral tests or to repeated FSS sessions) induce changes in the protein contents of opioid receptors and whether there is any adaptation to these possible stress effects.

The present study demonstrates that the simple fact that the animals performed the behavioral tests was able to induce an increase in the prefrontal cortical MOR protein content. Similarly, the repeated FSS sessions led to an increase in the MOR protein content when compared to that of the naïve group; however, this increase was even greater than that found in mice only exposed to the behavioral tests. The role of μ-opioid receptor in mood disorders is paradoxical because the pharmacological activation of μ receptor produces antidepressant-like effects in rodents [27–29], whereas μ-opioid receptor knockout mice have antidepressant-like behaviors [30, 31]. In agreement with our results, the increase of MOR expression in the limbic system has been reported after a single and repeated stress sessions [32–34]. Thus, it is possible that μ-opioid receptors are regulated according to the stimuli, and that both a decrease/blockage and an increase/activation of these receptors can have deleterious consequences on mood [35].

Regarding the modulation of DOR content by stress, our results showed that both the exposure to behavioral tests and to repeated FSS sessions reduced the prefrontal cortical DOR protein content when compared to that of the naïve mice. The decreased functioning of the encephalin-DOR system has been associated to the development of depression-like symptoms and, accordingly, an increase in encephalin-DOR signaling has been therapeutically explored in treatment of depression [5]. Therefore, in the present study, the decrease of the DOR protein content in mice subjected to the behavioral tests or to FSS is possibly associated to depression-like symptoms induced by stress, demonstrated in the FST, TST, and splash test. In addition, it is important to highlight that although the prefrontal cortical DOR protein contents of mice subjected to repeated FSS sessions were lower than those of the naïve mice, this content was higher than that found in mice exposed to the behavioral tests. This result could be interpreted as a possible partial adaptive response to stress because the encephalin-DOR system is an important mediator of stress adaptation in rodents [36].

Different from the results obtained for MOR and DOR protein contents, in the present study, the prefrontal cortical KOR protein content was increased only in mice subjected to repeated FSS sessions. This result is consistent with the findings showing that chronic or uncontrollable stress may lead to increased KOR signaling and consequent increase in core features of depressive disorders [37, 38], such as passive coping and anhedonia. In addition, the antidepressant-like effects of KOR antagonists, by ablation of the KORs or prodynorphin genes, have been reported as the most apparent after repeated stress. These are experimental evidence that the KOR system may be especially important in mediating the amplification or sensitization of stress responses [22, 39], and corroborates our findings that demonstrate that the mouse prefrontal cortical KOR protein content was increased after repeated FSS sessions.

Considering the pharmacological actions of $(m\text{-}CF_3\text{-}PhSe)_2$ in different depression-like models and the fact that the modulation of opioid system contributes to its action [15, 16], we hypothesized that $(m\text{-}CF_3\text{-}PhSe)_2$ treatment could regulate the prefrontal cortical opioid receptor protein contents in mice subjected to FSS sessions, contributing to abolish the depressive-like symptoms induced by repeated FSS. In the present study, the $(m\text{-}CF_3\text{-}PhSe)_2$ administration was effective against the increase of MOR and KOR protein contents induced by FSS, but it was ineffective against the increase in the DOR protein content promoted by this stress protocol. We recently demonstrated that pharmacological MOR and DOR activation and the KOR blockade contribute to the antidepressant-like action of $(m\text{-}CF_3\text{-}PhSe)_2$ [16]. In fact, MOR, DOR, and KOR receptors appear to be important and highly distinct players in the regulation of mood. The MOR activation exerts contrasting effects on mood, especially in stress situations [35, 40]. However, the DOR activation induces antidepressant-like effects in different behavioral paradigms in both rats and mice; on the other hand, KOR antagonists can reduce the depressant-like effects of stress [34, 39, 41–43]. The present study emphasizes that the $(m\text{-}CF_3\text{-}PhSe)_2$ opioidergic mechanism of action contributes to homeostasis maintenance in response to stress because this organoselenium compound normalized MOR and KOR protein contents and abolished depressant-like symptoms induced by repeated FSS sessions in mice. However, the lack of $(m\text{-}CF_3\text{-}PhSe)_2$ effect on the DOR protein content could be explained by the fact that repeated FSS sessions have already

induced an partial process of stress adaptation to behavioral tests exposure and because the activation of DOR is a molecular target of (m-CF_3-PhSe)$_2$ [16].

Recently, we demonstrated that (m-CF_3-PhSe)$_2$ regulates opioid receptor levels in the cerebral cortex of defeated mice and induces resilience to stress-induced depressive-like behaviors. In this study, the resilient mice and those treated with (m-CF_3-PhSe)$_2$ had similar protein contents of opioid receptors, indicating a relationship between the antidepressant-like effect of (mC-F_3-PhSe)$_2$ and the changes in these receptors [16]. The present study further confirmed that the opioid receptors are direct or indirect targets of (m-CF_3-PhSe)$_2$ and demonstrated that these receptors are, somehow, involved in the antidepressant-like action of this compound in different models of stress-induced depression. However, studies have shown that an interaction between these opioid and serotonergic systems contributes to the action of classical antidepressant drugs [44, 45]. Thus, given that (m-CF_3-PhSe)$_2$ is a multitarget compound, which elicits an antidepressant-like effect through the modulation of different neurotransmitter systems, including the serotonergic and opioid systems [14, 16], we cannot rule out the possibility that these neurotransmitter systems interact and contribute to the antidepressant-like effect of this compound. However, we acknowledge as a limitation of our study the absence of data to prove this hypothesis.

In conclusion, this study demonstrates the effectiveness of (m-CF_3-PhSe)$_2$ in abolishing the phenotype induced by repeated forced swim stress and indicates, for the first time, the effects of exposure to the behavioral tests and to repeated FSS on the prefrontal cortical protein contents of each opioid receptor in mice. In addition, the present study expands our knowledge about the role of opioidergic receptors in the (m-CF_3-PhSe)$_2$ antidepressant-like action and suggests that this compound regulates the changes in the prefrontal cortical protein contents of μ- and κ-opioid receptors of mice subjected to FSS sessions, which may contribute to the improvement of adaptive responses to stress.

Funding Information This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico - CNPq (441405/2014-2) for the financial support. C.W.N. is recipient of CNPq fellowship.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

References

1. Atwoli L, Stein DJ, Koenen KC, McLaughlin KA (2015) Epidemiology of posttraumatic stress disorder: prevalence, correlates and consequences. Curr Opin Psychiatry 28(4):307–311. https://doi.org/10.1097/YCO.0000000000000167
2. Koolhaas JM, de Boer SF, Coppens CM, Buwalda B (2010) Neuroendocrinology of coping styles: towards understanding the biology of individual variation. Front Neuroendocrinol 31(3):307–321. https://doi.org/10.1016/j.yfrne.2010.04.001
3. Binder EB, Nemeroff CB (2010) The CRF system, stress, depression and anxiety—insights from human genetic studies. Nature Publishing Group,
4. WHO (2012) Depression: a global crisis. Available at: http://www.who.int/mental_health/management/depression/wfmh_paper_depression_wmhd_2012.pdf
5. Bali A, Randhawa PK, Jaggi AS (2015) Stress and opioids: role of opioids in modulating stress-related behavior and effect of stress on morphine conditioned place preference. Neurosci Biobehav Rev 51:138–150. https://doi.org/10.1016/j.neubiorev.2014.12.018
6. Nummenmaa L, Tuominen L (2017) Opioid system and human emotions. Br J Pharmacol. https://doi.org/10.1111/bph.13812
7. Zalsman G, Molcho A, Huang Y, Dwork A, Li S, Mann JJ (2005) Postmortem mu-opioid receptor binding in suicide victims and controls. J Neural Transm (Vienna) 112(7):949–954. https://doi.org/10.1007/s00702-004-0239-3
8. Nogueira CW, Rocha JB (2011) Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. Arch Toxicol 85(11):1313–1359. https://doi.org/10.1007/s00204-011-0720-3
9. Ibrahim M, Muhammad N, Naeem M, Deobald AM, Kamdem JP, Rocha JB (2015) In vitro evaluation of glutathione peroxidase (GPx)-like activity and antioxidant properties of an organoselenium compound. Toxicol in Vitro 29(5):947–952. https://doi.org/10.1016/j.tiv.2015.03.017
10. Oliveira CE, Marcondes Sari MH, Zborowski VA, Prado VC, Nogueira CW, Zeni G (2016) Pain-depression dyad induced by reserpine is relieved by p,p′-methoxyl-diphenyl diselenide in rats. Eur J Pharmacol 791:794–802. https://doi.org/10.1016/j.ejphar.2016.10.021
11. Quines CB, Rosa SG, Velasquez D, Da Rocha JT, Neto JS, Nogueira CW (2016) Diphenyl diselenide elicits antidepressant-like activity in rats exposed to monosodium glutamate: a contribution of serotonin uptake and Na(+), K(+)-ATPase activity. Behav Brain Res 301:161–167. https://doi.org/10.1016/j.bbr.2015.12.038
12. Bruning CA, Martini F, Soares SM, Savegnago L, Sampaio TB, Nogueira CW (2015) Depressive-like behavior induced by tumor necrosis factor-alpha is attenuated by m-trifluoromethyl-diphenyl diselenide in mice. J Psychiatr Res 66-67:75–83. https://doi.org/10.1016/j.jpsychires.2015.04.019
13. Savegnago L, Jesse CR, Nogueira CW (2009) Structural modifications into diphenyl diselenide molecule do not cause toxicity in mice. Environ Toxicol Pharmacol 27(2):271–276. https://doi.org/10.1016/j.etap.2008.11.007
14. Bruning CA, Martini F, Soares SM, Sampaio TB, Gai BM, Duarte MM, Nogueira CW (2015) m-Trifluoromethyl-diphenyl diselenide, a multi-target selenium compound, prevented mechanical allodynia and depressive-like behavior in a mouse comorbid pain and depression model. Prog Neuro-Psychopharmacol Biol Psychiatry 63:35–46. https://doi.org/10.1016/j.pnpbp.2015.05.011
15. Bruning CA, Souza AC, Gai BM, Zeni G, Nogueira CW (2011) Antidepressant-like effect of m-trifluoromethyl-diphenyl diselenide in the mouse forced swimming test involves opioid and serotonergic systems. Eur J Pharmacol 658(2–3):145–149. https://doi.org/10.1016/j.ejphar.2011.02.039
16. Rosa SG, Pesarico AP, Tagliapietra CF, da Luz SCA, Nogueira CW (2017) Opioid system contribution to the antidepressant-like action of m-trifluoromethyl-diphenyl diselenide in mice: a compound devoid of tolerance and withdrawal syndrome. J Psychopharmacol 31:1250–1262. https://doi.org/10.1177/0269881117724353

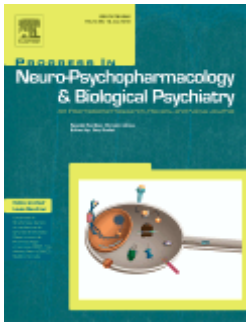
17. Paulmier C (1986) Selenoorganic functional groups. In: Paulmier C (ed) Selenium reagents and intermediates in organic synthesis, 1st edn. Prgamon Press, Oxford, pp. 25–51
18. Porsolt RD, Bertin A, Jalfre M (1977) Behavioral despair in mice—primary screening-test for antidepressants. Arch Int Pharmacod T 229(2):327–336
19. Steru L, Chermat R, Thierry B, Simon P (1985) The tail suspension test: a new method for screening antidepressants in mice. Psychopharmacology 85(3):367–370
20. Isingrini E, Camus V, Le Guisquet AM, Pingaud M, Devers S, Belzung C (2010) Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. PLoS One 5(4):e10404. https://doi.org/10.1371/journal.pone.0010404
21. York JM, Blevins NA, McNeil LK, Freund GG (2013) Mouse short- and long-term locomotor activity analyzed by video tracking software. J Vis Exp (76). doi:https://doi.org/10.3791/50252
22. Knoll AT, Carlezon WA Jr (2010) Dynorphin, stress, and depression. Brain Res 1314:56–73. https://doi.org/10.1016/j.brainres.2009.09.074
23. Rogoz Z, Kabzinski M, Sadaj W, Rachwalska P, Gadek-Michalska A (2012) Effect of co-treatment with fluoxetine or mirtazapine and risperidone on the active behaviors and plasma corticosterone concentration in rats subjected to the forced swim test. Pharmacol Rep 64(6):1391–1399
24. Rosa SG, Pesarico AP, Nogueira CW (2018) m-Trifluoromethyldiphenyl diselenide promotes resilience to social avoidance induced by social defeat stress in mice: contribution of opioid receptors and MAPKs. Prog Neuro-Psychopharmacol Biol Psychiatry 82:123–135. https://doi.org/10.1016/j.pnpbp.2017.11.021
25. Bodnar RJ (2017) Endogenous opiates and behavior: 2015. Peptides 88:126–188. https://doi.org/10.1016/j.peptides.2016.12.004
26. McEwen BS (2007) Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 87(3):873–904. https://doi.org/10.1152/physrev.00041.2006
27. Berrocoso E, Ikeda K, Sora I, Uhl GR, Sanchez-Blazquez P, Mico JA (2013) Active behaviours produced by antidepressants and opioids in the mouse tail suspension test. Int J Neuropsychopharmacol 16(1):151–162. https://doi.org/10.1017/S1461145711001842
28. Rojas-Corrales MO, Berrocoso E, Gibert-Rahola J, Mico JA (2002) Antidepressant-like effects of tramadol and other central analgesics with activity on monoamines reuptake, in helpless rats. Life Sci 72(2):143–152
29. Tejedor-Real P, Mico JA, Maldonado R, Roques BP, Gibert-Rahola J (1995) Implication of endogenous opioid system in the learned helplessness model of depression. Pharmacol Biochem Behav 52(1):145–152
30. Filliol D, Ghozland S, Chluba J, Martin M, Matthes HW, Simonin F, Befort K, Gaveriaux-Ruff C et al (2000) Mice deficient for delta- and mu-opioid receptors exhibit opposing alterations of emotional responses. Nat Genet 25(2):195–200. https://doi.org/10.1038/76061
31. Yoo JH, Lee SY, Loh HH, Ho IK, Jang CG (2004) Altered emotional behaviors and the expression of 5-HT1A and M1 muscarinic receptors in micro-opioid receptor knockout mice. Synapse 54(2):72–82. https://doi.org/10.1002/syn.20067
32. Komatsu H, Ohara A, Sasaki K, Abe H, Hattori H, Hall FS, Uhl GR, Sora I (2011) Decreased response to social defeat stress in mu-opioid-receptor knockout mice. Pharmacol Biochem Behav 99(4):676–682. https://doi.org/10.1016/j.pbb.2011.06.008
33. Nikulina EM, Hammer RP Jr, Miczek KA, Kream RM (1999) Social defeat stress increases expression of mu-opioid receptor mRNA in rat ventral tegmental area. Neuroreport 10(14):3015–3019
34. Yamamoto M, Komori T, Matsumoto T, Zhang K, Miyahara S, Shizuya K, Okazaki Y (2003) Effects of single and repeated prolonged stress on mu-opioid receptor mRNA expression in rat gross hypothalamic and midbrain homogenates. Brain Res 980(2):191–196
35. Lutz PE, Kieffer BL (2013) Opioid receptors: distinct roles in mood disorders. Trends Neurosci 36(3):195–206. https://doi.org/10.1016/j.tins.2012.11.002
36. Van Loon GR, Pierzchala K, Houdi AA, Kvetnansky R, Zeman P (1990) Tolerance and cross-tolerance to stress-induced increases in plasma met-enkephalin in rats with adaptively increased resting secretion. Endocrinology 126(4):2196–2204. https://doi.org/10.1210/endo-126-4-2196
37. Bruchas MR, Xu M, Chavkin C (2008) Repeated swim stress induces kappa opioid-mediated activation of extracellular signal-regulated kinase 1/2. Neuroreport 19(14):1417–1422. https://doi.org/10.1097/WNR.0b013e32830dd655
38. McLaughlin JP, Li S, Valdez J, Chavkin TA, Chavkin C (2006) Social defeat stress-induced behavioral responses are mediated by the endogenous kappa opioid system. Neuropsychopharmacology 31(6):1241–1248. https://doi.org/10.1038/sj.npp.1300872
39. McLaughlin JP, Marton-Popovici M, Chavkin C (2003) Kappa opioid receptor antagonism and prodynorphin gene disruption block stress-induced behavioral responses. J Neurosci 23(13):5674–5683
40. Wang Q, Long Y, Hang A, Zan GY, Shu XH, Wang YJ, Liu JG (2016) The anxiolytic- and antidepressant-like effects of ATPM-ET, a novel kappa agonist and mu partial agonist, in mice. Psychopharmacology 233(12):2411–2418. https://doi.org/10.1007/s00213-016-4292-z
41. Richards EM, Mathews DC, Luckenbaugh DA, Ionescu DF, Machado-Vieira R, Niciu MJ, Duncan WC, Nolan NM et al (2016) A randomized, placebo-controlled pilot trial of the delta opioid receptor agonist AZD2327 in anxious depression. Psychopharmacology 233(6):1119–1130. https://doi.org/10.1007/s00213-015-4195-4
42. Saitoh A, Sugiyama A, Nemoto T, Fujii H, Wada K, Oka J, Nagase H, Yamada M (2011) The novel delta opioid receptor agonist KNT-127 produces antidepressant-like and antinociceptive effects in mice without producing convulsions. Behav Brain Res 223(2):271–279. https://doi.org/10.1016/j.bbr.2011.04.041
43. Yamada K, Nabeshima T (1995) Stress-induced behavioral responses and multiple opioid systems in the brain. Behav Brain Res 67(2):133–145
44. Haj-Mirzaian A, Kordjazy N, Ostadhadi S, Amiri S, Dehpour A (2016) Fluoxetine reverses the behavioral despair induced by neurogenic stress in mice: role of N-methyl-d-aspartate and opioid receptors. Can J Physiol Pharmacol 94(6):599–612. https://doi.org/10.1139/cjpp-2015-0429
45. Berrocoso E, Mico JA (2009) Cooperative opioid and serotonergic mechanisms generate superior antidepressant-like effects in a mice model of depression. Int J Neuropsychopharmacol 12(8):1033–1044. https://doi.org/10.1017/S1461145709000236

3.3 ARTIGO 3

Disseleneto de *m*-Trifluometil-difenila promove resiliência à aversão social induzida pelo estresse de derrota social em camundongos: Contribuição dos receptores opioides e MAPKs

_m_-Trifluoromethyl-diphenyl diselenide promotes resilience to social avoidance induced by social defeat stress in mice: Contribution of opioid receptors and MAPKs

Suzan Gonçalves Rosa, Ana Paula Pesarico, Cristina Wayne Nogueira



Progress in Neuropsychopharmacology & Biological Psychiatry 82 (2018) 123–1352017,
DOI: 10.1016/j.pnpbp.2017.11.021

Progress in Neuropsychopharmacology & Biological Psychiatry 82 (2018) 123–135

Contents lists available at ScienceDirect

Progress in Neuropsychopharmacology & Biological Psychiatry

journal homepage: www.elsevier.com/locate/pnp

m-Trifluoromethyl-diphenyl diselenide promotes resilience to social avoidance induced by social defeat stress in mice: Contribution of opioid receptors and MAPKs

Suzan Gonçalves Rosa, Ana Paula Pesarico, Cristina Wayne Nogueira*

Laboratório de Síntese, Reatividade e Avaliação Farmacológica e Toxicológica de Organocalcogênios, Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria, Rio Grande do Sul CEP 97105-900, Brazil

ARTICLE INFO

Keywords:
Social avoidance
Social defeat stress
Opioid receptors
MAPK
Organoselenium

ABSTRACT

Depressive symptoms precipitated by stress are prevalent in population. In experimental models of social stress, endogenous opioids mediate different aspects of defensive and submissive behaviors. The present study investigated the opioid receptors, mitogen-activated protein kinase (MAPKs) and protein kinase B (Akt) contribution to *m*-trifluoromethyl-diphenyl diselenide [(*m*-CF_3-PhSe)$_2$] effects on social avoidance induced by social defeat stress (SDS). Adult Swiss mice were subjected to SDS and treated with (*m*-CF_3-PhSe)$_2$ (5 to 25 mg/kg) for 7 days. After that, the mice performed locomotor and social avoidance tests. The opioid receptors, MAPKs and Akt protein contents were determined in the prefrontal cortical samples of mice. Firstly, the mice were segregated in susceptible or resilient subpopulation based on their social avoidance induced by stress. (*m*-CF_3-PhSe)$_2$ (25 mg/kg) was effective against the stress-induced social avoidance and improved social interaction behavior in mice. SDS increased the μ and κ protein contents but reduced those of δ opioid receptors in susceptible mice. Resilient and (*m*-CF_3-PhSe)$_2$-treated mice had no alteration in the levels of opioid receptors. Moreover, (*m*-CF_3-PhSe)$_2$ was effective against the increase of c-Jun N-terminal kinase (JNK) and the decrease of Akt phosphorylation protein contents induced by SDS in susceptible mice. The protein content of extracellular signal–regulated kinase (ERK) phosphorylation was reduced in both susceptible and resilient mice, whereas p38 mitogen-activated protein kinase (p38 MAPK) phosphorylation was increased only in resilient mice. (*m*-CF_3-PhSe)$_2$ was partially effective against the pERK decrease and ineffective against the increase in p38 MAPK phosphorylation in mice subjected to SDS. These results suggest that the modulation of protein contents of opioid receptors, JNK and Akt phosphorylation is associated with resilience to SDS promoted by (*m*–CF_3–PhSe)$_2$ in mice.

1. Introduction

m-Trifluoromethyl-diphenyl diselenide [(*m*-CF_3-PhSe)$_2$], a lipophilic molecule, has been reported to have promising effects in the psychiatric disease models, such as a comorbid pain/depression, tumor necrosis factor-α induced-depressive-like behavior and mania induced by ouabain in rodents (Bruning et al., 2015a; Bruning et al., 2015b; Bruning et al., 2012). The blood-brain barrier permeant property of this compound allows it to act in the central nervous system, interacting with different neurotransmitter systems and exerting anxiolytic-, antidepressant- and antipsychotic-like effects (Bruning et al., 2009; Machado et al., 2006). The (*m*-CF_3-PhSe)$_2$ action in experimental models of depression is related to its anti-inflammatory property, and modulation of serotonergic and opioid systems (Bruning et al., 2015a; Bruning et al., 2015b; Bruning et al., 2011). Concerning the (*m*-CF_3-PhSe)$_2$ effects on the opioid system, recent pharmacological findings indicated that this compound elicits an antidepressant-like action by activating μ and δ opioid receptors and blocking the κ opioid receptor (Rosa et al., 2017). In addition, this organoselenium compound induces neither tolerance to its antidepressant-like effect in the forced swimming test nor physical signs of withdrawal precipitated by naloxone in mice (Rosa et al., 2017). However, even that (*m*-CF_3-PhSe)$_2$ has been reported to act in multiple biological targets, including the opioid system (Bruning et al., 2015a; Bruning et al., 2010; Rosa et al., 2017), molecular mechanisms of its effects remain largely unknown.

Endogenous opioid peptides are released in the central nervous system in response to stressful stimuli, playing an important role in emotional control (McLaughlin et al., 2006; Smith et al., 2012). The

* Corresponding author at: Departamento de Bioquímica e Biologia Molecular, Centro de Ciências Naturais e Exatas, Universidade Federal de Santa Maria, Santa Maria CEP 97105-900, Rio Grande do Sul, Brazil.
E-mail address: criswn@ufsm.br (C.W. Nogueira).

https://doi.org/10.1016/j.pnpbp.2017.11.021
Received 4 September 2017; Received in revised form 11 November 2017; Accepted 23 November 2017
Available online 24 November 2017

modulation of opioid receptors, which are densely distributed in stress-related regions, including the prefrontal cortex, mediates different kinds of neurochemical and behavioral stress responses, which vary according to the opioid receptor targeted (Al-Hasani and Bruchas, 2011; Mansour et al., 1995).

Studies have indicated that most depressive episodes, whether early or recurrent, are likely to be a consequence of a severe stressful experience or prolonged stressful life (Bosch et al., 2012; Hammen, 2005; Salavecz et al., 2014). In addition, changes in the opioid receptor expression could potentiate or prevent the stress consequences, inducing vulnerability or resilience to depression (Berube et al., 2013; Berube et al., 2014).

Models of depression based on social conflict (Golden et al., 2011; Iniguez et al., 2014) may be relevant for the study of stress-induced psychopathology in animals because they involve a social form of stress, the most common stressor in humans (Iniguez et al., 2014). Social defeat stress (SDS) is an animal depression model widely known to produce social avoidance, anhedonia and decreased ability to cope and mimic the neurobiological mechanisms of negative psychosocial experiences (Berton et al., 2006; Iniguez et al., 2014; Yin et al., 2015). Physiologically, SDS induces numerous molecular changes in the brain, including altered expression of proteins of MAPK pathway and opioid receptors (Iio et al., 2011; Nikulina et al., 2005).

Considering the opioid contribution to the antidepressant-like action of (m-CF_3-PhSe)$_2$ and the role of this system in stress-induced depressive-like symptoms, the purpose of this study was to investigate the (m-CF_3-PhSe)$_2$ effect on social avoidance induced by the mouse SDS model. We have also attempted to determine whether (m-CF_3-PhSe)$_2$ affects the levels of opioid receptors and MAPK in defeated mice. With this study we intend to better understand the contribution of opioid system in the (m-CF_3-PhSe)$_2$ action in a SDS model.

2. Materials and methods

2.1. Animals

The experiments were carried out using male adult Swiss mice: 25–35 g "intruders" and aggressive 43–50 g "residents" from our breeding colony. The animals were housed in cages (5 mice per cage) with free access to commercial diet (Guaiba, RS, Brazil) and tap water. They were kept in a separate animal room, on a 12-h light/12-h dark cycle; the lights were turned on every day at 7:00 a.m., in a controlled temperature environment (22 ± 2 °C). The experiments were performed according to a randomized schedule and each animal was used only once in each test. The present experimental study was approved by the Institutional Ethics Committee on Care and Use of Experimental Animal Resources from the Federal University of Santa Maria-RS - Brazil and registered under the number 7770060215. The procedures in this study were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animal suffering and to reduce the number of animals used in the experiments.

