**UNIVERSIDADE FEDERAL DE SANTA MARIA**
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**PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS BIOLÓGICAS: BIOQUÍMICA TOXICOLÓGICA**

# 3-(4-FLUOROFENILSELENIL)-2,5 DIFENILSELENOFENO PRODUZ AÇÃO DO TIPO-ANTIDEPRESSIVA EM DIFERENTES MODELOS DE DEPRESSÃO EM CAMUNDONGOS

**TESE DE DOUTORADO**

**Bibiana Mozzaquatro Gai**

**Santa Maria, RS, Brasil**

**2014**

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**Bibiana Mozzaquatro Gai**

Tese apresentada ao Curso de Doutorado do Programa de Pós-Graduação em Ciências Biológicas, Área de Concentração em Bioquímica Toxicológica, da Universidade Federal de Santa Maria (UFSM, RS), como requisito parcial para obtenção do grau de
**Doutora em Bioquímica Toxicológica**.

**Orientadora: Prof. Dra. Cristina Wayne Nogueira**

**Co-orientador: Prof. Dr. Gilson Rogério Zeni**

**Santa Maria, RS, Brasil**

**2014**

**Universidade Federal de Santa Maria**
**Centro de Ciências Naturais e Exatas**
**Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica**

A Comissão Examinadora, abaixo assinada,
aprova a Tese de Doutorado

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elaborada por
**Bibiana Mozzaquatro Gai**

como requisito parcial para obtenção do grau de
**Doutora em Bioquímica Toxicológica**

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Santa Maria, 11 de março de 2014.

*Dedico este estudo*

*à minha mãe Dolores,*

*meu pai Valmor*

*e minha irmã Rafaela,*

*que foram incansáveis no apoio e amor a mim dedicados*

*e que estiveram sempre ao meu lado,*

*mesmo quando a distância se fez presente.*

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*“Se você está deprimido,*

*Você está vivendo no passado;*

*Se você está ansioso,*

*Você está vivendo no futuro;*

*Se você está em paz*

*Você está vivendo no momento presente.”*

*(Lao Tzu)*

# RESUMO

Tese de Doutorado
Programa de Pós-Graduação em Ciências Biológicas: Bioquímica Toxicológica
Universidade Federal de Santa Maria

## 3-(4-FLUOROFENILSELENIL)-2,5 DIFENILSELENOFENO PRODUZ AÇÃO DO TIPO-ANTIDEPRESSIVA EM DIFERENTES MODELOS DE DEPRESSÃO EM CAMUNDONGOS

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ORIENTADORA: CRISTINA WAYNE NOGUEIRA
CO-ORIENTADOR: GILSON ROGÉRIO ZENI
Data e Local da Defesa: Santa Maria, 11 de março de 2014.

Os selenofenos são uma classe de compostos heterocíclicos aromáticos com promissoras propriedades farmacológicas. Tendo em vista o importante papel do selênio na regulação do humor e a grande prevalência populacional das doenças depressivas, o principal objetivo deste estudo foi investigar a ação do tipo antidepressiva de 3-(organosseleno)-2,5-difenil-selenofenos em camundongos. A fim de atender a este objetivo, o efeito farmacológico destes compostos foi analisado pelo uso de diferentes modelos experimentais de depressão e os resultados foram apresentados em três artigos científicos. Primeiramente, os resultados do Artigo 1 demonstraram a ação do tipo antidepressiva de cinco representantes da classe dos selenofenos. Os compostos **H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** e **$CF_3$-DPS** reduziram significantemente o tempo total de imobilidade de camundongos avaliados no teste do nado forçado (TNF), efeito este que parece estar relacionado às suas estruturas químicas. A ação do tipo antidepressiva do **F-DPS** foi observada em menores doses em comparação aos outros selenofenos e envolve a fosforilação da proteína quinase regulada por sinal extracelular (ERK), cuja via de sinalização é comumente modulada por drogas antidepressivas. Os Artigos 2 e 3 investigaram o efeito farmacológico do **F-DPS** em modelos crônicos de depressão induzida pela dor neuropática e pela administração crônica de corticosterona em camundongos, respectivamente. Tanto o tratamento agudo como o subcrônico com **F-DPS** reverteram significantemente o comportamento do tipo depressivo produzido pela ligação parcial do nervo ciático (LPNC), enquanto que a sensibilidade à dor foi reduzida somente após a terapia prolongada com este composto. Por sua vez, a administração repetida do hormônio glicocorticoide corticosterona induziu alterações comportamentais, endócrinas e neuroquímicas similares às observadas clinicamente na depressão e que também foram revertidas pelo tratamento dos animais com **F-DPS**. Com base nestes resultados, acredita-se que os mecanismos de ação farmacológica deste composto orgânico de selênio envolvam, pelo menos em parte, a modulação dos sistemas glutamatérgico e serotonérgico, a regulação da atividade do eixo hipotálamo-pituitária-adrenal (HPA) e modificações em vias neuronais relacionadas à plasticidade sináptica. Juntos os resultados apresentados nesta tese de doutorado sugerem que o estudo das propriedades farmacológicas de compostos selenofenos, particularmente do **F-DPS**, parece ser interessante no desenvolvimento de futuras terapias para o tratamento dos distúrbios neurológicos relacionados às doenças depressivas.

**Palavras-chave:** Selênio. Selenofenos. Depressão. Estresse. Dor crônica. Camundongos.

# ABSTRACT

Doctor Course Thesis
Professional Graduation Program in Biological Sciences: Toxicological Biochemistry
Federal University of Santa Maria

## 2,5-DIPHENYL-3-(4-FLUOROPHENYLSELENO) SELENOPHENE PRODUCES ANTIDEPRESSANT-LIKE ACTION IN DIFFERENT MODELS OF DEPRESSION IN MICE

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CO-ADVISER: GILSON ROGÉRIO ZENI
Defense Place and Date: Santa Maria, March 11th, 2014.

Selenophenes are a promising class of heterocyclic selenium-containing compounds presenting important pharmacological properties. Based on selenium well-described role on mood regulation and since depression is a serious and prevalent disease affecting a wide part of the world's population, the main aim of this work was to investigate de antidepressant-like action of 3-(organosseleno)-2,5-diphenyl-selenophenes in mice. The pharmacological effect of these compounds was analyzed by using different experimental models of depression and results were shown by three scientific articles. Firstly, results of Article 1 demonstrated the antidepressant-like action of five selenophene compounds. **H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** and **$CF_3$-DPS** reduced the total immobility time of mice evaluated in the forced swimming test (FST), which seems to be related to their chemical structure. The antidepressant-like action of **F-DPS** was observed at lower doses than other selenophenes e involves the phosphorylation of extracellular-signal-regulated kinases (ERK), whose signaling pathway is commonly modulated by antidepressant drugs. Articles 2 and 3 investigated the pharmacological effect of **F-DPS** in mouse models of depression induced by both neuropathic pain and chronic corticosterone administration, respectively. Both the acute and subchronic treatments with **F-DPS** significantly reversed the depression-related behavior produced by partial sciatic nerve ligation (PSNL), whereas pain sensibility was only reduced after repeated treatment with this selenophene. Besides, repeated administration of the glucocorticoid hormone corticosterone induced behavior, endocrinal and neurochemical changes similar to those clinically observed in depression, which were also reversed by treatment of animals with **F-DPS**. Based on these data, the mechanisms of pharmacological action of this organoselenium compound seem to involve, at least in part, a modulation of glutamatergic and serotonergic systems, the hypothalamic-pituitary-adrenal (HPA) axis regulation and changes on neuronal pathways related to the synaptic plasticity. Together, the results shown in this thesis suggest the pharmacological properties of selenophene compounds, particularly **F-DPS**, as an interesting tool in the study and development of future therapies for depressive disorders.

**Keywords**: Selenium. Selenophenes. Depression. Stress. Chronic pain. Mice.

# LISTA DE FIGURAS

# LISTA DE ABREVIATURAS E SIGLAS

**3-ASP** – 3-alquinilselenofeno

**5-HT** – 5-hidroxitriptamina, serotonina

**ACTH** – Hormônio adrenocorticotrófico

**ADTs** – Antidepressivos tricíclicos

**AMPA** – Alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiónico

**BDNF** – Fator neurotrófico derivado do cérebro

**$CF_3$-DPS –** 3-(4-trifluorofenilselenil)-2,5 difenilselenofeno

**CFR –** Hormônio liberador de corticotrofina

**$CH_3$-DPS –** 3-(4-metilfenilselenil)-2,5 difenilselenofeno

**Cl-DPS –** 3-(4-clorofenilselenil)-2,5 difenilselenofeno

**CREB** – Proteína ligadora ao elemento responsivo ao AMP cíclico

**ECIM** – Estresse crônico imprevisível moderado

**ERK** – proteína quinase regulada por sinal extracelular

**F-DPS –** 3-(4-fluorofenilselenil)-2,5 difenilselenofeno

**GLT-1** – Transportador de glutamato do tipo 1

**H-DPS –** 3-fluorofenilselenil-2,5 difenilselenofeno

**HPA** – Hipotálamo-pituitária-adrenal

**IMAOs** – Inibidores da monoaminoxidase

**ISRSs** – Inibidores seletivos da recaptação de serotonina

**LPNC** – Ligação parcial do nervo ciático

**MAO** – Monoaminoxidase

**MAPK** – proteína quinase regulada por mitógeno

**NA** – Noradrenalina

**NMDA** – N-metil D-aspartato

**PCPA** – paraclorofenilalanina

**PKA** – Proteína quinase A

**PKC** – Proteína quinase C

**TCE** – Teste do claro-escuro

**TNF** – Teste do nado forçado

**TSC** – Teste da suspensão da cauda

# LISTA DE APÊNDICES

## SUMÁRIO

# INTRODUÇÃO

## 1 A depressão e suas causas

A depressão é uma doença heterogênea e multifatorial, que acomete cerca de 21% da população (desenvolvimento de pelo menos um episódio depressivo ao longo da vida) e que envolve uma complexa interação entre fatores genéticos, desenvolvimentais e ambientais (Millan, 2006). O estado depressivo é uma alteração psicológica em que a pessoa tem um sentimento de culpa, nunca bem definido, e se sente fracassada, desesperançosa, pessimista, com auto-estima baixa, triste, melancólica e anedônica, isto é, sem interesse nos prazeres da vida. O indivíduo apresenta também alterações cognitivas, perda de interesse, diminuição da capacidade de concentração, associado em geral à fadiga e lentidão psicomotora (Thase, 2013). Além do humor depressivo, que varia pouco de dia para dia ou segundo as circunstâncias, o paciente pode apresentar sintomas somáticos como fraqueza muscular, perda do apetite, perda de peso, perda da libido, distúrbios do sono, cefaléia e aumento na sensação de dor (Demyttenaere et al., 2005; Thase, 2013).

As causas da depressão ainda permanecem desconhecidas. Estudos sugerem o envolvimento de várias vias neurais no desenvolvimento dessa doença, mas a hipótese das monoaminas é comumente utilizada para explicar a gênese dos transtornos depressivos (Millan, 2004, 2006, 2013). A Hipótese Monoaminérgica da depressão postula que esta doença se deve à deficiência de neurotransmissores em sinapses monoaminérgicas (Schildkraut, 1965). Nos anos 60, após a introdução no mercado dos primeiros medicamentos com efeito antidepressivo, constatou-se que eles interagiam com sistemas de monoaminas (principalmente serotonina e noradrenalina), que atuam como neurotransmissores em sinapses nervosas. A serotonina (5-HT) e a noradrenalina (NA) são liberadas em todo o encéfalo por neurônios de regiões específicas e interagem com múltiplos tipos de receptores cerebrais para regular a vigília, alerta, atenção, processos sensoriais, apetite e também o humor, dentre outras funções (Murphy and Lesch, 2008). Esses neurotransmissores são removidos das sinapses após sua liberação, por um processo de recaptação pelo neurônio pré-sináptico. Após a recaptação, são degradados no neurônio pré-sináptico pela ação da enzima monoaminoxidase (MAO), ou são "re-embalados" em vesículas, para serem liberados na fenda sináptica novamente (Figura 1). Os dois mecanismos produzem um controle nos níveis de neurotransmissores presentes na fenda sináptica. Esta constatação sugeriu inicialmente que

os antidepressivos atuassem por aumento da transmissão serotoninérgica e noradrenérgica, compensando um possível estado de deficiência de neurotransmissores (McLeod and McLeod, 1971; Schildkraut, 1965).

Apesar da relevância da hipótese das monoaminas na investigação da depressão, atualmente existe certa resistência para sua plena aceitação, especialmente devido ao fato de que todos os medicamentos antidepressivos aumentam, de imediato, o nível desses neurotransmissores na fenda sináptica, porém seu efeito clínico só ocorre algumas semanas depois (Millan, 2004, 2006, 2013). Outras substâncias, como por exemplo, a cocaína, também elevam os níveis das monoaminas, mas não apresentam efeito antidepressivo (Millan, 2004). Desse modo, o conhecimento atual da complexa inter-relação entre os sistemas de neurotransmissão cerebrais restringiu a hipótese do déficit de monoaminas na fenda sináptica a concepções simplistas e uma de suas consequências foi que o foco das hipóteses biológicas da depressão também foi abrangido para outros neurotransmissores e seus receptores (Blier, 2013; Millan, 2004, 2006, 2013; Villanueva, 2013).

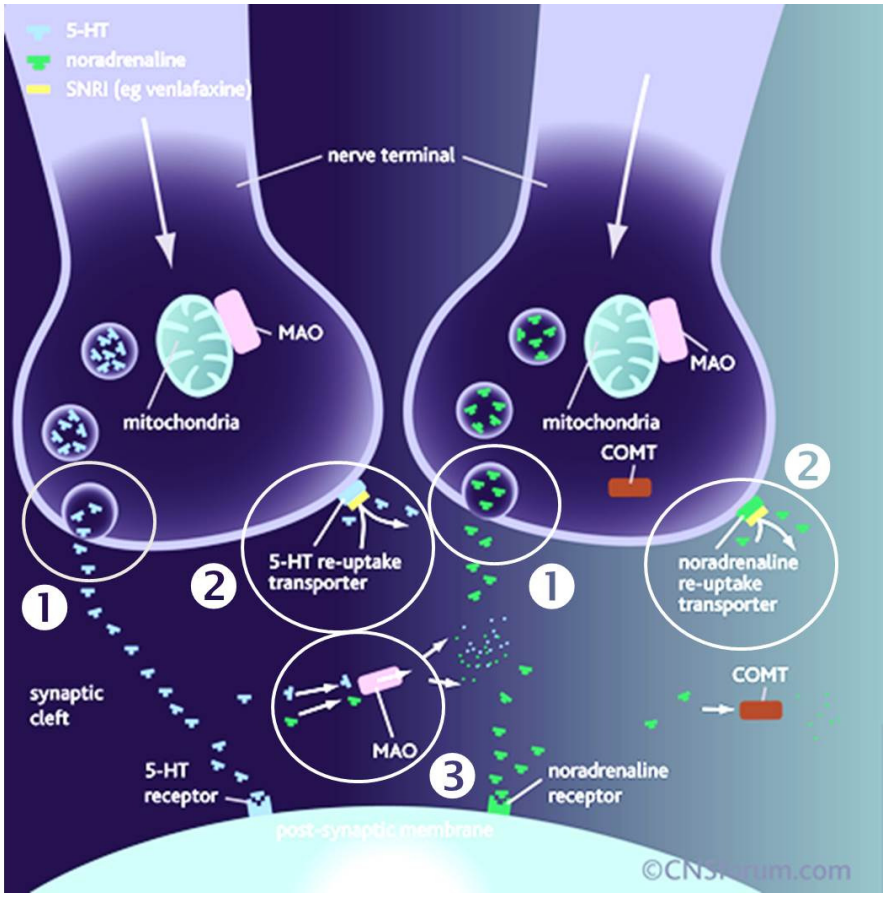


**Figura 1.** Alvos de fármacos monoaminérgicos no controle da sinapse serotoninérgica. Representação da liberação de serotonina (5-HT) e noradrenalina na fenda sináptica (1), do transporte destas monoaminas pelo neurônio pré-sináptico (2) e da ação da monoaminoxidase (MAO) (3). Adaptado de CNSforum.com.

Recentemente foram descobertas novas drogas clinicamente efetivas que desempenham seu efeito antidepressivo por mecanismos não-monoaminérgicos (Bosker et al., 2004; Browne and Lucki, 2013; Millan, 2006, 2013; Racagni and Popoli, 2010). Outros neurotransmissores, incluindo o L-glutamato, neuropeptídeos e o hormônio liberador de corticotropina, têm sido amplamente estudados e também parecem estar envolvidos com os transtornos depressivos (Aubry, 2013; Browne and Lucki, 2013; Kormos and Gaszner, 2013; Musazzi et al., 2013). O glutamato é o principal neurotransmissor excitatório do sistema nervoso central e, nas últimas décadas, estudos reforçam a hipótese de que alterações na estrutura e função do circuito excitatório/inibitório poderiam representar um importante papel no desenvolvimento das doenças do humor, particularmente a depressão (Musazzi et al., 2013). A excitotoxicidade neuronal, causada pelo aumento dos níveis cerebrais de glutamato, tem sido apontada como a principal consequência deste desequilíbrio. Os principais alvos que podem sofrer modulação e, portanto, interferir na transmissão glutamatérgica estão representados na Figura 2 e consistem, basicamente, na liberação pelo neurônio pré-sináptico, a ativação dos receptores metabotrópicos e ionotrópicos de glutamato, o processo de recaptação realizado pelos transportadores glutamatérgicos gliais e o metabolismo do glutamato a partir da glutamina. Baseado em evidências pré-clínicas, foi demonstrado que a hiperestimulação glutamatérgica decorrente de prejuízos nos mecanismos de transmissão promove alterações na estrutura e função dos neurônios, que parecem estar envolvidas com o aparecimento de distúrbios de comportamento (Musazzi et al., 2012; Sanacora et al., 2012). No entanto, o fato que culmina com a alteração dos sistemas de neurotransmissão envolvidos no desenvolvimento da depressão, sejam eles monoaminérgicos ou não, ainda não é bem entendido.

Os eventos desencadeantes da depressão são amplamente estudados e não é recente a ideia de que existe uma forte relação entre o acometimento do indivíduo por doenças que levam à dor crônica e o início de um episódio depressivo (Bagnato et al., 2014; Dufton, 1990; Patten et al., 2013; Rudy et al., 1988; Walker et al., 2014; Yavuz et al., 2013). A dor é uma experiência complexa que é constituída tanto por fatores sensoriais como afetivo-emocionais e cognitivos. Fisiologicamente, a dor tem um papel protetor e adaptativo, servindo como um sistema de alerta do corpo contra danos teciduais iminentes ou reais e auxiliando na reparação da ferida. No entanto, ao contrário da dor fisiológica, a dor patológica crônica tem grande relevância clínica, produzindo grandes problemas sociais e econômicos (Liu and Chen, 2014). Estudos demonstram que em pacientes com dor crônica, a prevalência de

depressão varia entre 22 e 78%, sendo o diagnóstico psiquiátrico mais comum entre os doentes com afecções clínico-cirúrgicas (Cassem, 1990), câncer (Faller et al., 2013) e fibromialgia (Dunne and Dunne, 2012). Por outro lado, queixas dolorosas persistentes ocorrem entre 30 e 100% dos indivíduos deprimidos, o que estreita ainda mais os limites entre a dor crônica e o comportamento depressivo (Patten et al., 2013). No entanto, embora a comorbidade dor-depressão seja bem conhecida, os mecanismos neurofisiológicos que levam ao aparecimento de depressão em pacientes acometidos de dor crônica ainda não são bem entendidos (Liu and Chen, 2014).

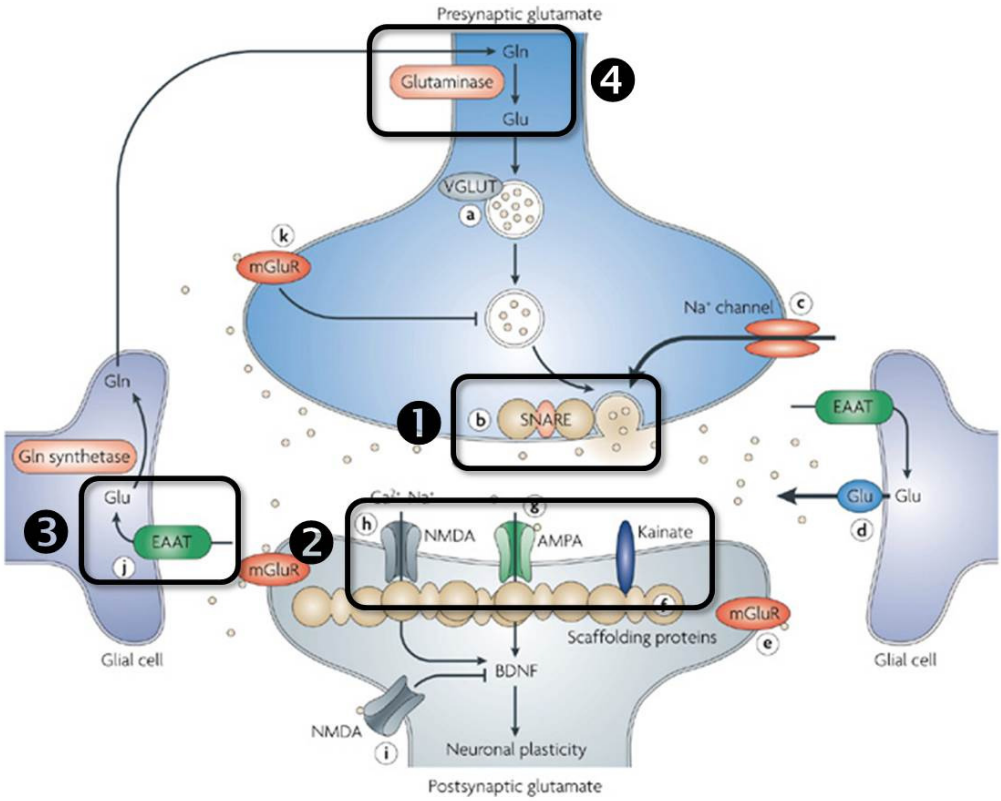


**Figura 2.** Alvos de regulação da sinapse glutamatérgica. Representação da liberação de glutamato pelo neurônio pré-sináptico (1), ativação dos receptores ionotrópicos de glutamato localizados na membrana pós-sináptica (2), processo de recaptação realizado pelos transportadores de glutamatos localizados na membrana das células gliais (3) e ciclo glutamato/glutamina (4). Adaptado de Sanacora et al. (2008), *Nature Reviews Drug Discovery*.

O desencadeamento de um transtorno depressivo também é, muitas vezes, consequente a fatores estressantes, que são denominados estressores sociais (North and Pfefferbaum, 2013; Renoir et al., 2013). Eles são os acontecimentos vitais, os estresses crônicos e os problemas cotidianos. Os acontecimentos vitais são mudanças claras nos padrões de vida que alteram o comportamento habitual e ameaçam o bem-estar do indivíduo. O luto é um exemplo típico de acontecimento vital, assim como a aposentadoria. Os estresses

crônicos compreendem aquelas situações de longa duração que desafiam o indivíduo, tais como dificuldade financeira, perda do emprego, conflito interpessoal constante, como problemas matrimoniais, e ameaça persistente à integridade, como viver em um ambiente perigoso e o acometimento por doenças graves. Os problemas cotidianos são acontecimentos comuns, porém estressantes, que fazem parte da vida moderna, como o trânsito, vizinhos desagradáveis, etc. Os eventos estressantes da vida cotidiana provavelmente "disparam" o desenvolvimento da depressão (Timmermans et al., 2013). Eles atuam via ativação do eixo hipotálamo-pituitária-adrenal (HPA), induzindo a liberação de hormônios glicocorticoides (Figura 3), cujas ações servem para preparar o organismo para desafios fisiológicos ou ambientais e são importantes para a consolidação da resposta ao estresse. A persistência e a intensidade exagerada do estresse, no entanto, bem como a incapacidade do organismo em terminar sua resposta, podem tornar o eixo hiperreativo, com prejuízos potenciais ao organismo, culminando com o desenvolvimento da depressão (North and Pfefferbaum, 2013; Renoir et al., 2013; Timmermans et al., 2013). De fato, já foi demonstrada a existência de uma estreita associação entre o aparecimento de um episódio depressivo e a desregulação do eixo HPA. Em pacientes deprimidos, o controle inibitório da atividade do eixo HPA (Figura 3) parece estar comprometido (Braquehais et al., 2012; Martocchia et al., 2013). O envolvimento do eixo HPA na neurobiologia da depressão é apoiado, ainda, pela observação de que indivíduos com síndrome de Cushing apresentam déficits cognitivos e alterações na estrutura e função hipocampais, semelhantes àquelas encontradas em pacientes deprimidos (Arnaldi et al., 2012). Sabe-se que a hiperativação descontrolada deste eixo causa uma elevação da liberação de hormônios glicocorticoides que, via ativação de receptores específicos, induz a liberação de mediadores sinápticos envolvidos no prejuízo das funções neurais. Por este motivo, recentemente, muitos autores têm se dedicado a estudar a relação entre distúrbios neuroendócrinos envolvendo o estresse e o desenvolvimento de depressão (Musazzi et al., 2011; Musazzi et al., 2013; Popoli et al., 2012; Sanacora et al., 2012).

Achados recentes provocaram a reformulação das bases fisiopatológicas dos transtornos de humor. Nesse novo paradigma, propõe-se um envolvimento direto das disfunções da plasticidade sináptica e das vias de resiliência celulares na fisiopatologia dos transtornos de humor (Kerman, 2012; Manji and Duman, 2001; Marsden, 2013; Millan, 2006, 2009). A plasticidade neuronal é caracterizada como a capacidade do cérebro produzir respostas adaptativas por meio da geração de novas conexões neurais secundariamente a estímulos internos ou externos (Marsden, 2013). A relevância do estudo da neuroplasticidade

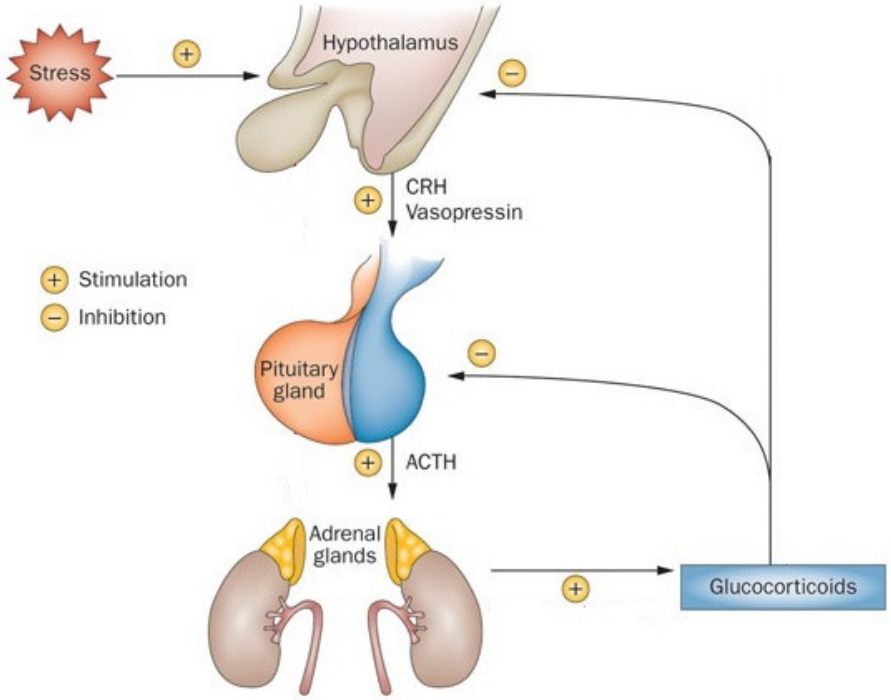


**Figura 3.** Ativação do eixo hipotálamo-pituitária-adrenal (HPA) pelo estresse e sua regulação pelo mecanismo de *feedback* negativo. Adaptado de Vitale et al. (2013), *Nature Reviews Endocrinology*.

nos transtornos de humor baseia-se no fato de que este processo integrador está envolvido de forma crítica nos fatores de risco mais importantes dos transtornos de humor: a vulnerabilidade genética e os estressores psicossociais (Kerman, 2012; Manji and Lenox, 2000; Mansell et al., 2005). Os alvos bioquímicos e moleculares promissores na terapia das doenças relacionadas ao humor envolvem as proteínas reguladoras das cascatas neurotróficas, como proteína ligadora ao elemento responsivo ao AMP cíclico (CREB)/ fator neurotrófico derivado do cérebro (BDNF) e proteína quinase regulada por sinal extracelular (ERK)/ proteína quinase regulada por mitógeno (MAPK) (Figura 4) (Chen et al., 2001; First et al., 2013; Kerman, 2012; Marsden, 2013; Zarate et al., 2005). Têm sido amplamente relatados níveis alterados de neurotrofinas em estudos envolvendo doenças como depressão e ansiedade, assim como a normalização desses níveis após tratamento farmacológico e melhoria clínica (Kerman, 2012; Manji and Duman, 2001; Marsden, 2013). Diversos estabilizadores de humor e antidepressivos demonstraram melhorar a plasticidade neural e a conectividade sináptica por meio da atuação nos circuitos moduladores-chave relacionados à sobrevivência celular e à regulação do estresse (Manji and Lenox, 2000; Mansell et al., 2005). De fato, o tratamento com estabilizadores do humor como lítio e ácido valpróico, e com drogas antidepressivas como fluoxetina e reboxetina, demonstrou hiper-regular a via de

fosforilação da ERK (Chen and Manji, 2006; First et al., 2011, 2013). Similarmente, a fosforilação de CREB, o produto final da via da ERK, desempenha um papel direto na neuroplasticidade, na sobrevivência celular e na regulação comportamental por meio dos hormônios moduladores, fatores de crescimento e vias da plasticidade sináptica (Figura 4) (Marsden, 2013), que se mostraram reduzidas em pacientes com depressão e hiper-reguladas após o tratamento crônico com antidepressivos (Chen and Manji, 2006). Nesse contexto, os agentes com alvo nas vias da plasticidade têm sido considerados como opções terapêuticas emergentes para as doenças relacionadas ao humor (Marsden, 2013).

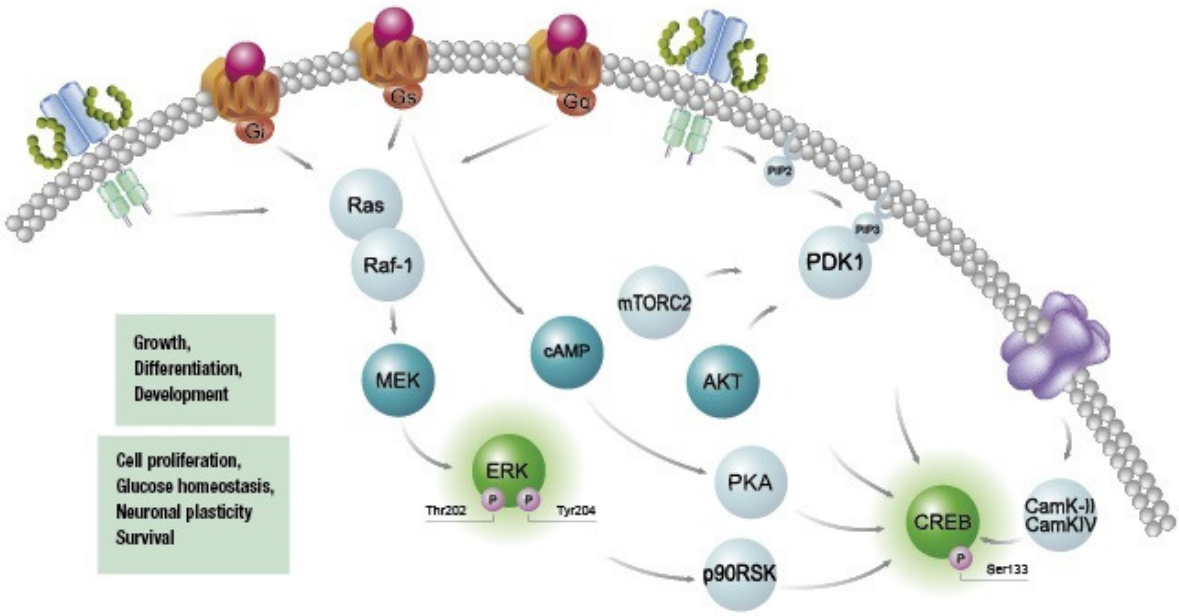


**Figura 4.** Representação das vias de sinalização que levam à fosforilação de CREB. Destaque para a via de ERK/MAPK. Adaptado de Cisbio Assays®.

## 2 Terapias antidepressivas

Os principais objetivos do tratamento da depressão são eliminar os sintomas, restaurando a atividade psicossocial e ocupacional ao estado pré-sintomático, e reduzir a probabilidade de recaída e recorrência. O tratamento antidepressivo eficaz deve eliminar gradual e completamente os sintomas, melhorar o funcionamento ocupacional e interpessoal, reduzir o risco potencial de suicídio, racionalizar os recursos e reduzir o uso dos serviços de saúde (Millan, 2006, 2013). Existem várias opções para o tratamento da depressão, incluindo

terapia cognitiva comportamental, psicoterapia interpessoal, medicamentos, terapia eletroconvulsiva e a combinação das terapias com os antidepressivos.

A terapia eletroconvulsiva, introduzida na década de 30, foi o primeiro tratamento efetivo no combate à depressão (Hargrove et al., 1953; Millet, 2009). O choque elétrico provoca uma convulsão de curto período, que induz a liberação de neurotransmissores, promovendo a estimulação direta de estruturas encefálicas (Dukart et al., 2013). Todavia, hoje é utilizada como opção de tratamento se os medicamentos existentes não oferecerem uma resposta satisfatória (Baweja and Singareddy, 2013; Kellner et al., 2012). Em depressões graves com risco de suicídio, com características psicóticas e em grávidas (Leiknes et al., 2013; van Waarde et al., 2013), a eletroconvulsoterapia é uma excelente opção desde que seja administrada de forma ética com anestesia, bloqueio da transmissão neuromuscular, pessoal treinado e ambiente apropriado e com o consentimento do paciente.

A terapia farmacológica é, atualmente, a forma mais utilizada para o tratamento da depressão (Millan, 2006, 2009, 2013; Racagni and Popoli, 2010). Os antidepressivos são um grupo heterogêneo de medicamentos com efeitos terapêuticos em comum e, embora a maioria desses fármacos também seja eficaz no tratamento de outras doenças (Olatunji et al., 2008; Powers et al., 2013; Zylicz et al., 1998), os efeitos mais importantes estão relacionados à terapia da depressão. Os antidepressivos produzem, em média, uma melhora dos sintomas depressivos de 60% a 70% enquanto a taxa de placebo é em torno de 30%. Esta taxa de melhora dificilmente é encontrada em outras abordagens terapêuticas de depressão, motivo que faz com que a terapia medicamentosa seja a mais usada (Millan, 2004, 2006). Outros medicamentos como, por exemplo, os anticonvulsivantes, não apresentam efeitos antidepressivos, mas podem ser úteis em alguns casos em combinação com antidepressivos (Millan, 2006). A escolha do medicamento e/ou sua associação é baseada nas características da depressão, presença de efeitos adversos, risco de suicídio, terapia concomitante, tolerabilidade, custo, danos cognitivos, etc (Millan, 2004).

O desenvolvimento de drogas específicas para o tratamento da depressão ocorreu no final da década de 50 do século passado, com a descoberta dos inibidores da monoaminoxidase (IMAOs) e os antidepressivos tricíclicos (ADT). Ambas as classes aumentam a disponibilidade de NA e 5-HT em certas estruturas do encéfalo, sendo mais potentes e mais eficazes em formas graves de depressão. Porém, estas duas classes de drogas antidepressivas, além de apresentarem muitos efeitos adversos, estão associadas com efeitos secundários devido ao risco de interação com outros medicamentos e alimentos. Além dos

efeitos colaterais, em superdose, os ADT são cardiotóxicos, e têm apresentado risco potencial em pacientes com tendência suicida. Além disso, têm posologia bastante variável, sendo que a dose é ajustada individualmente, e devem ser introduzidos de forma gradual e lenta. Particularmente os IMAOs, por causa de sua toxicidade e risco, são reservados a pacientes refratários (Millan, 2006).