2.2. Drugs

(m–CF_3–PhSe)$_2$ was prepared and characterized in our laboratory by the method previously described (Paulmier, 1986). Analysis of the ^{1}H NMR and ^{13}C NMR spectra (Fig. 1S A–B) showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of (m–CF_3–PhSe)$_2$ (99.9%) was determined by gas chromatography-mass spectrometry (Shimadzu QP2010PLUS GC/MS combination). (m–CF_3–PhSe)$_2$ was dissolved in canola oil and administered to mice by the intragastric route (i.g) in a constant volume of 10 ml/kg body weight. Groups of mice treated with vehicle (canola oil) were simultaneously assessed.

2.3. Experimental procedure

This study was divided in three experimental protocols, performed independently.

In the first experimental protocol (Fig. 1A), the mice were subjected to SDS procedure for 7 days and, after that, they were evaluated in the social avoidance test and spontaneous locomotor activity monitored (control group, n = 12 animals, SDS group, n = 20 animals). In this protocol, the mice were segregated in susceptible (n = 12 mice) or resilient (n = 8 mice) subpopulation based on their social avoidance induced by SDS according to Krishnan et al. (2007). Mice were assigned to each of three experimental groups: I- Control, mice that were not subjected to the SDS; II- Susceptible, mice that were subjected to the SDS and developed social avoidance and III- Resilient, mice that were subjected to the SDS and did not develop social avoidance.

The second experimental protocol (Fig. 1B) aimed to investigate whether (m–CF_3–PhSe)$_2$ treatment induces resilience to SDS. For this purpose, vehicle or (m–CF_3–PhSe)$_2$ at doses of 5, 10 and 25 mg/kg was administered intragastrically (i.g.) to unstressed or defeated mice, once a day, for 7 days concomitant with SDS (n = 12 animals/group). Twenty-four h after (m–CF_3–PhSe)$_2$ treatment/SDS, the mice were evaluated in social avoidance and spontaneous locomotor tests. For this experimental protocol, the animals were assigned to each of eight experimental groups: I- Vehicle, unstressed mice treated with vehicle (canola oil); II, III and IV- (m–CF_3–PhSe)$_2$ 5 to 25 mg/kg, unstressed mice treated with (m–CF_3–PhSe)$_2$ at a dose of 5, 10 or 25 mg/kg, respectively; V- SDS, mice subjected to SDS and treated with vehicle (canola oil); VI, VII and VIII- SDS + (m–CF_3–PhSe)$_2$ 5 to 25 mg/kg, mice subjected to SDS and treated with (m–CF_3–PhSe)$_2$ at a dose of 5, 10 or 25 mg/kg, respectively.

The third experimental protocol (Fig.1C) was designed to determine whether an effective dose of (m–CF_3–PhSe)$_2$ in the social avoidance test regulates the motivation for natural social interaction between unstressed mice, independent of negative or positive stimuli. In this protocol, unstressed mice were administered with vehicle (canola oil) or (m–CF_3–PhSe)$_2$ at a dose of 25 mg/kg once a day for 7 days. Twenty-four h after treatment, the mice were subjected to the social interaction test (n = 7 animals/group). Immediately after social avoidance test (protocols 1 and 2), the mice were killed by cervical dislocation and samples of prefrontal cortex were excised and stored at − 80 °C for the western-blot analyses.

2.4. SDS

The procedure of SDS was performed as previously reported by Bartolomucci et al. (2001) with few modifications. Aggressor mice were selected for their attack latencies reliably shorter than 30 s upon 3 consecutive screening tests. Intruder mice were subjected to SDS for 7 consecutive days. Every day each intruder mouse was introduced into the home cage of an unfamiliar aggressor mouse to physical interaction for approximately 5 min. Following the daily defeat, the intruder mouse remained in the home cage of aggressor on the other side of a perforated translucent Plexiglas partition to allow visual, auditory and olfactory interaction with the aggressor for 24 h until the next defeat (Fig. 2A). Each defeated mouse (intruder) was placed in the home cage of a new aggressor every day for 7 days. The control mice (unstressed) were housed by pair, one on each side of a perforated plexiglas partition, and were handled daily. The social avoidance test was performed 24 h after the last SDS procedure.

2.5. Behavioral tests

2.5.1. Social avoidance test

The social avoidance test was performed as previously described by Berton et al. (2006). This test was composed of two phases of 2.5 min each in which the mouse was evaluated in an open field arena (42 cm

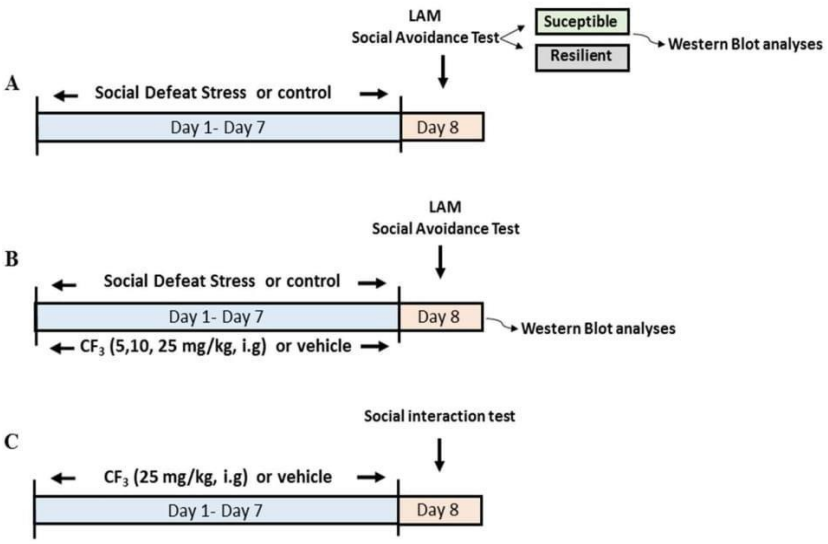


Fig. 1. Schedule of SDS and (m–CF_3–PhSe)$_2$ treatment. Repeated social defeat stress was performed for 7 days (day 1-day 7). Social avoidance test was used to segregate defeated mice into susceptible and resilient subpopulations (A). Another set of experiments was performed to evaluate the effect of (m–CF_3–PhSe)$_2$ at different doses (5, 10 and 25 mg/kg, i.g) on behavioral and molecular alterations induced by social defeat stress (B). Effect of (m-CF_3-PhSe)$_2$ (25 mg/kg, i.g) administration for 7 days on social interaction between unstressed mice (C). CF_3: (m-CF_3-PhSe)$_2$; SDS: social defeat stress. LAM: locomotor activity monitor.

wide, 42 cm depth, and 42 cm height). In the first phase, the mice were allowed to habituate and to explore the testing arena that contained an empty wire mesh cage (non-target) (10 × 6.5 cm) located at one end of the field. During the second session, the conditions were identical except that an unfamiliar aggressor was placed into the wire mesh cage (target). Between the 2 sessions, the experimental mouse was removed from the arena and was placed back into its home cage for 1 min. The time spent in the "interaction zone" (8 cm perimeter surrounding the cage) and "corner zone" (opposite to the location of the box) during the "no target" and "target" conditions were recorded (Fig. 2A).

The segregation of susceptible and resilient mice was based on the interaction ratio of animals in the social avoidance test, which was calculated as 100 × (interaction time, target present) / (interaction time, target absent). Mice with interaction ratio < 100 were defined as "susceptible" and those with interaction ratio ≥ 100 were defined as "resilient" (Krishnan et al., 2007) (Fig. 2B).

2.5.2. Social interaction test

The social interaction test was performed as previously described by Goeldner et al. (2011). Initially, each mouse was habituated individually in an open field for 15 min. Immediately after the habituation time, pairs of unstressed mice of same treatment [control or (m–CF_3–PhSe)$_2$] and weight, but from different home cages were placed simultaneously in the arena, for 10 min. The time spent grooming, sniffing, following or in physical contact with the partner were recorded as social interaction (Fig. 4A).

2.5.3. Spontaneous locomotor activity

In order to discard sedative or motor abnormality induced by SDS or (m–CF_3–PhSe)$_2$ treatment the mice performed spontaneous locomotor

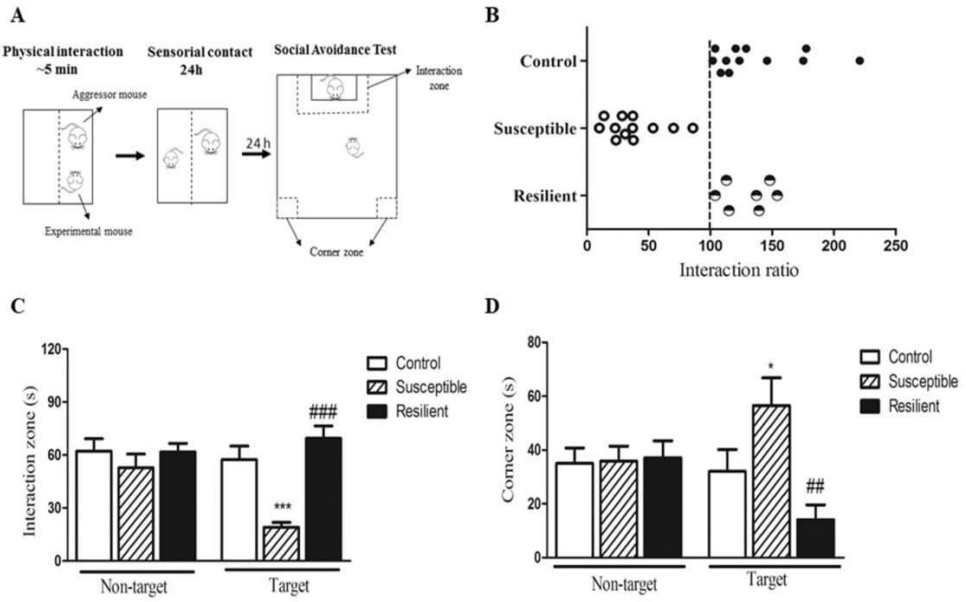


Fig. 2. Effect of SDS on social avoidance test: segregation of defeated mice into susceptible and resilient. Scheme of SDS procedure and social avoidance test (A). Horizontal scatterplot depicting the distribution of interaction ratio for control, susceptible, and resilient mice over multiple social defeat stress (B). Time in the interaction zone (C) and time in the corner zone (D) of susceptible and resilient mice subjected to the SDS. Values are expressed as mean ± S.E.M. of 12 animals (control and susceptible groups) and 8 animals (resilient group). Asterisks denote the significance levels when compared to the control group: (*) $P < 0.05$ and (***) $P < 0.001$. Hashtags denote the significance levels when compared to the susceptible group: ($^{\#\#}$)$P < 0.01$ and ($^{\#\#\#}$) $P < 0.001$ (one-way ANOVA followed by the Newman Keuls test). SDS: social defeat stress.

activity test. Testing took place in a clear Plexiglas cage (500 × 480 × 500 mm) equipped with 16 infrared sensors for the automatic recording of horizontal activity (Model EP149, Insight Instruments Ltda, São Paulo, BR). Each animal was placed in the center of the apparatus and allowed to freely explore the arena while being tracked by an automated tracking system (Software activity monitor IR, Insight Instruments Ltda). The data (crossings, distance traveled and velocity) were collected and recorded for 4 min.

2.6. Ex vivo analyses

2.6.1. Western blot assay

Samples of prefrontal cortices (n = 7) were homogenized in ice-cold sucrose buffer (pH 7.4) containing 1 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 0.1 mM ethylene glycol tetraacetic acid (EGTA) and 0.1 mM phenylmethylsulfonyl fluoride in the presence of commercial protein inhibitor cocktail (Sigma-Aldrich Company, St. Louis, Missouri, United States). Tissue extracts were diluted to a final protein concentration 2 μg/μl. The samples (40 μg of protein) and pre stained molecular weight standards (Sigma-Aldrich Company, St. Louis, Missouri, United States) were separated on 10% resolving with 4% concentrating SDS-PAGE electrophoresis gels. Proteins were transferred to nitrocellulose membrane using Transfer-Blot® Turbo™ Transfer System (1.0 mA; 45 min). After blocking with 3% bovine serum albumin solution, the blots were incubated overnight at 4 °C with primary antibodies as shown in Table 1. β-actin was stained as additional control of the protein loading. After primary antibodies incubation, membranes were washed and incubated with secondary antibodies conjugated with horseradish peroxidase (Bio-Rad Laboratories, Hercules, CA, USA) for 1 h at room temperature and developed with chemiluminescence kit (Amersham, São Paulo/Brazil). Optical density (OD) of the Western blotting bands was quantified using Image J (NIH, Bethesda, MD, USA) software for Windows. Each value was derived from the ratio between arbitrary units obtained by the protein band and the respective β-actin band. The results are shown as % of control.

2.6.2. Protein determination

Protein concentration was determined by the method previously described (Bradford, 1976) using bovine serum albumin (1 mg/ml) as a standard.

2.7. Statistical analysis

All experimental results are presented as the mean ± S.E.M. First, normality of data was analyzed using a D'Agostino and Pearson omnibus normality test. Comparisons between susceptible and resilient mice (behavioral tests and western blot analyses) were performed by one-way ANOVA variance followed by the Newman–Keuls test. Results of (m–CF_3–PhSe)$_2$ × SDS effect on social avoidance test and western blot were analyzed using two-way ANOVA of variance followed by the Newman–Keuls test. Unpaired Student's t-test was used to analyze the effects of (m–CF_3–PhSe)$_2$ in the social interaction test. Main effects are presented only when the first order interaction was non-significant. All analyses were performed by blinded investigator by using the STATISTICA for Windows software Version 7 (Stat Soft, Oklahoma, USA). Probability values < 0.05 ($P < 0.05$) were considered as statistically significant.

3. Results

3.1. SDS effect on social avoidance test: susceptible and resilient mice segregation

Mice subjected to SDS were segregated in susceptible or resilient subpopulation based on the interaction ratio of animals in the social avoidance test. Control and resilient mice showed interaction ratio in the social avoidance test higher than 100, whereas susceptible mice displayed interaction score lower than 100 (Fig. 2B). The percentage of 40 (8/20) of mice subjected to this paradigm was in the resilient group in this study. The one-way ANOVA of time in the interaction and corner zones (non-target) of the arena revealed that, in the absence of an aggressor (non-target), all experimental groups spent similar amount of time in the interaction [$F_{(2,31)} = 0.561$, $P > 0.05$] and corner zones [$F_{(2,31)} = 0.974$, $P > 0.05$] (Fig. 2C and D).

By contrast, the one-way ANOVA revealed a significant effect of time in the interaction zone (target) [$F_{(2,31)} = 18.61$, $P < 0.001$]. The results illustrated in Fig. 2C show that susceptible mice spent significantly less time in the interaction zone in the presence of an aggressor (target) when compared to the control group ($P < 0.001$), phenomenon absent in resilient mice ($P > 0.05$). In addition, susceptible mice spent more time in the corner zone (target) [$F_{(2,31)} = 5.32$, $P < 0.05$] when compared to the control group ($P < 0.05$), whereas resilient mice spent less time in the corner zones, in the presence of an aggressor (target), when compared to those in the susceptible group ($P < 0.01$) (Fig. 2D).

3.2. (m–CF_3–PhSe)$_2$ treatment induces resilience to SDS in social avoidance test

The two-way ANOVA analyses of time in the interaction [$F_{(1,88)} = 0.017$, $P > 0.05$] and corner [$F_{(1,88)} = 0.937$, $P > 0.05$] zones of the arena, in the absence of an aggressor (non-target), showed no statistically significant effect of SDS and (m–CF_3–PhSe)$_2$. All experimental groups spent equal time in the interaction and corner zones (non-target) (Fig. 3A and 3C).

Table 1
List of primary antibodies and their dilutions.

Antibody name	Molecular weight (kDa)	Type	Company	Dilution
MOR	55	Goat	Santa Cruz Biotechnology	1:1000
DOR	92	Rabbit	Santa Cruz Biotechnology	1:1000
KOR	75	Rabbit	Santa Cruz Biotechnology	1:1000
*p*Akt	60	Rabbit	Cell Signaling Technology	1:1000
Akt	60	Rabbit	Cell Signaling Technology	1:1000
*p*ERK	42/44	Rabbit	Cell Signaling Technology	1:1000
ERK	42/44	Rabbit	Cell Signaling Technology	1:1000
*p*JNK	46/54	Mouse	Santa Cruz Biotechnology	1:1000
JNK	46/54	Rabbit	Santa Cruz Biotechnology	1:1000
*p*p38 MAPK	43	Rabbit	Cell Signaling Technology	1:1000
p38 MAPK	43	Rabbit	Cell Signaling Technology	1:1000
β-actin	42	Mouse	Abcam	1:5000

MOR (μ-opioid receptor); DOR (δ-opioid receptor); KOR (κ-opioid receptor); Akt (protein kinase B); ERK (extracelular signal-regulated kinase); JNK (c-Jun N-terminal kinase); p38 MAPK (mitogen-activated protein kinase).

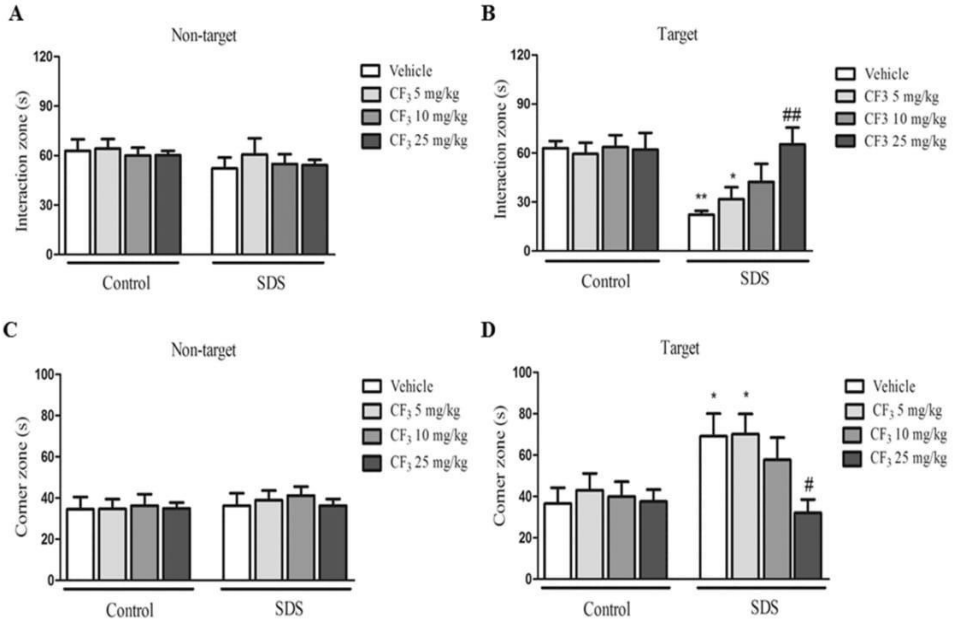


Fig. 3. (m–CF_3–PhSe)$_2$ treatment induces resilience to SDS in social avoidance test. Effects of (m–CF_3–PhSe)$_2$ (5, 10 and 25 mg/kg, i.g.) on time in the interaction zone [non-target (A) and target (B)] and in the corner zone [non-target (C) and target (D)] of mice subjected to the SDS. Values are expressed as mean ± S.E.M. of 12 animals/group. Asterisks denote the significance levels when compared to the control group: (*)$P < 0.05$ and (**)$P < 0.01$. Hashtags denote the significance levels when compared to the SDS group: ($^{\#}$) $P < 0.05$ and ($^{\#\#}$) $P < 0.01$ (Two-way ANOVA followed by the Newman Keuls test). CF_3: (m-CF_3-PhSe)$_2$; SDS: social defeat stress. Control means unstressed mice.

However, when an aggressor was introduced into the cage (target), the two-way ANOVA analyses of time spent in the interaction zone revealed a significant SDS x (m–CF_3–PhSe)$_2$ interaction [$F_{(1,88)} = 2.78$, $P < 0.05$]. Defeated mice spent less time in the interaction zone (target) than control mice (unstressed mice treated with vehicle) ($P > 0.01$). Treatment of defeated mice with (m–CF_3–PhSe)$_2$ at the dose of 25 mg/kg was effective against social avoidance induced by SDS ($P < 0.01$), whereas doses of 5 and 10 mg/kg of this compound were ineffective ($P > 0.05$). In addition, the administration of (m–CF_3–PhSe)$_2$ at all tested doses to unstressed mice did not change the time in the interaction zone of the arena, in the presence of an aggressor ($P > 0.05$) (Fig. 3B).

The two-way ANOVA analyses of time spent in the corner zone (target) revealed a significant (m–CF_3–PhSe)$_2$ x SDS interaction [$F_{(1,88)} = 2.76$, $P < 0.05$]. Defeated mice spent more time in the corner zone in the presence of an aggressor ($P < 0.05$), and treatment with (m–CF_3–PhSe)$_2$ only at the dose of 25 mg/kg reduced this time ($P < 0.05$). The administration of (m–CF_3–PhSe)$_2$ at all doses tested to unstressed mice did not change the time in the corner zone of the arena, in the presence of an aggressor ($P > 0.05$) (Fig. 3D).

Fig. 4 shows that unstressed mice that received vehicle, and the mice subjected to SDS and treated with (m–CF_3–PhSe)$_2$ displayed interaction ratio higher than 100, whereas those subjected to SDS and treated with vehicle showed interaction score lower than 100.

3.3. (m–CF_3–PhSe)$_2$ treatment improves the social natural interaction between unstressed mice

Fig. 5B shows the effect of treatment with (m–CF_3–PhSe)$_2$ on social interaction test. An unpaired Student's t-test revealed that unstressed mice treated with (m–CF_3–PhSe)$_2$ at the dose of 25 mg/kg spent more time in social natural interaction when compared to those of the vehicle group ($P < 0.01$).

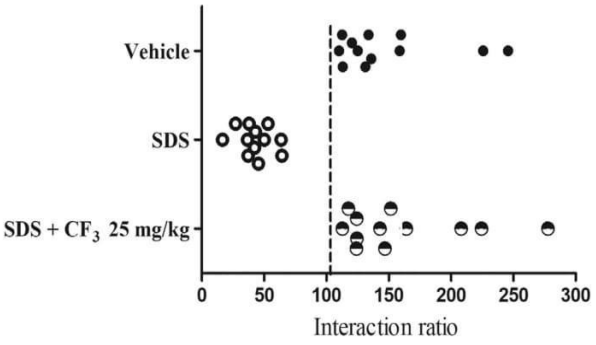


Fig. 4. Horizontal scatterplot depicting the distribution of interaction ratios for vehicle (unstressed, n = 12), SDS (susceptible, n = 12), and SDS + CF_3 25 mg/kg (n = 12) groups over multiple SDS. SDS: social defeat stress.

3.4. (m–CF_3–PhSe)$_2$ treatment and SDS do not alter the mouse spontaneous locomotor activity

Tables 2 and 3 show the effect of SDS or (m–CF_3–PhSe)$_2$ treatment on the mouse spontaneous locomotor activity. SDS and (m–CF_3–PhSe)$_2$ did not alter the number of crossings, distance traveled (mm) and velocity (m/s) of mice ($P > 0.05$).

3.5. (m–CF_3–PhSe)$_2$ treatment modulates protein contents of opioid receptors altered by SDS

The two-way ANOVA of prefrontal cortical μ-opioid receptor (MOR) levels revealed a significant (m–CF_3–PhSe)$_2$ x SDS interaction [$F_{(1,24)} = 5.79$, $P < 0.05$]. SDS induced an increase of MOR levels when compared to those of the vehicle group ($P < 0.05$), which was reduced by (m–CF_3–PhSe)$_2$ treatment in defeated mice ($P < 0.05$) (Fig. 6A).

The insert of Fig. 6A shows the prefrontal cortical MOR protein contents in susceptible and resilient defeated mice. Susceptible defeated mice showed an increase of MOR levels when compared to those of the

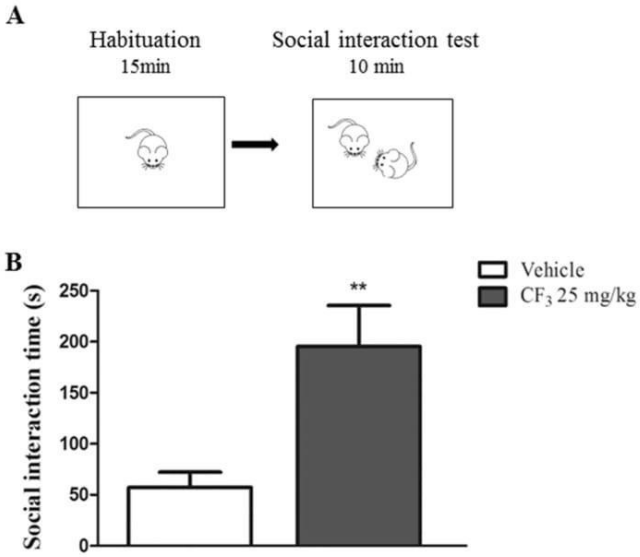


Fig. 5. (m–CF_3–$PhSe)_2$ treatment improves the social natural interaction between unstressed mice. Scheme of social interaction test (A). Effect of (m–CF_3–$PhSe)_2$ administration to mice on social interaction test (B). Values are expressed as mean ± S.E.M. of 7 animals/group. Asterisks denote the significance levels when compared to the vehicle group: (**) $P < 0.01$ (unpaired Student's t-test). CF_3: (m-CF_3-$PhSe)_2$.

Table 2
Spontaneous locomotor parameters of mice after social defeat.

Groups	Number of crossings	Distance (mm)	Velocity (mm/s)
Control	445.70 ± 49.12	8052.00 ± 1072.31	37.48 ± 4.31
Susceptible	436.40 ± 45.65	7874.90 ± 1123.64	37.14 ± 3.81
Resilient	458.00 ± 61.19	8164.20 ± 1174.14	35.56 ± 3.21

Values are reported as means ± S.E.M. for 8 (resilient) to 12 (control and susceptible) animals per group. Data were analyzed by using a one-way analysis of variance (ANOVA). Control - mice that were not subjected to the SDS; Susceptible - mice that were subjected to the SDS and developed social avoidance; Resilient - mice that were subjected to the SDS and did not develop social avoidance.

Table 3
Spontaneous locomotor parameters of mice after social defeat and/or treatment with (m-CF_3-$PhSe)_2$.