A busca de novos medicamentos foi alcançada na década de 80, com os antidepressivos de segunda geração, denominados inibidores seletivos da recaptação de serotonina (ISRSs), sendo atualmente os mais empregados para o tratamento da depressão. Os ISRSs aumentam a concentração extracelular de 5-HT ao inibir a sua recaptação pelo neurônio pré-sináptico, aumentando o nível de 5-HT disponível para se ligar ao receptor pós-sináptico (Figura1) (Millan, 2006). Recentemente descobriu-se que, além de interferir com o sistema monoaminérgico, esses fármacos também modulam a transmissão de outras moléculas, como melatonina, neuropeptídeos e glutamato (Ballesteros-Zebadua et al., 2013; Racagni and Popoli, 2010); o sistema glutamatérgico é um dos mais estudados e é alvo de diversos estudos no desenvolvimento de terapias antidepressivas (Musazzi et al., 2013; Pehrson and Sanchez, 2013; Racagni and Popoli, 2010; Sanacora et al., 2012).

De acordo com recentes estudos, antidepressivos como imipramina, citalopram, fluoxetina e reboxetina, diminuem a transmissão do glutamato por reduzir a liberação pré-sináptica deste neurotransmissor assim como a função dos receptores glutamatérgicos (Figura 2) (Musazzi et al., 2013; Wolak et al., 2013). De fato, atualmente um número crescente de estudos tem apontado para a relevância clínica de substâncias cujo alvo específico é o sistema glutamatérgico (Lapidus et al., 2013; Murrough et al., 2013). Uma única administração de uma dose subanestésica de quetamina, um antagonista de receptores glutamatérgicos do tipo ionotrópicos (NMDA), produz uma rápida e prolongada ação antidepressiva, que foi observada tanto em estudos pré-clínicos e clínicos quanto em pacientes refratários à terapia com drogas monoaminérgicas (Murrough et al., 2013). Os efeitos adversos da quetamina, que são principalmente relacionados à piora cognitiva, no entanto, limitam o uso clínico desta droga (Musazzi et al., 2013).

A busca, portanto, por uma droga antidepressiva ideal ainda é alvo de estudos. O antidepressivo ideal deveria ser eficaz em todas as formas de depressão, inclusive as severas, não ter qualquer efeito adverso, ter baixo custo, poucas interações medicamentosas, poder ser aplicado em todas as idades, melhorar a qualidade do sono, ter posologia fácil e efeito

ansiolítico. No entanto, uma droga com todas essas atribuições ainda não está presente no mercado.

## 3 Modelos experimentais para o estudo da depressão

Os modelos animais são importantes ferramentas que permitem o estudo de teorias relacionadas à etiologia de depressão, assim como o desenvolvimento de novos alvos terapêuticos para o seu tratamento e a triagem de novas drogas com efeito antidepressivo (Duman, 2010; Overstreet, 2012). Os sintomas da depressão em humanos, no entanto, são muito difíceis de reproduzir em animais de laboratório e considerar que um animal está deprimido não é tarefa fácil. Entretanto, algumas espécies animais podem exibir alterações de comportamento do tipo depressivo (do inglês, *depressive-like*), ou seja, parecido com alguns comportamentos apresentados pelos humanos (Duman, 2010; Stewart and Kalueff, 2013).

O comportamento de desânimo, a redução de peso corporal e a falta de atração por experiências que geralmente causam prazer, denominada anedonia, são condições comuns em pessoas acometidas pela depressão e que podem ser mimetizadas pelo uso de modelos experimentais de depressão (Duman, 2010). Além disso, a exemplo do que ocorre na depressão, é comum que os modelos experimentais também induzam comportamentos relacionados a ansiedade, o que reforça ainda mais a utilidade destes modelos no entendimento das doenças afetivas (Stewart and Kalueff, 2013). Não se pode observar, no entanto, o sentimento de tristeza, culpa ou tendências suicidas, que são comuns aos pacientes depressivos.

Até a década de 90, mais de 20 modelos animais de depressão já tinham sido desenvolvidos e vários modelos experimentais têm sido validados para o estudo do comportamento depressivo, principalmente em roedores (Duman, 2010; Willner and Mitchell, 2002). Os modelos diferem no grau em que eles produzem características que se assemelham a um estado do tipo depressivo e testes que incluem a exposição ao estresse são amplamente utilizados (Duman, 2010). Em geral, os animais são expostos a um evento estressante inescapável e o comportamento deste animal frente a esse evento é avaliado. Paradigmas que empregam a exposição ao estresse agudo incluem o desamparo aprendido, o teste do nado forçado (TNF) e o teste da suspensão da cauda (TSC), que empregam a exposição a curto prazo ao estresse inevitável e incontrolável e podem predizer a resposta dos animais a uma substância com ações antidepressivas.

Modelos a longo prazo incluem o estresse crônico, estresse na infância e modelos de conflito social, que podem simular os processos que levam à depressão (Duman, 2010). Além disso, algumas linhagens específicas de roedores, como os ratos Flinders, Wistar Kyoto e *fawn-hooded* (ou ratos do capuz castanho), são consideradas bons modelos de depressão, uma vez que são relativamente mais sensíveis ao estresse e apresentam alterações neuroquímicas e comportamentais que mimetizam em parte as alterações presentes em pacientes depressivos (Overstreet, 2012). Atualmente, modelos experimentais nos quais as alterações comportamentais são induzidas pela dor crônica e pela administração intermitente de hormônios glicocorticoides têm sido comumente usados para o estudo da depressão e na pesquisa por novas drogas antidepressivas (Ago et al., 2013; Hache et al., 2012; Jesse et al., 2010; Matsuzawa-Yanagida et al., 2008; Suzuki et al., 2007; Wu et al., 2013). Particularmente, animais submetidos à ligação parcial do nervo ciático desenvolvem uma dor neuropática que, com o passar das semanas, leva ao aparecimento de um comportamento do tipo depressivo (Matsuzawa-Yanagida et al., 2008). Por sua vez, a administração de corticosterona (o principal hormônio glicocorticoide dos roedores) simula uma situação de estresse continuada (Ago et al., 2013). Além de mimetizar alguns dos sintomas da depressão apresentados por humanos, sabe-se que estes modelos induzem alterações patofisiológicas similares àquelas que ocorrem em pacientes depressivos, como desregulação do eixo HPA, aumento nos níveis de glicocorticoides, deficiência na transmissão monoaminérgica e glutamatérgica e atrofia de regiões cerebrais importantes na regulação do humor (Ago et al., 2013; Hache et al., 2012; Jesse et al., 2010; Matsuzawa-Yanagida et al., 2008; Suzuki et al., 2007; Wu et al., 2013). Tais alterações podem ser revertidas pela terapia com fármacos antidepressivos, o que valida estes modelos para o estudo da depressão (Ago et al., 2013; Matsuzawa-Yanagida et al., 2008).

## 4 Compostos orgânicos contendo selênio: propriedades biológicas

O selênio é um micronutriente essencial para todas as formas de vida, cuja concentração pode ocasionar deficiência ou toxicidade. Esse elemento é encontrado principalmente em alimentos como a castanha-do-pará, alho, cebola, brócolis, cogumelos, cereais, pescados, ovos e carnes. A ingestão diária recomendada pela Junta de Alimentação e Nutrição da Academia de Ciências dos Estados Unidos para adultos é de 50-200 μg; porém,

de fato, quando a ingestão diária ultrapassa 400 μg, excedendo a capacidade corporal de eliminação, o selênio pode provocar efeito tóxicos, denominados selenoses.

No ano de 1930, o selênio foi reconhecido como uma substância tóxica quando cavalos do oeste da China, que se alimentaram de plantas com grande potencial de acumular este elemento, apresentaram sintomas de envenenamento, como perda de cascos, pêlos e anemia (Zhang et al., 1993). No entanto, a partir da descoberta do papel essencial do selênio, o conceito sobre esse elemento modificou-se, sendo intensificada a pesquisa por suas propriedades farmacológicas.

Este elemento apresenta importantes ações biológicas, destacando-se os efeitos antioxidantes e imunomoduladores. A deficiência de selênio tem sido relacionada com uma ampla variedade de patologias que incluem doenças endócrinas, câncer e transtornos do humor (Fairweather-Tait et al., 2011). Sabe-se que baixos níveis de selênio na dieta humana (32-36 μg/dia) têm sido associados com o aumento na incidência de depressão, ansiedade e agressividade (Sher, 2000, 2002) e que a suplementação da dieta com este elemento (226 μg/dia) melhora o humor e a qualidade de vida (Benton, 2002; Benton and Cook, 1990), o que reforça o papel do selênio nas doenças afetivas.

O selênio é raramente encontrado em seu estado natural, podendo tanto combinar-se com metais ou não metais para formar compostos inorgânicos, quanto apresentar-se sob a forma de compostos orgânicos. As moléculas orgânicas de selênio são alvo de estudos do nosso grupo de pesquisa, que tem se dedicado a investigar as propriedades farmacológicas e toxicológicas desses compostos (Nogueira and Rocha, 2011; Nogueira et al., 2004). Diversos estudos envolvendo as ações do tipo antidepressiva e ansiolítica de compostos de selênio já foram realizados, tendo demonstrado seus efeitos em diferentes modelos experimentais (Bortolatto et al., 2012; Bruning et al., 2011; Gay et al., 2010; Jesse et al., 2010; Posser et al., 2009; Rocha et al., 2012). O disseleneto de difenila, $(PhSe)_2$, e o ebselen são moléculas lipofílicas contendo selênio muito estudadas pelo nosso grupo de pesquisa. Dentre as inúmeras propriedades farmacológicas dessas moléculas (Nogueira and Rocha, 2011), com grande relevância destaca-se a atividade do tipo antidepressiva (Acker et al., 2009; Posser et al., 2009; Rocha et al., 2012). Além disso, compostos dissubstituídos derivados do $(PhSe)_2$ também apresentam propriedades semelhantes (Bruning et al., 2011). Em estudos envolvendo modelos experimentais de depressão como TSC e TNF, o efeito do tipo antidepressivo dos destes compostos parece ser mediado por uma interação com o sistema monoaminérgico (Bruning et al., 2011; Posser et al., 2009).

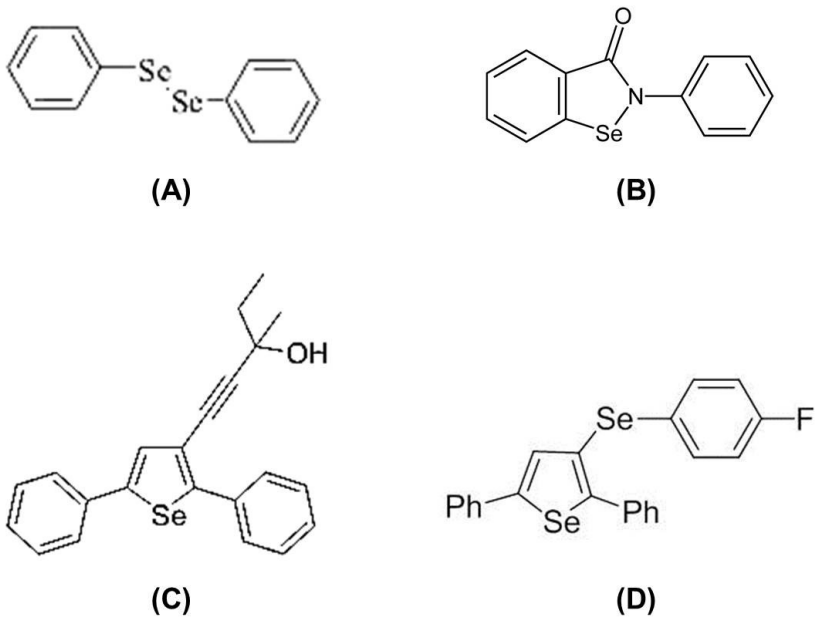


**Figura 5.** Estrutura química de compostos orgânicos de selênio com importância biológica. (**A**) Disseleneto de difenila, (**B**) Ebselen, (**C**) 3-alquinilselenofeno (**3-ASP**) e (**D**) 3-(4-p-fluorofenilselenil)-2,5-difenilselenofeno (**F-DPS**).

Os selenofenos são uma classe de compostos heterocíclicos aromáticos amplamente estudada, cujo anel de cinco membros apresenta um átomo de selênio. Alguns representantes desta classe apresentam propriedades antimicrobianas e antitumorais (Juang et al., 2007; Luo et al., 2011; Shiah et al., 2007; Wiles et al., 2011). Nosso grupo de pesquisa já demonstrou as ações farmacológicas do 3-alquinilselenofeno (**3-ASP**), dentre elas as propriedades anticonvulsivantes e antinociceptivas, que parecem envolver uma modulação dos sistemas gabaérgico e glutamatérgico (Wilhelm et al., 2012a; Wilhelm et al., 2009a, b; Wilhelm et al., 2010; Wilhelm et al., 2009c; Wilhelm et al., 2012b). No entanto, poucos estudos acerca das propriedades antidepressivas de compostos selenofenos são encontrados na literatura (Gai et al., 2012; Gay et al., 2010).

A hipótese de que 3-(organosseleno)-2,5-difenil-selenofenos apresentam ação do tipo antidepressiva foi recentemente levantada pelo nosso grupo de pesquisa (Gai et al., 2012). Já foi demonstrado que o composto 3-(4-p-fluorofenilselenil)-2,5-difenilselenofeno (**F-DPS**) apresenta ação do tipo antidepressiva em camundongos e que este selenofeno parece interagir com o sistema serotoninérgico. O efeito anti-imobilidade do **F-DPS** no teste do nado forçado foi abolido pelo tratamento dos animais com um composto capaz de depletar os níveis cerebrais de serotonina, o paraclorofenilalanina (PCPA). Além disso, antagonistas dos receptores de serotonina também bloquearam de modo significativo a ação do tipo antidepressiva deste composto (Gay et al., 2010). No mesmo estudo, o tratamento de

camundongos com uma dose aguda de **F-DPS** inibiu a captação de serotonina em sinaptossomas de cérebro, o que confirma a modulação do sistema serotoninérgico por esta droga.

Desse modo, com base nas promissoras propriedades farmacológicas apresentadas por essa classe de compostos e tendo em vista a importância do selênio na regulação do humor, torna-se interessante ampliar os estudos envolvendo os selenofenos e seus efeitos em modelos experimentais de transtornos afetivos.

## 5 Objetivos

### 5.1 Objetivo geral

Tendo em vista o mencionado acima, o principal objetivo do presente estudo foi investigar a ação do tipo antidepressiva do composto **F-DPS** em modelos experimentais de depressão induzida pela dor crônica e administração de corticosterona em camundongos.

### 5.2 Objetivos específicos

- Analisar a ação do tipo antidepressiva de 3-(organosseleno)-2,5-difenil-selenofenos (**H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** e **$CF_3$-DPS**) no TNF em camundongos;
- Investigar o envolvimento da via de sinalização da ERK na ação do tipo antidepressiva produzida pelo tratamento agudo com **F-DPS** em camundongos;
- Investigar se os tratamentos agudo e subcrônico com **F-DPS** são capazes de reverter o comportamento do tipo depressivo induzido pela dor crônica em camundongos;
- Analisar o efeito dos tratamentos agudo e subcrônico com **F-DPS** sobre a sensibilidade à dor em um modelo de dor crônica em camundongos;
- Determinar se o tratamento subcrônico com **F-DPS** é capaz de reverter os comportamentos do tipo depressivo e ansiogênico induzidos pela administração de corticosterona em camundongos;
- Analisar os efeitos endócrinos do tratamento subcrônico com **F-DPS** após a administração de corticosterona em camundongos;
- Investigar o envolvimento dos sistemas glutamatérgico e serotonérgico na ação farmacológica do **F-DPS** em animais expostos cronicamente à corticosterona.

## 6 Desenvolvimento desta tese de doutorado

O desenvolvimento desta tese está apresentado sob a forma de dois artigos científicos e um manuscrito. Os itens Materiais e Métodos, Resultados, Discussão e Referências Bibliográficas encontram-se no próprio artigo. Os artigos estão dispostos da mesma forma que foram publicados ou submetidos.

Os dados apresentados nos apêndices A, B e C, sob a forma de material suplementar, são complementares aos resultados dos artigos científicos e estão divididos em Objetivos, Materiais e Métodos, Resultados e Considerações Finais. Estes resultados estão expostos ao final desta tese de doutorado, após o item Referências Bibliográficas.

Os resultados presentes no manuscrito 1 bem como os dados apresentados no apêndice C são oriundos de uma colaboração entre a Universidade Federal de Santa Maria e a Universidade de Florença, na Itália, que foi possível graças ao Programa de Doutorado Sanduíche no Exterior (PDSE) da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes).

## MANUSCRITO 1:

**ERK1/2 phosphorylation is involved in the antidepressant-like action of 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene in mice**

(Manuscrito submetido)

Bibiana Mozzaquatro Gai, Maria Domenica Sanna, André Luiz Stein, Gilson Zeni, Nicoletta Galeotti e Cristina Wayne Nogueira

# ERK1/2 phosphorylation is involved in the antidepressant-like action of 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene in mice

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**Abstract**

In this study we investigated the antidepressant-like action of five compounds belonging to the selenophene class. The involvement of ERK and CREB activation in this action was also demonstrated. In the experiment 1, time-course and dose-response effect of **H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** and **$CF_3$-DPS** were accompanied in the mouse forced swimming test (FST). Firstly, animals received compounds at a dose of 50 mg/kg, by intragastric (i.g.) route, at different times (15-240 min) before test. Results showed that the peak of maximum anti-despair behavior induced by **Cl-DPS**, **F-DPS** and **$CF_3$-DPS** was at 30 min; maximum effect of **H-DPS** and **$CH_3$-DPS** was found at 60 min, which was maintained until 120 min. Regarding dose-response effect, all compounds reduced immobility time and increased latency for the first episode of immobility at a dose of 50 mg/kg. In addition, **F-DPS** also showed antidepressant-like action at a dose of 25 mg/kg, whilst **H-DPS**, **$CH_3$-DPS**, **Cl-DPS** and **$CF_3$-DPS** were not effective at lower doses. Thus, **F-DPS** was chosen for further investigation of its mechanism of action. Experiment 2 showed that treatment of animals with **F-DPS** (50 mg/kg, i.g.) significantly increased phosphorylated ERK1/2 levels in the prefrontal cortex and hippocampus; however, pCREB levels were not affected. Additionally, in the experiment 3 anti-immobility effect of **F-DPS** was completely blocked by pretreatment of animals with PD 98,059, an inhibitor of ERK phosphorylation, suggesting that ERK signaling activation is involved in its antidepressant-like action in mice. Together our data appoint **F-DPS** as a promising molecule for the development of a new antidepressant therapy.

**Keywords:** selenium, selenophene, mice, antidepressant-like, ERK phosphorylation.

# 1 Introduction

Organic forms of selenium have been suggested as relevant biologic agents (Nogueira and Rocha, 2011). In fact, over the last decade preclinical studies have demonstrated that several organoselenium compounds have pharmacological properties including antioxidant, anticonvulsant, neuroprotective and antidepressant actions (Bruning et al., 2011; Dias et al., 2014; Gai et al., 2013; Mahadevan et al., 2013; Pinton et al., 2013; Wilhelm et al., 2012b).

In this study we highlight the selenophene class, a specific group of heterocyclic selenium-containing molecules whose members have been shown to produce antidepressant-like properties (Gai et al., 2013; Gai et al., 2012; Gay et al., 2010). A recent study performed by our research group showed that the administration of a single dose of 3-chalcogen selenophenes (50 mg/kg) reduced the immobility time in the mouse forced swimming test (FST) (Gai et al., 2012). Additionally, structure–activity relationship studies demonstrated that the phenyl group at the 2-position and an organoselenium group at the 3-position of the five-member ring are essential for the antidepressant-like activity of selenophenes in the FST (Gai et al., 2012). However, although the antidepressant-like action selenophene compounds is known, the pharmacological profile of 3-chalcogen selenophenes in the FST was not further investigated. Furthermore, mechanisms involved in the antidepressant-like action of selenophenes remain still unclear.

Regarding mechanisms of antidepressant action, although most studies focus on serotonergic and noradrenergic systems, recent studies have identified modifications of intracellular signaling proteins and target genes that could contribute to the pharmacological action of antidepressant therapy (Blendy, 2006; Carreno and Frazer, 2014; Kuo et al., 2013; Nair and Vaidya, 2006; Reus et al., 2011). Modulation of mitogen-activated protein kinase (MAPK) pathways and the transcription factor CREB (cAMP-response element-binding

protein) are molecular targets for antidepressants (Blendy, 2006; Kuo et al., 2013). CREB is one of the most important transcription factors and has been shown to play an important role in depression. CREB is upregulated by antidepressant treatment, and increasing CREB levels in rodent models results in antidepressant-like behaviors (Blendy, 2006; Carreno and Frazer, 2014; Nair and Vaidya, 2006; Reus et al., 2011). Phosphorylation, and therefore activation of CREB can be induced by a number of upstream signaling cascades, including MAPK/ERK (Blendy, 2006; Nair and Vaidya, 2006). The mitogen activated protein kinase (MAPK) pathway is a major signaling system that regulates cellular responses and activation of the MAPK cascade plays a critical role in the pathophysiology of depression (Roux and Blenis, 2004). Accordingly, it has been shown that inhibition of MAPK signaling produces a depressive-like phenotype and blocks behavioral actions of antidepressants. Extracellular signal-regulated protein kinases (ERKs) are MAPKs that are involved in cell proliferation and neuroprotection (Mebratu and Tesfaigzi, 2009). Although the role of ERK in depression is unclear, previous reports showing decreased phosphorylated levels of ERK1/2 in the post-mortem brain of depressed suicide subjects and in rodent models of depression suggest an important involvement of this protein in mood disorders (Dwivedi et al., 2001; Feng et al., 2003). Furthermore, recent studies have demonstrated a positive modulation of ERK isoforms by antidepressant drugs such as fluoxetine and reboxetine (First et al., 2013; Kuo et al., 2013).

Taking into account the above mentioned points, the main objective of this study was to demonstrate the profile of antidepressant-like action of selenophene compounds in mice as well as to investigate the participation of ERK and CREB phosphorylation in this effect.

## 2 Materials and Methods

### 2.1 Animals

The experiments were conducted using male Swiss mice (25-30g) maintained at 22-25°C with free access to water and food, under a 12:12 hour light/dark cycle, with lights on at 7:00 a.m. All manipulations were carried out between 08.00 a.m. and 04.00 p.m and mice were acclimated to the behavioral room at least 2 hours before the test. The experiments were performed according to a randomized schedule and each animal was used only once in each test. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil (# 124/2010). The procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

### 2.2 Drugs

2,5-Diphenyl-3-(phenylseleno)-selenophene **(H-DPS)**, 2,5-diphenyl-3-(4-methylphenylseleno)-selenophene **($CH_3$-DPS)**, 2,5-diphenyl-3-(4-chlorophenylseleno)-selenophene **(Cl-DPS)**, 2,5-diphenyl-3-(4-fluorophenylseleno)-selenophene **(F-DPS)** and 2,5-diphenyl-3-(3-trifluoromethylphenylseleno)-selenophene **($CF_3$-DPS)** (Figure 1), were prepared and characterized in our laboratory by the method previously described (Stein et al., 2008). Analysis of the $^{1}$HNMR and $^{13}$CNMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of studied compound (99.9%) was determined by GC/MS.

Specific antibody against ERK1/2 phosphorylated (pERK1/2) was obtained from Cell Signaling Technology®. Antibody against CREB phosphorylated on Ser133 (pCREB) and β-

actin were obtained from Santa Cruz Biothechnology®. PD98,059 was purchased from Sigma®. All other chemicals were obtained from analytical grade and standard commercial suppliers.

### 2.3 Experimental design

*Experiment 1*

This experiment was performed in order to analyze the pharmacological profile of antidepressant-like action of selenophene compounds (**H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** and **$CF_3$-DPS**) in the mouse forced swimming test (FST).

To assess the time-course of antidepressant-like action of selenophene compounds, separate groups of mice received a single administration of canola oil (10 ml/kg) or **H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** and **$CF_3$-DPS** at a dose of 50 mg/kg, administered by intragastric (i.g.) route. At different times after treatment (15, 30, 60, 120, 180 or 240 min) animals were evaluated in the FST (n = 6-8 animals/group). Pretreatment time in which compounds presented maximum anti-immobility effect in the FST was chosen for performing the subsequent step (Figure 1).

To obtain the dose-response assessment**,** compounds were administered at doses of 10, 25 and 50 mg/kg, i.g., 30 min (**H-DPS** and **$CH_3$-DPS**) or 60 min (**Cl-DPS**, **F-DPS** and **$CF_3$-DPS**) before FST (n = 6-8 animals/group). Animals were also evaluated in the locomotor activity monitor (LMA) in order to rule out any interference of locomotion in the FST (Figure 1).

Since **F-DPS** produced antidepressant-like action at lower doses than other organoselenium compounds, it was chosen for performing experiments 2 and 3.

*Experiment 2*

To investigate whether ERK1/2 phosphorylation is modulated by **F-DPS**, firstly mice received a single i.g. dose of **F-DPS** 50 mg/kg or canola oil (10 ml/kg; n = 4/group). Thirty min after treatment animals were then killed by decapitation and hippocampi and prefrontal cortices were removed for determination of pERK1/2 (Figure 1). Since MAP kinases, like ERK, regulate the activities of several transcription factors, such as CREB, pCREB expression was also determined. Because total protein levels are generally no modulated after a short period, only phosphorylated proteins were quantified.

Additionally, since single treatment did not modify pCREB levels, we performed a repeated treatment with **F-DPS** in order to analyze this protein. Compound was administered at a dose of 50 mg/kg, i.g, during 3 consecutive days and hippocampus and prefrontal cortex of mice were removed 30 min after the last dose for pCREB expression (Figure 1).

In a separate group of animals, **F-DPS** (50 mg/kg, i.g.) or canola oil (10 ml/kg; n = 4/group) were administered to mice. After 30 min, mice were anesthetized and brains were perfused with paraformaldehyde solution for pERK1/2 staining (Figure 1).

*Experiment 3*

In order to assess the antidepressant-like action of **F-DPS** in the tail suspension test (TST), mice were previously treated with canola oil (10 ml/kg, n = 6-8) or F-DPS at doses of 10, 25 and 50 mg/kg (i.g.; n= 6-8) and subjected to the TST after 30 min.

To test the hypothesis that the antidepressant-like action of F-DPS is mediated through a modulation of ERK phosphorylation, a separated group of animals was treated with saline (5 μl/site) or PD98,059 (a MEK inhibitor, 20 μg/site), by i.c.v. route, one hour before the canola oil or F-DPS administration (50 mg/kg. i.g.; n = 6/group). The TST was carried out 30 min after F-DPS treatment (Figure 1).

**2.4 Spontaneous locomotor activity**

To discard non-specific effects of treatments, spontaneous locomotor activity of mice was performed in the locomotor activity monitor (LMA). LMA is a Plexiglas cage (45 × 45 × 45 cm) surrounded by a frame consisting of 32 photocells mounted on opposite walls (16 L x 16 W, spaced 2 cm apart) that continuously tracks the animal's movement. Animals were placed in the center of the apparatus and allowed to freely explore the arena during 4 min. Motor activity was monitored with the Insight® Monitor Activity System. Data were collected in the form of photobeam breaks as an indication of activity within different predetermined "zones" in the open field using Monitor Activity® software (Insight). Number of crossings and rearings, average velocity (mm/s) and total distance traveled (dm) were recorded.

**2.5 FST**

The FST is one of the most widely used tools for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at rendering or preventing depressive-like states. The procedure used in this study was based on that previously described (Porsolt et al., 1979). Mice were gently placed in an inescapable cylindrical container (10 × 25 cm) that was filled with water (19 cm, 25±1ºC) and their escape related mobility behavior (latency for the first immobility episode and total duration of floating) was measured by a blinded observer during a 6 min period by using a stopwatch. Latency was defined as the amount of time that elapsed between placing the mouse in the tank and the first instance of each behavioral occurrence. 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.

**2.6 TST**

The TST was performed in a quiet experimental room according to the method reported by Steru and collaborators (Steru et al., 1985). Each mouse was suspended by its tail to an horizontal wooden bar located inside a yellow plastic box (40 cm × 46 cm × 40 cm) approximately 30 cm above the floor. The mouse was secured to the bar by adhesive tape placed 1 cm from the tip of the tail, such that the mouse's head was about 20 cm above the floor. The trial was conducted for 6 min during which a blinded observer scored the latency for the first immobility episode and total duration of immobility by using a stopwatch. The mouse was considered immobile only when it hung passively and completely motionless. Mice that climbed their tails were eliminated from further analyses.

**2.7 Intracerebroventricular (i.c.v.) injection**

The i.c.v. injection was carried out as previously described (Haley and McCormick, 1957) and modified (Laursen and Belknap, 1986), with the bregma fissure as a reference point. A 0.4 mm external diameter hypodermic needle was briefly attached to a cannula, which was linked to a 25 μl Hamilton syringe, inserted perpendicularly through the skull no more than 2 mm into the brain of the mouse. A volume of 5 μl was then administered in the left lateral ventricle. The injection was given over 30 s, and the needle remained in place for another 30 s in order to avoid the reflux of the substances injected. The injection site was 1

mm to the right or left from the mid-point on a line drawn through to the anterior base of the ears.

**2.8 Western blot analysis**

The hippocampi and prefrontal cortices were homogenized in ice-cold lysis buffer containing 25 mM TrisHCl (pH 7.5), 25 mM NaCl, 5 mM EGTA, 2.5 mM EDTA, 2 mM NaPP, 4 mM PNFF, 1 mM $Na_3VO_4$, 1 mM PMSF, 20 µg/ml leupeptin, 50 µg/ml aprotinin, 0.1 % SDS. The homogenate was centrifuged at 9,000 × g for 20 min at 4 °C, the low speed pellet was discarded. The supernatant (whole cell lysate) was separated on 10 % SDS-PAGE and transferred onto nitrocellulose membranes (120 min at 100 V) using standard procedures. Membranes were blocked in PBST (PBS containing 0.1 % Tween) containing 5 % nonfat dry milk for 120 min. Following washes, blots were incubated overnight at 4ºC with specific antibodies against pERK1/2 (1:1,000), pCREB (1:500) and ß-actin (1:3,000 dilution). After being washed with PBS containing 0.1 % Tween, the nitrocellulose membrane was incubated with a horseradish peroxidase-conjugated anti-rabbit IgG secondary antibody (1:5,000) and left for 2 h at room temperature. Blots were then extensively washed according to the manufacturer's instruction and developed using enhanced chemiluminescence detection system (Pierce, Milan, Italy). Exposition and developing time used was standardized for all the blots. Optical density measurements were performed by dividing the intensity of the bands by the intensity of the housekeeping protein ß-actin, used as loading control, at each time point. Measurements in control samples were assigned a relative value of 100 %.

### 2.9 Protein determination

Protein concentration was measured by biuret method using bovine serum albumin (1mg/ml) as the standard (Gornall et al., 1949).

### 2.10 Tissue preparation and immunofluorescence

Mice were perfused through the left cardiac ventricle with 10 ml of a cold fixative (4% paraformaldehyde in 100 mM phosphate buffer). After perfusion, the brain tissues were quickly removed, postfixed for 18 h with the same fixative at 4ºC, and transferred to 10%, then 20%, and then 30% sucrose solution. After preincubation in 5 mg/ml BSA/0.3% Triton-X-100/PBS, sections were incubated overnight at 4°C with primary antibody at optimized working dilution. pERK antibody (1:50, SantaCruz Biothechnology Inc, CA, USA) was detected by Alexa 488-conjugated rabbit secondary antibody (1:200; Invitrogen, Carlsbad, CA). Sections were coverslipped using Vectorshield mounting medium with DAPI (Vector Laboratories, Burlingame, CA). A Leica DF 350 FX microscope with appropriate excitation and emission filters for each fluorophore was used to acquire representative images. Images were acquired with x 20 to x 40 objectives using a digital camera.

### 2.11 Statistical analysis

All experimental results are given as the mean ± S.E.M. First, we evaluated the normality of data using the D'Agostino and Pearson omnibus normality test. Comparisons between experimental and control groups in Experiments 1 (FST and LMA) and 3 (TST) were performed by one-way analysis of variance (ANOVA) followed by Newman-Keuls test for

post hoc comparison when appropriate. Results from pERK1/2 and pCREB levels (Experiment 2) were analyzed by using unpaired t-test. Blocking effect of PD98,059 on the antidepressant-like action of F-DPS in the TST was analyzed by two-way ANOVA followed by Newman-Keuls test. Main effects of first order interactions are presented only when interaction was not significant. All analyses were performed using the STATISTICA for Windows software Version 7 (StatSoft, Oklahoma, USA). Probability values less than 0.05 ($P < 0.05$) were considered as statistically significant.

## 3 Results

### 3.1 Selenophene compounds produce antidepressant-like action in the FST without any change on locomotor activity of animals

Our results showed that all selenophene compounds tested in this study showed antidepressant-like actions in the mouse FST. However, the pharmacological profile (dose and pretreatment time) was different for each drug.

As shown in Figures 2A and 2B, acute treatment with **H-DPS** (50 mg/kg, i.g.) significantly modified total immobility time [$F_{(5,34)} = 15.49$, $P < 0.001$; Figure 2A] and latency for the first episode of immobility in the FST [$F_{(5,34)} = 2.56$, $P < 0.05$; Figure 2B]. Post hoc analyzes showed that **H-DPS** decreased the immobility duration at 60 (64.3%, $P < 0.001$) and 120 (53.8%, $P < 0.001$) min after a single administration. Latency was increased at 60 min after treatment (47.2%, $P < 0.01$). Treatment of animals with **H-DPS** at doses of 10 and 25 mg/kg, 60 min before FST, did not produce any significant difference in the immobility time and latency for the first episode of immobility when compared to the control group ($P > 0.05$; Table 1).

In a similar way to **H-DPS**, treatment of mice with C**H$_3$-DPS**, at a dose of 50 mg/kg, produced a significant effect on immobility time [$F_{(5,36)}$ = 7.40, $P < 0.001$; Figure 2C] and latency in the FST [$F_{(5,36)}$ = 3.01, $P < 0.05$; Figure 2D]. Total immobility time of mice was decreased around of 30% ($P < 0.01$) after 60 and 120 min of C**H$_3$-DPS** treatment whilst latency for the first episode was increased around of 57.5% ($P < 0.05$) at the pretreatment time of 60 min when compared to the vehicle-treated group. Doses of 10 and 25 mg/kg, 60 min before FST, did not produce significant effect ($P > 0.05$; Table 1).

Treatment of animals with **Cl-DPS** produced the longest antidepressant-like action when compared with other selenophene compounds (Figure 2). Latency [$F_{(6,48)}$ = 6.56, $P < 0.001$; Figure 2E] and immobility duration [$F_{(6,47)}$ = 5.09, $P < 0.001$; Figure 2F] were significantly modified after a single dose of 50 mg/kg **Cl-DPS**. Post hoc analyzes showed that **Cl-DPS** decreased immobility at pretreatment times of 30 min (22.6%, $P < 0.05$), 60 min (31.4%, $P < 0.05$;), 120 min (34.5%, $P < 0.01$) and 180 min (32.7%, $P < 0.01$). Latency for the first episode of immobility was increased around of 47.6% ($P < 0.01$; 30 min), 50.5% ($P < 0.001$; 60 min), 36.9% ($P < 0.05$; 120 min) and 35.9% ($P < 0.05$; 180 min) after single treatment with **Cl-DPS**. Administration of **Cl-DPS** at doses of 10 and 25 mg/kg, 60 min before FST, did not produce significant antidepressant-like action ($P > 0.05$; Table 1).

Figures 2G and 2H show the pharmacological profile of **F-DPS** in the FST. One-way ANOVA yielded a significant effect of **F-DPS** on total immobility time [$F_{(3,23)}$ = 17.97, $P < 0.001$; Figure 2G] and latency for the first episode of immobility [$F_{(3,23)}$ = 6.86, $P < 0.01$; Figure 2H]. At 30 min after a single dose of 50 mg/kg, animals treated with **F-DPS** showed a decrease in the total immobility duration (47.5%, $P < 0.001$) and an increase in latency (103.2%, $P < 0.01$). Moreover, whilst a dose of 10 mg/kg was not effective in the FST ($P > 0.05$), administration of **F-DPS** at a dose of 25 mg/kg produced a significant antidepressant-like action in the FST: immobility time was decreased at around 67.3% and latency was

increased at approximately 53% when compared to the control group treated with vehicle (Table 1).