Groups	Number of Crossings	Distance (mm)	Velocity (mm/s)
Vehicle	484.25 ± 59.53	8642.99 ± 984.57	40.90 ± 3.08
CF_3 5 mg/kg	468.76 ± 57.20	8947.14 ± 1031.31	42.34 ± 4.37
CF_3 10 mg/kg	510.83 ± 34.85	9264.18 ± 693.76	39.63 ± 2.78
CF_3 25 mg/kg	507.50 ± 47.70	9091.06 ± 970.63	38.56 ± 4.08
SDS	508.75 ± 44.79	8559.55 ± 1122.74	40.44 ± 3.16
SDS + CF_3 5 mg/kg	486.25 ± 75.15	9178.30 ± 1023.30	41.91 ± 3.53
SDS + CF_3 10 mg/kg	475.50 ± 59.38	8620.30 ± 896.12	38.05 ± 3.65
SDS + CF_3 25 mg/kg	504.50 ± 45.56	8710.55 ± 811.34	40.97 ± 3.43

Values are reported as means ± S.E.M. for 12 animals per group. Data were analyzed by using a two-way analysis of variance (ANOVA). Vehicle - unstressed mice treated with vehicle (canola oil); SDS - mice subjected to social defeat stress and treated with vehicle (canola oil). CF_3: (m-CF_3-PhSe).

control group ($P < 0.01$), whereas resilient defeated mice had reduced protein content of MOR when compared to those of control ($P < 0.05$) and susceptible groups ($P < 0.001$) (One-way ANOVA [$F_{(2,20)} = 15.95, P < 0.001$]).

The two-way ANOVA of prefrontal cortical δ-opioid receptor (DOR) protein content revealed a significant SDS x (m–CF_3–$PhSe)_2$ interaction [$F_{(1,24)} = 6.10$, $P < 0.05$]. Defeated mice showed a decrease in the DOR levels when compared to those of the control group ($P < 0.05$) and treatment of defeated mice with (m–CF_3–$PhSe)_2$ was effective against this alteration ($P < 0.01$) (Fig. 6B).

The insert of Fig. 6B shows the prefrontal cortical DOR levels of susceptible and resilient defeated mice. Susceptible defeated mice showed a decrease of DOR levels when compared to those of the control group ($P < 0.05$), whereas resilient defeated mice showed an increase of DOR protein content when compared to that of susceptible groups ($P < 0.05$) (One-way ANOVA [$F_{(2,20)} = 4.34$, $P < 0.01$]).

The two-way ANOVA of the prefrontal cortical κ-opioid receptor (KOR) levels showed a significant SDS x (m–CF_3–$PhSe)_2$ interaction [$F_{(1,24)} = 5.64$, $P < 0.05$]. SDS induced an increase of KOR levels when compared to those of the control group ($P < 0.05$), which was reduced by (m–CF_3–$PhSe)_2$ treatment in defeated mice ($P < 0.05$) (Fig. 6C).

The insert of Fig. 6C shows the prefrontal cortical KOR protein content of susceptible and resilient defeated mice. Susceptible defeated mice showed higher levels of KOR when compared to those of the control group ($P < 0.05$), this effect was not observed in resilient defeated mice ($P > 0.05$). One-way ANOVA [$F_{(2,20)} = 4.20$, $P < 0.05$].

3.6. (m–CF_3–$PhSe)_2$ treatment is effective against the increase of JNK phosphorylation induced by SDS

The two-way ANOVA of JNK data showed a significant (m–CF_3–$PhSe)_2$ x SDS interaction in the pJNK/JNK ratio [$F_{(1,24)} = 4.20$, $P < 0.05$] and in the pJNK levels [$F_{(1,24)} = 5.05$, $P < 0.05$]. Defeated mice showed an increase in the pJNK/JNK ratio and pJNK levels when compared to those of the control group ($P < 0.05$). Treatment of defeated mice with (m–CF_3–$PhSe)_2$ was effective against the increase in these protein levels induced by SDS ($P < 0.05$) (Fig. 7A and 7B). Statistical analysis of the JNK levels revealed a significant main effect of SDS [$F_{(1,24)} = 5.93$, $P < 0.05$]. Total JNK levels were decreased only in SDS group (Fig. 7C).

The inserts of Fig.7A and 7B show the pJNK/JNK ratio and pJNK levels, respectively, in the prefrontal cortices of susceptible and resilient defeated mice. SDS induced an increase of pJNK/JNK. One-way ANOVA [$F_{(2,20)} = 8.60$, $P < 0.01$] ratio and pJNK. One-way ANOVA [$F_{(2,20)} = 5.28$, $P < 0.05$] levels in the prefrontal cortices of susceptible mice when compared to those of the control group ($P < 0.05$). By contrast, resilient defeated mice showed a decrease in the pJNK/JNK ratio ($P < 0.01$) and pJNK levels ($P < 0.05$) when compared to those of the susceptible group. In addition, whereas total JNK levels were reduced in susceptible mice when compared to the control mice ($P > 0.05$), the content of this protein was increased in resilient mice when compared to control ($P < 0.05$) and susceptible groups ($P > 0.001$) (Fig. 7C, insert).

3.7. (m–CF_3–$PhSe)_2$ treatment is partially effective against the decrease of ERK phosphorylation induced by SDS

The two-way ANOVA of pERK/ERK ratio [$F_{(1,24)} = 7.25$, $P < 0.05$] and pERK protein content [$F_{(1,24)} = 5.27$, $P < 0.05$] revealed a significant (m–CF_3–$PhSe)_2$ x SDS interaction. Susceptible defeated mice showed the pERK/ERK ratio and pERK levels reduced when compared to those of the control group ($P < 0.05$). Treatment of defeated mice with (m–CF_3–$PhSe)_2$ was partially effective against the reduction in the pERK/ERK ratio and pERK protein content induced by SDS (Fig. 8A and 8B). In addition, the total ERK levels in the prefrontal cortices of mice were similar in all experimental groups [$F_{(2,24)} = 0.011$, $P > 0.05$] (Fig.8C).

The inserts of Fig. 8A and 8B show, respectively, the pERK/ERK ratio and pERK levels in the prefrontal cortices of susceptible and resilient defeated mice. SDS induced a decrease of prefrontal cortical

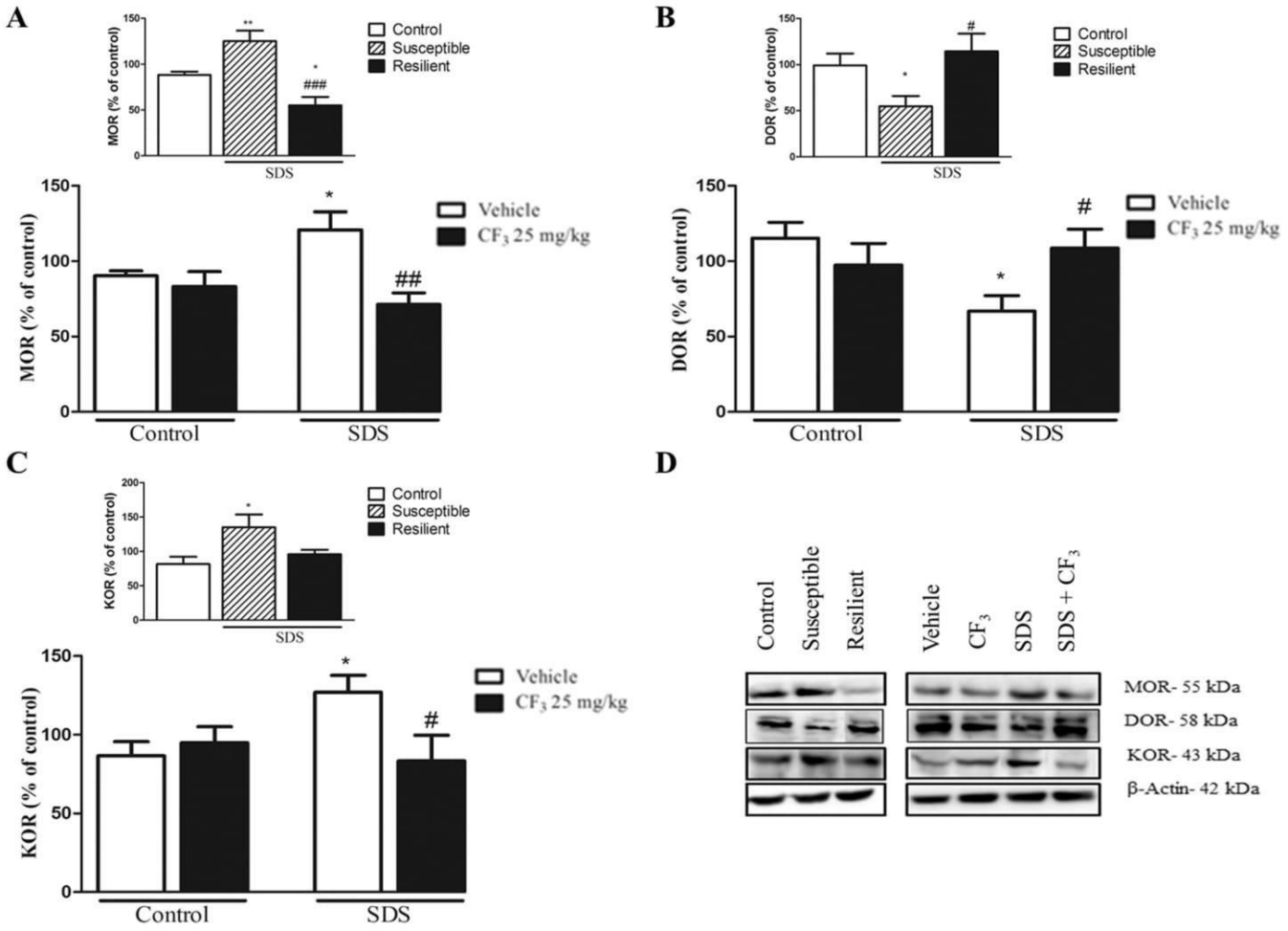


Fig. 6. (m–CF_3–PhSe)$_2$ treatment modulates opioid receptor levels altered by SDS in mice. Effect of (m–CF_3–PhSe)$_2$ treatment (25 mg/kg, i.g) on MOR (A), DOR (B), and KOR (C) levels in the prefrontal cortices of mice subjected to the SDS. Inserts show MOR (A), DOR (B) and KOR (C) levels in the prefrontal cortices of susceptible and resilient mice. Absolute values expressed as optical density (OD): control group: 9,694,000 ± 1,009,000 (MOR); 8,897,000 ± 1,455,000 (DOR); 3,311,000 ± 592,900 (KOR). Values are expressed as mean ± S.E.M. of 7 animals/group. Asterisks denote the significance levels when compared to the control group: (*) $P < 0.05$ and (**) $P < 0.01$ Hashtags denote the significance levels when compared to the SDS or susceptible group: ($^{\#}$) $P < 0.05$ and ($^{\#\#\#}$) $P < 0.001$ (One way or two-way ANOVA followed by the Newman Keuls test). Photographs are representation of qualitative Western blotting analysis (D). CF_3: (m-CF_3-PhSe)$_2$, SDS: social defeat stress.

*p*ERK/ERK ratio. One-way ANOVA [$F_{(2,20)} = 7.43$, $P < 0.01$] and *p*ERK. One-way ANOVA[$F_{(2,20)} = 6.34$, $P < 0.01$] levels in both susceptible ($P < 0.05$) and resilient mice ($P < 0.01$) when compared to those of the control group. Statistical analysis of total ERK levels revealed that there was no significant difference among the groups [$F_{(2,20)} = 0.252$, $P > 0.05$] (Fig.8C, insert).

3.8. SDS induces an increase in pp38/p38MAPK ratio in resilient mice

The two-way ANOVA analyses of *p*p38/p38 MAPK ratio, *p*p38 MAPK and total p38 MAPK levels showed no statistically significant effect of SDS and (m–CF_3–PhSe)$_2$. Mice of all experimental groups showed similar *p*p38/p38 MAPK ratio [$F_{(1,24)} = 0.858$, $P > 0.05$], *p*p38 MAPK [$F_{(1,24)} = 0.867$, $P > 0.05$] and p38 MAPK [$F_{(1,24)} = 0.418$, $P > 0.05$] contents (Fig. 9A, B and C).

SDS induced an increase in *p*p38/p38 MAPK ratio (One-way ANOVA [$F_{(2,20)} = 5.07$, $P < 0.05$] and *p*p38 MAPK levels) (One-way ANOVA [$F_{(2,20)} = 5.85$, $P < 0.05$] in the prefrontal cortices of resilient mice when compared to those of control ($P < 0.05$) and susceptible groups ($P < 0.05$)). However, the levels of these proteins in the prefrontal cortices of susceptible mice were not changed by SDS ($P > 0.05$) (Fig. 9A and B, insert). The total p38 MAPK levels were similar in all experimental groups [$F_{(2,20)} = 0.034$, $P > 0.05$] (Fig. 9C, insert).

3.9. (m–CF_3–PhSe)$_2$ treatment is partially effective against the decrease of Akt phosphorylation induced by SDS

The two-way ANOVA of *p*Akt/Akt ratio [$F_{(1,24)} = 6.70$, $P < 0.05$] and *p*Akt levels [$F_{(1,24)} = 4.40$, $P < 0.05$] revealed a (m–CF_3–PhSe)$_2$ × SDS interaction. The defeated mice showed a decrease in *p*Akt/Akt ratio and *p*Akt levels when compared to those of the control group ($P < 0.05$). (m–CF_3–PhSe)$_2$ treatment in defeated mice was partially effective against the decrease of *p*Akt/Ak tratio and *p*Akt levels induced by SDS (Fig. 10A and 10B). Statistical analysis of total Akt levels revealed that there was no significant difference in these protein levels [$F_{(2,24)} = 0.010$, $P > 0.05$] among the groups (Fig. 10C).

The insert of Fig. 10A and 10B shows, respectively, the *p*Akt/Akt ratio and *p*Akt levels in the prefrontal cortices of susceptible and resilient defeated mice. Susceptible defeated mice showed a reduction in the prefrontal cortical *p*Akt/Akt ratio (One-way ANOVA [$F_{(2,20)} = 7.88$, $P < 0.01$]) and *p*Akt levels (One-way ANOVA [$F_{(2,20)} = 7.19$, $P < 0.01$]) when compared to those of the control group ($P < 0.05$). By contrast, resilient defeat mice showed an increase in *p*Akt/Akt ratio

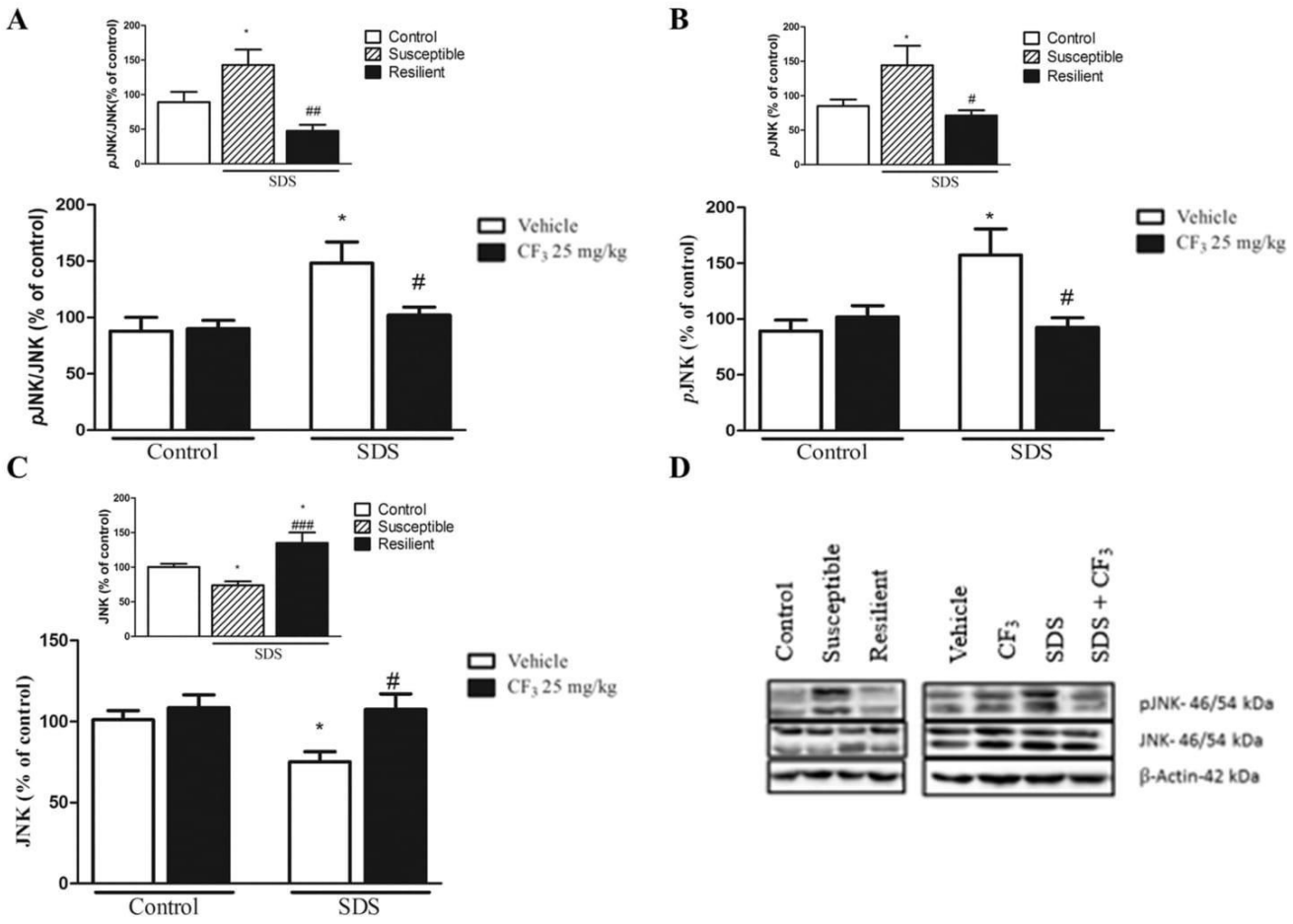


Fig. 7. (m–CF_3–PhSe)$_2$ treatment is effective against the increase of JNK phosphorylation induced by SDS in mice. Effect of (m–CF_3–PhSe)$_2$ treatment (25 mg/kg, i.g) on *p*JNK/JNK ratio (A), *p*JNK (B), and JNK (C) levels in the prefrontal cortices of mice subjected to the SDS. Inserts show the *p*JNK/JNK ratio (A), *p*JNK (B), and JNK (C) levels in the prefrontal cortices of susceptible and resilient mice. Absolute values expressed as optical density (OD): control group: 22,070,000 ± 1,923,000 (*p*JNK); 35,590,000 ± 5,567,000 (JNK). Values are expressed as mean ± S.E.M. of 7 animals/group. Asterisk denotes the significance levels when compared to the control group: (*) $P < 0.05$. Hashtag denotes the significance levels when compared to the social defeat or susceptible group: ($^{\#}$) $P < 0.05$ ($^{\#\#}$) $P < 0.01$ and ($^{\#\#\#}$) $P < 0.001$(One-way or two-way ANOVA followed by the Newman Keuls test). Photographs are representation of qualitative Western blotting analysis (D). CF_3: (m-CF_3-PhSe)$_2$; SDS: social defeat stress.

($P < 0.001$) and *p*Akt levels ($P < 0.001$) when compared to those of the susceptible mice (Fig. 10C). In addition, the total Akt levels in the prefrontal cortices of mice were similar in all experimental groups [$F_{(2,20)} = 0.270$, $P > 0.05$] (Fig. 10C, insert).

4. Discussion

The present study contributes to a better understanding the molecular basis of (m–CF_3–PhSe)$_2$ antidepressant-like action in a model of SDS. The results reported here show clearly that (m–CF_3–PhSe)$_2$ treatment was effective against social avoidance induced by SDS and promoted an increase of natural social interaction between mice, without changing their spontaneous locomotor activity. The molecular findings of this study provide further evidence of μ, δ and κ opioid receptors, JNK, ERK and Akt contribution to the (m–CF_3–PhSe)$_2$ action in the depression-like behavior induced by SDS.

The depressant symptoms precipitated by stress occur with high prevalence in general population (Bjorkqvist, 2001) and are consistently reproduced by SDS in rodents (Iniguez et al., 2014; Yin et al., 2015). Individuals exhibit a wide variation in responses to stress, which are related to fail to adopt active responses to stressful situations, increasing the likelihood of developing psychiatric disorders (susceptibility) or ability to adapt to stressful situations, avoiding its negative consequences (resilience) (Russo et al., 2012). It has been demonstrated that, like humans, not all mice subjected to stressful conditions develop depression-like symptoms. Concerning SDS, it is reported that approximately 66% of defeated mice display social avoidance, which can be characterized as susceptible mice (Bonanno, 2004; Krishnan et al., 2007). In agreement with these data, the current results demonstrated that 60% of mice subjected to SDS developed social avoidance, as shown by interaction ratio lower than 100. On the other hand, a part of defeated mice (40%) did not show social avoidance, indicating a possible stress adaptation, which characterizes the resilience.

The findings of the present study demonstrate the effectiveness of (m–CF_3–PhSe)$_2$ treatment at the dose of 25 mg/kg against the social avoidance induced by SDS, producing behavioral responses to stress similar to those of resilient mice. In this context, it has been reported the (m–CF_3–PhSe)$_2$ action in various models of depression and behavioral tests used for screening antidepressants (Bruning et al., 2015a; Bruning et al., 2015b; Bruning et al., 2011). The present study is the first to demonstrate the (m–CF_3–PhSe)$_2$ action in a depressive symptom of social nature, which is of particular importance because the psychological or social stressors are the main factors involved in depression induced by stress (Bjorkqvist, 2001).

Natural social interactions are spontaneous relationships that do not depend on positive or negative stimuli, such as sexual and maternal or defeat and fear, respectively. Social interactions are a fundamental and adaptive component of the biology of rodents, and the natural

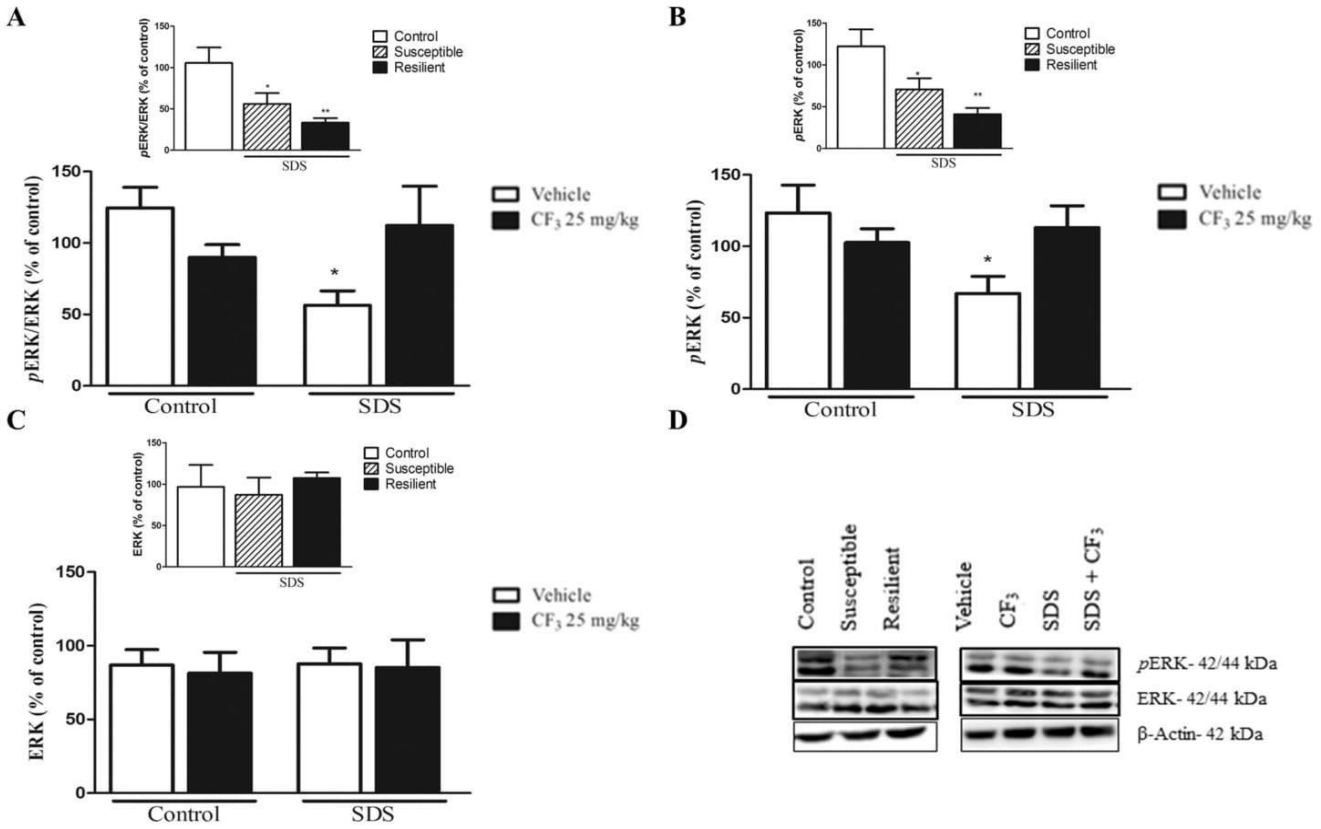


Fig. 8. (m–CF_3–PhSe)$_2$ treatment is partially effective against the decrease of ERK phosphorylation induced by SDS in mice. Effect of (m–CF_3–PhSe)$_2$ treatment (25 mg/kg, i.g) on *p*ERK/ERK ratio (A), *p*ERK (B)and ERK (C) levels in the prefrontal cortices of mice subjected to the SDS. Inserts show the *p*ERK/ERK ratio (A), *p*ERK (B), and ERK (C) levels in the prefrontal cortices of susceptible and resilient mice. Absolute values expressed as optical density (OD): control group: 2,451,000 ± 1,595,000 optical density (*p*ERK); 3,223,000 ± 1,049,000 (ERK). Values are expressed as mean ± S.E.M. of 7 animals/group. Asterisks denote the significance levels when compared to the control group: (*) $P < 0.05$ and (**) $P < 0.01$ (One-way or two-way ANOVA followed by the Newman Keuls test). Photographs are representation of qualitative Western blotting analysis (D). CF_3: (m-CF_3-PhSe)$_2$; SDS: social defeat stress.

sociability between them can be an indicative of psychological well-being of animal (Robinson et al., 2005). In addition, disruptions in natural social behavior characterized by a lack of desire to interact socially without a definite cause are common symptoms of a variety of neuropsychiatric disorders, such as depression (Miyoshi and Morimura, 2010). In the present study, (m–CF_3–PhSe)$_2$ treatment, at an effective dose in the social avoidance test, increased the interaction time between mice without any negative or positive stimuli, indicating that (m–CF_3–PhSe)$_2$ can regulate the motivation for social interactions, independent of whether it occurs naturally or during stressful situations, such as in a depressed state.