Finally, Figures 2I and 2J show the antidepressant-like action of **$CF_3$-DPS**. Similar to the other selenophene compounds, our results demonstrated that acute treatment with **$CF_3$-DPS** produced a significant anti-immobility effect in the FST [$F_{(4,29)} = 4.50$, $P < 0.01$; Figure 2I]. Latency for the first episode of immobility was also modified by treatment with this organoselenium compound [$F_{(4,29)} = 2.28$, $P < 0.05$; Figure 2J]. Post hoc comparison showed that at 30 min after **$CF_3$-DPS** treatment (50 mg/kg) immobility duration of mice in the FST was significantly decreased (50.6%, $P < 0.05$) whilst latency was increased (57.1%, $P < 0.01$). Despair parameters in the FST were not modified by lower doses of **$CF_3$-DPS** (10 and 25 mg/kg, $P > 0.05$; Table 1).

Regarding locomotor activity of mice acutely treated with selenophene compounds, statistical analysis showed that neither the number of crossings and rearings, velocity nor total distance travelled were modified by **H-DPS, $CH_3$-DPS, Cl-DPS, F-DPS** or **$CF_3$-DPS** at doses of 10, 25 and 50 mg/kg ($P > 0.05$; Table 2).

### 3.2 F-DPS increases ERK phosphorylation in hippocampus and prefrontal cortex of mice

Figure 3 shows the effect of acute treatment with **F-DPS** (50 mg/kg, i.g., 30 min) on ERK1/2 phosphorylation in hippocampus of mice. An unpaired t-test revealed a significant difference between Control and **F-DPS** groups on pERK1 [$t_{(6)} = 3.86$, $P < 0.01$] and pERK2 [$t_{(6)} = 7.45$, $P < 0.001$] levels in hippocampus of mice. ERK1 phosphorylation was increased around of 100% whilst pERK2 levels suffered an increase of 121%.

In a similar way, pERK1/2 levels were also increased in prefrontal cortex after treatment with **F-DPS** [$t_{(6)} = 5.50$, $P < 0.01$ and $t_{(6)} = 17.96$, $P < 0.001$, respectively; Figure 4]. A single dose of 50 mg/kg **F-DPS**, given by i.g. route 30 min earlier, was effective in increasing phosphorylated ERK1 (61%) and ERK2 (111%) levels in prefrontal cortex of mice.

The increase on phosphorylation of ERK1/2 after a single dose of **F-DPS** was not accompanied by CREB phosphorylation. Phosphorylated CREB levels were not modified by single **F-DPS** treatment both in hippocampus [$t_{(6)} = 0.53$, $P > 0.05$; Figure 3] and prefrontal cortex [$t_{(6)} = 2.12$, $P > 0.05$; Figure 4]. Similarly, there was not any modulation on pCREB expression after repeated treatment with **F-DPS**. An unpaired t-test revealed no difference between vehicle and **F-DPS** administration (3 doses of 50 mg/kg) on phosphorylation of CREB in hippocampus [$t_{(6)} = 2.12$, $P > 0.05$] and prefrontal cortex [$t_{(6)} = 1.31$, $P > 0.05$] (Figure 5).

We performed immunofluorescence experiments to detect the expression and localization of p-ERK in the hippocampus after acute F-DPS administration. p-ERK immunolabeling was detected in both CA3 (Fig. 6A,C) region and dentate gyrus (Fig. 6E,G). F-DPS selectively increased ERK phosphorylation within the dentate gyrus (Fig. 6F,H) whereas in the CA3 region the number of p-ERK positive cells following F-DPS treatment (Fig. 6B,D) were comparable to that of control (Fig. 6A,C).

### 3.3 Inhibition of ERK phosphorylation blocks antidepressant-like action of F-DPS in the mouse TST

Figure 7 shows the effect of acute treatment with **F-DPS** on immobility time and latency for the first episode of immobility in the TST. One-way ANOVA yielded a significant

effect of **F-DPS** on total immobility time [$F_{(3,27)}$ = 21.47, $P < 0.001$; Figure 7A] and latency for the first episode of immobility [$F_{(3,7)}$ = 10.73, $P < 0.001$; Figure 7B]. Animals treated with **F-DPS** at doses of 25 and 50 mg/kg, given 30 min earlier by i.g. route, showed a reduction in the total immobility duration around of 52.4%, ($P < 0.001$) and 62.4%, ($P < 0.001$), respectively. Latency in the TST was increased after acute treatment with **F-DPS** at doses of 25 mg/kg (42.5%, $P < 0.01$) and 50 mg/kg (58.4%, $P < 0.001$). A dose of 10 mg/kg was not effective in the TST ($P > 0.05$).

Two-way ANOVA of total immobility time in the TST revealed a significant PD98,059 × **F-DPS** interaction [$F_{(1,20)}$ = 4.97, $P<0.05$]. Results depicted in Figure 8 show that pretreatment of mice with an inhibitor of MEK, PD98,059 (20 µg/site, i.c.v, 60 min before **F-DPS** treatment), was effective in reversing the anti-immobility effect of **F-DPS** (50 mg/kg, i.g.) in the mouse TST ($P < 0.01$).

## 4 Discussion

In the present study, we provided further evidence that acute administration of 3-chalcogen selenophenes exerts antidepressant-like effect in mice. **H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** and **$CF_3$-DPS** significantly reduced immobility in the FST without any locomotor-enhancing effect. A close inspection of the results also revealed that the fluorophenyl portion appears to contribute for the potency of selenophene compounds since **F-DPS** was effective at lower doses than other compounds. Furthermore, acute treatment with **F-DPS** promoted a significant increase on phosphorylated ERK 1/2 levels in prefrontal cortex and hippocampus of mice. Besides, the antidepressant-like effect of **F-DPS** evaluated in the TST was blocked by PD98,059, an inhibitor of ERK 1/2 phosphorylation (MEK inhibitor).

Taken together, the results of this study indicated that ERK phosphorylation is involved in the acute antidepressant-like action of **F-DPS** in mice.

Among all animal models, the FST remains one of the most used tools for screening antidepressants (Petit-Demouliere et al., 2005). Immobility is thought to reflect either a failure to persist in escape directed behavior after persistent stress or the development of passive behavior that disengages the animal from active forms of coping with stressful stimuli. This immobility, referred to as behavioral despair in animals, is claimed to reproduce a condition similar to human depression and is reduced by several agents therapeutically effective in this disorder (Lucki, 1997; Petit-Demouliere et al., 2005). Latency corresponds to a time period (starting at 0 min) in which mice showed movements before the first episode of immobility and it is also commonly used by researchers as an additional parameter of depression-like behavior (Costa et al., 2013). In fact, selenophenes tested in this study were effective in reducing total immobility duration and increasing latency in the FST. However, some compounds that alter motor activity may give false positive effects in this test, in particular psychomotor stimulants and drugs enhancing motor activity, which decrease immobility time by stimulating locomotor activity (Petit-Demouliere et al., 2005). In order to discard this effect, in this study animals treated with the tested compounds were observed in the locomotor activity monitor, which demonstrated that selenophenes did not change the number of crossings, rearings, velocity and total distance travelled. Thus it is unlikely that the effect of selenophenes observed in the FST is based on stimulation of general motor activity. This study provides evidence that 3-chalcogen selenophenes have antidepressant-like effect in mice.

Accordingly, as previously demonstrated by our research group, the administration of selenophenes **Cl-DPS**, **F-DPS** and **$CF_3$-DPS** (50 mg/kg, i.g. route) reduced significantly the total immobility time of mice when they were administered at 30 min before FST (Gai et al.,

2012). Besides confirming these data, in the present study, our results showed that the anti-immobility effect of **Cl-DPS** was maintained up to 180 min after administration, whilst the antidepressant-like action induced by both **F-DPS** and **$CF_3$-DPS** was significant at only 30 min. Latency for the first episode of immobility was also modified by **Cl-DPS**, **F-DPS** and **$CF_3$-DPS**, and the time-course of reducing latency was similar to that observed for anti-immobility effect. Regarding effects of **H-DPS** and **$CH_3$-DPS**, in the present study we showed for the first time the ability of these selenophenes in reducing the despair behavior in the mouse FST. Recently, we have already demonstrated that administration of **H-DPS** and **$CH_3$-DPS** at a dose of 50 mg/kg, 30 min before test, was not effective in reducing the total immobility duration in the FST (Gai et al., 2012). In fact, these data were confirmed in the present study. However, when the test was carried out at 60 and 120 min after treatment, both compounds showed a significant antidepressant-like action elicited by reduction on immobility time and increase on latency for the first episode of immobility. Together these data further appoint to a significant structure-activity relationship and suggest that chemical modifications performed on selenophene structures could alter their pharmacokinetic properties and then modify their absorption, distribution, metabolism and/or excretion. In order to confirm this hypothesis, our research group is already performing a research project to investigate the pharmacokinetic profile of selenophene compounds.

Despite the fast and short-acting of **F-DPS**, this compound produced antidepressant-like action at lower doses than **H-DPS**, **$CH_3$-DPS**, **Cl-DPS** and **$CF_3$-DPS**. While other selenophenes were effective at only 50 mg/kg, animals treated with **F-DPS** at a dose of 25 mg/kg showed a significant reduction in the total immobility duration and increase in the latency for the first episode of immobility in the FST; also, our results confirm previous data of Gay et al. (2010) showing that immobility time was also reduced in the TST when F-DPS was administered at doses of 25 and 50 mg/kg. In fact, fluorine has played a particularly

important and historical role in the development of biologically active agents and the presence of 4-fluorophenyl group appears to be essential for optimum potency of some neuroleptic agents (Granger and Albu, 2005; Kirk, 2006). Fluorophenyl group is also found in the structure of other antidepressant drugs such as serotonine reuptake inhibitors, like paroxetine, citalopram and escitalopram. Interestingly, recent studies have demonstrated the involvement of serotonergic system in the antidepressant-like action of **F-DPS** (Gai et al., 2013; Gay et al., 2010). Acute anti-immobility effect of this selenophene compound was significantly blocked by depletion of serotonin using p-chlorophenylalanine and antagonists of serotonin receptors (5-$HT_{1A}$, 5-$HT_{2A/2C}$ and 5-$HT_3$ subtypes) (Gay et al., 2010). In addition, both acute and subchronic treatments with **F-DPS** inhibited serotonin uptake in a synaptosomal preparation from prefrontal cortex and hippocampus of mice (Gai et al., 2013; Gay et al., 2010). Thus, although the mechanisms regarding the pharmacological action of **H-DPS**, **$CH_3$-DPS**, **Cl-DPS** and **$CF_3$-DPS** are also being investigated by our research group, in this study we considered **F-DPS** as the most promising selenophene to further investigate the mechanisms involved on its antidepressant-like action.

In addition to effects on monoamines, novel theories propose that signal pathway related to synaptic plasticity may be the mechanism of antidepressant action and the pathophysiology of depression. It is now well established that antidepressants affect different signaling pathways like that producing phosphorylation of CREB (Blendy, 2006; Carreno and Frazer, 2014; First et al., 2013; Kuo et al., 2013; Nair and Vaidya, 2006; Reus et al., 2011). Previous researches indicated that the ERK-CREB signal system may be involved in the molecular mechanism of depression (Qi et al., 2006) and some antidepressant therapies increase both phosphorylated ERK and CREB levels in brain of rodents (Carreno and Frazer, 2014; Musazzi et al., 2010a; Qi et al., 2008; Tardito et al., 2009). Wherever, while early peak of ERK activation is commonly observed, CREB phosphorylation is generally seen only after

days or weeks after antidepressant treatment (Di Benedetto et al., 2012). Although **F-DPS** did not modulate pCREB levels, even after repeated administration, pERK levels were significantly increased after acute treatment. ERK is the most-studied member of the MAPK family, and the ERK pathway is the major convergence point in all signal pathways, regulating cellular growth and differentiation and neuronal plasticity. ERK1 and ERK2 are prominently found in hippocampus and prefrontal cortex which are brain regions most likely to be implicated in stress response and depression (Ortiz et al., 1995). Interestingly, we observed a significant increase (around 2 times) of both pERK1 and pERK2 isoforms in prefrontal cortex and hippocampus of mice after acute treatment with this selenophene compound. Even though it has been demonstrated that some antidepressants do not alter phosphorylation of ERK (Carreno and Frazer, 2014), it was previously reported that fluoxetine, a classical antidepressant drug, increases phosphorylation of ERK1/2 but not non-phosphorylated proteins in prefrontal cortex and hippocampus of rats (Qi et al., 2008; Tardito et al., 2009; Tiraboschi et al., 2004). Accordingly, First et al. (2013) have demonstrated that reboxetine-treated rats also presented an increase in hippocampal ERK phosphorylation. In addition, lithium and valproate, two medications largely used for the treatment of bipolar disorder illness, also stimulate the ERK pathway (Einat et al., 2003a; Einat et al., 2003b). Immunofluorescence experiments further confirmed the ERK1/2 phosphorylation within hippocampus following F-DPS treatment and supported the hypothesis of an involvement of hippocampal ERK-mediated pathways in the antidepressant mechanism of F-DPS. Hippocampal plasticity is integrally involved in the pathophysiology of major depressive disorder (MDD). Clinical evidence includes reports of reduced hippocampal volume in magnetic resonance imaging studies and post-mortem studies of MDD patients. Meta-analysis of 32 publications found that volume is significantly reduced with greater than one lifetime major depressive episode or greater than 2 years of illness, suggesting that the observed

atrophy is resultant from the burden of illness rather than being a pre-existing risk factor (McKinnon et al., 2009). Specifically, we detected a selective F-DPS-induced ERK overphosphorylation in the dentate gyrus. This appeared of particular importance since the dentate gyrus is the area where the adult hippocampal neurogenesis, a process downregulated by stress conditions and depressive-like behaviours and upregulated by antidepressants, such as SSRI (David et al., 2009; Santarelli et al., 2003), occurred. Since our results clearly demonstrated that **F-DPS** modulates ERK activation, the next step in this research was to investigate whether this effect could be responsible for its antidepressant-like action. In fact, pretreatment of mice with PD98,059, an inhibitor of ERK phosphorylation, was effective in blocking the acute anti-immobility effect of F-DPS in the TST. Based on these data our results suggest that the ERK pathway may be the potential target of this organoselenium compound and participate in the molecular mechanism of its antidepressant-like action.

ERK activity is able to induce phosphorylation of CREB at a specific serine residue, serine 133, producing an active transcription complex enabling target gene activation. The ability to detect phosphorylation of CREB has been an important means to monitor signaling pathways that trigger CREB activation; however, data suggest that CREB-mediated gene expression might occur in the absence of serine 133 phosphorylation (Conkright et al., 2003). Thus, although phosphorylation is an indicator of CREB activation, the ultimate measure of CREB function is gene transcription. As we did not perform mRNA studies, one cannot exclude the modulation in the function of CREB after treatment with **F-DPS**. Nevertheless, it is possible that short-term antidepressant-like action of this organoselenium compound is independent of CREB modulation. Recent studies have demonstrated that some antidepressant drugs increase expression and release of neurotrophic factors via ERK activity (First et al., 2013; Hisaoka et al., 2007). Besides, serotonin has been showed to increase ERK activation and neurotrophines release via 5-$HT_{2A}$ receptors (Hisaoka et al., 2007). Trophic

factors such as brain-derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) regulate neurogenesis and neuronal plasticity, which are also improved after antidepressant treatment (Mahar et al., 2014; Ruan et al., 2013). Thus, these data allow us to hypothesize that antidepressant-like action of **F-DPS** might be due phosphorylation of ERK via activation of serotonergic receptors leading to neurotrophic factors expression/release. In order to confirm it, the effect of selenophene compounds on the hippocampal expression of BDNF and GDNF is target of our research group in future investigations.

In conclusion, our results confirm that the acute antidepressant-like action of 3-chalcogen selenophenes (**H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** and **$CF_3$-DPS**) is linked to their chemical structure. **F-DPS** was the most promising selenophene tested and its antidepressant-like action seems to involve ERK signaling activation, particularly in the prefrontal cortex and dentate gyrus of the hippocampus, without inducing CREB phosphorylation. Our research group is engaged on investigating the pharmacokinetics properties as well as understanding the molecular effects of selenophene compounds that could be further involved on their antidepressant-like action.

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## Conflict of interest statement

The authors declare no conflict of interests in the present study.

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**Figure Captions**

**Figure 1:** Schematic representation of the experimental design of this study.

**Figure 2:** Effect of acute treatment with selenophene compounds on depression-related behavior in the FST. (**A**), (**C**), (**E**), (**G**) and (**I**) represent the total immobility time; (**B**), (**D**), (**F**), (**H**) and (**J**) represent the latency to the first episode of immobility. **H-DPS (A and B)**, **$CH_3$-DPS (C and D)**, **Cl-DPS (E and F)**, **F-DPS (G and H)** and **$CF_3$-DPS (I and J)** were administered to mice at a dose of 50 mg/kg, i.g. Animals performed FST at different times (15-240 min) after treatment. Values are expressed as mean ± S.E.M. of 6-8 animals. Data were analyzed by using a one-way analysis of variance (ANOVA), followed by the Newman-Keuls test. Asterisks denote the significance level when compared to the control group treated with vehicle: (*) $P < 0.05$, (**) $P < 0.01$ and (***) $P < 0.001$.

**Figure 3:** Effect of acute treatment with **F-DPS** on phosphorylation of ERK1/2 and CREB in hippocampus of mice. **F-DPS** was administered at a dose of 50 mg/kg, i.g and hippocampus was removed after 30 min of treatment. Values are expressed as mean ± S.E.M. of 4 animals. Data were analyzed by using unpaired t-test. Asterisk denotes the significance level when compared to the control group: (*) $P < 0.05$. Representative qualitative Western blotting analysis at the top of the figure, graphic shows representative quantification of the proteins immunocontent normalized to β-actin protein.