The opioid system has become a target of studies on the variability of psychobiological responses to stress (Cabib et al., 2012). Endogenous opioids mediate different aspects of defensive and submissive behaviors in social stress models and play a pivotal role in neurochemical changes induced by stress, according to the type of opioid receptors with which they interact (Lutz and Kieffer, 2013).

In this context, the present study revealed the involvement of each opioid receptor in behavioral consequences of SDS. Susceptible defeated mice showed higher protein levels of μ and κ opioid receptors than those of unstressed mice, whereas δ opioid receptor levels were decreased in the prefrontal cortex of defeated mice. In line with our data, a previous study demonstrated that repeated SDS induces an increase of MOR mRNA in the ventral tegmental area, which is accompanied by a long-lasting neural expression of FosB/ΔFosB in mesocorticolimbic projection areas, such as the nucleus accumbens, prefrontal cortex, and amygdala, important stress-related brain regions (Nikulina et al., 2008). Moreover, the activation of dynorphin/KOR system has been reported as necessary and sufficient for stress-induced behavioral responses in animal models of anxiety and depression, including SDS (Bérubé et al., 2013). Although the antidepressant-like action of DOR agonists is most well documented (Javelot et al., 2010; Richards et al., 2016), to our knowledge, the current study is the first to demonstrate the SDS effect on the DOR protein content in susceptible and resilient mice. Consistent with the decrease of prefrontal cortical DOR levels in susceptible defeated mice found in the present study, depressive-like behavior has been shown in DOR knockout mice (Jutkiewicz, 2013).

Moreover, there is compelling experimental evidence indicating that genetic or pharmacological opioid interventions are effective against behavioral and biochemistry changes induced by SDS. Mice lacking a functional MOR and KOR gene, or pretreated with KOR antagonists, displayed less total time in defeat postures than their control non-stressed (Komatsu et al., 2011; McLaughlin et al., 2006), whereas DOR agonists elicited antidepressant-like effects across behavioral paradigms, which involve environmental stress, as forced swimming and tail suspension tests (Naidu et al., 2007; Nozaki et al., 2014). In the present study, the (m–CF_3–PhSe)$_2$ administration was effective against the alterations in the levels of opioid receptors induced by SDS. The contribution of each opioid receptor to the (m–CF_3–PhSe)$_2$ antidepressant-like action was recently reported by us in a pharmacological protocol (Rosa et al., 2017), and it was molecularly confirmed by the present results. Interestingly, western blot analyses showed that (m–CF_3–PhSe)$_2$-treated and resilient mice subjected to SDS had similar prefrontal cortical levels of opioid receptors, indicating that these receptors can mediate the resilience to social avoidance induced by (m–CF_3–PhSe)$_2$.

In addition to stress effects on the opioid system, some authors have

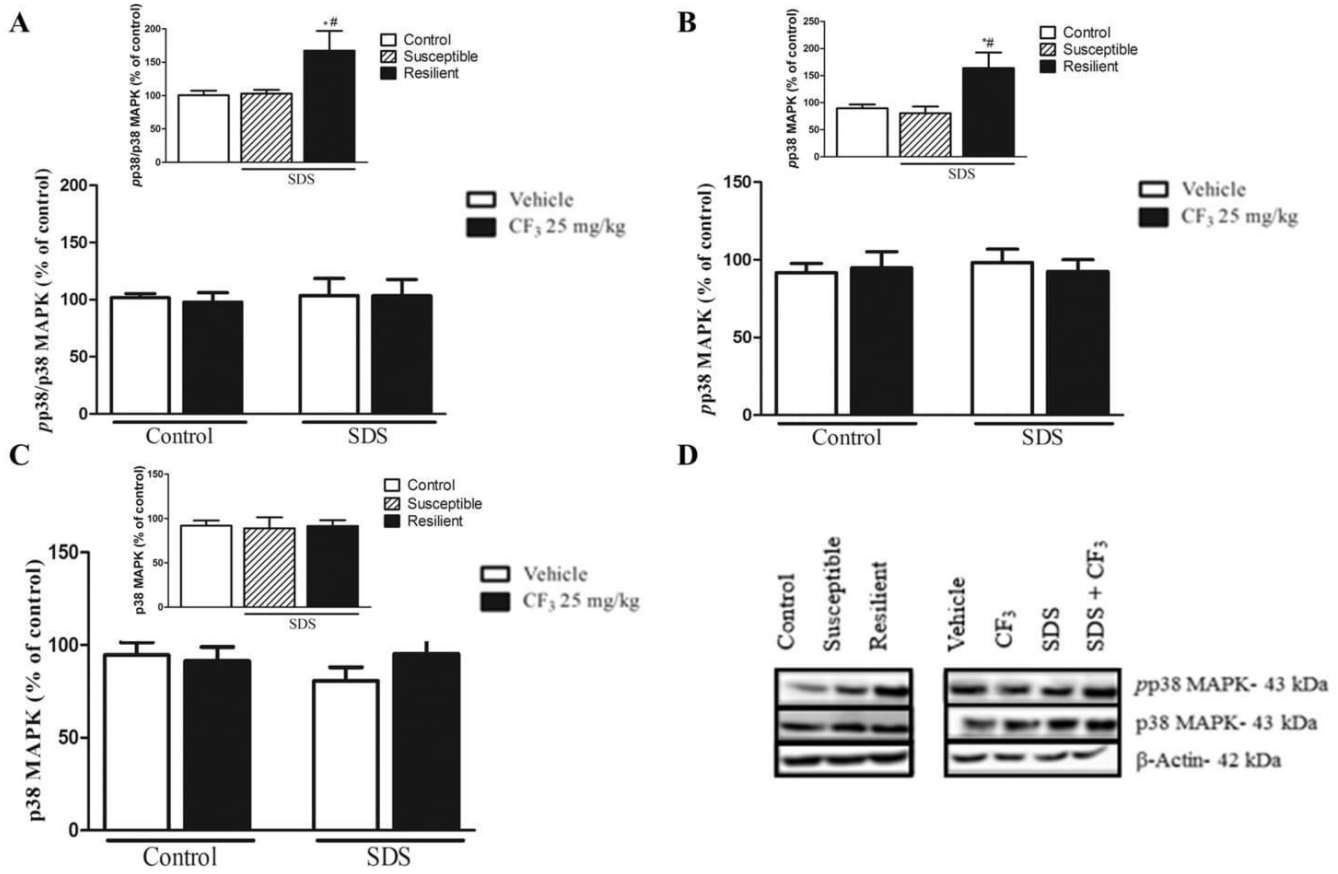


Fig. 9. SDS induces an increase in pp38/p38MAPK ratio in resilient mice. Effect of $(m\text{–}CF_3\text{–}PhSe)_2$ treatment (25 mg/kg, i.g) on *p*p38/p38 MAPK ratio (A), *p*p38 MAPK (B), and p38 MAPK (C) levels in the prefrontal cortices of mice subjected to the SDS. Inserts show the *p*p38/p38 MAPK ratio (A), *p*p38 MAPK (B), and p38 MAPK (C) levels in the prefrontal cortices of susceptible and resilient mice. Absolute values expressed as optical density (OD): control group: 1,130,000 ± 191,900 (*p*p38 MAPK); 9,972,000 ± 921,900 (p38 MAPK). Values are expressed as mean ± S.E.M. of 7 animals/group. Asterisk denotes the significance levels when compared to the control group: (*) $P < 0.05$. Hashtag denotes the significance levels when compared to the susceptible group: (#) $P < 0.05$ (One-way or Two-way ANOVA followed by the Newman Keuls test). Photographs are representation of qualitative Western blotting analysis (D). CF_3: $(m\text{-}CF_3\text{-}PhSe)_2$; SDS: social defeat stress.

proposed that MAPK pathways contribute to behavioral stress responses and may be a mechanism of antidepressant action (Iio et al., 2011; Naidu et al., 2007). In the present study, $(m\text{–}CF_3\text{–}PhSe)_2$ was effective against the increase of JNK phosphorylation and the decrease of ERK phosphorylation in the prefrontal cortex of susceptible defeated mice. Moreover, it is important to highlight that the increase in JNK phosphorylation induced by stress occurred only in the susceptible defeated mice, whereas the decrease in ERK phosphorylation was independent of susceptibility or resilience to SDS, suggesting the involvement of JNK pathway on depressive-like behavior induced by social defeat stress. In fact, environmental triggers, including stress, activate the JNK pathway (Johnson and Nakamura, 2007; Liu et al., 2004). In addition, opioid drugs, such as nor BNI and morphine, can regulate the JNK phosphorylation by acting in KOR and MOR, respectively (Melief et al., 2010). Therefore, the modulation of opioid receptors and JNK phosphorylation may be dependent on or independent of each other and can contribute to the action of $(m\text{–}CF_3\text{–}PhSe)_2$ in this model of depression induced by stress.

Although the ERK phosphorylation seems not to be directly related to resilience to SDS in this study, this protein plays a role in long-term adaptive responses to opioids, and the decrease in the ERK expression is reported in the cerebral cortex of depressive-like mice and depressed suicide victims (Dwivedi et al., 2001; Qi et al., 2006). Given that $(m\text{–}CF_3\text{–}PhSe)_2$ was effective against the decrease of ERK phosphorylation induced by SDS, the modulation of ERK levels can indirectly contribute to this compound antidepressant-like action.

The present study also investigated the SDS effect on the p38 MAPK content, one of the main MAPK involved in cellular and behavioral responses to stress (Lemos et al., 2012). In fact, it has been reported that p38 MAPK is activated during the stressful events (Bruchas et al., 2007; Bruchas et al., 2011; Su et al., 2017). In addition, an increase in p38 MAPK phosphorylation is associated to social avoidance caused by SDS and can be prevented by blocking opioid receptors (Bruchas et al., 2011). However, in the present study, no change in p38 MAPK content was observed in the prefrontal cortex of susceptible defeated mice. A possible explanation for this divergent results is that there is, at least, four isoforms of p38 MAPK, α, βγ and δ, which show different substrate specificities, tissue distribution (Wang et al., 1997) and function in stress signaling (Huwiler et al., 2000). The deletion of p38α and lack of compensation by p38β has been demonstrated by causing significant behavioral effects in models of stress-induced depression (Bruchas et al., 2011; Lemos et al., 2012). In the current study, the antibody used to detect p38 MAPK has no specificity for the isoforms of this protein, which may have made difficult to observe the well-known effect of stress on p38 MAPK activation. In addition, a compensatory decrease of any p38 isoform in response to increase of p38α induced by stress could be regulating the prefrontal cortical p38 MAPK levels in susceptible defeated mice. This way, the intriguing increase of the prefrontal cortical p38 phosphorylation of resilient defeat mice could indicate compensatory effects to stress or even a deregulation of other p38 MAPK isoforms, less involved in this type of stress. However, the absence of validation of this hypothesis is a weak point of our study.

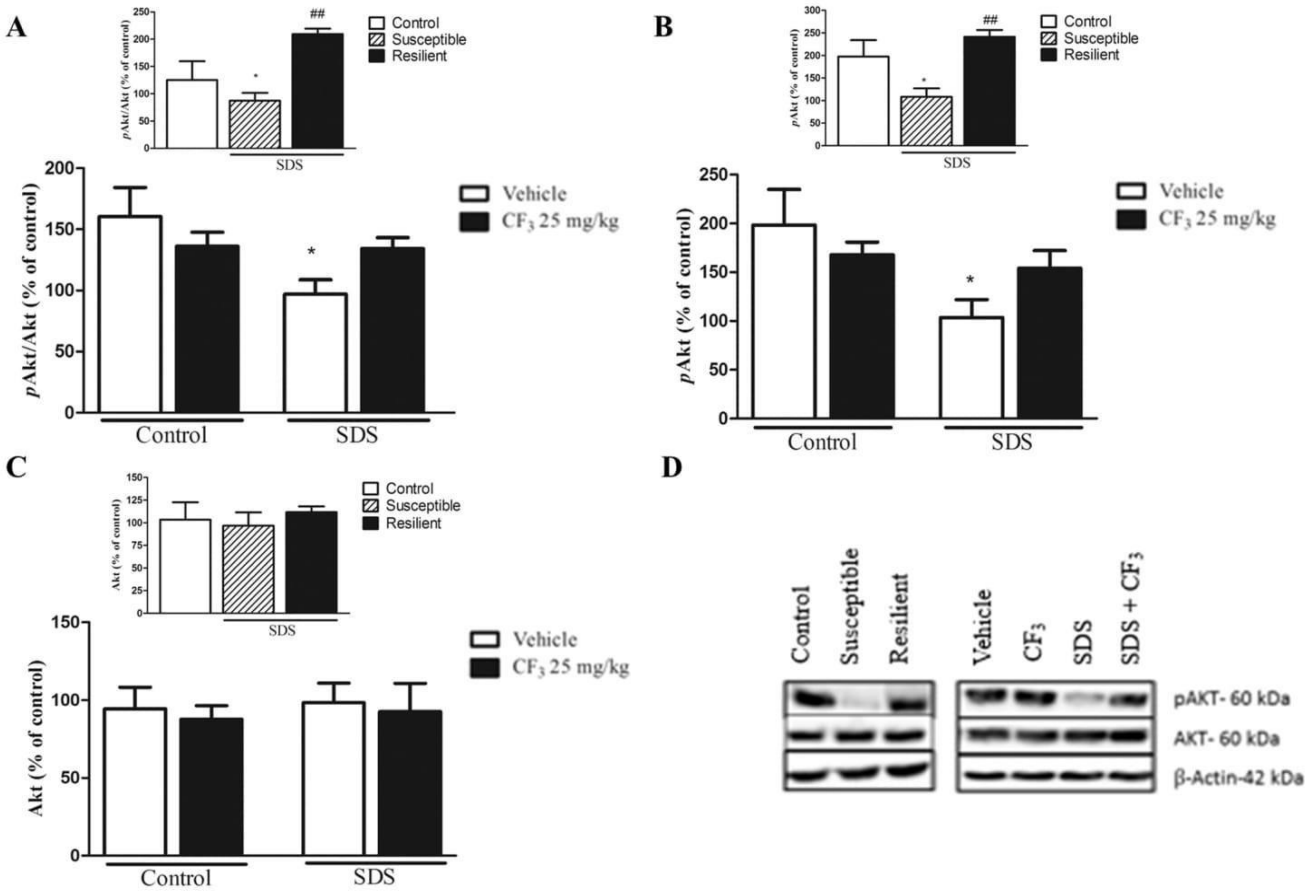


Fig. 10. (m–CF_3–PhSe)$_2$ treatment is partially effective against the decrease of Akt phosphorylation induced by SDS in mice. Effect of (m–CF_3–PhSe)$_2$ treatment (25 mg/kg, i.g) on *p*Akt/Akt ratio (A), *p*Akt (B), and Akt (C) levels in the prefrontal cortices of mice subjected to the SDS. Inserts show the *p*Akt/Akt ratio (A), *p*Akt (B), and Akt (C) levels in the prefrontal cortices of susceptible and resilient mice. Absolute values expressed as optical density (OD): control group: 8,757,000 ± 4,102,000 (*p*Akt); 16,840,000 ± 765,700 (Akt). Values are expressed as mean ± S.E.M. of 7 animals/group. Asterisk denotes the significance levels when compared to the control group: (*) $P < 0.05$. Hashtag denotes the significance levels when compared to the susceptible group: ($^{\#\#}$) $P < 0.01$ (One-way or two-way ANOVA followed by the Newman Keuls test). Photographs are representation of qualitative Western blotting analysis (D). CF_3: (m-CF_3-PhSe)$_2$; SDS: social defeat stress.

Krishnan et al. (2008) demonstrated that decreased Akt phosphorylation is a crucial mediator of stress vulnerability to develop depressive behaviors in both mouse and rat models. In addition, postmortem studies of cortical areas from depressed humans have also reported reductions in the Akt function (Hsiung et al., 2003). In agreement with these studies, our results demonstrated that (m–CF_3–PhSe)$_2$ was effective against the reduction of Akt phosphorylation induced by SDS. Similar content of *p*Akt and *p*akt/Akt ratio was found in defeated mice treated with (m–CF_3–PhSe)$_2$ and defeated resilient mice, confirming the important role of this protein to social stress susceptibility and indicating that Akt can be a mediator of resilience to SDS induced by (m–CF_3–PhSe)$_2$.

Researchers have found evidence that stress, particularly the stress of social defeat, induces symptoms similar to those found in depressive patients, such as social avoidance, which are associated with modulation of μ, κ and δ opioid receptors (Iniguez et al., 2014; McLaughlin et al., 2006; Nikulina et al., 2008). The opioid receptors and other G protein-coupled receptors have been shown to activate MAPKs, which play an important role in the regulation of stress responses and depression (Kim and Choi, 2010; Ogura and Egan, 2013). Therefore, we hypothesize that modifications in the contents of opioid receptors induced by SDS could modulate the activation of MAPKs and, consequently, lead to social avoidance behavior. Although the present results do not support a direct relationship between behavioral and molecular effects of SDS, our results suggest that social avoidance induced by SDS is associated, at least in part, with alterations in the levels of opioid receptors and MAPKs in the prefrontal cortices of stressed mice. By a combination of molecular and behavioral tests, we show that (m–CF_3–PhSe)$_2$-treated stressed mice have a similar profile to the resilient mice.

5. Conclusion

In conclusion, the present results demonstrate that (m–CF_3–PhSe)$_2$ treatment promotes resilience to social avoidance induced by SDS and induces an increase in natural social interaction between mice. The molecular findings suggest that this effect is mediated through modulation of opioid system and regulation of prefrontal cortical JNK and Akt protein contents in mice. Thus, this study contributes to better understand the basis of (m-CF_3-PhSe)$_2$ antidepressant-like action in a depression model and indicates that this multitarget compound could become an interesting approach to treat depressive disorders.

Conflict of interest

The authors declare that there is no conflict of interest in the present study.

Acknowledgments

We gratefully acknowledge CNPq (441405/2014-2) for the financial support. C.W·N is recipient of CNPq fellowship.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.pnpbp.2017.11.021.

References

Al-Hasani, R., Bruchas, M.R., 2011. Molecular mechanisms of opioid receptor-dependent signaling and behavior. Anesthesiology 115, 1363–1381.
Bartolomucci, A., Palanza, P., Gaspani, L., Limiroli, E., Panerai, A.E., Ceresini, G., Poli, M.D., Parmigiani, S., 2001. Social status in mice: behavioral, endocrine and immune changes are context dependent. Physiol. Behav. 73, 401–410.
Berton, O., McClung, C.A., Dileone, R.J., Krishnan, V., Renthal, W., Russo, S.J., Graham, D., Tsankova, N.M., Bolanos, C.A., Rios, M., Monteggia, L.M., Self, D.W., Nestler, E.J., 2006. Essential role of BDNF in the mesolimbic dopamine pathway in social defeat stress. Science 311, 864–868.
Berube, P., Laforest, S., Bhatnagar, S., Drolet, G., 2013. Enkephalin and dynorphin mRNA expression are associated with resilience or vulnerability to chronic social defeat stress. Physiol. Behav. 122, 237–245.
Bérubé, P., Laforest, S., Bhatnagar, S., Drolet, G., 2013. Enkephalin and dynorphin mRNA expression are associated with resilience or vulnerability to chronic social defeat stress. Physiol. Behav. 122, 237–245.
Berube, P., Poulin, J.F., Laforest, S., Drolet, G., 2014. Enkephalin knockdown in the basolateral amygdala reproduces vulnerable anxiety-like responses to chronic unpredictable stress. Neuropsychopharmacology 39, 1159–1168.
Bjorkqvist, K., 2001. Social defeat as a stressor in humans. Physiol. Behav. 73, 435–442.
Bonanno, G.A., 2004. Loss, trauma, and human resilience: have we underestimated the human capacity to thrive after extremely aversive events? Am Psychol 59, 20–28.
Bosch, O.G., Seifritz, E., Wetter, T.C., 2012. Stress-related depression: neuroendocrine, genetic, and therapeutical aspects. World J Biol Psychiatry 13, 556–568.
Bradford, M.M., 1976. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal. Biochem. 72, 248–254.
Bruchas, M.R., Land, B.B., Aita, M., Xu, M., Barot, S.K., Li, S., Chavkin, C., 2007. Stress-induced p38 mitogen-activated protein kinase activation mediates kappa-opioid-dependent dysphoria. J. Neurosci. 27, 11614–11623.
Bruchas, M.R., Schindler, A.G., Shankar, H., Messinger, D.I., Miyatake, M., Land, B.B., Lemos, J.C., Hagan, C.E., Neumaier, J.F., Quintana, A., Palmiter, R.D., Chavkin, C., 2011. Selective p38alpha MAPK deletion in serotonergic neurons produces stress resilience in models of depression and addiction. Neuron 71, 498–511.
Bruning, C.A., Prigol, M., Roehrs, J.A., Nogueira, C.W., Zeni, G., 2009. Involvement of the serotonergic system in the anxiolytic-like effect caused by m-trifluoromethyl-diphenyl diselenide in mice. Behav. Brain Res. 205, 511–517.
Bruning, C.A., Prigol, M., Roehrs, J.A., Zeni, G., Nogueira, C.W., 2010. Evidence for the involvement of mu-opioid and delta-opioid receptors in the antinociceptive effect caused by oral administration of m-trifluoromethyl-diphenyl diselenide in mice. Behav. Pharmacol. 21, 621–626.
Bruning, C.A., Souza, A.C., Gai, B.M., Zeni, G., Nogueira, C.W., 2011. Antidepressant-like effect of m-trifluoromethyl-diphenyl diselenide in the mouse forced swimming test involves opioid and serotonergic systems. Eur. J. Pharmacol. 658, 145–149.
Bruning, C.A., Prigol, M., Luchese, C., Pinton, S., Nogueira, C.W., 2012. Diphenyl diselenide ameliorates behavioral and oxidative parameters in an animal model of mania induced by ouabain. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 38, 168–174.
Bruning, C.A., Martini, F., Soares, S.M., Sampaio, T.B., Gai, B.M., Duarte, M.M., Nogueira, C.W., 2015a. m-Trifluoromethyl-diphenyl diselenide, a multi-target selenium compound, prevented mechanical allodynia and depressive-like behavior in a mouse comorbid pain and depression model. Prog. Neuro-Psychopharmacol. Biol. Psychiatry 63, 35–46.
Bruning, C.A., Martini, F., Soares, S.M., Savegnago, L., Sampaio, T.B., Nogueira, C.W., 2015b. Depressive-like behavior induced by tumor necrosis factor-alpha is attenuated by m-trifluoromethyl-diphenyl diselenide in mice. J. Psychiatr. Res. 66-67, 75–83.
Cabib, S., Campus, P., Colelli, V., 2012. Learning to cope with stress: psychobiological mechanisms of stress resilience. Rev. Neurosci. 23, 659–672.
Dwivedi, Y., Rizavi, H.S., Roberts, R.C., Conley, R.C., Tamminga, C.A., Pandey, G.N., 2001. Reduced activation and expression of ERK1/2 MAP kinase in the post-mortem brain of depressed suicide subjects. J. Neurochem. 77, 916–928.
Goeldner, C., Lutz, P.E., Darcq, E., Halter, T., Clesse, D., Ouagazzal, A.M., Kieffer, B.L., 2011. Impaired emotional-like behavior and serotonergic function during protracted abstinence from chronic morphine. Biol. Psychiatry 69, 236–244.
Golden, S.A., Covington III, H.E., Berton, O., Russo, S.J., 2011. A standardized protocol for repeated social defeat stress in mice. Nat. Protoc. 6, 1183–1191.
Hammen, C., 2005. Stress and depression. Annu. Rev. Clin. Psychol. 1, 293–319.
Hsiung, S.C., Adlersberg, M., Arango, V., Mann, J.J., Tamir, H., Liu, K.P., 2003. Attenuated 5-HT1A receptor signaling in brains of suicide victims: involvement of adenylyl cyclase, phosphatidylinositol 3-kinase, Akt and mitogen-activated protein kinase. J. Neurochem. 87, 182–194.
Huwiler, A., Wartmann, M., van den Bosch, H., Pfeilschifter, J., 2000. Extracellular nucleotides activate the p38-stress-activated protein kinase cascade in glomerular mesangial cells. Br. J. Pharmacol. 129, 612–618.
Iio, W., Matsukawa, N., Tsukahara, T., Kohari, D., Toyoda, A., 2011. Effects of chronic social defeat stress on MAP kinase cascade. Neurosci. Lett. 504, 281–284.
Iniguez, S.D., Riggs, L.M., Nieto, S.J., Dayrit, G., Zamora, N.N., Shawhan, K.L., Cruz, B., Warren, B.L., 2014. Social defeat stress induces a depression-like phenotype in adolescent male c57BL/6 mice. Stress 17, 247–255.
Javelot, H., Messaoudi, M., Garnier, S., Rougeot, C., 2010. Human opiorphin is a naturally occurring antidepressant acting selectively on enkephalin-dependent delta-opioid pathways. J. Physiol. Pharmacol. 61, 355–362.
Johnson, G.L., Nakamura, K., 2007. The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. Biochim. Biophys. Acta 1773, 1341–1348.
Jutkiewicz, E.M., 2013. S. 7.1-the role of the delta-opioid receptor in regulating mood and affective states. Behav. Pharmacol. 24, e8.
Kim, E.K., Choi, E.-J., 2010. Pathological roles of MAPK signaling pathways in human diseases. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease 1802, 396–405.
Komatsu, H., Ohara, A., Sasaki, K., Abe, H., Hattori, H., Hall, F.S., Uhl, G.R., Sora, I., 2011. Decreased response to social defeat stress in mu-opioid-receptor knockout mice. Pharmacol. Biochem. Behav. 99, 676–682.
Krishnan, V., Han, M.H., Graham, D.L., Berton, O., Renthal, W., Russo, S.J., Laplant, Q., Graham, A., Lutter, M., Lagace, D.C., Ghose, S., Reister, R., Tannous, P., Green, T.A., Neve, R.L., Chakravarty, S., Kumar, A., Eisch, A.J., Self, D.W., Lee, F.S., Tamminga, C.A., Cooper, D.C., Gershenfeld, H.K., Nestler, E.J., 2007. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. Cell 131, 391–404.
Krishnan, V., Han, M.H., Mazei-Robison, M., Iniguez, S.D., Ables, J.L., Vialou, V., Berton, O., Ghose, S., Covington III, H.E., Wiley, M.D., Henderson, R.P., Neve, R.L., Eisch, A.J., Tamminga, C.A., Russo, S.J., Bolanos, C.A., Nestler, E.J., 2008. AKT signaling within the ventral tegmental area regulates cellular and behavioral responses to stressful stimuli. Biol. Psychiatry 64, 691–700.
Lemos, J.C., Roth, C.A., Messinger, D.I., Gill, H.K., Phillips, P.E., Chavkin, C., 2012. Repeated stress dysregulates kappa-opioid receptor signaling in the dorsal raphe through a p38alpha MAPK-dependent mechanism. J. Neurosci. 32, 12325–12336.
Liu, Y.F., Bertram, K., Perides, G., McEwen, B.S., Wang, D., 2004. Stress induces activation of stress-activated kinases in the mouse brain. J. Neurochem. 89, 1034–1043.
Lutz, P.E., Kieffer, B.L., 2013. Opioid receptors: distinct roles in mood disorders. Trends Neurosci. 36, 195–206.
Machado, M.S., Rosa, R.M., Dantas, A.S., Reolon, G.K., Appelt, H.R., Braga, A.L., Henriques, J.A., Roesler, R., 2006. An organic selenium compound attenuates apomorphine-induced stereotypy in mice. Neurosci. Lett. 410, 198–202.
Mansour, A., Fox, C.A., Akil, H., Watson, S.J., 1995. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. Trends Neurosci. 18, 22–29.
McLaughlin, J.P., Li, S., Valdez, J., Chavkin, T.A., Chavkin, C., 2006. Social defeat stress-induced behavioral responses are mediated by the endogenous kappa opioid system. Neuropsychopharmacology 31, 1241–1248.
Melief, E.J., Miyatake, M., Bruchas, M.R., Chavkin, C., 2010. Ligand-directed c-Jun N-terminal kinase activation disrupts opioid receptor signaling. Proc. Natl. Acad. Sci. U. S. A. 107, 11608–11613.
Miyoshi, K., Morimura, Y., 2010. Clinical Manifestations of Neuropsychiatric Disorders, Neuropsychiatric Disorders. Springer, pp. 1–14.
Naidu, P.S., Lichtman, A.H., Archer, C.C., May, E.L., Harris, L.S., Aceto, M.D., 2007. NIH 11082 produces anti-depressant-like activity in the mouse tail-suspension test through a delta-opioid receptor mechanism of action. Eur. J. Pharmacol. 566, 132–136.
Nikulina, E.M., Miczek, K.A., Hammer Jr., R.P., 2005. Prolonged effects of repeated social defeat stress on mRNA expression and function of mu-opioid receptors in the ventral tegmental area of rats. Neuropsychopharmacology 30, 1096–1103.
Nikulina, E.M., Arrillaga-Romany, I., Miczek, K.A., Hammer Jr., R.P., 2008. Long-lasting alteration in mesocorticolimbic structures after repeated social defeat stress in rats: time course of mu-opioid receptor mRNA and FosB/DeltaFosB immunoreactivity. Eur. J. Neurosci. 27, 2272–2284.
Nozaki, C., Nagase, H., Nemoto, T., Matifas, A., Kieffer, B.L., Gaveriaux-Ruff, C., 2014. In vivo properties of KNT-127, a novel delta opioid receptor agonist: receptor internalization, antihyperalgesia and antidepressant effects in mice. Br. J. Pharmacol. 171, 5376–5386.
Ogura, T., Egan, T.D., 2013. Chapter 15 - Opioid Agonists and Antagonists, Pharmacology and Physiology for Anesthesia. W.B. Saunders, Philadelphia, pp. 253–271.
Paulmier, C., 1986. Selenoorganic functional groups. In: Selenium Reagents and Intermediates in Organic Synthesis. vol. 1. pp. 25–51.
Qi, X., Lin, W., Li, J., Pan, Y., Wang, W., 2006. The depressive-like behaviors are correlated with decreased phosphorylation of mitogen-activated protein kinases in rat brain following chronic forced swim stress. Behav. Brain Res. 175, 233–240.
Richards, E.M., Mathews, D.C., Luckenbaugh, D.A., Ionescu, D.F., Machado-Vieira, R., Niciu, M.J., Duncan, W.C., Nolan, N.M., Franco-Chaves, J.A., Hudzik, T., Maciag, C., Li, S., Cross, A., Smith, M.A., Zarate Jr., C.A., 2016. A randomized, placebo-controlled pilot trial of the delta opioid receptor agonist AZD2327 in anxious depression. Psychopharmacology 233, 1119–1130.
Robinson, G.E., Grozinger, C.M., Whitfield, C.W., 2005. Sociogenomics: social life in molecular terms. Nat. Rev. Genet. 6, 257–270.
Rosa, S.G., Pesarico, A.P., Tagliapietra, C.F., da Luz, S.C.A., Nogueira, C.W., 2017. Opioid system contribution to the antidepressant-like action of m-trifluoromethyl-diphenyl diselenide in mice: A compound devoid of tolerance and withdrawal syndrome. J. Psychopharmacol. 269881117724353.
Russo, S.J., Murrough, J.W., Han, M.H., Charney, D.S., Nestler, E.J., 2012. Neurobiology