**Figure 4:** Effect of acute treatment with **F-DPS** on phosphorylation of ERK1/2 and CREB in prefrontal cortex of mice. **F-DPS** was administered at a dose of 50 mg/kg, i.g and prefrontal cortex was removed after 30 min of treatment. Values are expressed as mean ± S.E.M. of 4

animals. Data were analyzed by using unpaired t-test. Asterisk denotes the significance level when compared to the control group: (*) $P < 0.05$. Representative qualitative Western blotting analysis at the top of the figure, graphic shows representative quantification of the proteins immunocontent normalized to β-actin protein.

**Figure 5:** Effect of repeated treatment with **F-DPS** on CREB phosphorylation in hippocampus and prefrontal cortex of mice. **F-DPS** was administered at the dose of 50 mg/kg, i.g, during 3 days. Hippocampus and prefrontal cortex were removed 30 minutes after the last dose of **F-DPS**. Values are expressed as mean ± S.E.M. of 4 animals. Data were analyzed by using unpaired t-test. Representative qualitative Western blotting analysis at the top of the figure, graphic shows representative quantification of the proteins immunocontent normalized to β-actin protein.

**Figure 6:** Phospho-ERK (pERK) labeling in the dorsal hippocampal formation of F-DPS-treated mice. **F-DPS** was administered at the dose of 50 mg/kg, thirty minutes before brain perfusion. In CA3, F-DPS-induced pERK immunostaining (B, low magnification; D, high magnification) is comparable to that of control mice (A, low magnification; C, high magnification). In the dentate gyrus (DG), the number of labeled neurons is substantially increased in the F-DPS-treated mice (F, low magnification; H, high magnification) in comparison with control mice (E, low magnification; G, high magnification). Scale bars 100 μm.

**Figure 7:** Effect of acute treatment with **F-DPS** on depression-related behavior in the TST. (**A**) represents latency to the first episode of immobility and (**B**) shows the total immobility time. F-DPS (10-50 mg/kg, i.g.) was administered 30 min before testing. Values are expressed

as mean ± S.E.M. of 6 animals. Data were analyzed by using a one-way analysis of variance (ANOVA), followed by the Newman-Keuls test. Asterisks denote the significance level when compared to the control group: (**) $P < 0.01$ and (***) $P < 0.001$.

**Figure 8:** Effect of ERK phosphorylation inhibition on **F-DPS**-induced reduction in total immobility time in the TST. F-DPS was administered by intragastric (i.g.) route 60 min after PD98,059 (20 µg/site) and 30 min before the test. PD98,059 was injected by intracerebroventricular (i.c.v.) way. Values are expressed as mean ± S.E.M. of 6 animals. Asterisk denotes the significance levels when compared to the control group treated with oil: (*) $P < 0.05$. Hashtags denote the significance levels when compared to the **F-DPS**-treated group: (##) $P < 0.01$ (two-way ANOVA followed by the Newman-Keuls test).

# Figures

## Figure 1

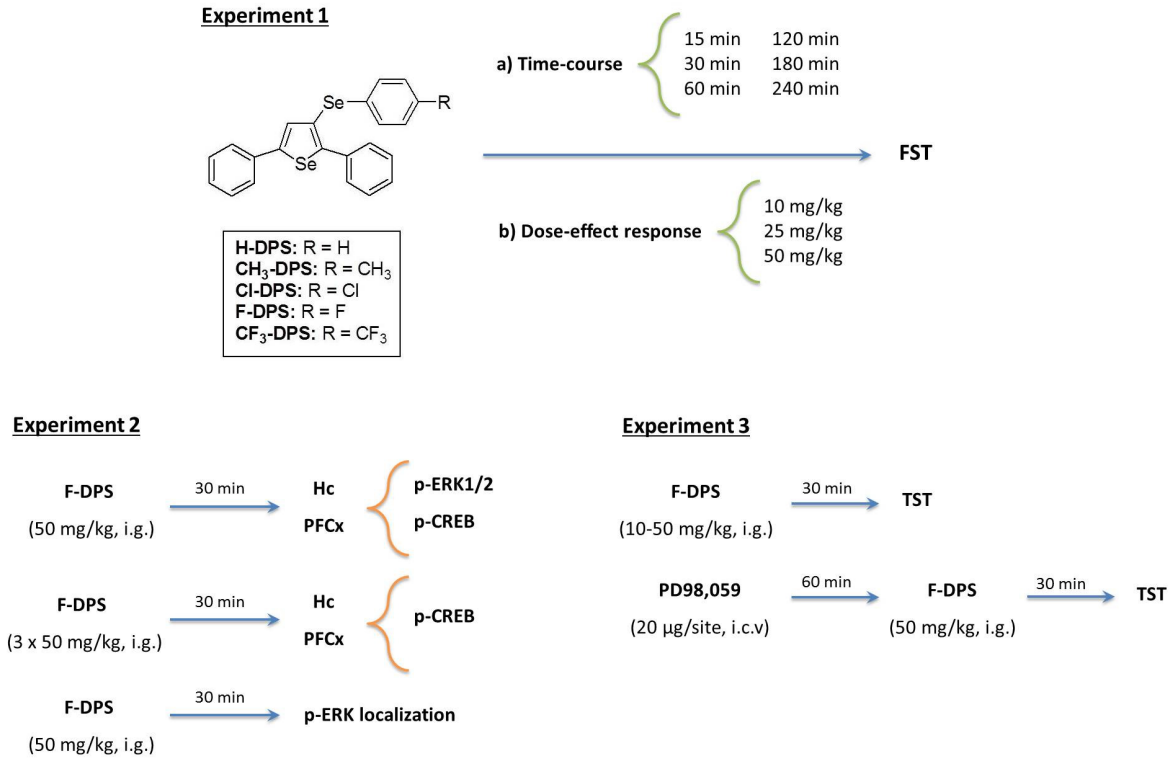
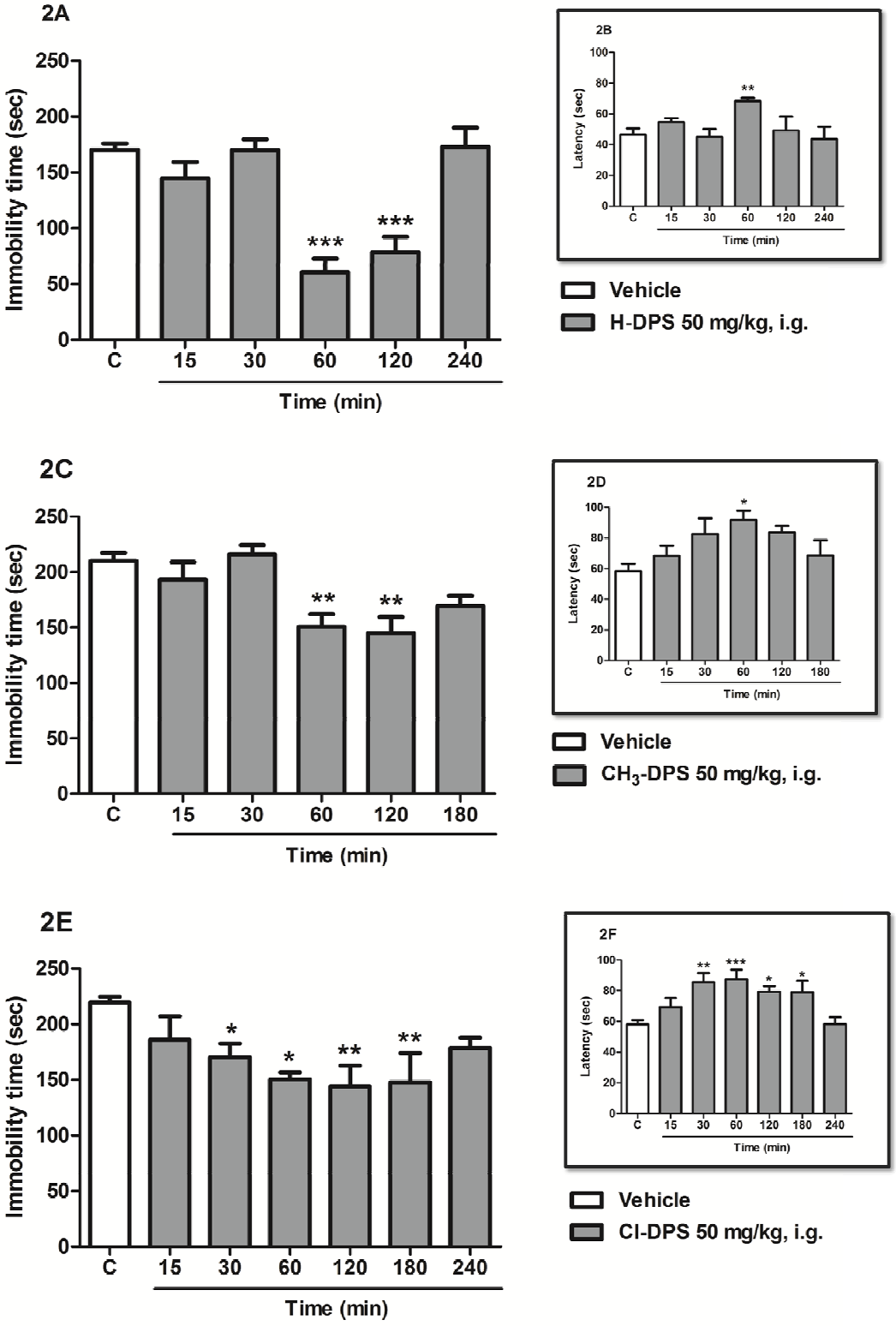


**Figure 2**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Immobility time (sec)
250
200
150
100
50
0
C
15
30
60
120
240
Time (min)
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***
2B
Latency (sec)
100
80
60
40
20
0
**
C
15
30
60
120
240
Time (min)
Vehicle
H-DPS 50 mg/kg, i.g.
2C
Immobility time (sec)
250
200
150
100
50
0
C
15
30
60
120
180
Time (min)
**
**
2D
Latency (sec)
100
80
60
40
20
0
*
C
15
30
60
120
180
Time (min)
Vehicle
CH3-DPS 50 mg/kg, i.g.
2E
Immobility time (sec)
250
200
150
100
50
0
C
15
30
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Time (min)
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Latency (sec)
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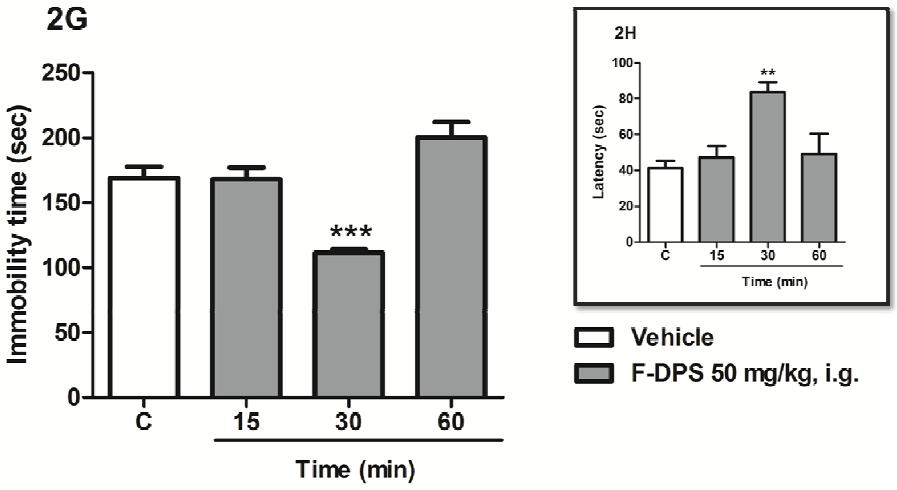
180
240
Time (min)
Vehicle
Cl-DPS 50 mg/kg, i.g.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Immobility time (sec)
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Time (min)
2H
Latency (sec)
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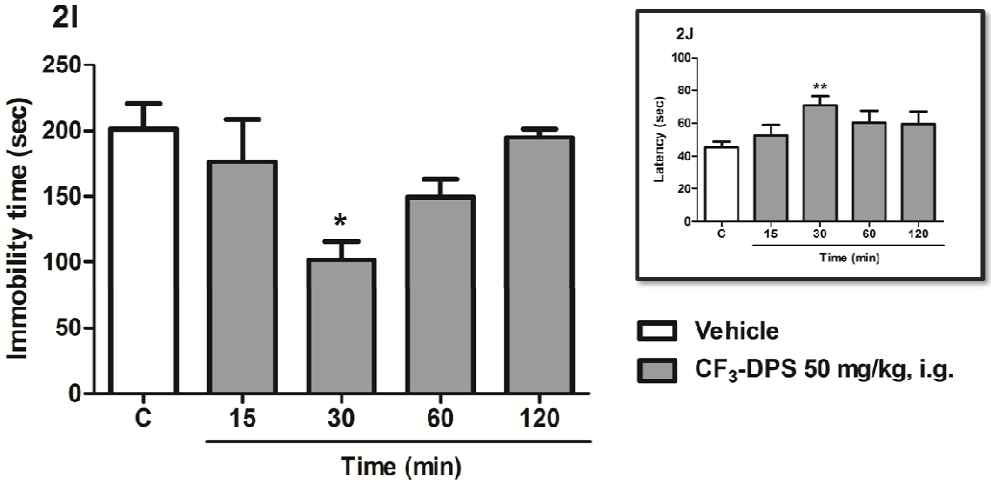
Time (min)
Vehicle
F-DPS 50 mg/kg, i.g.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Immobility time (sec)
250
200
150
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C
15
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Time (min)
2J
Latency (sec)
100
80
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0
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C
15
30
60
120
Time (min)
Vehicle
CF3-DPS 50 mg/kg, i.g.

**Figure 3**

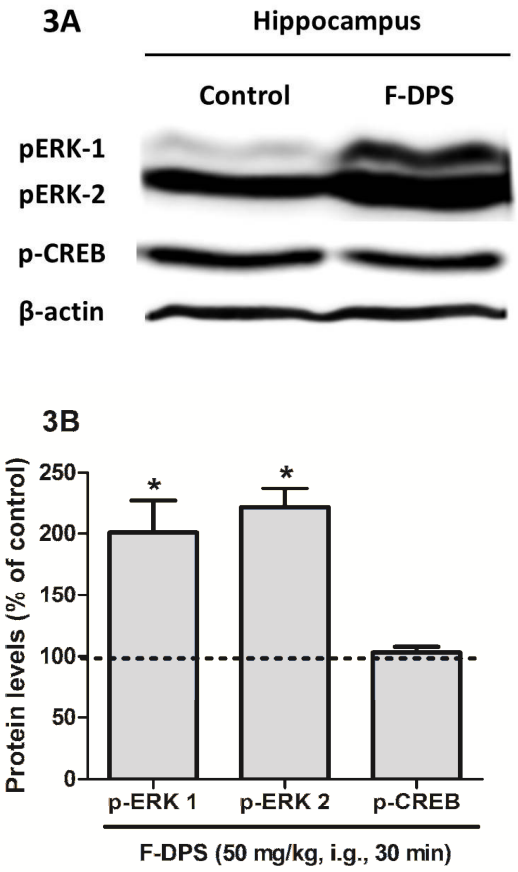

3A
Hippocampus
Control
F-DPS
pERK-1
pERK-2
p-CREB
β-actin
3B
Protein levels (% of control)
250
200
150
100
50
0
*
*
p-ERK 1
p-ERK 2
p-CREB
F-DPS (50 mg/kg, i.g., 30 min)

**Figure 4**

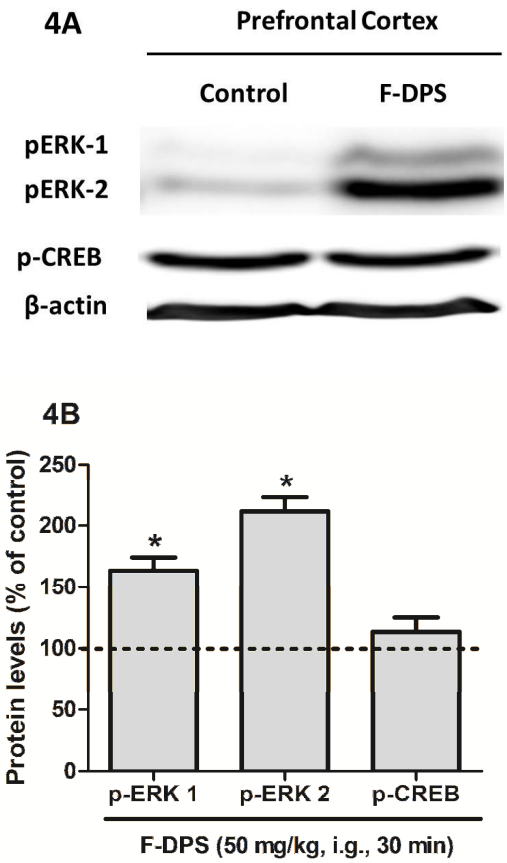

4A
Prefrontal Cortex
Control
F-DPS
pERK-1
pERK-2
p-CREB
β-actin
4B
Protein levels (% of control)
250
200
150
100
50
0
*
*
p-ERK 1
p-ERK 2
p-CREB
F-DPS (50 mg/kg, i.g., 30 min)

**Figure 5**

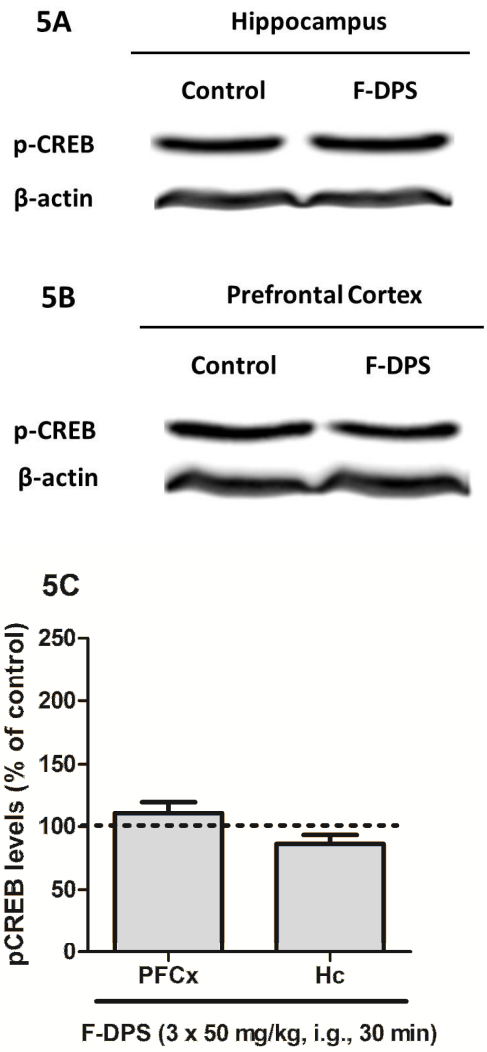

5A
Hippocampus
Control
F-DPS
p-CREB
β-actin
5B
Prefrontal Cortex
Control
F-DPS
p-CREB
β-actin
5C
pCREB levels (% of control)
250
200
150
100
50
0
PFCx
Hc
F-DPS (3 x 50 mg/kg, i.g., 30 min)

**Figure 6**

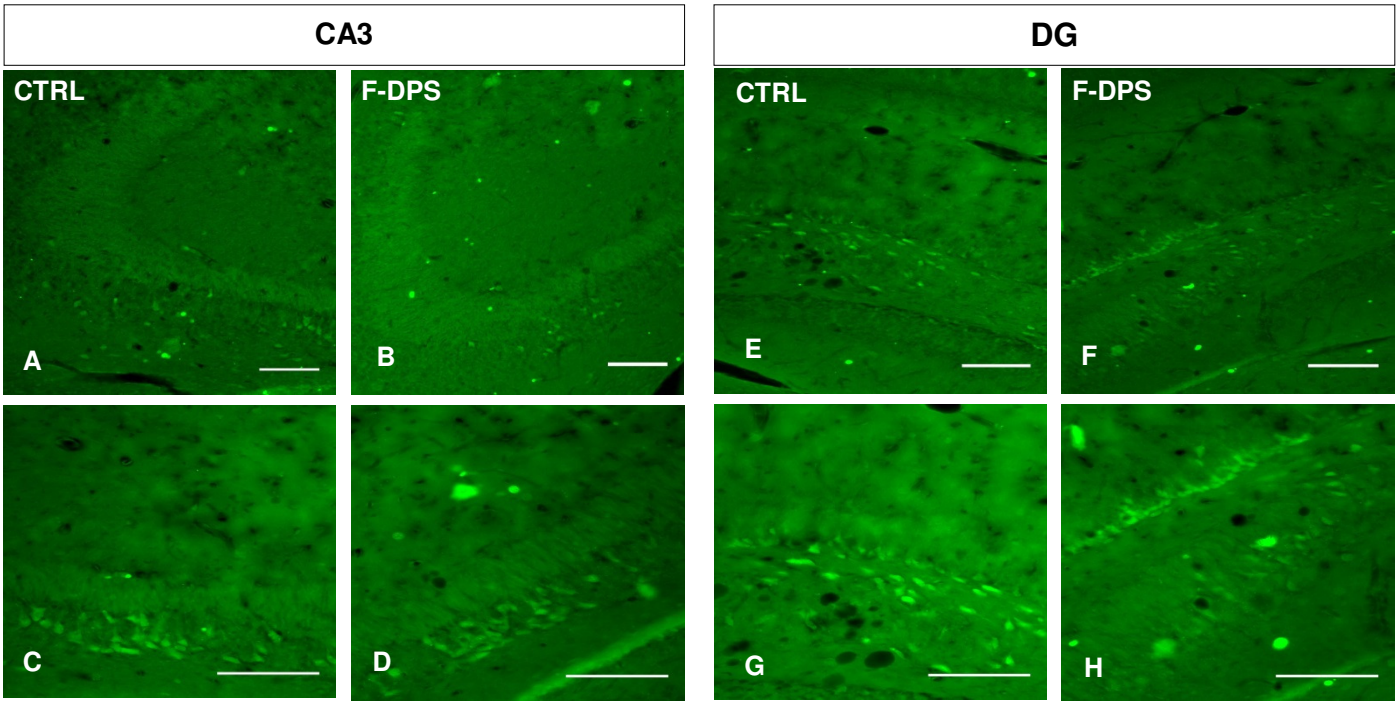


**Figure 7**

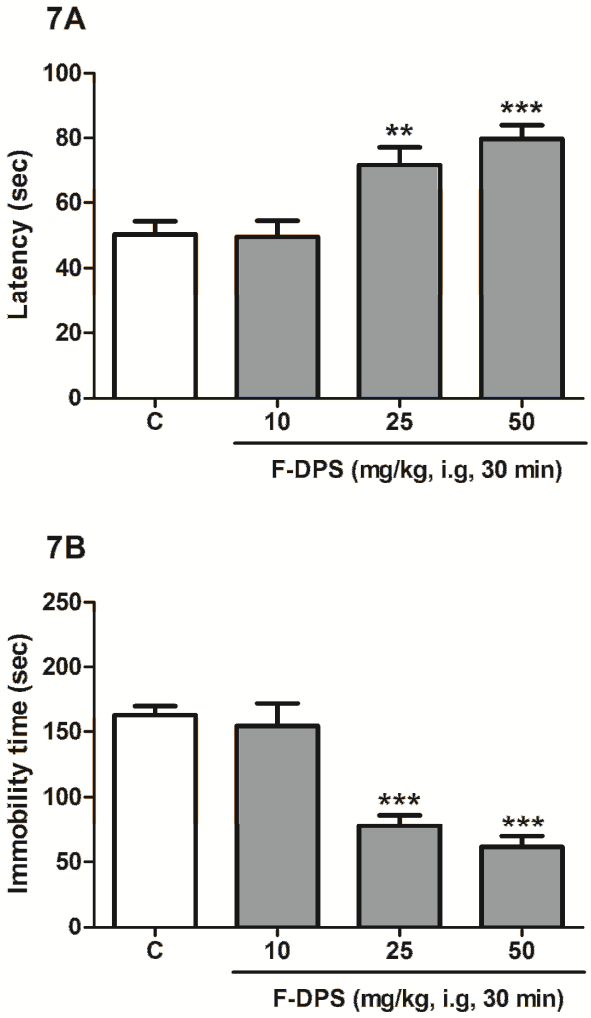
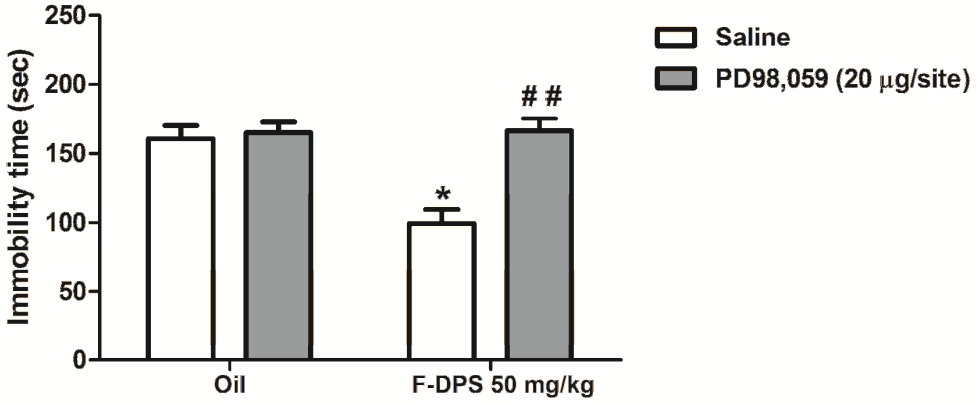

7A
Latency (sec)
100
80
60
40
20
0
**
***
C
10
25
50
F-DPS (mg/kg, i.g, 30 min)
7B
Immobility time (sec)
250
200
150
100
50
0
***
***
C
10
25
50
F-DPS (mg/kg, i.g, 30 min)

**Figure 8**


250
200
150
100
50
0
Immobility time (sec)
Oil
F-DPS 50 mg/kg
*
# #
Saline
PD98,059 (20 μg/site)

## Tables

**Table 1.** Effect of acute treatment with selenophene compounds (dose-range: 10-50 mg/kg) on depression-related behavior in the mouse forced swimming test (FST).

| | **Latency (sec)** | **Immobility time (sec)** |
|---|---|---|
| **H-DPS** (mg/kg, i.g., 60 min) | | |
| 0 | 47.8 ± 3.5 | 170.4 ± 5.6 |
| 10 | 51.5 ± 3.6 | 167.5 ± 12.7 |
| 25 | 52.8 ± 4.5 | 174.5 ± 24.6 |
| 50 | 68.3 ± 2.0** | 60.8 ± 12.0*** |
| **$CH_3$-DPS** (mg/kg, i.g., 60 min) | | |
| 0 | 55.5 ± 3.8 | 221.2 ± 8.0 |
| 10 | 56.3 ± 3.9 | 197.5 ± 11.8 |
| 25 | 69.7 ± 6.3 | 181.2 ± 12.5 |
| 50 | 90.0 ± 7.9** | 135.7 ± 15.0*** |
| **Cl-DPS** (mg/kg, i.g., 30 min) | | |
| 0 | 58.0 ± 5.5 | 210.0 ± 6.0 |
| 10 | 61.3 ± 5.3 | 164.7 ± 26.6 |
| 25 | 72.8 ± 8.0 | 174.7 ± 16.7 |
| 50 | 88.8 ± 6.8* | 107.2 ± 14.0** |
| **F-DPS** (mg/kg, i.g., 30 min) | | |
| 0 | 50.5 ± 4.7 | 163.2 ± 8.0 |
| 10 | 50.0 ± 5.6 | 145.2 ± 24.4 |
| 25 | 77.2 ± 8.1* | 53.3 ± 4.6*** |
| 50 | 80.0 ± 5.0* | 54.8 ± 7.9*** |
| **$CF_3$-DPS** (mg/kg, i.g., 30 min) | | |
| 0 | 51.3 ± 4.4 | 201.3 ± 19.4 |
| 10 | 57.6 ± 5.1 | 172.5 ± 11.0 |
| 25 | 51.8 ± 2.5 | 191.2 ± 13.3 |
| 50 | 75.5 ± 5.2** | 101.8 ± 13.8** |

Selenophene compounds were administered by intragastric (i.g.) route 30 or 60 min before evaluation in the FST. Values are expressed as mean ± S.E.M of 6-8 animals/group. Data were analysed by using a one-way analysis of variance (ANOVA), followed by Newman-Keuls test. Asterisks denote the significance levels when compared to the vehicle group: (*) $P < 0.05$, (**) $P < 0.01$ and (***) $P < 0.001$.

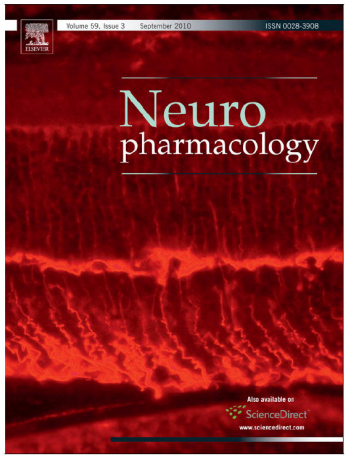
**Table 2.** Locomotor parameters of mice acutely treated with selenophene compounds (dose-range: 10-50 mg/kg).

| | Number of Crossings | Number of Rearings | Velocity (mm/s) | Distance (dm) |
|---|---|---|---|---|
| **H-DPS** (mg/kg, i.g., 60 min) | | | | |
| 0 | 358.7 ± 42.0 | 10.0 ± 1.8 | 25.1 ± 3.8 | 62.2 ± 9.0 |
| 10 | 364.4 ± 47.7 | 10.4 ± 1.6 | 29.5 ± 4.2 | 70.0 ± 9.1 |
| 25 | 319.8 ± 62.4 | 8.0 ± 1.6 | 23.9 ± 5.0 | 55.1 ± 12.2 |
| 50 | 366.3 ± 42.7 | 12.6 ± 2.5 | 30.5 ± 1.0 | 64.2 ± 5.7 |
| **$CH_3$-DPS** (mg/kg, i.g., 60 min) | | | | |
| 0 | 359.7 ± 40.5 | 9.2 ± 2.5 | 25.5 ± 5.9 | 57.1 ± 13.5 |
| 10 | 346.7 ± 37.2 | 11.8 ± 2.1 | 27.5 ± 2.4 | 64.6 ± 5.6 |
| 25 | 327.8 ± 62.4 | 11.6 ± 2.8 | 34.1 ± 2.5 | 70.3 ± 12.7 |
| 50 | 373.7 ± 55.8 | 10.7 ± 2.7 | 29.2 ± 2.6 | 60.8 ± 9.5 |
| **Cl-DPS** (mg/kg, i.g., 30 min) | | | | |
| 0 | 404.8 ± 55.9 | 11.5 ± 2.3 | 31.6 ± 4.8 | 73.8 ± 11.9 |
| 10 | 406.5 ± 65.9 | 12.3 ± 1.4 | 35.2 ± 4.8 | 84.2 ± 11.6 |
| 25 | 401.5 ± 36.2 | 15.5 ± 1.7 | 36.9 ± 2.7 | 82.2 ± 10.9 |
| 50 | 406.0 ± 49.0 | 15.3 ± 1.4 | 39.2 ± 3.4 | 90.0 ± 9.8 |
| **F-DPS** (mg/kg, i.g., 30 min) | | | | |
| 0 | 349.8 ± 35.1 | 10.3 ± 1.1 | 24.9 ± 3.2 | 53.3 ± 9.0 |
| 10 | 367.7 ± 54.6 | 10.0 ± 2.3 | 32.2 ± 4.9 | 62.8 ± 6.6 |
| 25 | 365.8 ± 49.5 | 12 ± 2.6 | 27.5 ± 4.4 | 70.2 ± 10.9 |
| 50 | 332.7 ± 49.7 | 12.5 ± 2.0 | 26.5 ± 3.3 | 66.9 ± 9.0 |
| **$CF_3$-DPS** (mg/kg, i.g., 30 min) | | | | |
| 0 | 389.7 ± 40.9 | 11.1 ± 1.6 | 27.2 ± 4.0 | 60.9 ± 9.1 |
| 10 | 402.2 ± 42.0 | 9.0 ± 1.4 | 24.4 ± 3.7 | 46.7 ± 7.9 |
| 25 | 394.3 ± 56.6 | 9.9 ± 1.8 | 24.3 ± 3.2 | 53.4 ± 7.9 |
| 50 | 364.8 ± 43.1 | 10.2 ± 1.2 | 28.3 ± 3.5 | 60.8 ± 9.8 |

Selenophene compounds were administered by intragastric (i.g.) route 30 or 60 min before evaluation in the locomotor activity monitor. Values are expressed as mean ± S.E.M of 6-8 animals/group. Data were analysed by using a one-way analysis of variance (ANOVA), followed by Newman-Keuls test.

# ARTIGO 1:

## 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

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# 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

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ABSTRACT

Clinically, it is suggested that chronic pain might induce mood disorders like depression and anxiety. Based on this antidepressant drugs have emerged as a new therapy for pain. In this study, the effect of acute and subchronic treatments with 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene (F-DPS) on behavioral changes induced by partial sciatic nerve ligation (PSNL) was evaluated. At the 4th week after surgery, PSNL caused a significant depression-like behavior in mice evaluated in the forced swimming test (FST) and the tail suspension test (TST), which was accompanied by increased pain sensitivity. The anxiety-like behavior assessed in the light–dark test (LDT) was not modified by PSNL. Acute treatment with F-DPS, at a dose of 1 mg/kg, intragastrically (i.g.) administered 30 min before the FST, produced a significant anti-immobility effect in PSNL mice. The antidepressant drug paroxetine showed acute antidepressant-like action at a dose 10 times higher than F-DPS. Subchronic treatment with F-DPS (0.1 mg/kg, i.g.) reversed depression-like behavior of sciatic nerve-ligated mice in the TST and FST and produced a significant anxiolytic-like action in both sham-operated and PSNL animals. Although the acute F-DPS treatment did not produce anti-allodynic effect, F-DPS subchronic treatment significantly reduced pain sensitivity in PSNL mice. These findings demonstrated that F-DPS blocked behavioral changes induced by neuropathic pain, suggesting that it might be attractive in the pharmacological approach of pain-emotion diseases.



## 1. Introduction

Mood disorders such as depression and anxiety are frequently observed in patients suffering from chronic pain (Goldberg and McGee, 2011). This comorbidity leads to serious clinical problems and has a larger negative impact on the quality of life (Arnow et al., 2006; Goldberg and McGee, 2011). Although the mechanisms concerning pain-emotion diseases have not been defined, preclinical studies have demonstrated a relationship between neuropathic pain and mood disorders in animal models (Arnow et al., 2006; Matsuzawa-Yanagida et al., 2008; Yalcin et al., 2011). In fact, previous studies have shown depression-related behavior in rodents subjected to sciatic nerve injury, a well-recognized model for neuropathic pain (Fukuhara et al., 2012; Jesse et al., 2010). The partial sciatic nerve ligation (PSNL), model of Narita et al. (2005), incorporates a less peripheral inflammatory component compared with other peripheral nerve injury models and has a robust mechanical hypersensitivity and high responsiveness to analgesic drugs and novel therapies for chronic pain (Crisp et al., 2003; Dowdall et al., 2005; Narita et al., 2005; Roeska et al., 2008; Seltzer et al., 1990).

In the last decade, antidepressant drugs have emerged as first-line drugs for neuropathic pain, a chronic condition, severe, and resistant to most analgesics (Attal et al., 2006; Mico et al., 2006). It has been clinically and pre clinically demonstrated that treatment with drugs that increase extracellular concentrations of serotonin (5-HT), dopamine (DA), and noradrenaline (NA) seems to be effective in improving both pain and depression symptoms (Attal et al., 2006; Blier and Abbott, 2001; Hauser et al., 2013; Thaler

*Abbreviations:* F-DPS, 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene; PSNL, partial sciatic nerve ligation; FST, forced swimming test; LAM, locomotor activity monitor; LDT, light–dark test; SRI, serotonin reuptake inhibitor; TST, tail suspension test; VFH, Von-Frey hair.

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et al., 2012). These actions are mainly related to changes on cellular signaling pathways by inhibiting reuptake transporters and interacting with monoaminergic membrane receptors (Blier and Abbott, 2001). However, exact mechanisms underlying their effectiveness in anti-nociception/pain are unknown.