of resilience. Nat. Neurosci. 15, 1475–1484.
Salavecz, G., Stauder, A., Purebl, G., 2014. Work Related Stress and Depression.
Smith, J.S., Schindler, A.G., Martinelli, E., Gustin, R.M., Bruchas, M.R., Chavkin, C., 2012. Stress-induced activation of the dynorphin/kappa-opioid receptor system in the amygdala potentiates nicotine conditioned place preference. J. Neurosci. 32, 1488–1495.
Su, W.-J., Zhang, Y., Chen, Y., Gong, H., Lian, Y.-J., Peng, W., Liu, Y.-Z., Wang, Y.-X., You, Z.-L., Feng, S.-J., 2017. NLRP3 gene knockout blocks NF-κB and MAPK signaling pathway in CUMS-induced depression mouse model. Behav. Brain Res. 322, 1–8.
Wang, X.S., Diener, K., Manthey, C.L., Wang, S., Rosenzweig, B., Bray, J., Delaney, J., Cole, C.N., Chan-Hui, P.Y., Mantlo, N., Lichenstein, H.S., Zukowski, M., Yao, Z., 1997. Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. J. Biol. Chem. 272, 23668–23674.
Yin, Y.Q., Zhang, C., Wang, J.X., Hou, J., Yang, X., Qin, J., 2015. Chronic caffeine treatment enhances the resilience to social defeat stress in mice. Food Funct. 6, 479–491.

Supplementary Material

Fig 1S.

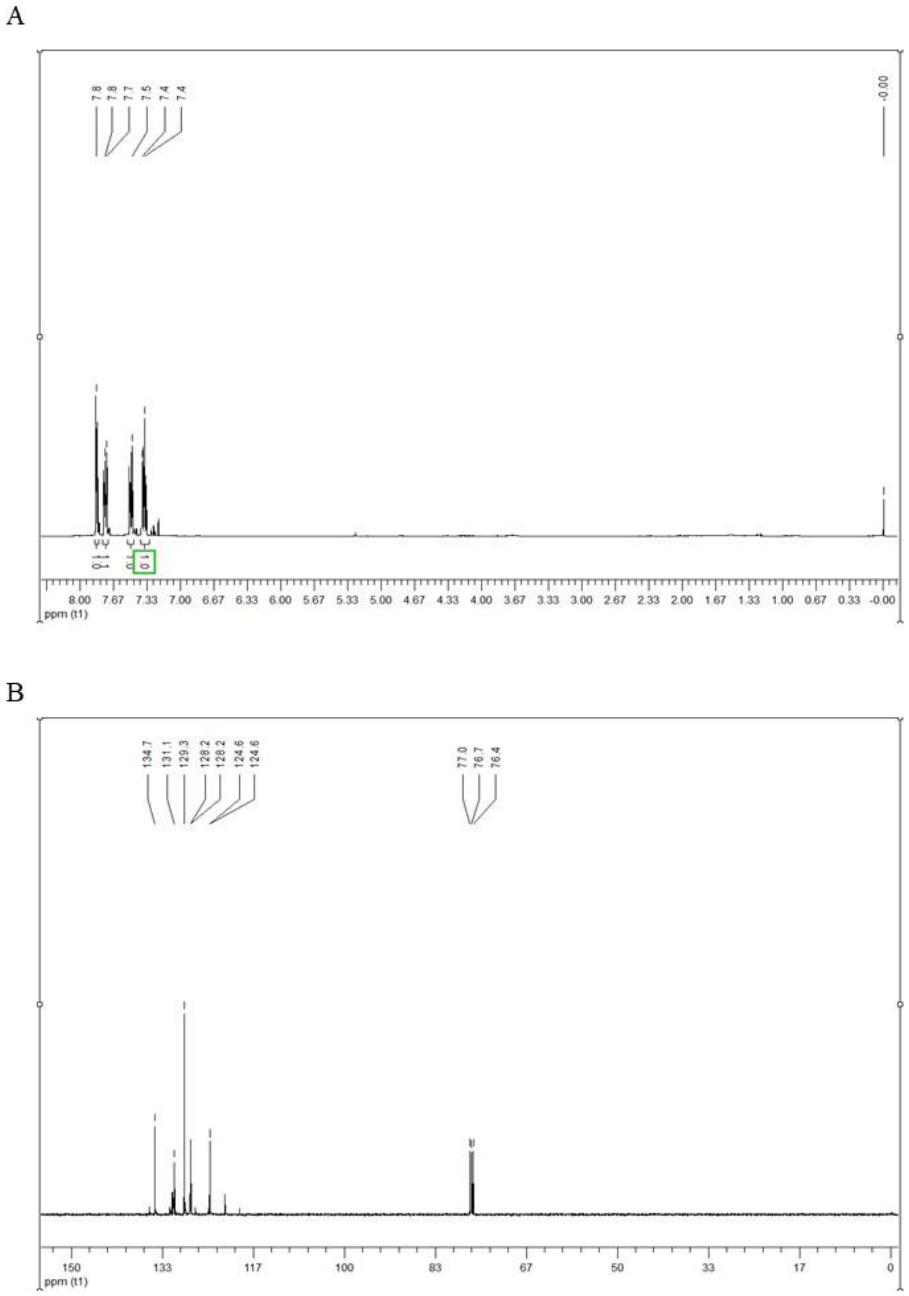


Fig 1S. ^{1}H NMR (A) and ^{13}C NMR (B) spectra of *m*-trifluoromethyl-diphenyl diselenide (400 MHz, CDCl3).

4 DISCUSSÃO

A depressão é uma doença heterogênea e com patofisiologia complexa, a qual se manifesta em múltiplos sintomas fisiológicos, comportamentais e psicológicos, o que dificulta o tratamento desta doença (SAVEANU e NEMEROFF, 2012). Desta forma, a propriedade multi-alvo de moléculas tem sido considerada uma abordagem apropriada na pesquisa e desenvolvimento de novos fármacos para a prevenção e tratamento da depressão. Além disso, o uso da monoterapia para o tratamento simultâneo dos diferentes sintomas depressivos é importante para aumentar a adesão ao tratamento, a segurança terapêutica e reduzir os efeitos adversos (MILLAN, 2014).

Neste sentido, além dos diferentes efeitos farmacológicos do composto orgânico de selênio (*m*-CF_3-PhSe)$_2$, como ansiolítico, antinociceptivo e do tipo antidepressivo, estudos têm demonstrado que este composto possui múltiplos alvos de ação (BRUNING et al., 2015a).

O efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ foi demonstrado, pela primeira vez, no TNF (BRUNING et al., 2011). Neste estudo, antagonistas de receptores serotoninérgicos WAY100635 (antagonista de receptores 5-HT_{1A}), ritanserina (antagonista de receptores 5-$HT_{2A/2C}$) e ondansetrona (antagonista de receptores 5-HT_3) bloquearam o efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ no TNF, evidenciando o envolvimento do sistema serotoninérgico neste efeito do composto. Além disso, a administração de naloxona também bloqueou o efeito do (*m*-CF_3-PhSe)$_2$ em reduzir o tempo de imobilidade dos animais no TNF, demonstrando que além do sistema serotoninérgico, o sistema opioide também está envolvido no mecanismo antidepressivo deste composto (BRUNING et al., 2011).

A contribuição do sistema serotoninérgico para ações farmacológicas do (*m*-CF_3- PhSe)$_2$ também já foi reportada em outros estudos, os quais caracterizaram os efeitos deste composto sobre diferentes pontos da transmissão serotoninérgica. Um estudo *in vitro* demonstrou que o (*m*-CF_3-PhSe)$_2$ inibe a recaptação de 5-HT em sinaptossomas de ratos (BORGES et al., 2009). Somado a isso, este composto apresenta efeito ansiolítico *per se* associado à modulação de receptores serotoninérgicos 5-HT_{1A}, 5-$HT_{2A/2C}$ e 5-HT_3, e inibição da atividade da MAO-A *ex vivo* em camundongos (BRUNING et al., 2009). Em modelos experimentais de depressão, a administração aguda e subcrônica do (*m*-CF_3-PhSe)$_2$ reduziu a captação de 5-HT alterada pela constrição parcial do nervo ciático (BRUNING et al., 2015a).

Posteriormente, BRUNING et al. (2015b) expandiram os conhecimentos sobre o efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ por meio de um estudo no qual o tratamento agudo

e subcrônico com o composto foi efetivo em reduzir comportamentos do tipo depressivo induzidos por TNF-α, por meio da regulação de proteínas inflamatórias como o fator nuclear kB (NFkB) e a proteína quinase ativada por mitógeno p38 (p38 MAPK). Destacando assim, a contribuição da propriedade anti-inflamatória para o efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$. Com os resultados obtidos nestes estudos foram elucidados os mecanismos serotoninérgicos e anti-inflamatórios que medeiam o efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$. No entanto, apesar do fato de ter sido demonstrado que a naloxona bloqueia a ação do tipo antidepressiva do (*m*-CF_3-PhSe)$_2$, não havia outra evidência da contribuição dos receptores opioides para esse efeito. Deste modo, o principal objetivo desta tese foi investigar o envolvimento de cada receptor opioide na ação do tipo antidepressiva do (*m*-CF_3-PhSe)$_2$.

Os resultados apresentados no **artigo 1** primeiramente estenderam os estudos sobre o efeito deste composto no TNF e TSC, investigando seu perfil farmacológico em diferentes tempos de pré-administração e doses, bem como o efeito do tipo antidepressivo da administração aguda e repetida do (*m*-CF_3-PhSe)$_2$ e seus possíveis efeitos toxicológicos. Neste estudo, a dose de 50 mg/kg do composto foi escolhida para a realização de uma curva de tempo no TNF baseado em dados prévios que demonstraram que esta é a menor dose efetiva do (*m*-CF_3-PhSe)$_2$ neste teste, quando administrado de forma aguda (BRUNING et al., 2011). Desta forma, o **artigo 1** revelou pela primeira vez o efeito prolongado (até 24 horas) de uma única administração do (*m*-CF_3-PhSe)$_2$ no TNF. Além disso, a contribuição de cada receptor opioide para o efeito do tipo antidepressivo do composto foi farmacologicamente demonstrada, por meio do uso de antagonistas seletivos destes receptores. O bloqueio do efeito do (*m*-CF_3-PhSe)$_2$ no TNF pelos antagonistas de receptores μ e δ (naloxonazina e naltrindole, respectivamente) e o efeito sinérgico entre o (*m*-CF_3-PhSe)$_2$ e o antagonista de receptores κ (nor-binaltorfimina) confirmaram o envolvimento do sistema opioide no efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ e demonstraram a contribuição de cada receptor opioide para este efeito. O fato da ativação dos receptores μ e δ e o bloqueio de receptores κ opioides estarem associados ao efeito do tipo antidepressivo do composto está de acordo com vários estudos que demonstram o efeito antidepressivo de fármacos que atuam diretamente no sistema opioide, por meio da ligação com os receptores opioides, como a buprenorfina, agonista parcial de receptores μ e antagonista de receptores κ, tianeptina, agonista de receptores μ, e agonistas de receptores δ, como o SNC 80 (DRIPPS, I. J. et al., 2018; GASSAWAY et al., 2014; KARP et al., 2014). Além disso, a ativação dos receptores μ e δ e/ou o bloqueio de receptores κ contribui indiretamente para o efeito do tipo antidepressivo de fármacos como a agmatina, a adenosina, o ácido fólico e a

fluoxetina (BROCARDO et al., 2009; HAJ-MIRZAIAN et al.,2016; KASTER et al., 2007; ZOMKOWSKI et al., 2005). Estas evidências também corroboram um estudo prévio do nosso grupo de pesquisa que demonstra o envolvimento dos receptores μ e δ, e descarta a contribuição do receptor κ, no efeito antinociceptivo do (*m*- CF_3-PhSe)$_2$ (BRUNING et al., 2010). De fato, o receptor κ pode estar associado somente ao efeito do tipo antidepressivo do composto, porém mais estudos seriam necessários para confirmar se o receptor κ não está envolvido na ação antinociceptiva do (*m*-CF_3-PhSe)$_2$, principalmente, considerando-se o fato de que apenas uma dose do antagonista destes receptores foi testada.

Em conjunto, os resultados obtidos com ferramentas farmacológicas descritos no **artigo 1** são relevantes por representarem os primeiros passos para a compreensão da contribuição de cada receptor opioide para o efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$. No entanto, eles não nos permitem afirmar se o efeito do composto sobre estes receptores é direto ou indireto, uma vez que o ensaio de ligação específica com estes receptores não foi realizado, uma limitação deste estudo. Desta forma, algumas hipóteses sobre os efeitos do (*m*- CF_3-PhSe)$_2$ no sistema opioide podem ser formuladas: (I) o composto pode se ligar aos receptores opioides, agindo como um agonista, no caso dos receptores μ e δ, ou antagonista, no caso de receptores κ; (II) o (*m*-CF_3-PhSe)$_2$ pode ter efeitos indiretos nestes receptores agindo sobre a liberação de opioides endógenos, ou ainda, levando em conta os múltiplos alvos biológicos do composto, seu efeito sobre o sistema opioide pode ser indireto e o resultado da interação entre sistemas neurotransmissores, como por exemplo, opioide e serotoninérgico (III). A possibilidade de que o (*m*-CF_3-PhSe)$_2$ atue de acordo com mais de uma destas hipóteses também não pode ser descartada, uma vez existem diversos relatos na literatura de compostos antidepressivos que agem em diferentes sistemas e receptores de modo cooperativo. Como é o caso da tianeptina, um antidepressivo com ações mediadas indiretamente pelos sistemas serotoninérgico e glutamatérgico e com alta afinidade por receptores opioides μ, ou a fluoxetina, inibidor da recaptação de serotonina com ação indireta sobre os receptores de NMDA e opioides (GASSAWAY et al., 2014; HAJ-MIRZAIAN et al., 2016. Além disso, uma ação agonista direta em diferentes receptores opioides tem sido demonstrada com antidepressivos tricíclicos (ONALI et al., 2010).

Ainda no **artigo 1** o envolvimento do sistema opioide no efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ foi investigado em outro paradigma comportamental, o TSC modificado. Este teste foi previamente validado por BERROCOSO et al. (2013) para o *screening* da atividade do tipo antidepressiva de compostos que modulam a neurotransmissão opioide ou

monoaminérgica. Os resultados do **artigo 1** demonstraram que o (*m*-CF_3-PhSe)$_2$ aumentou o comportamento ondulatório dos animais, assim como a morfina, utilizada como controle positivo neste estudo, e como outros fármacos opioides (BERROCOSO et al., 2013). Além disso, este estudo evidenciou a eficácia antidepressiva do tratamento repetido com o (*m*-CF_3-PhSe)$_2$ a partir de baixas doses e o envolvimento do sistema opioide também no efeito repetido do composto no TSC modificado, um importante resultado, tendo em vista a necessidade de tratamento prolongado para transtornos depressivos.

Tendo em vista o mecanismo de ação opioide do (*m*-CF_3-PhSe)$_2$ e a grande limitação do uso de opioides na clínica, qual seja, o desenvolvimento de tolerância e os sintomas de abstinência, o próximo passo do **artigo 1** foi avaliar se o tratamento repetido com o (*m*-CF_3-PhSe)$_2$ apresenta estas limitações. Para isso, um modelo de indução de tolerância aos efeitos antinociceptivos da morfina foi adaptado para avaliação do comportamento do tipo depressivo no teste do nado forçado (ABDEL-ZAHER et al., 2010). Os resultados deste estudo revelaram o desenvolvimento de tolerância à morfina a partir do quinto dia de tratamento, por outro lado, quando o (*m*-CF_3-PhSe)$_2$ foi administrado aos camundongos nas mesmas doses e protocolo de tratamento, não houve indução de tolerância ao efeito do tipo antidepressivo. Além disso, a falta de efeito da primeira administração do composto, em uma baixa dose, confirma que a ação do tipo antidepressiva do tratamento agudo com este composto ocorre somente em doses altas (BRUNING et al., 2011) e que, no entanto, em um tratamento repetido o (*m*-CF_3-PhSe)$_2$ diminui a imobilidade dos animais no TNF em menores doses.

A administração de naloxona a animais tratados repetidamente com morfina mimetiza a retirada abrupta de opioides e induz, rapidamente, ao aparecimento dos sinais físicos de abstinência (ABDEL-ZAHER et al., 2010; HOOSHMANDI et al., 2017), como os observados neste estudo, a saber, elevações, pulos, ranger dos dentes, tremor das patas e diarreia. Os comportamentos observados nos animais correlacionam-se com os sintomas de abstinência, tremores, inquietação e nervosismo, encontrados em pacientes (KRANTZ e MEHLER, 2004). Muitos estudos demonstram que o bloqueio de receptores opioides e a consequente diminuição dos efeitos inibitórios destes leva a uma maior atividade noradrenérgica e aumenta a sensibilidade de receptores adrenérgicos, ampliando os efeitos autonômicos que caracterizam a síndrome de abstinência (CHAIJALE et al., 2013). No entanto, o **artigo 1** demonstrou que diferentemente dos animais tratados com a morfina, a administração de naloxona não induziu sinais físicos de abstinência em animais tratados com o (*m*-CF_3-PhSe)$_2$. A ausência destes sinais pode estar relacionada com o fato de que a captação e a liberação de glutamato em córtex e hipocampo,

regiões importantes para a regulação do humor (LIU, W. et al., 2017a), permaneceu inalterada após a retirada induzida por naloxona em camundongostratados com o (*m*-CF_3-PhSe)$_2$, o que pode indicar uma transmissão glutamatérgica normal. Por outro lado, uma diminuição da captação de glutamato associada a um aumento na liberação deste neurotransmissor excitatório, possivelmente, contribuiu para o aumento de sinais de abstinência em animais tratados com morfina, uma vez que a excitabilidade neuronal decorrente do bloqueio dos efeitos inibitórios de opioides está relacionada com o aumento da transmissão noradrenérgica e dos sinais físicos de abstinência em roedores, bem como o aumento da captação de glutamato por meio da administração de um ativador de transportadores de glutamato está relacionada com uma diminuição da tolerância e dependência em roedores (NAKAGAWA et al., 2001). Ainda, o fato da naloxona não induzir sinais de abstinência em camundongos tratados com o (*m*-CF_3-PhSe)$_2$ pode ser um indicativo de que a ativação de receptores μ e δ ocorre de forma indireta e não pela ligação do (*m*-CF_3- PhSe)$_2$ a estes receptores. No entanto, mesmo fármacos que se ligam aos receptores opioides podem apresentar menores ou nenhum efeito de tolerância e retirada, sugerindo que a sinalização *downstream* dos receptores opioides está envolvida no desenvolvimento de tolerância e dependência aos opioides (SAMUELS et al., 2017; TOMPKINS et al., 2014).

Neste contexto, a noção de "agonismo tendencioso" ou "seletividade funcional" tem emergido, revelando que a natureza do GPCR sinalização não é tão rígida e que diferentes ligantes que atuam o mesmo sítio alostérico de um receptor podem estabilizar distintas conformações ativas que sinalizam preferencialmente subtipos de proteína G ou arrestinas envolvendo respostas de sinalização por diferentes vias (WHALEN et al., 2011). Dessa forma, o "agonismo tendencioso" poderia separar os efeitos terapêuticos e adversos, orientando assim os estímulos para respostas desejadas (GUI e WONG, 2018). No entanto, nossos resultados são bastante preliminares no que diz respeito aos efeitos do (*m*-CF_3-PhSe)$_2$ no desenvolvimento de tolerância e dependência. Desta forma, além do ensaio de ligação específica aos receptores opioides, o estudo de cascatas de sinalização decorrentes da ativação de receptores opioides e outros mecanismos de tolerância e dependência, como a dessensibilização, desacoplamento e internalização de receptores, após a administração do (*m*- CF_3-PhSe)$_2$ são pontos que ainda precisam ser elucidados para a compreensão dos mecanismos pelos quais este composto não induz tolerância e sinais físicos de abstinência.

Embora muitos estudos tenham reportado os efeitos farmacológicos do (*m*-CF_3-PhSe)$_2$ (BRUNING et al., 2014; BRUNING et al., 2015a; BRUNING et al., 2015b; MAGNI et al.,

2012; PRIGOL et al., 2009), as propriedades toxicológicas deste composto têm sido pouco exploradas, principalmente, após administração repetida. Dados anteriores demonstram que o *m*-CF_3-PhSe)$_2$ apresenta baixa toxicidade em camundongos em doses agudas, com uma DL50 (dose letal em 50% dos animais) de 278 mg/kg, sem alteração de parâmetros bioquímicos, tais como a alanina aminotransferase (ALT), a aspartato aminotransferase (AST), a ureia e a creatinina (SAVEGNAGO et al., 2009). Complementando estes dados, o **artigo 1** desta tese revelou que o tratamento repetido com (*m*-CF_3-PhSe)$_2$ em diferentes doses não altera parâmetros de toxicidade hepática (ALT e AST), renal (ureia) e cardíaca (creatina quinase, CK) em camundongos, mesmo na maior dose (50 mg/kg) administrada. Além disso, a administração repetida do composto na maior dose utilizada neste estudo não induziu alterações morfológicas em fígado e rim de camundongos. Desta forma, pode-se considerar que este composto apresenta segurança terapêutica em doses agudas e repetidas em camundongos.

Levando em conta a ação do tipo antidepressiva dos tratamentos agudo e repetido com o (*m*-CF_3-PhSe)$_2$ e o envolvimento dos receptores opioides μ, δ e κ nesse efeito, demonstrados no **artigo 1**, buscou-se investigar mecanismos moleculares que contribuam para melhor esclarecer como este composto interage com o sistema opioide. Desta forma, modelos de depressão induzida por estresse foram escolhidos para dar continuidade aos estudos desta tese, uma vez que os opioides endógenos exercem um papel importante em respostas adaptativas ou patológicas desencadeadas por estresse e que muitos estudos têm demonstrado o efeito do estresse em alterações moleculares no sistema opioide, como a expressão de receptores, a liberação de peptídeos endógenos e a fosforilação de proteínas que fazem parte da via de (BALI et al., 2015; BERUBE et al., 2014; BRUCHAS et al., 2008) sinalização opioide . Somado a isso, o fato de que o estresse é um dos principais fatores envolvidos no desenvolvimento da depressão justifica a importância do estudo dos efeitos do (*m*-CF_3-PhSe)$_2$ em modelos de depressão induzida por estresse (DEMPSEY, 2018; TAFET e NEMEROFF, 2016).

Como mencionado anteriormente, o TNF é amplamente utilizado em estudos pré-clínicos para o *screening* de novos fármacos antidepressivos (YANKELEVITCH-YAHAV et al., 2015). No entanto, a exposição a este teste é considerada um estresse de natureza física e psicológica (DAYAS et al., 2001), o qual pode produzir uma variedade de alterações no SNC que se assemelham a depressão em humanos (BRUCHAS et al., 2007; DAL-ZOTTO et al., 2000; QI, X. et al., 2006a; SHISHKINA et al., 2008). Estudos têm demonstrado que uma única exposição ao ENF aumenta os níveis séricos de corticosterona e a liberação do opioide endógeno dinorfina com a subsequente ativação de receptores κ (ROGOZ et al., 2012), importantes mediadores de respostas negativas ao estresse (HOMBERGER et al., 2015;

KNOLL e CARLEZON, 2010). Além disso, o ENF aumenta respostas analgésicas induzidas por estresse de uma maneira dependente dos receptores opioides μ (RUBINSTEIN et al., 1996). Já a exposição repetida ao TNF aumenta a imobilidade dos animais gradualmente e induz alterações neuroquímicas e moleculares que podem estar associadas a uma adaptação ao estresse ou ao desenvolvimento de transtornos psiquiátricos, principalmente a depressão (BRUCHAS et al., 2007; MCLAUGHLIN et al., 2006a; QI, X. et al., 2008; YANAGIDA et al., 2016).

Neste contexto, os resultados apresentados no **artigo 2** demonstraram que a exposição repetida ao ENF induz um comportamento do tipo depressivo evidenciado pelo aumento na imobilidade dos animais no TNF e TSC e a redução do comportamento de autolimpeza no *splash* teste. Notavelmente, o tratamento com o (*m*-CF_3-PhSe)$_2$, em baixas doses, reduziu os comportamentos do tipo depressivo induzidos pelo ENF repetido, demonstrando que este composto também apresenta ação do tipo antidepressiva frente a situações estressantes. De particular importância, os resultados apresentados no **artigo 2** revelaram, pela primeira vez, a eficácia do composto contra a redução do comportamento de autolimpeza dos animais, o qual mimetiza um importante sintoma da depressão em humanos, a falta de autocuidado (WHO, 2012).

Tendo em vista que o estresse agudo e/ou repetido pode gerar respostas adaptativas ou patológicas no SNC, procurou-se avaliar o efeito de uma única ou repetidas exposições ao ENF sobre os níveis dos receptores opioides μ, δ e κ em córtex pré-frontal de camundongos. Interessantemente, a exposição ao estresse, tanto aguda quanto repetida, produziu efeitos particulares sobre cada tipo de receptor opioide. Um aumento gradual foi observado no conteúdo de proteína dos receptores μ após a primeira e as subsequentes sessões de nado forçado. Por outro lado, o estresse diminuiu os níveis de receptores δ, no entanto, os níveis destes receptores foram maiores em animais submetidos repetidamente ao estresse do nado forçado em comparação aos animais submetidos ao estresse agudo. Ainda, somente o estresse repetido aumentou os níveis dos receptores κ em córtex pré-frontal de camundongos. Estas alterações nos níveis de receptores opioides podem estar associadas ao aumento de comportamentos do tipo depressivo induzidos pelo ENF, uma vez que como discutido anteriormente, o sistema opioide exerce importante papel regulatório em transtornos depressivos e em respostas ao estresse (BALI et al., 2015; LUTZ e KIEFFER, 2013).

Corroborando os resultados descritos no **artigo 2**, um aumento da expressão de receptores μ em várias regiões do sistema límbico tem sido demonstrada após o estresse único e repetido em roedores (KOMATSU et al., 2011; NIKULINA et al., 2005; YAMAMOTO et

al., 2003). No entanto, uma relação entre o aumento nos níveis destes receptores e o comportamento do tipo depressivo induzido por estresse ainda não foi estabelecida, principalmente, porque estudos demonstram o efeito antidepressivo de agonistas de receptores μ em modelos de depressão induzida por estresse e que a redução nos níveis de β-endorfina está relacionada a respostas aversivas ao estresse (JOSHI et al., 2014; SHAW e AL'ABSI, 2008; WANG, F.-R. et al., 2015). Neste contexto, pode-se levar em conta que, como mencionado anteriormente, os receptores opioides μ são regulados de acordo com estímulos, e que tanto uma diminuição ou um aumento destes receptores pode ter consequências prejudiciais sobre o humor (LUTZ e KIEFFER, 2013). Além disso, um aumento dos níveis destes receptores pode ser uma resposta adaptativa para compensar o aumento da liberação de peptídeos opioides induzido por estresse (BALI et al., 2015).

Dados da literatura indicam que camundongos com deleção genética dos ligantes endógenos de receptores δ, as encefalinas, estão associados ao desenvolvimento de sintomas do tipo depressivo e as alterações induzidas por estresse (BERUBE et al., 2013; MELO et al., 2014). Logo, a diminuição dos níveis de receptores δ por estresse agudo e repetido, possivelmente, está relacionado com os comportamentos do tipo depressivo induzidos pelo ENF observados no **artigo 2**. No entanto, os maiores níveis de receptores δ em animais expostos repetidamente à natação forçada em relação aos submetidos ao estresse agudo sugerem um mecanismo adaptativo, uma vez que o sistema de encefalina-receptor δ já foi reportado como um importante mediador da adaptação ao estresse em ratos (BERUBE et al., 2014; HENRY et al., 2017).

Surpreendentemente, os resultados do **artigo 2** revelaram um aumento dos níveis de receptores κ somente em animais estressados repetidamente. Estudos têm demonstrado um aumento da liberação de dinorfinas e a ativação dos receptores κ após uma única exposição ao estresse (CHUNG et al., 2014; SHIRAYAMA et al., 2004). Por outro lado, corroborando os resultados do **artigo 2**, algumas pesquisas reportaram que os efeitos antidepressivos de antagonistas de receptores κ ou da deleção genética destes receptores e seus ligantes são mais evidentes após o estresse repetido, uma vez que a ativação do sistema dinorfina- receptores κ é especialmente importante na amplificação ou sensibilização das respostas ao estresse (KNOLL e CARLEZON, 2010; MCLAUGHLIN et al., 2003). Logo, uma primeira exposição ao estresse do nado forçado e um consequente aumento da liberação de dinorfinas e a ativação dos receptores κ reportados na literatura, podem ter contribuído para a sensibilização dos receptores κ e posterior aumento dos níveis destes receptores em resposta ao estresse repetido. Vale ressaltar que, em conjunto, estes resultados indicam que o simples fato de os animais serem

submetidos a testes comportamentais, como o TNF, pode induzir alterações em importantes sistemas envolvidos no controle do comportamento e de respostas ao estresse como o sistema opioide e, portanto, devem ser considerados no delineamento experimental e na interpretação dos resultados de um estudo.

Assim, tendo em vista que as alterações nos níveis de receptores opioides poderiam contribuir para o desenvolvimento de comportamentos do tipo depressivo induzidos pelo ENF, os quais foram prevenidos pelo tratamento com (*m*-CF_3-PhSe)$_2$, avaliou-se o efeito deste composto sobre os níveis de receptores opioides em córtex pré-frontal de camundongos estressados. Os resultados do **artigo 2** demonstraram que o tratamento com (*m*-CF_3-PhSe)$_2$ normalizou os níveis de receptores opioides μ e κ alterados pela exposição repetida ao ENF. Além disso, a administração do composto na dose de 5 mg/kg, a qual apresenta efeito do tipo antidepressivo agudo no TNF (BRUNING et al., 2011), não alterou os níveis de receptores μ e κ após uma exposição a este teste. Somado a isso, quando o (*m*-CF_3-PhSe)$_2$ foi administrado em animais repetidamente estressados ele não só apresentou efeito do tipo antidepressivo, como também normalizou os níveis destes receptores em córtex pré-frontal de camundongos estressados. Juntos, estes dados sugerem uma importante relação entre a regulação dos níveis de receptores μ e κ e o efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$. Neste sentido, levando-se em conta que tanto uma regulação positiva quanto negativa dos receptores μ pode ter efeitos prejudiciais sobre o humor, enquanto que, o aumento nos níveis ou a ativação de receptores κ está relacionado a respostas aversiva ao estresse e a regulação negativa do humor (LUTZ e KIEFFER, 2013), a normalização nos níveis de receptores μ e κ pelo composto pode contribuir para a sua ação do tipo antidepressiva. De fato, a ativação de receptores μ e o bloqueio de receptores κ são amplamente relacionados com efeitos do tipo antidepressivo de inúmeros compostos (LI et al., 2016; LUTZ e KIEFFER, 2013; ROBINSON, S. A. et al., 2017), incluindo o (*m*-CF_3-PhSe)$_2$, como demonstrado nos resultados obtidos no **artigo 1**. Porém, ainda não está claro se a ativação dos receptores μ e/ou o bloqueio de receptores κ está relacionado com a normalização dos níveis destes receptores pelo composto em animais estressados.

Embora os dados farmacológicos demonstrados no **artigo 1** sugiram que a ativação de receptores opioides δ contribui para o efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$, este composto não foi efetivo em normalizar os níveis destes receptores em camundongos submetidos ao ENF repetido. Uma possível explicação para este resultado poderia ser o fato de que o ENF repetido *per se* já induziu um processo parcial de adaptação ao estresse em relação a uma única exposição ao nado forçado. Além disso, este resultado pode sugerir que a

contribuição dos receptores δ para a ação do tipo antidepressiva do (*m*-CF_3-PhSe)$_2$, demonstrada no **artigo 1**, está relacionada com um aumento nos níveis destes receptores ou ainda que estes receptores não estão envolvidos com o efeito do tipo antidepressivo do composto em modelos de depressão induzida por estresse.

Em suma, os resultados mostrados no **artigo 2** confirmam que o efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ envolve a modulação do sistema opioide, como indicado no artigo 1 e sugerem que o composto contribui para a manutenção da homeostase em resposta ao estresse por meio da regulação dos níveis de receptores opioides, principalmente μ e κ.

Sabe-se que os efeitos do estresse são dependentes de muitos fatores, como o tempo de exposição e o tipo de estressor (TAFET e NEMEROFF, 2016). Desta forma, com base nos resultados comportamentais apresentados no artigo 2, tornou-se importante investigar se o (*m*-CF_3-PhSe)$_2$ também seria efetivo contra os efeitos prejudiciais do estresse em um modelo de estresse de diferente natureza e mais prolongado que o ENF. Logo, o EDS foi escolhido para dar continuidade ao estudo do efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ e do seu mecanismo de ação opioide, uma vez que o conflito social é um dos principais estressores envolvidos no desenvolvimento da depressão em humanos e estudos têm demonstrado que este modelo é capaz de induzir alterações no sistema opioide (BJORKQVIST, 2001; LAREDO et al., 2015; MCLAUGHLIN et al., 2006b).

O modelo de depressão induzida pelo EDS baseia-se principalmente no desenvolvimento de aversão social, um recorrente sintoma depressivo em humanos (HOLLIS e KABBAJ, 2014). Este modelo induz alterações em diferentes aspectos comportamentais e fisiológicos, os quais levam ao desenvolvimento de respostas adaptativas ou patológicas que caracterizam a resiliência ou a suscetibilidade ao estresse (KRISHNAN et al., 2007). Indivíduos resilientes apresentam a habilidade de enfrentar e se adaptar a situações estressantes, mantendo o funcionamento psicológico e físico quando exposto a um estresse prolongado ou trauma severo. Por outro lado, a dificuldade de enfrentar o estresse e a maior facilidade de desenvolver transtornos psiquiátricos caracterizam os indivíduos suscetíveis (RUSSO et al., 2012). De forma semelhante, roedores também podem ser classificados como suscetíveis e resilientes baseado em suas respostas comportamentais e fisiológicas ao estresse. No **artigo 3**, o teste de interação social foi utilizado para avaliar a resiliência ou a suscetibilidade dos camundongos derrotados ao EDS. Corroborando a literatura, os resultados apresentados no **artigo 3** demonstraram que as sucessivas agressões físicas e o subsequente contato sensorial com camundongos agressores induziram aversão social em camundongos derrotados, caracterizada pela diminuição do tempo gasto na zona de interação do teste de interação social. Neste mesmo

teste, também foi possível observar um comportamento do tipo ansioso induzido pelo EDS e indicado pelo aumento do tempo gasto por camundongos derrotados nas zonas de esquina. É importante destacar que não houve alterações no tempo gasto pelos animais derrotados na zona de interação e na zona de esquina na ausência do camundongo agressor desconhecido, confirmando a aversão social induzida pelo EDS e classificando estes animais como suscetíveis ao estresse. Por outro lado, também de acordo com os dados descritos na literatura, em torno de 40% dos animais expostos ao EDS não apresentaram comportamentos de aversão social e foram classificados como resilientes. Interessantemente, a resiliência ou a suscetibilidade ao EDS, avaliados no **artigo 3**, são comparáveis a ampla variabilidade de respostas ao estresse encontrada em humanos, o que torna este modelo ainda mais atrativo para o estudo da depressão relacionada ao estresse e de possíveis tratamentos para esta doença.

Com o objetivo de mimetizar o tratamento de doenças psiquiátricas, o qual ocorre durante o curso da doença, o (*m*-CF_3-PhSe)$_2$ foi administrado nas doses de 5 a 25 mg/kg, concomitantemente com o protocolo de EDS. Neste modelo, o tratamento com o composto na maior dose foi efetivo em reduzir a aversão social e a ansiedade nos animais derrotados. De fato, o EDS é um estresse mais severo que o ENF, o que explicaria a necessidade de doses maiores e maior tempo de tratamento com o (*m*-CF_3-PhSe)$_2$ para a redução dos sintomas depressivos induzidos pelo EDS. Em conjunto, os dados comportamentais dos **artigos 2 e 3** demonstraram que o composto é efetivo em dois diferentes modelos de depressão induzida por estresse, um ambiental e de menor duração (ENF) e outro de natureza social, prolongado e mais severo.

Ademais, os resultados do **artigo 3** demonstraram que a administração do (*m*-CF_3-PhSe)$_2$ na dose efetiva em reduzir a aversão social induzida por EDS também é capaz de aumentar a sociabilidade natural dos animais. Este resultado é particularmente interessante, uma vez que as interações sociais são componentes fundamentais da natureza dos roedores e a sociabilidade natural entre eles pode ser um sinal de bem-estar psicológico (ROBINSON, G. E. et al., 2005), além de que, a diminuição da sociabilidade ou o isolamento social são comumente reportados em pacientes depressivos (SANTINI et al., 2015). Desta forma, o aumento da sociabilidade em animais tratados com (*m*-CF_3-PhSe)$_2$ está possivelmente relacionado com a ação do tipo antidepressiva do composto.

Muitos estudos têm apontado o papel do sistema opioide nos efeitos do EDS, sendo que, a modulação deste sistema pode induzir resiliência ou suscetibilidade ao EDS em roedores (KRISHNAN et al., 2007). No **artigo 3**, camundongos suscetíveis ao EDS apresentaram maiores níveis de receptores opioides μ e κ e menores níveis de receptores δ, enquanto que

nenhuma alteração nos níveis de receptores opioides foi observada no córtex pré-frontal de camundongos resilientes e de camundongos tratados com (*m*-CF_3-PhSe)$_2$ na dose de 25 mg/kg. Estes resultados sugerem que o composto promove resiliência ao EDS por meio da regulação dos níveis de receptores opioides em córtex pré-frontal de camundongos estressados.

Os efeitos do EDS e do tratamento com (*m*-CF_3-PhSe)$_2$ sobre os níveis de receptores opioides foram semelhantes aos observados no modelo do ENF (**artigo 2**), principalmente, no que diz respeito aos receptores μ e κ. Além disso, embora no **artigo 2** o ENF repetido tenha aumentado os níveis de receptores δ comparado com o ENF agudo, esses níveis ainda foram menores do que os dos animais controle, um resultado similar ao encontrado no modelo do EDS (**artigo 3**). Ainda, o efeito do (*m*-CF_3-PhSe)$_2$ em regular os níveis de receptores δ no EDS e não no ENF pode ser explicado pelo maior tempo de tratamento e dose utilizados no protocolo do EDS e também pode sugerir um menor envolvimento dos receptores δ nos efeitos do (*m*-CF_3-PhSe)$_2$ em situações de estresse. Corroborando estes resultados, estudos têm demonstrado um aumento na expressão de receptores μ após o EDS, e uma diminuição dos efeitos do EDS em camundongos com deleção genética destes receptores (KOMATSU et al., 2011; NIKULINA et al., 2005). De forma semelhante, o EDS induz um aumento na expressão de dinorfina em camundongos estressados, enquanto a administração de antagonistas de receptores κ ou a deleção destes receptores diminui a postura submissa de animais derrotados (DONAHUE et al., 2015; MCLAUGHLIN et al., 2003). Por outro lado, uma diminuição na expressão de encefalina está relacionada com a susceptibilidade ao EDS e a ativação dos receptores δ diminui as respostas depressivas ao estresse social (BERUBE et al., 2013; HENRY et al., 2018). Em conjunto, os dados dos **artigos 2 e 3** demonstram a contribuição dos receptores opioides para os sintomas do tipo depressivos induzidos pelos dois tipos de estresse testados nesta tese e confirmam que a ação do tipo antidepressiva do composto envolve a modulação do sistema opioide, tanto em situações não estressantes, quanto na exposição a estresse de menor e maior duração.

Levando-se em conta que as MAPKs fazem parte da via de sinalização de receptores opioides e estão envolvidas em respostas comportamentais ao estresse e no mecanismo antidepressivo de diversos fármacos, procurou-se investigar se estas proteínas contribuem para a resiliência ao EDS promovida peçp (*m*-CF_3-PhSe)$_2$. Diferentes resultados foram encontrados para cada uma das MAPK avaliadas. Os animais suscetíveis ao EDS apresentaram maiores níveis de fosforilação da quinase c-Jun N-terminal (JNK). No entanto, estes níveis não foram alterados em animais resilientes e foram normalizados pelo tratamento com (*m*-CF_3-PhSe)$_2$,

sugerindo o envolvimento da JNK na aversão social induzida por EDS e indicando a contribuição desta proteína para a resiliência promovida pelo tratamento com (*m*-CF_3-PhSe)$_2$. De fato, a ativação da JNK pode ser alterada tanto por estímulos estressantes, quanto pela modulação de receptores opioides (JOHNSON e NAKAMURA, 2007; KUHAR et al., 2015; LIU, Y. F. et al., 2004) . Desta forma, a fosforilação da JNK demonstrada no **artigo 3** pode estar relacionada com as alterações nos receptores opioides induzidas pelo EDS e a regulação desta via de sinalização parece contribuir para os efeitos do tipo antidepressivos do (*m*-CF_3-PhSe)$_2$ neste modelo.

Por outro lado, a redução dos níveis de fosforilação da quinase regulada por sinal extracelular (ERK) foi independente da suscetibilidade ou resiliência ao EDS e parcialmente bloqueada pelo (*m*-CF_3-PhSe)$_2$. Embora estudos demonstrem diferentes efeitos do estresse sobre os níveis e expressão desta proteína, os resultados demonstrados no **artigo 3** indicam que ela não está diretamente relacionada aos efeitos do EDS. No entanto, o envolvimento da ERK no efeito do tipo antidepressivo do (*m*-CF_3-PhSe)$_2$ não pode ser descartado, uma vez que a diminuição da fosforilação desta proteína está associada a sintomas depressivos (FENG et al., 2003; QI, X. et al., 2008) e que o composto foi parcialmente efetivo contra a diminuição da fosforilação da ERK em animais derrotados.

Surpreendentemente, no **artigo 3**, o EDS induziu um aumento na fosforilação da p38 MAPK somente em animais resilientes. A ativação da p38 MAPK por diferentes tipos de estresse, incluindo o EDS, tem sido amplamente demonstrada em roedores (BRUCHAS et al., 2007; BRUCHAS et al., 2011; CAI et al., 2008; OBATA et al., 2000). Além disso, a fosforilação mediada pela ativação de receptores opioides, especialmente o receptor κ, está envolvida no comportamento do tipo depressivo e aversivo induzido por estresse em camundongos (BRUCHAS et al., 2007; BRUCHAS et al., 2008). No entanto, estudos têm demonstrado que diferentes isoformas da p38 MAPK apresentam variadas especificidades de substrato, distribuição tecidual e que a deleção da p38α e a falta de compensação pela p38β causa profundos efeitos comportamentais do tipo depressivo no modelo de EDS em camundongos. Desta forma, a falta de efeito do EDS nos níveis de fosforilação da p38 MAPK demonstrada no **artigo 3** poderia ser explicada pelo fato de que o anticorpo usado para detectar p38 MAPK não tem especificidade para as isoformas desta proteína, o que pode ter dificultado a observação do efeito bem conhecido do estresse na ativação do MAPK p38. Além disso, o aumento intrigante da fosforilação p38 MAPK no córtex pré-frontal de camundongos resilientes pode indicar um efeito compensatório ao estresse ou ainda uma desregulação de outras isoformas da p38 MAPK, menos envolvidas com este tipo de estresse. Porém, a ausência

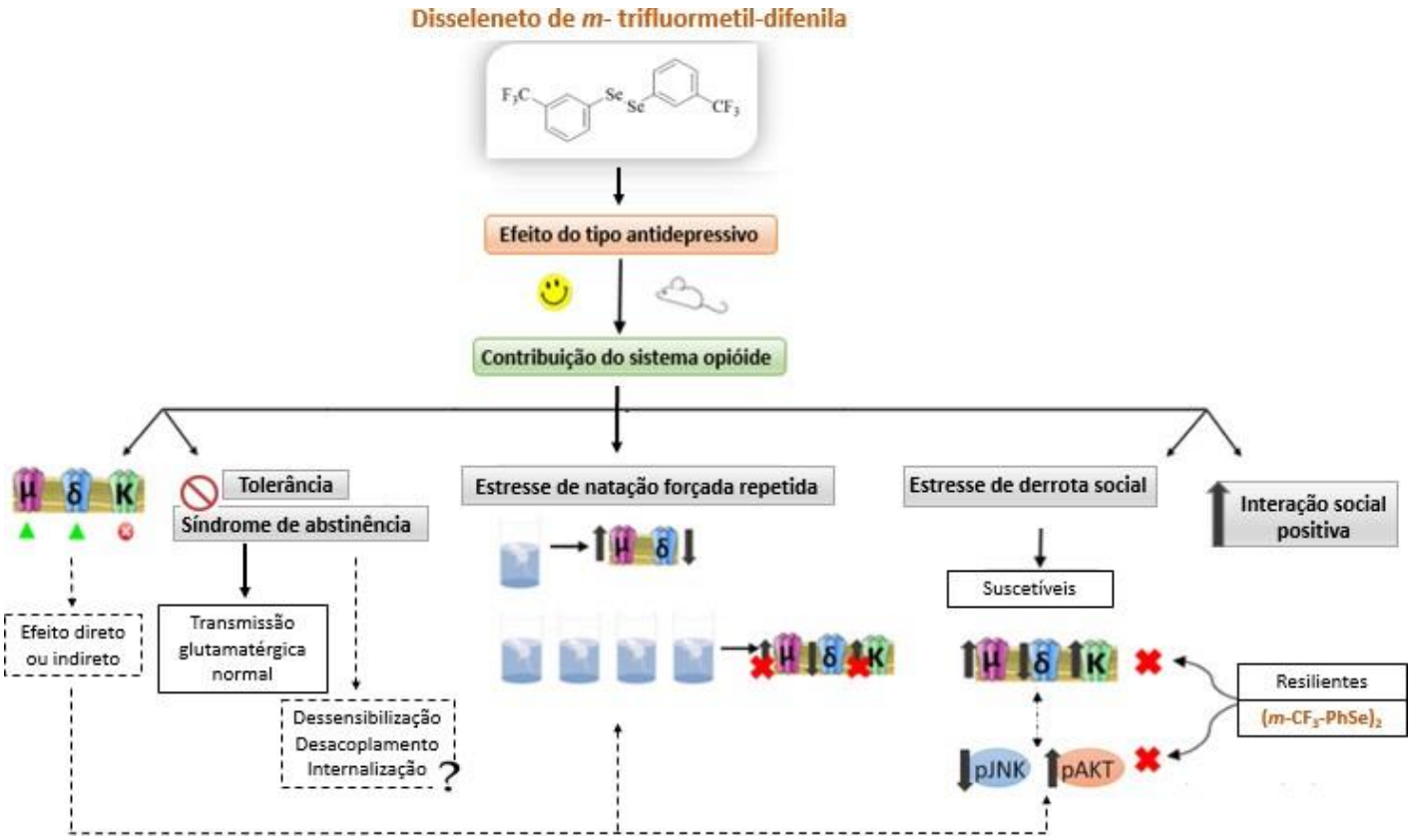
de validação desta hipótese é um ponto fraco deste estudo.

Assim como as MAPKs, a proteína quinase B (Akt) também está envolvida em respostas ao estresse e na depressão (KAREGE et al., 2007; WILLOCK e FRANKE, 2015). Somado a isso, estudos já demonstraram que a ativação desta proteína pode ser mediada por receptores opioides (OLIANAS et al., 2011; SANCHEZ-BLAZQUEZ et al., 2010). No **artigo 3**, o envolvimento da fosforilação da Akt na aversão social induzida pelo EDS e a sua contribuição para a resiliência promovida pelo (*m*-CF_3-PhSe)$_2$ foi sugerida devido a diminuição da fosforilação desta proteína demonstrada apenas em camundongos susceptíveis e também pela normalização destes níveis pelo (*m*-CF_3-PhSe)$_2$. De acordo com estes resultados, (KRISHNAN et al., 2008)demonstraram que a diminuição da ativação de Akt é necessária e suficiente para conferir vulnerabilidade ao EDS. Em conjunto, os resultados descritos no **artigo 3** confirmam a eficácia do (*m*-CF_3-PhSe)$_2$ na depressão induzida por um estresse prolongado e sugerem que este composto promove resiliência ao EDS por regular os níveis de receptores opioides, a fosforilação da JNK e da Akt em córtex pré-frontal de camundongos estressados.

Por fim, é importante salientar que nenhum dos tratamentos realizados no decorrer desta tese alterou a atividade locomotora dos animais, o que descarta que as análises comportamentais possam ter sido influenciadas por alterações na atividade locomotora.

Assim, com base nos dados demonstrados até aqui, pode-se inferir que o composto orgânico de selênio (*m*-CF_3-PhSe)$_2$ apresentou um efeito do tipo antidepressivo em situações não estressantes e frente ao estresse curto ou prolongado. Além disso, os resultados desta tese sugerem que estas ações farmacológicas do composto podem ser atribuídas à modulação do sistema opioide (Figura 5). No entanto, esta molécula precisa ser melhor estudada, principalmente, no que tange a compreensão dos mecanismos pelos quais a molécula interage com os receptores opioides.

Figura 5- Esquema geral dos resultados obtidos nesta tese e perspectivas



Fonte: arquivo pessoal do autor. As flechas cheias demonstram os resultados obtidos nesta tese, enquanto que as linhas pontilhadas indicam as perspectivas para este estudo.

5 CONCLUSÃO

Os resultados apresentados nesta tese indicam que o composto orgânico de selênio (*m*-CF_3-PhSe)$_2$ apresenta um efeito do tipo antidepressivo mediado pela modulação do sistema opioide, possivelmente, por meio da ativação de receptores μ e δ e do bloqueio de receptores κ, sem induzir a tolerância, os sinais físicos de abstinência e toxicidade sistêmica.

Além disto, o presente estudo demonstra a eficácia do (*m*-CF_3-PhSe)$_2$ contra os sintomas do tipo depressivo induzidos por estresse de natação forçada e do estresse de derrota social, por meio da normalização dos níveis de receptores opioides e proteínas relacionadas a sinalização opioide em córtex pré-frontal de camundongos estressados.

Finalmente, esta tese contribui para o esclarecimento dos mecanismos opioides envolvidos na ação do tipo antidepressiva do (*m*-CF_3-PhSe)$_2$ e reforça a hipótese de que este composto pode ser uma interessante alternativa terapêutica para o tratamento da depressão.

6 PERSPECTIVAS

A seguir perspectivas para trabalhos futuros, as quais estão representadas em linhas pontilhadas na figura 5 desta tese:

- Investigar se o (*m*-CF_3-PhSe)$_2$ exerce efeito do tipo antidepressivo por ação direta ou indireta sobre receptores opioides;
-Avaliar os mecanismos pelos quais a administração repetida do (*m*-CF_3-PhSe)$_2$ não induz tolerância e síndrome de abstinência;
- Investigar se existe alguma relação entre o efeito do (*m*-CF_3-PhSe)$_2$ na ativação/bloqueio de receptores opioides e a regulação dos níveis destes receptores pelo composto em situações estressantes;
-Avaliar se o efeito do (*m*-CF_3-PhSe)$_2$ sobre a fosforilação da JNK e Akt está relacionado com a modulação dos receptores opioides pelo composto.

REFERÊNCIAS BIBLIOGRÁFICAS

ABDEL-ZAHER, A. O. et al. Blockade of nitric oxide overproduction and oxidative stress by Nigella sativa oil attenuates morphine-induced tolerance and dependence in mice. **Neurochemical Research,** v. 35, n. 10, p. 1557-65, Oct 2010.

ABELAIRA, H. M. et al. Animal models as tools to study the pathophysiology of depression. **Rev Bras Psiquiatr,** v. 35 Suppl 2, p. S112-20, 2013.

AL-HASANI, R.; BRUCHAS, M. R. Molecular mechanisms of opioid receptor-dependent signaling and behavior. **Anesthesiology,** v. 115, n. 6, p. 1363-81, Dec 2011.

ALLOUCHE, S. et al. Opioid receptor desensitization: mechanisms and its link to tolerance. 2014.

AMERICAN PSYCHIATRIC ASSOCIATION. **Diagnostic and statistical manual of mental disorders (DSM-5®)**. 5th. Arlington, VA: American Psychiatric Publishing, 2013.

BAGOT, R. C. et al. Ketamine and Imipramine Reverse Transcriptional Signatures of Susceptibility and Induce Resilience-Specific Gene Expression Profiles. **Biol Psychiatry,** v. 81, n. 4, p. 285-295, Feb 15 2017.

BALE, T. L. Stress sensitivity and the development of affective disorders. **Horm Behav,** v. 50, n. 4, p. 529-33, Nov 2006.

BALI, A. et al. Stress and opioids: role of opioids in modulating stress-related behavior and effect of stress on morphine conditioned place preference. **Neurosci Biobehav Rev,** v. 51, p. 138-50, Apr 2015.

BENTLEY, S. M. et al. Major depression. **Med Clin North Am,** v. 98, n. 5, p. 981-1005, Sep 2014.

BERROCOSO, E. et al. Active behaviours produced by antidepressants and opioids in the mouse tail suspension test. **Int J Neuropsychopharmacol,** v. 16, n. 1, p. 151-62, Feb 2013.

BERROCOSO, E. et al. Opiates as Antidepressants. **Current Pharmaceutical Design,** v. 15, n. 14, p. 1612-1622, May 2009.

BERSHAD, A. K. et al. Effects of Buprenorphine on Responses to Emotional Stimuli in Individuals with a Range of Mood Symptomatology. **Int J Neuropsychopharmacol,** v. 21, n. 2, p. 120-127, Feb 12018.

BERUBE, P. et al. Enkephalin and dynorphin mRNA expression are associated with resilience or vulnerability to chronic social defeat stress. **Physiol Behav,** v. 122, p. 237-45, Oct 02 2013.

BERUBE, P. et al. Enkephalin knockdown in the basolateral amygdala reproduces vulnerable anxiety-like responses to chronic unpredictable stress. **Neuropsychopharmacology,** v. 39, n. 5, p. 1159-68, Apr 2014.

BISAGA, A. et al. The NMDA antagonist memantine attenuates the expression of opioid physical dependence in humans. **Psychopharmacology (Berl),** v. 157, n. 1, p. 1-10, Aug 2001.

BJORKQVIST, K. Social defeat as a stressor in humans. **Physiol Behav,** v. 73, n. 3, p. 435-42, Jun 2001.

BODNAR, R. J. Endogenous opiates and behavior: 2012. **Peptides,** v. 50, p. 55-95, Dec 2013.

_____. Endogenous Opiates and Behavior: 2015. **Peptides,** v. 88, p. 126-188, Feb 2017.

BONDAR, N. et al. Molecular Adaptations to Social Defeat Stress and Induced Depression in Mice. **Mol Neurobiol**, May 12 2017.

BORGES, V. C. et al. Disubstituted diaryl diselenides inhibit [3H]-serotonin uptake in rats. **Neurotox Res,** v. 15, n. 1, p. 57-61, Jan 2009.

BROCARDO, P. S. et al. Evidence for the involvement of the opioid system in the antidepressant-like effect of folic acid in the mouse forced swimming test. **Behavioural brain research,** v. 200, n. 1, p. 122-127, Jun 8 2009.

BRUCHAS, M. R. et al. Stress-induced p38 mitogen-activated protein kinase activation mediates kappa-opioid-dependent dysphoria. **J Neurosci,** v. 27, n. 43, p. 11614-23, Oct 24 2007.

BRUCHAS, M. R. et al. Selective p38alpha MAPK deletion in serotonergic neurons produces stress resilience in models of depression and addiction. **Neuron,** v. 71, n. 3, p. 498-511, Aug 11 2011.

BRUCHAS, M. R. et al. Repeated swim stress induces kappa opioid-mediated activation of extracellular signal-regulated kinase 1/2. **Neuroreport,** v. 19, n. 14, p. 1417-22, Sep 17 2008.

BRUNING, C. A. et al. Serotonergic systems are implicated in antinociceptive effect of m-trifluoromethyl diphenyl diselenide in the mouse glutamate test. **Pharmacol Biochem Behav,** v. 125, p. 15-20, Oct 2014.

BRUNING, C. A. et al. m-Trifluoromethyl-diphenyl diselenide, a multi-target selenium compound, prevented mechanical allodynia and depressive-like behavior in a mouse comorbid pain and depression model. **Prog Neuropsychopharmacol Biol Psychiatry,** v. 63, p. 35-46, Dec 3 2015a.

BRUNING, C. A. et al. Depressive-like behavior induced by tumor necrosis factor-alpha is attenuated by m-trifluoromethyl-diphenyl diselenide in mice. **J Psychiatr Res,** v. 66-67, p. 75-83, Jul-Aug 2015b.

BRUNING, C. A. et al. Diphenyl diselenide ameliorates behavioral and oxidative parameters in an animal model of mania induced by ouabain. **Prog Neuropsychopharmacol Biol Psychiatry,** v. 38, n. 2, p. 168-74, Aug 07 2012.

BRUNING, C. A. et al. Involvement of the serotonergic system in the anxiolytic-like effect caused by m-trifluoromethyl-diphenyl diselenide in mice. **Behav Brain Res,** v. 205, n. 2, p. 511-7, Dec 28 2009.

BRUNING, C. A. et al. Evidence for the involvement of mu-opioid and delta-opioid receptors in the antinociceptive effect caused by oral administration of m-trifluoromethyl-diphenyl diselenide in mice. **Behavioural pharmacology,** v. 21, n. 7, p. 621-626, Oct 2010.

BRUNING, C. A. et al. Antidepressant-like effect of m-trifluoromethyl-diphenyl diselenide in the mouse forced swimming test involves opioid and serotonergic systems. **Eur J Pharmacol,** v. 658, n. 2- 3, p. 145-9, May 11 2011.

CAI, Q. et al. The effects of prenatal stress on expression of p38 MAPK in offspring hippocampus. **Int J Dev Neurosci,** v. 26, n. 6, p. 535-40, Oct 2008.

CALLAGHAN, C. K. et al. Antidepressant-like effects of 3-carboxamido seco-nalmefene (3CS-nalmefene), a novel opioid receptor modulator, in a rat IFN-alpha-induced depression model. **Brain Behav Immun,** v. 67, p. 152-162, Jan 2018.

CARLEZON, W. A., JR. et al. Depressive-like effects of the kappa-opioid receptor agonist salvinorin A on

behavior and neurochemistry in rats. **J Pharmacol Exp Ther,** v. 316, n. 1, p. 440-7, Jan 2006.

CARVALHO, A. F. et al. The safety, tolerability and risks associated with the use of newer generation antidepressant drugs: a critical review of the literature. **Psychotherapy and psychosomatics,** v. 85, n. 5, p. 270-288, 2016.

CHAIJALE, N. N. et al. Social stress engages opioid regulation of locus coeruleus norepinephrine neurons and induces a state of cellular and physical opiate dependence. **Neuropsychopharmacology,** v. 38, n. 10, p. 1833-1843, 2013.

CHAN, P.; LUTFY, K. Molecular Changes in Opioid Addiction: The Role of Adenylyl Cyclase and cAMP/PKA System. **Prog Mol Biol Transl Sci,** v. 137, p. 203-27, 2016.

CHEN, Z. et al. Morphine postconditioning protects against reperfusion injury via inhibiting JNK/p38 MAPK and mitochondrial permeability transition pores signaling pathways. **Cellular Physiology and Biochemistry,** v. 39, n. 1, p. 61-70, 2016.

CHUNG, S. et al. Desipramine and citalopram attenuate pretest swim-induced increases in prodynorphin immunoreactivity in the dorsal bed nucleus of the stria terminalis and the lateral division of the central nucleus of the amygdala in the forced swimming test. **Neuropeptides,** v. 48, n. 5, p. 273-280, 2014.

CONNER, T. S. et al. Optimal serum selenium concentrations are associated with lower depressive symptoms and negative mood among young adults. **J Nutr,** v. 145, n. 1, p. 59-65, Jan 2015.

DAL-ZOTTO, S. et al. Influence of single or repeated experience of rats with forced swimming on behavioural and physiological responses to the stressor. **Behavioural brain research,** v. 114, n. 1, p. 175-181, 2000.

DARKO, D. F. et al. Association of beta-endorphin with specific clinical symptoms of depression. **Am J Psychiatry,** v. 149, n. 9, p. 1162-7, Sep 1992.

DAYAS, C. V. et al. Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment patterns in the amygdala and in medullary noradrenergic cell groups. **Eur J Neurosci,** v. 14, n. 7, p. 1143-52, Oct 2001.

DEMPSEY, L. A. Stress-induced depression. **Nature immunology**, p. 1, 2018.

DETKE, M. J. et al. Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. **Psychopharmacology (Berl),** v. 121, n. 1, p. 66-72, Sep 1995.

DONAHUE, R. J. et al. Effects of acute and chronic social defeat stress are differentially mediated by the dynorphin/kappa-opioid receptor system. **Behavioural pharmacology,** v. 26, n. 7 0 0, p. 654, 2015.

DRIPPS, I. J. et al. Role of signalling molecules in behaviours mediated by the delta opioid receptor agonist SNC80. **Br J Pharmacol,** v. 175, n. 6, p. 891-901, Mar 2018.

DRIPPS, I. J.; JUTKIEWICZ, E. M. Delta Opioid Receptors and Modulation of Mood and Emotion. 2017.

DROLET, G. et al. Role of endogenous opioid system in the regulation of the stress response. **Prog Neuropsychopharmacol Biol Psychiatry,** v. 25, n. 4, p. 729-41, May 2001.

DUMAN, C. H. Models of depression. **Vitam Horm,** v. 82, p. 1-21, 2010.

DUMONT, E. et al. Selenium speciation from food source to metabolites: a critical review. **Anal Bioanal Chem,** v. 385, n. 7, p. 1304-23, Aug 2006.

DUNTAS, L. H.; BENVENGA, S. Selenium: an element for life. **Endocrine,** v. 48, n. 3, p. 756-75, Apr 2015.

FAIRWEATHER-TAIT, S. J. et al. Selenium in human health and disease. **Antioxid Redox Signal,** v. 14, n. 7, p. 1337-83, Apr 01 2011.

FALCON, E. et al. Antidepressant-like effects of buprenorphine are mediated by kappa opioid receptors. **Neuropsychopharmacology**, 2016.

FARRAR, J. T. et al. A Novel 12-Week Study, with Three Randomized, Double-Blind Placebo- Controlled Periods to Evaluate Fentanyl Buccal Tablets for the Relief of Breakthrough Pain in Opioid- Tolerant Patients with Noncancer-Related Chronic Pain. **Pain Medicine,** v. 11, n. 9, p. 1313-1327, 2010.

FENG, P. et al. Impairments of ERK signal transduction in the brain in a rat model of depression induced by neonatal exposure of clomipramine. **Brain research,** v. 991, n. 1, p. 195-205, 2003.

FRODL, T.; O'KEANE, V. How does the brain deal with cumulative stress? A review with focus on developmental stress, HPA axis function and hippocampal structure in humans. **Neurobiol Dis,** v. 52, p. 24-37, Apr 2013.

FUJISHIMA, T. et al. Synthesis and biological activity of fluorinated vitamin D. **Current Organic Chemistry,** v. 14, n. 9, p. 962-976, 2010.

GAO, S. et al. Selenium level and depressive symptoms in a rural elderly Chinese cohort. **BMC Psychiatry,** v. 12, p. 72, 2012.

GASSAWAY, M. M. et al. The atypical antidepressant and neurorestorative agent tianeptine is a mu-opioid receptor agonist. **Translational Psychiatry,** v. 4, Jul 2014.

GAVIN, N. I. et al. Perinatal depression: a systematic review of prevalence and incidence. **Obstetrics and Gynecology,** v. 106, n. 5 Pt 1, p. 1071-83, Nov 2005.

GOLDEN, S. A. et al. A standardized protocol for repeated social defeat stress in mice. **Nat Protoc,** v. 6, n. 8, p. 1183-91, Jul 21 2011.

GUI, C.; WONG, S. Biased mu-opioid receptor agonists confer analgesia with reduced side effects. **University of Western Ontario Medical Journal,** v. 87, n. 1, p. 62-64, 2018.

GUTSTEIN, H. B. et al. Opioid effects on mitogen-activated protein kinase signaling cascades. **The Journal of the American Society of Anesthesiologists,** v. 87, n. 5, p. 1118-1126, 1997.

HAJ-MIRZAIAN, A. et al. Fluoxetine reverses the behavioral despair induced by neurogenic stress in mice: role of N-methyl-d-aspartate and opioid receptors. **Can J Physiol Pharmacol,** v. 94, n. 6, p. 599-612, Jun 2016.

HARRIS, A. C. et al. Effects of the NMDA receptor antagonist memantine on the expression and development of acute opiate dependence as assessed by withdrawal-potentiated startle and hyperalgesia. **Psychopharmacology (Berl),** v. 196, n. 4, p. 649-60, Mar 2008.

HENRIQUES-ALVES, A. M.; QUEIROZ, C. M. Ethological evaluation of the effects of social defeat stress in mice: beyond the social interaction ratio. **Frontiers in behavioral neuroscience,** v. 9, 2015.

HENRY, M. S. et al. Delta Opioid Receptor Signaling Promotes Resilience to Stress Under the Repeated Social Defeat Paradigm in Mice. **Front Mol Neurosci,** v. 11, p. 100, 2018.

HENRY, M. S. et al. Enkephalins: Endogenous Analgesics with an Emerging Role in Stress Resilience. **Neural Plast,** v. 2017, p. 1546125, 2017.

HIRSCHFELD, R. History and evolution of the monoamine hypothesis of depression. **The Journal of clinical psychiatry**, 2000.

HOLLIS, F.; KABBAJ, M. Social defeat as an animal model for depression. **ILAR J,** v. 55, n. 2, p. 221-32, 2014.

HOMBERGER, B. et al. Distinct responses of baseline and stress-induced corticosterone levels to genetic and environmental factors. **General and comparative endocrinology,** v. 210, p. 46-54, 2015.

HOOSHMANDI, M. et al. Antagonism of orexin type-1 receptors (OX1Rs) attenuates naloxone-precipitated morphine withdrawal syndrome in rat dorsal hippocampus. **Pharmacol Biochem Behav**, Jun 02 2017.

HSU, D. T. et al. It still hurts: altered endogenous opioid activity in the brain during social rejection and acceptance in major depressive disorder. **Mol Psychiatry,** v. 20, n. 2, p. 193-200, Feb 2015.

HURST, R. et al. Establishing optimal selenium status: results of a randomized, double-blind, placebo-controlled trial. **Am J Clin Nutr,** v. 91, n. 4, p. 923-31, Apr 2010.

IBARRA, O. et al. The Mediterranean diet and micronutrient levels in depressive patients. **Nutr Hosp,** v. 31, n. 3, p. 1171-5, 2014.

IBGE. Pesquisa Nacional de Saúde 2013: percepção do estado de saúde, estilos de vida e doenças crônicas: Instituto Brasileiro de Geografia e Estatística Rio de Janeiro 2014.

JOHNSON, G. L.; NAKAMURA, K. The c-jun kinase/stress-activated pathway: regulation, function and role in human disease. **Biochim Biophys Acta,** v. 1773, n. 8, p. 1341-8, Aug 2007.

JOSHI, J. C. et al. Differential modulatory effects of morphine on acute and chronic stress induced neurobehavioral and cellular markers in rats. **Eur J Pharmacol,** v. 729, p. 17-21, Apr 15 2014.

JUTKIEWICZ, E. M. et al. Behavioral and neurobiological effects of the enkephalinase inhibitor RB101 relative to its antidepressant effects. **Eur J Pharmacol,** v. 531, n. 1-3, p. 151-9, Feb 15 2006.

KAREGE, F. et al. Alteration in kinase activity but not in protein levels of protein kinase B and glycogen synthase kinase-3beta in ventral prefrontal cortex of depressed suicide victims. **Biol Psychiatry,** v. 61, n. 2, p. 240-5, Jan 15 2007.

KARP, J. F. et al. Safety, tolerability, and clinical effect of low-dose buprenorphine for treatment-resistant depression in mid-life and older adults. **The Journal of clinical psychiatry,** v. 75, n. 8, p. e785, 2014.

KASTER, M. P. et al. Pharmacological evidence for the involvement of the opioid system in the antidepressant-like effect of adenosine in the mouse forced swimming test. **European Journal of Pharmacology,** v. 576, n. 1-3, p. 91-8, Dec 8 2007.

KJÆR, A. et al. Histamine-and stress-induced secretion of ACTH and β-endorphin: involvement of corticotropin-releasing hormone and vasopressin. **Neuroendocrinology,** v. 56, n. 3, p. 419-428, 1992.

KNOLL, A. T.; CARLEZON, W. A., JR. Dynorphin, stress, and depression. **Brain Res,** v. 1314, p. 56-73, Feb 16 2010.

KOMATSU, H. et al. Decreased response to social defeat stress in mu-opioid-receptor knockout mice. **Pharmacol Biochem Behav,** v. 99, n. 4, p. 676-82, Oct 2011.

KOPNISKY, K. L.; HYMAN, S. E. Molecular and cellular biology of addiction. **Neuropsychopharmacology: The fifth generation of progress**, p. 1368-1379, 2002.

KRANTZ, M. J.; MEHLER, P. S. Treating opioid dependence. Growing implications for primary care. **Arch Intern Med,** v. 164, n. 3, p. 277-88, Feb 09 2004.

KRISHNAN, V. et al. Molecular adaptations underlying susceptibility and resistance to social defeat in brain reward regions. **Cell,** v. 131, n. 2, p. 391-404, Oct 19 2007.

KRISHNAN, V. et al. AKT signaling within the ventral tegmental area regulates cellular and behavioral responses to stressful stimuli. **Biol Psychiatry,** v. 64, n. 8, p. 691-700, Oct 15 2008.

KUHAR, J. R. et al. Mu opioid receptor stimulation activates c-Jun N-terminal kinase 2 by distinct arrestin-dependent and independent mechanisms. **Cell Signal,** v. 27, n. 9, p. 1799-806, Sep 2015.

LAREDO, S. A. et al. Effects of defeat stress on behavioral flexibility in males and females: modulation by the mu-opioid receptor. **Eur J Neurosci,** v. 41, n. 4, p. 434-41, Feb 2015.

LAW, P. Y. et al. Molecular mechanisms and regulation of opioid receptor signaling. **Annu Rev Pharmacol Toxicol,** v. 40, p. 389-430, 2000.

LI, W. et al. Major Depressive Disorder and Kappa Opioid Receptor Antagonists. **Transl Perioper Pain Med,** v. 1, n. 2, p. 4-16, 2016.

LIN, C.-P. et al. Role of Spinal CXCL1 (GROα) in Opioid Tolerance: A Human-to-rodent Translational Study. **Anesthesiology,** v. 122, n. 3, p. 666, 2015.

LIPINSKI, C. A. et al. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. **Advanced drug delivery reviews,** v. 23, n. 1-3, p. 3-25, 1997.

LIU, W. et al. The Role of Neural Plasticity in Depression: From Hippocampus to Prefrontal Cortex. **Neural Plast,** v. 2017, p. 6871089, 2017a.

LIU, Y. F. et al. Stress induces activation of stress-activated kinases in the mouse brain. **J Neurochem,** v. 89, n. 4, p. 1034-43, May 2004.

LIU, Y. Y. et al. Social defeat stress causes depression-like behavior with metabolite changes in the prefrontal cortex of rats. **PLoS One,** v. 12, n. 4, p. e0176725, 2017b.

LOVELL, K. M. et al. structure–activity relationship studies of functionally selective kappa opioid receptor agonists that modulate ERK 1/2 phosphorylation while preserving G protein over βarrestin2 signaling bias. **ACS chemical neuroscience,** v. 6, n. 8, p. 1411-1419, 2015.

LU, W. Y. et al. G-protein-coupled receptors act via protein kinase C and Src to regulate NMDA receptors. **Nat Neurosci,** v. 2, n. 4, p. 331-8, Apr 1999.

LUTZ, P. E.; KIEFFER, B. L. Opioid receptors: distinct roles in mood disorders. **Trends Neurosci,** v. 36, n. 3, p. 195-206, Mar 2013.

MACHADO MDA, S. et al. 3'3-ditrifluoromethyldiphenyl diselenide: a new organoselenium compound

with interesting antigenotoxic and antimutagenic activities. **Mutat Res,** v. 673, n. 2, p. 133-40, Mar 17 2009.

MACHADO, M. S. et al. An organic selenium compound attenuates apomorphine-induced stereotypy in mice. **Neurosci Lett,** v. 410, n. 3, p. 198-202, Dec 27 2006.

MAGNI, D. V. et al. m-Trifluoromethyl diphenyl diselenide attenuates glutaric acid-induced seizures and oxidative stress in rat pups: involvement of the gamma-aminobutyric acidergic system. **J Neurosci Res,** v. 90, n. 9, p. 1723-31, Sep 2012.

MANJI, H. K.; ZARATE, C. A. Molecular and cellular mechanisms underlying mood stabilization in bipolar disorder: implications for the development of improved therapeutics. **Mol Psychiatry,** v. 7 Suppl 1, p. S1-7, 2002.

MANSOUR, A. et al. Opioid-receptor mRNA expression in the rat CNS: anatomical and functional implications. **Trends Neurosci,** v. 18, n. 1, p. 22-9, Jan 1995.

MAO, J. et al. Chronic morphine induces downregulation of spinal glutamate transporters: implications in morphine tolerance and abnormal pain sensitivity. **Journal of Neuroscience,** v. 22, n. 18, p. 8312-8323, 2002.

MARCUS, M. et al. Depression: A global public health concern. **WHO Department of Mental Health and Substance Abuse,** v. 1, p. 6-8, 2012.

MAZEI-ROBISON, M. S.; NESTLER, E. J. Opiate-induced molecular and cellular plasticity of ventral tegmental area and locus coeruleus catecholamine neurons. **Cold Spring Harbor Perspectives in Medicine,** v. 2, n. 7, p. a012070, 2012.

MCLAUGHLIN, J. P. et al. Prior activation of kappa opioid receptors by U50,488 mimics repeated forced swim stress to potentiate cocaine place preference conditioning. **Neuropsychopharmacology,** v. 31, n. 4, p. 787-94, Apr 2006a.

MCLAUGHLIN, J. P. et al. Social defeat stress-induced behavioral responses are mediated by the endogenous kappa opioid system. **Neuropsychopharmacology,** v. 31, n. 6, p. 1241-8, Jun 2006b.

MCLAUGHLIN, J. P. et al. Kappa opioid receptor antagonism and prodynorphin gene disruption block stress-induced behavioral responses. **J Neurosci,** v. 23, n. 13, p. 5674-83, Jul 02 2003.

MELO, I. et al. Enkephalin knockout male mice are resistant to chronic mild stress. **Genes Brain Behav,** v. 13, n. 6, p. 550-8, Jul 2014.

MENDEZ, I. A.; TRUJILLO, K. A. NMDA receptor antagonists inhibit opiate antinociceptive tolerance and locomotor sensitization in rats. **Psychopharmacology (Berl),** v. 196, n. 3, p. 497-509, Feb 2008.

MILLAN, M. J. On 'polypharmacy' and multi-target agents, complementary strategies for improving the treatment of depression: a comparative appraisal. **Int J Neuropsychopharmacol,** v. 17, n. 7, p. 1009-37, Jul 2014.

MUL, J. D. et al. Validity assessment of 5 day repeated forced-swim stress to model human depression in young-adult C57BL/6J and BALB/cJ mice. **eNeuro,** v. 3, n. 6, p. ENEURO. 0201-16.2016, 2016.

NABESHIMA, T. et al. Stress-induced changes in brain Met-enkephalin, Leu-enkephalin and dynorphin concentrations. **Life Sci,** v. 51, n. 3, p. 211-7, 1992.

NAKAGAWA, T. et al. Inhibition of morphine tolerance and dependence by MS-153, a glutamate

transporter activator. **European Journal of Pharmacology,** v. 419, n. 1, p. 39-45, 2001.

NAKAMURO, K. et al. Metabolism of Selenoamino Acids and Contribution of Selenium Methylation to Their Toxicity. **Journal of Health Science,** v. 46 (6), p. 418-421, 2000.

NARAJJI, C. et al. Biological importance of organoselenium compounds. **Indian Journal of Pharmaceutical Sciences,** v. 69, p. 344-351, 2007.

NIKULINA, E. M. et al. Prolonged effects of repeated social defeat stress on mRNA expression and function of mu-opioid receptors in the ventral tegmental area of rats. **Neuropsychopharmacology,** v. 30, n. 6, p. 1096-103, Jun 2005.

NOGUEIRA, C. W.; ROCHA, J. B. Toxicology and pharmacology of selenium: emphasis on synthetic organoselenium compounds. **Arch Toxicol,** v. 85, n. 11, p. 1313-59, Nov 2011.

NOGUEIRA, C. W. et al. Organoselenium and organotellurium compounds: toxicology and pharmacology. **Chem Rev,** v. 104, n. 12, p. 6255-85, Dec 2004.

OBATA, T. et al. MAP kinase pathways activated by stress: the p38 MAPK pathway. **Critical care medicine,** v. 28, n. 4, p. N67-N77, 2000.

OGURA, T.; EGAN, T. D. Chapter 15 - Opioid Agonists and Antagonists. In: (Ed.). **Pharmacology and Physiology for Anesthesia**. Philadelphia: W.B. Saunders, 2013. p.253-271. ISBN 978-1-4377-1679-5.

OLIANAS, M. C. et al. Regulation of PI3K/Akt signaling by N-desmethylclozapine through activation of delta-opioid receptor. **Eur J Pharmacol,** v. 660, n. 2-3, p. 341-50, Jun 25 2011.

OLIVEIRA, C. E. et al. Pain-depression dyad induced by reserpine is relieved by p,p'-methoxyl- diphenyl diselenide in rats. **Eur J Pharmacol,** v. 791, p. 794-802, Nov 15 2016.

ONALI, P. et al. Direct agonist activity of tricyclic antidepressants at distinct opioid receptor subtypes. **J Pharmacol Exp Ther,** v. 332, n. 1, p. 255-65, Jan 2010.

PARLATO, R. et al. Effects of the cell type-specific ablation of the cAMP-responsive transcription factor in noradrenergic neurons on locus coeruleus firing and withdrawal behavior after chronic exposure to morphine. **J Neurochem,** v. 115, n. 3, p. 563-73, Nov 2010.

PASCO, J. A. et al. Dietary selenium and major depression: a nested case-control study. **Complement Ther Med,** v. 20, n. 3, p. 119-23, Jun 2012.

PFEIFFER, A. et al. Psychotomimesis mediated by kappa opiate receptors. **Science,** v. 233, n. 4765, p. 774-6, Aug 15 1986.

PORSOLT, R. D. et al. Behavioral Despair in Mice - Primary Screening-Test for Antidepressants. **Archives Internationales De Pharmacodynamie Et De Therapie,** v. 229, n. 2, p. 327-336, 1977.

POULIN, J. F. et al. Enkephalin downregulation in the nucleus accumbens underlies chronic stress-induced anhedonia. **Stress,** v. 17, n. 1, p. 88-96, Jan 2014.

PRIGOL, M. et al. m-Trifluoromethyl-diphenyl diselenide attenuates pentylenetetrazole-induced seizures in mice by inhibiting GABA uptake in cerebral cortex slices. **Pharmacol Rep,** v. 61, n. 6, p. 1127-33, Nov-Dec 2009.

PURSER, S. et al. Fluorine in medicinal chemistry. **Chem Soc Rev,** v. 37, n. 2, p. 320-30, Feb 2008.

QI, X. et al. Fluoxetine increases the activity of the ERK-CREB signal system and alleviates the

depressive-like behavior in rats exposed to chronic forced swim stress. **Neurobiol Dis,** v. 31, n. 2, p. 278-85, Aug 2008.

QI, X. et al. The depressive-like behaviors are correlated with decreased phosphorylation of mitogen-activated protein kinases in rat brain following chronic forced swim stress. **Behav Brain Res,** v. 175, n. 2, p. 233-40, Dec 15 2006a.

QI, X. et al. The depressive-like behaviors are correlated with decreased phosphorylation of mitogen-activated protein kinases in rat brain following chronic forced swim stress. **Behavioural brain research,** v. 175, n. 2, p. 233-240, 2006b.

QUINES, C. B. et al. Homeostatic effect of p-chloro-diphenyl diselenide on glucose metabolism and mitochondrial function alterations induced by monosodium glutamate administration to rats. **Amino Acids,** v. 48, n. 1, p. 137-48, Jan 2016a.

QUINES, C. B. et al. Diphenyl diselenide elicits antidepressant-like activity in rats exposed to monosodium glutamate: A contribution of serotonin uptake and Na(+), K(+)-ATPase activity. **Behav Brain Res,** v. 301, p. 161-7, Mar 15 2016b.

RAYMAN, M. P. The importance of selenium to human health. **Lancet,** v. 356, n. 9225, p. 233-41, Jul 15 2000.

RITTER, S. L.; HALL, R. A. Fine-tuning of GPCR activity by receptor-interacting proteins. **Nature reviews. Molecular cell biology,** v. 10, n. 12, p. 819, 2009.

ROBINSON, G. E. et al. Sociogenomics: social life in molecular terms. **Nat Rev Genet,** v. 6, n. 4, p. 257-70, Apr 2005.

ROBINSON, S. A. et al. A role for the mu opioid receptor in the antidepressant effects of buprenorphine. **Behav Brain Res,** v. 319, p. 96-103, Feb 15 2017.

ROGOZ, Z. et al. Effect of co-treatment with fluoxetine or mirtazapine and risperidone on the active behaviors and plasma corticosterone concentration in rats subjected to the forced swim test. **Pharmacol Rep,** v. 64, n. 6, p. 1391-9, 2012.

ROMAN, D. L. et al. Interactions of antidepressants with the serotonin transporter: a contemporary molecular analysis. **Eur J Pharmacol,** v. 479, n. 1-3, p. 53-63, Oct 31 2003.

ROSA, S. G. et al. Antinociceptive action of diphenyl diselenide in the nociception induced by neonatal administration of monosodium glutamate in rats. **Eur J Pharmacol,** v. 758, p. 64-71, Jul 5 2015.

ROSA, S. G. et al. Diphenyl diselenide ameliorates monosodium glutamate induced anxiety-like behavior in rats by modulating hippocampal BDNF-Akt pathway and uptake of GABA and serotonin neurotransmitters. **Physiol Behav,** v. 155, p. 1-8, Mar 1 2016.

RUBINSTEIN, M. et al. Absence of opioid stress-induced analgesia in mice lacking beta-endorphin by site-directed mutagenesis. **Proc Natl Acad Sci U S A,** v. 93, n. 9, p. 3995-4000, Apr 30 1996.

RUSSO, S. J. et al. Neurobiology of resilience. **Nat Neurosci,** v. 15, n. 11, p. 1475-84, Nov 2012.

SAITOH, A. et al. The novel delta opioid receptor agonist KNT-127 produces antidepressant-like and antinociceptive effects in mice without producing convulsions. **Behav Brain Res,** v. 223, n. 2, p. 271-9, Oct 1 2011.

SALAVECZ, G. et al. Work related stress and depression. 2014.

SAMUELS, B. A. et al. The Behavioral Effects of the Antidepressant Tianeptine Require the Mu-Opioid

Receptor. **Neuropsychopharmacology**, Apr 19 2017.

SANCHEZ-BLAZQUEZ, P. et al. Mu-opioid receptors transiently activate the Akt-nNOS pathway to produce sustained potentiation of PKC-mediated NMDAR-CaMKII signaling. **PLoS One,** v. 5, n. 6, p. e11278, Jun 23 2010.

SANTINI, Z. I. et al. The association between social relationships and depression: a systematic review. **Journal of affective disorders,** v. 175, p. 53-65, 2015.

SARTORI, G. et al. Antiviral Action of Diphenyl Diselenide on Herpes Simplex Virus 2 Infection in Female BALB/c Mice. **J Cell Biochem,** v. 117, n. 7, p. 1638-48, Jul 2016.

SAVEANU, R. V.; NEMEROFF, C. B. Etiology of depression: genetic and environmental factors. **Psychiatr Clin North Am,** v. 35, n. 1, p. 51-71, Mar 2012.

SAVEGNAGO, L. et al. Structural modifications into diphenyl diselenide molecule do not cause toxicity in mice. **Environ Toxicol Pharmacol,** v. 27, n. 2, p. 271-6, Mar 2009.

SCARONE, S. et al. Asymmetrical distribution of beta-endorphin in cerebral hemispheres of suicides: preliminary data. **Psychiatry Res,** v. 32, n. 2, p. 159-66, May 1990.

SCHILDKRAUT, J. J. The catecholamine hypothesis of affective disorders: a review of supporting evidence. **Am J Psychiatry,** v. 122, n. 5, p. 509-22, Nov 1965.

SCHWARTZ, J.-C. et al. Endogenous Enkephalins, Depression and Antidepressants. In: BRILEY, M. e FILLION, G. (Ed.). **New Concepts in Depression**. London: Macmillan Education UK, 1988. p.247-259. ISBN 978-1-349-09506-3.

SHARP, B. M. Opioid receptor expression and function. **Journal of neuroimmunology**, 2004.

SHAW, D.; AL'ABSI, M. Attenuated beta endorphin response to acute stress is associated with smoking relapse. **Pharmacol Biochem Behav,** v. 90, n. 3, p. 357-62, Sep 2008.

SHIRAYAMA, Y. et al. Stress increases dynorphin immunoreactivity in limbic brain regions and dynorphin antagonism produces antidepressant-like effects. **Journal of Neurochemistry,** v. 90, n. 5, p. 1258-68, Sep 2004.

SHISHKINA, G. T. et al. Serotonergic changes produced by repeated exposure to forced swimming: correlation with behavior. **Ann N Y Acad Sci,** v. 1148, p. 148-53, Dec 2008.

SIGGINS, G. R. et al. Glutamatergic transmission in opiate and alcohol dependence. **Ann N Y Acad Sci,** v. 1003, p. 196-211, Nov 2003.

SMITH, J. S. et al. Stress-induced activation of the dynorphin/kappa-opioid receptor system in the amygdala potentiates nicotine conditioned place preference. **J Neurosci,** v. 32, n. 4, p. 1488-95, Jan 25 2012.

STERU, L. et al. The Tail Suspension Test - a New Method for Screening Antidepressants in Mice. **Psychopharmacology,** v. 85, n. 3, p. 367-370, 1985.

STEWART, A. M.; KALUEFF, A. V. Developing better and more valid animal models of brain disorders. **Behav Brain Res,** v. 276, p. 28-31, Jan 01 2015.

TAFET, G. E.; NEMEROFF, C. B. The Links Between Stress and Depression: Psychoneuroendocrinological, Genetic, and Environmental Interactions. **J Neuropsychiatry Clin Neurosci,** v. 28, n. 2, p. 77-88, Spring 2016.

THOMAS, G. M.; HUGANIR, R. L. MAPK cascade signalling and synaptic plasticity. **Nat Rev Neurosci,** v. 5, n. 3, p. 173-83, Mar 2004.

TOMPKINS, D. A. et al. A double blind, within subject comparison of spontaneous opioid withdrawal from buprenorphine versus morphine. **J Pharmacol Exp Ther,** v. 348, n. 2, p. 217-26, Feb 2014.

TREADWAY, M. T. et al. Illness progression, recent stress, and morphometry of hippocampal subfields and medial prefrontal cortex in major depression. **Biological psychiatry,** v. 77, n. 3, p. 285- 294, 2015.

WAKIM, J. H. Alleviating symptoms of withdrawal from an opioid. **Pain Ther,** v. 1, n. 1, p. 4, Dec 2012.

WANG, F.-R. et al. Possible Involvement of μ opioid receptor in the antidepressant-like effect of shuyu formula in restraint stress-induced depression-like rats. **Evidence-Based Complementary and Alternative Medicine,** v. 2015, 2015.

WANG, X. F. et al. Agmatine modulates neuroadaptations of glutamate transmission in the nucleus accumbens of repeated morphine-treated rats. **European Journal of Pharmacology,** v. 650, n. 1, p. 200-5, Jan 10 2011.

WANG, X. S. et al. Molecular cloning and characterization of a novel p38 mitogen-activated protein kinase. **J Biol Chem,** v. 272, n. 38, p. 23668-74, Sep 19 1997.

WHALEN, E. J. et al. Therapeutic potential of beta-arrestin- and G protein-biased agonists. **Trends Mol Med,** v. 17, n. 3, p. 126-39, Mar 2011.

WHO. Depression: A Global Crisis. 2012. Disponível em: < http://www.who.int/mental_health/management/depression/wfmh_paper_depression_wmhd_2012.pdf >.

_____. Depression and other common mental disorders: global health estimates. March 2018 2017. Disponível em: < http://apps.who.int/iris/bitstream/handle/10665/254610/WHO-MSD-MER-2017. >.

WILLIAMS, A. V. et al. Acute inhibition of kappa opioid receptors before stress blocks depression-like behaviors in California mice. **Progress in Neuro-Psychopharmacology and Biological Psychiatry**, 2018.

WILLNER, P.; MITCHELL, P. J. The validity of animal models of predisposition to depression. **Behav Pharmacol,** v. 13, n. 3, p. 169-88, May 2002.

WILLNER, P. et al. The neurobiology of depression and antidepressant action. **Neurosci Biobehav Rev,** v. 37, n. 10 Pt 1, p. 2331-71, Dec 2013.

WILLOCK, C.; FRANKE, T. Akt signaling in fear memory processing and depression-like behaviors. **The FASEB Journal,** v. 29, n. 1 Supplement, p. 931.5, 2015.

YAMAMOTO, M. et al. Effects of single and repeated prolonged stress on mu-opioid receptor mRNA expression in rat gross hypothalamic and midbrain homogenates. **Brain Res,** v. 980, n. 2, p. 191-6, Aug 08 2003.

YANAGIDA, S. et al. Effect of acute imipramine administration on the pattern of forced swim-induced c-Fos expression in the mouse brain. **Neurosci Lett,** v. 629, p. 119-24, Aug 26 2016.

YANG, X. et al. The Role of MAPK and Dopaminergic Synapse Signaling Pathways in Antidepressant Effect of Electroacupuncture Pretreatment in Chronic Restraint Stress Rats. **Evid Based Complement**

Alternat Med, v. 2017, p. 2357653, 2017.

YANKELEVITCH-YAHAV, R. et al. The forced swim test as a model of depressive-like behavior. **J Vis Exp**, n. 97, Mar 02 2015.

YIN, X. et al. Stress-based animal models of depression: Do we actually know what we are doing? **Brain research,** v. 1652, p. 30-42, 2016.

YOUDIM, M. B. et al. The therapeutic potential of monoamine oxidase inhibitors. **Nat Rev Neurosci,** v. 7, n. 4, p. 295-309, Apr 2006.

ZALSMAN, G. et al. Postmortem mu-opioid receptor binding in suicide victims and controls. **J Neural Transm (Vienna),** v. 112, n. 7, p. 949-54, Jul 2005.

ZBOROWSKI, V. A. et al. p-Chloro-diphenyl diselenide reverses memory impairment-related to stress caused by corticosterone and modulates hippocampal [(3)H]glutamate uptake in mice. **Physiol Behav,** v. 164, n. Pt A, p. 25-33, Oct 01 2016.

ZOMKOWSKI, A. D. E. et al. Evidence for the involvement of the opioid system in the agmatine antidepressant-like effect in the forced swimming test. **Neuroscience Letters,** v. 381, n. 3, p. 279-283, Jun 24 2005.

ANEXOS

ANEXO A- CARTA DE APROVAÇÃO DO PROJETO DE PESQUISA PELA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE FEDERAL DE SANTA MARIA

Comissão de Ética no Uso de Animais
da
Universidade Federal de Santa Maria

Santa Maria, 8th May 2015

CERTIFIED

We certify that the Research "Evaluation of the opioid system involvement in antidepressant-like effect of diselenide m-trifluoromethyl-diphenyl in mice", protocol number CEUA 7770060215, utilizing 460 Heterogenics mice (460 males), under the responsibility Cristina W. Nogueira, was approved in the meeting of day 04/29/2015, and agree with Ethical Principles in Animal Research adopted by Ethic Committee on Animal Use of Federal University of Santa Maria.

Certificamos que o Projeto intitulado "Avaliação do envolvimento do sistema opióide no efeito do tipo antidepressivo do disseleneto de m-trifluormetil-difenila em camundongos", protocolado sob o CEUA nº 7770060215, utilizando 460 Camundongos heterogênicos (460 machos), sob a responsabilidade de Cristina W. Nogueira, foi aprovado na reunião de 29/04/2015, e está de acordo com os princípios éticos de experimentação animal da Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria.

yours faithfully,

Vânia Lucia Loro
Coordinator of the Ethics Committe on Animal Use
Federal University of Santa Maria

Avenida Roraima, 1000, Reitoria, 2º andar - CEP 97105-900 Santa Maria, RS - tel: 55 (55) 3220-9362 / fax: 55 (55) 3220-8009
Horário de atendimento: das 8:30 ás 11:30 e 14:00 ás 16:30hs : e-mail:ceua.ufsm@gmail.com
CEUA Nº 7770060215

ANEXO B- Autorização para reprodução do artigo científico "Opioid system contribution to the antidepressant-like action of *m*-trifluoromethyl-diphenyl diselenide in mice: A compound devoid of tolerance and withdrawal syndrome" publicado na revista *Journal of Psychopharmacology 31(9) (2017) 1250– 1262*

Copyright Clearance Center RightsLink®

Home | Account Info | Help

SAGE Publishing

Title:	Opioid system contribution to the antidepressant-like action of m-trifluoromethyl-diphenyl diselenide in mice: A compound devoid of tolerance and withdrawal syndrome
Author:	Suzan G Rosa, Ana P Pesarico, Carolina F Tagliapietra, et al
Publication:	Journal of Psychopharmacology
Publisher:	SAGE Publications
Date:	09/01/2017

Copyright © 2017, © SAGE Publications

Logged in as: Suzan Rosa
Account #: 3001279050
LOGOUT

If you are a SAGE journal author requesting permission to reuse material from your journal article, please note you may be able to reuse your content without requiring permission from SAGE. Please review SAGE's author re-use and archiving policies at https://us.sagepub.com/en-us/nam/journal-author-archiving-policies-and-re-use for more information.

If your request does not fall within SAGE's reuse guidelines, please proceed with submitting your request by selecting one of the other reuse categories that describes your use. Please note, a fee may be charged for reuse of content requiring permission. Please contact permissions@sagepub.co.uk if you have questions.

ANEXO C- Autorização para reprodução do artigo científico "*m*-Trifluoromethyl-diphenyl Diselenide Regulates Prefrontal Cortical MOR and KOR Protein Levels and Abolishes the Phenotype Induced by Repeated Forced Swim Stress in Mice" aceito para publicacão na revista *Molecular Neurobiology, 2018.* doi: 10.1007/s12035-018-1024-x.

SPRINGER NATURE LICENSE TERMS AND CONDITIONS

Jun 13, 2018

This Agreement between Ms. Suzan Rosa ("You") and Springer Nature ("Springer Nature") consists of your license details and the terms and conditions provided by Springer Nature and Copyright Clearance Center.

License Number	4367381170120
License date	Jun 13, 2018
Licensed Content Publisher	Springer Nature
Licensed Content Publication	Molecular Neurobiology
Licensed Content Title	m-Trifluoromethyl-diphenyl Diselenide Regulates Prefrontal Cortical MOR and KOR Protein Levels and Abolishes the Phenotype Induced by Repeated Forced Swim Stress in Mice
Licensed Content Author	Suzan Gonçalves Rosa, Ana Paula Pesarico, Franciele Martini et al
Licensed Content Date	Jan 1, 2018
Type of Use	Thesis/Dissertation
Requestor type	academic/university or research institute
Format	print and electronic
Portion	full article/chapter
Will you be translating?	no
Circulation/distribution	<501
Author of this Springer Nature content	yes
Title	CONTRIBUIÇÃO DO SISTEMA OPIÓIDE PARA O EFEITO DO TIPO ANTIDEPRESSIVO DO DISSELENETO DE m-TRIFLUORMETIL-DIFENILA EM CAMUNDONGOS
Instructor name	Cristina Nogueira
Institution name	UFSM
Expected presentation date	Jul 2018
Requestor Location	Ms. Suzan Rosa Av Roraima 1000 SANTA MARIA, 97115900 Brazil Attn: Ms. Suzan Rosa
Billing Type	Invoice
Billing Address	Ms. Suzan Rosa Rua Dr Bozano 1100 SANTA MARIA, Brazil 97015002 Attn: Ms. Suzan Rosa
Total	0.00 USD
Terms and Conditions	

Springer Nature Terms and Conditions for RightsLink Permissions

Springer Customer Service Centre GmbH (the Licensor) hereby grants you a non-exclusive, world-wide licence to reproduce the material and for the purpose and

ANEXO D- Autorização para reprodução do artigo científico "*m*-Trifluoromethyl-diphenyl diselenide promotes resilience to social avoidance induced by social defeat stress in mice: Contribution of opioid receptors and MAPKs" publicado na revista *Progress in Neuropsychopharmacology & Biological Psychiatry 82 (2018) 123–135.*

ELSEVIER LICENSE
TERMS AND CONDITIONS

Apr 25, 2018

This Agreement between Ms. Suzan Rosa ("You") and Elsevier ("Elsevier") consists of your license details and the terms and conditions provided by Elsevier and Copyright Clearance Center.

License Number	4336010882308
License date	Apr 25, 2018
Licensed Content Publisher	Elsevier
Licensed Content Publication	Progress in Neuro-Psychopharmacology and Biological Psychiatry
Licensed Content Title	m-Trifluoromethyl-diphenyl diselenide promotes resilience to social avoidance induced by social defeat stress in mice: Contribution of opioid receptors and MAPKs
Licensed Content Author	Suzan Gonçalves Rosa,Ana Paula Pesarico,Cristina Wayne Nogueira
Licensed Content Date	Mar 2, 2018
Licensed Content Volume	82
Licensed Content Issue	n/a
Licensed Content Pages	13
Start Page	123
End Page	135
Type of Use	reuse in a thesis/dissertation
Portion	full article
Format	both print and electronic
Are you the author of this Elsevier article?	No
Will you be translating?	No
Title of your thesis/dissertation	CONTRIBUIÇÃO DO SISTEMA OPIÓIDE PARA O EFEITO DO TIPO ANTIDEPRESSIVO DO DISSELENETO DE m-TRIFLUORMETIL-DIFENILA EM CAMUNDONGOS
Expected completion date	Jul 2018
Estimated size (number of pages)	100
Requestor Location	Ms. Suzan Rosa Av Roraima 1000 SANTA MARIA, 97115900 Brazil Attn: Ms. Suzan Rosa
Publisher Tax ID	GB 494 6272 12
Total	0.00 USD
Terms and Conditions	