Over the past decade, it has been shown the efficacy of organoselenium compounds in many experimental models of chronic pain and depression-like behavior (Gai et al., 2012; Gay et al., 2010; Jesse et al., 2010; Nogueira and Rocha, 2011). 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene (F-DPS) is an organoselenium drug belonging to the selenophene class which has shown antidepressant-like action (Gai et al., 2012; Gay et al., 2010). Its mechanism of action still remains unclear; however, a previous study showed that 5-HT receptor antagonists block its acute antidepressant-like effect on the mouse forced swimming test (FST). Further, F-DPS seems to increase serotonergic neurotransmission by inhibiting presynaptic 5-HT transport (Gay et al., 2010).

Taking into account the abovementioned points, the main objective of this study was to investigate whether acute and subchronic F-DPS treatments could be effective in reducing depression-related behavior and pain sensitivity in sciatic nerve-ligated mice.

## 2. Materials and methods

### 2.1. Animals

The experiments were conducted using male Swiss mice (25–30 g) maintained at 22–25 °C with free access to water and food, under a 12:12 h light/dark cycle with lights on at 7:00 a.m. All manipulations were carried out between 08:00 a.m. and 04:00 p.m. and mice were acclimated to the behavioral room at least 2 h before the test. The experiments were performed according to a randomized schedule and each animal was used only once in each test. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil (# 124/2010). The procedures in this study were performed in accordance with the 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

3-(4-fluorophenylselenyl)-2,5-diphenylselenophene (F-DPS, Fig. 1) was prepared and characterized in our laboratory based on a previous study (Stein et al., 2008). Analysis of the $^{1}$HNMR and $^{13}$CNMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of studied compound (99.9%) was determined by GC/MS.

Paroxetine was purchased from Sigma–Aldrich (St. Louis, MO, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

F-DPS was dissolved in canola oil and administered by intragastric (i.g.) route. I.g. procedure is commonly used by our research group for administration of organoselenium compounds and oil-soluble drugs (Gai et al., 2012; Gay et al., 2010), compounds are administered by using a gastroesophageal probe that releases them directly into the stomach. Paroxetine was dissolved in saline with dimethyl sulfoxide (DMSO) 1% and administered intraperitoneally (i.p.) (Gay et al., 2010).

### 2.3. Surgical procedure

PSNL has been shown to produce neuropathic pain and increased depressive-like behavior in rodents (Jesse et al., 2010; Yalcin et al., 2011). PSNL was performed based on the original description (Narita et al., 2005) under intraperitoneal ketamine/xylazine (150 and 10 mg/kg, respectively) anesthesia. Briefly, the right sciatic nerve was exposed after the incision of skin and blunt separation of the muscle. The sciatic nerve was freed of the adhering tissue gently for about 7 mm, and one ligature (8/0 Ethicon GmbH, Norderstedt, Germany) was made around approximately 1/3–1/2 the diameter of the sciatic nerve. Great care was taken to tie the ligatures so that the diameter of the nerve was just barely constricted. Sham operation was performed by exposing sciatic nerve except for nerve ligation.

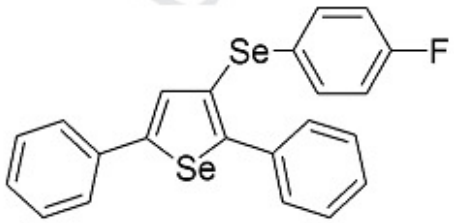


**Fig. 1.** Chemical structure of 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene (F-DPS).

### 2.4. Experimental design

This study was divided into 2 experimental protocols (Fig. 2). The first protocol aimed to investigate the acute effect of F-DPS on the depression-like behavior and mechanical allodynia induced by PSNL in mice. In this experiment, we used the classical antidepressant drug, paroxetine, as a positive control. In the Experiment 2, we investigated whether an acute subeffective dose of F-DPS would be effective if subchronically administered to mice. Thus, we treated sham and PSNL-subjected animals with F-DPS at a dose of 0.1 mg/kg, during 1 or 2 weeks.

#### 2.4.1. Experiment 1

At the end of the 4th week after surgery, PSNL mice were treated with vehicle (10 ml/kg) or F-DPS (dose range: 0.1–10 mg/kg) by the intragastric (i.g.) route ($n = 9$ animals/group). Thirty minutes after treatment, mice were then tested in the forced swimming test (FST). In order to investigate changes on the mouse locomotion, before the FST mice were observed in the locomotor activity monitor (LAM). The F-DPS pretreatment time was based on a previous study from our research group, which established 30 min as the maximum acute F-DPS antidepressant-like effect (Gay et al., 2010).

In order to compare the antidepressant-like effect of F-DPS with a classical antidepressant drug, PSNL mice received vehicle (10 ml/kg) or paroxetine at doses of 1 and 10 mg/kg ($n = 7$ animals/group), intraperitoneally (i.p.), forty five minutes before the FST. Spontaneous locomotor activity of mice was also observed into LAM.

For the purpose of investigating the effect of F-DPS on the neuropathic pain induced by PSNL, a separate group of animals received vehicle (10 ml/kg) or F-DPS at a dose of 10 mg/kg, i.g., and was evaluated in the mechanical allodynia test 30 min after treatment ($n = 7$ animals/group). The anti-allodynic effect of paroxetine at doses of 1 and 10 mg/kg ($n = 7$ animals/group), intraperitoneally (i.p.), forty five minutes before VFH, was also investigated.

#### 2.4.2. Experiment 2

The second part of this study investigated the antidepressant-like effect of F-DPS after subchronic treatment. For this purpose, animals were divided into six groups ($n = 10$ animals/group) and a subeffective dose of F-DPS, selected in the Experiment 1 (0.1 mg/kg), or vehicle (10 ml/kg), was administered daily to sham and sciatic nerve-ligated mice during the 3rd and/or 4th weeks after surgery. At the end of the 4th week, twenty-four hours after the last dose of F-DPS, mice were evaluated in the LAM, tail suspension test (TST) and FST. Pain sensitivity of mice was accompanied by using Von-Frey Hair (VFH) paradigm. Further, the possible anxiolytic-like action of F-DPS was performed in the light–dark test (LDT).

### 2.5. Behavioral testing

#### 2.5.1. Spontaneous locomotor activity

To discard non-specific effects of treatments, spontaneous locomotor activity of mice was performed in the locomotor activity monitor (LMA). LMA is a Plexiglas cage (45 × 45 × 45 cm) surrounded by a frame consisting of 32 photocells mounted on opposite walls (16 L × 16 W, spaced 2 cm apart) that continuously tracks the animal's movement. Animals were placed in the center of the apparatus and allowed to freely explore the arena during 4 min. Motor activity was monitored with the Insight® Monitor Activity System. Data were collected in the form of photobeam breaks as an indication of activity within different predetermined "zones" in the open field using Monitor Activity® software (Insight). Number of crossings and rearings, average velocity (mm/s) and total distance traveled (dm) were recorded.

#### 2.5.2. Tail suspension test (TST)

The TST was performed in a quiet experimental room according to the method reported by Steru and collaborators (Steru et al., 1985). Each mouse was suspended by its tail to a horizontal wooden bar located inside a yellow plastic box (40 cm × 46 cm × 40 cm) approximately 30 cm above the floor. The mouse was secured to the bar by adhesive tape placed 1 cm from the tip of the tail, such that the mouse's head was about 20 cm above the floor. The trial was conducted for 6 min during which a blinded observer scored the latency for the first immobility episode and total duration of immobility by using a stopwatch. The mouse was considered immobile only when it hung passively and completely motionless. Mice that climbed their tails were eliminated from further analyses.

#### 2.5.3. Forced swimming test (FST)

The FST is one of the most widely used tools for evaluation of antidepressant drugs, antidepressant efficacy of new compounds, and experimental manipulations that are aimed at rendering or preventing depressive-like states. The procedure used in this study was based on that previously described (Porsolt et al., 1979). Mice were



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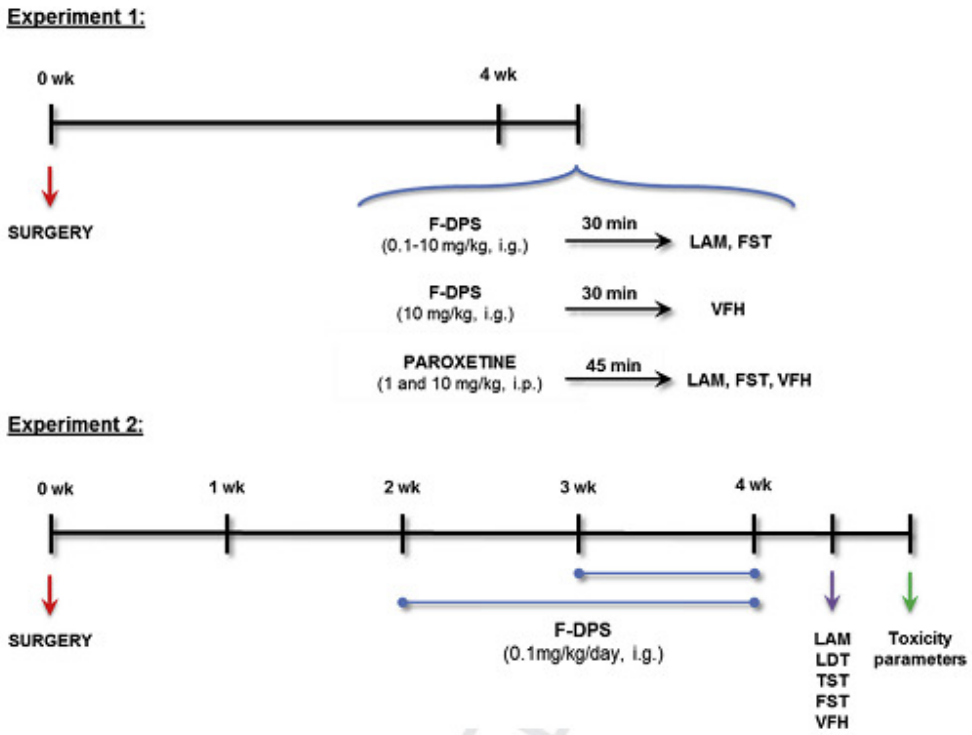


**Fig. 2.** Schematic representation of the experimental design of this study. All experiments were performed at the end of the 4th week after PSNL surgical procedure. F-DPS was administered by the intragastric (i.g.) route, whereas mice received an intraperitoneal (i.p.) administration of paroxetine. LAM: locomotor activity monitor; FST: forced swimming test; TST: tail suspension test; VFH: Von-Frey hair.

gently placed in an inescapable cylindrical container (10 × 25 cm) that was filled with water (19 cm, 25 ± 1 °C) and their escape related mobility behavior (latency for the first immobility episode and total duration of floating) was measured by a blinded observer during a 6 min period by using a stopwatch. Latency was defined as the amount of time that elapsed between placing the mouse in the tank and the first instance of each behavioral occurrence. 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.

#### 2.5.4. Light–dark test (LDT)

The LDT is based on the innate aversion of rodents to brightly illuminated areas and on the spontaneous exploratory behavior of rodents in response to mild stressors, that is, novel environment and light. Classic anxiolytics as well as the newer anxiolytic-like compounds (e.g. serotonergic drugs) can be detected using this paradigm (Bourin and Hascoet, 2003). The light–dark box apparatus consists of a white–black acrylic rectangular box (46 cm × 27 cm × 30 cm), which is divided into two compartments (light and large: 27 cm × 27 cm, dark and small: 18 cm × 27 cm) by a partition. These areas are connected by a small central open door (7.5 cm × 7.5 cm) located in the center of the partition at floor level. The large compartment was open at the top, illuminated by a 100 W bulb located 90 cm above the apparatus and the small compartment had a removable black lid at the top. To start the test, each mouse was placed at the center of the light compartment, facing away from the door and the animal was allowed to explore freely both compartments for 5 min and their behavior was recorded during this time. The following parameters were recorded by a blinded observer during a 6 min period by using a stopwatch: latency to enter into the dark compartment, the amount of time spent into the light and dark compartments (all four paws) and number of zone transitions.

#### 2.5.5. Mechanical allodynia test

The mechanical allodynia was measured as described before (Bortalanza et al., 2002). The response frequency was measured after ten applications (duration of 1–2 s each) of 0.6 g Von-Frey Hair (VFH, Stoelting, Chicago, IL). To this end, mice were further habituated in individual clear Plexiglas boxes (9 × 7 × 11 cm) on an elevated wire mesh platform to allow access to the ventral surface of the hind paws. A previous study of our research group indicated that 0.6 g VFH produced a mean withdrawal frequency of ipsilateral hind paw of approximately 70% in PSNL-subjected mice (Savegnago et al., 2007), which was considered to be an adequate value for studying the anti-allodynic effect of organoselenium compounds. Therefore, 0.6 g VFH was used in these experiments. The test was carried on by an observer who was blinded to treatments.

Both the ipsilateral (right hind paw) and the contralateral hind paws were tested in order to evaluate the occurrence of mirror-image pain. The contralateral allodynia (bilateral allodynia or mirror-image pain) to an injury has been described both in humans and various models of neuropathic and inflammatory pain in animals (Huang and Yu, 2010; Jaggi and Singh, 2011).

### 2.6. Statistical analysis

All experimental results are given as the mean ± S.E.M. First, we evaluated the normality of data using the D'Agostino and Pearson omnibus normality test. In the Experiment 1, for data from the FST, comparisons between sham and PSNL groups were performed by unpaired *t*-test; statistical differences between PSNL and F-DPS- or paroxetine-treated groups were performed by a one-way analysis of variance (ANOVA) followed by the Newman–Keuls test for post hoc comparison. The unpaired *t*-test was considered appropriate to compare the response frequency of VFH stimulation between PSNL group and F-DPS- treated group.

In the Experiment 2, comparisons between groups were performed by two-way ANOVA followed by the Newman–Keuls test. The main effects of first order interactions are presented only when interaction was not significant. All analyses were performed by using the STATISTICA for Windows software Version 7 (StatSoft, Oklahoma, USA). Probability values less than 0.05 ($P < 0.05$) were considered as statistically significant.

## 3. Results

### 3.1. Acute antidepressant-like action of F-DPS in PSNL mice is not accompanied by anti-allodynic effect

Data from the FST of PSNL mice treated with F-DPS are shown in Fig. 3. An unpaired *t*-test revealed a significant difference between Sham and PSNL groups on latency for the first immobility episode [$t_{(16)} = 2.92$, $P < 0.01$; Fig. 3A] and total immobility time [$t_{(16)} = 3.43$, $P < 0.01$; Fig. 3B] in the FST. PSNL decreased the latency of mice in the FST at about 21.5%, whereas total immobility time was increased 50.0%.

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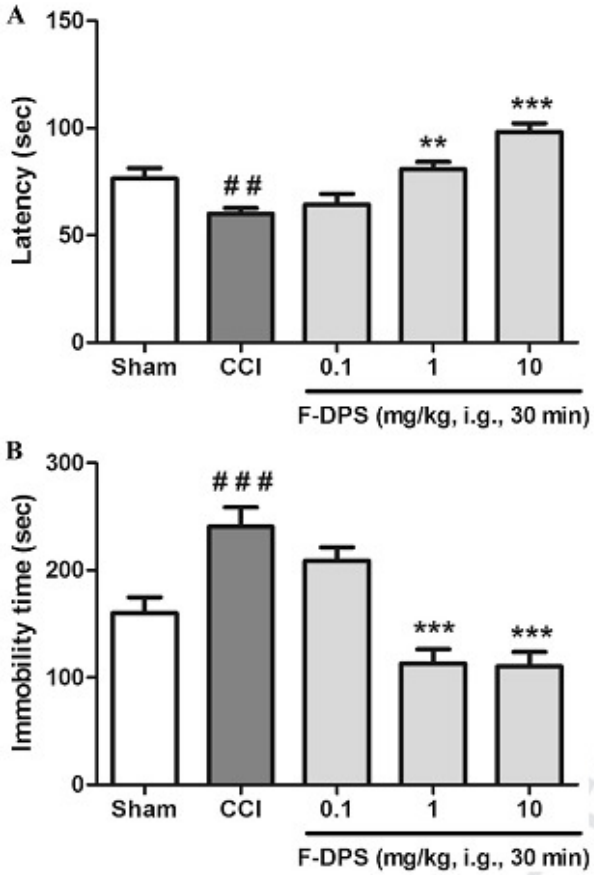


**Fig. 3.** Effect of acute treatment with F-DPS (dose range: 0.1–10 mg/kg) on depression-like behavior induced by PSNL in the mouse FST. (**A**) represents latency to the first episode of immobility and (**B**) shows the total immobility time. F-DPS was administered intragastrically (i.g.) 30 min before test. Values are expressed as mean ± S.E.M. of 9 animals. Hashtags denote the significance levels when compared to the sham group: (##) $P < 0.01$ and (###) $P < 0.001$ (unpaired *t*-test). Asterisks denote the significance levels when compared to the PSNL group: (**) $P < 0.01$ and (***) $P < 0.001$ (one-way ANOVA followed by the Newman–Keuls test).

A one-way ANOVA revealed a significant effect of F-DPS treatment on latency [$F_{(3,35)} = 20.30$, $P < 0.001$] and immobility time [$F_{(3,35)} = 21.07$, $P < 0.001$] of PSNL mice in the FST (Fig. 3). F-DPS increased the latency for the first immobility episode at doses of 1 and 10 mg/kg ($P < 0.01$ and $P < 0.001$, respectively; Fig. 3A). In addition, total immobility time of PSNL mice was decreased by F-DPS administration at doses of 1 and 10 mg/kg ($P < 0.001$; Fig. 3B). The number of crossings, rearings, velocity and distance traveled were not changed by treatments ($P > 0.05$, Table 1).

Effects of the classical antidepressant, paroxetine, on the depression-like behavior induced by PSNL are depicted in Fig. 4. Paroxetine showed a significant effect on latency for the first immobility episode [$F_{(2,20)} = 9.18$, $P < 0.01$] and total immobility time [$F_{(2,20)} = 14.55$, $P < 0.001$] of PSNL mice in the FST. Mice treated with paroxetine at a dose of 10 mg/kg showed higher latency ($P < 0.01$; Fig. 4A) and lower immobility time ($P < 0.001$; Fig. 4B) when compared to the PSNL group treated with vehicle. A dose of 1 mg/kg did not produce effect in the FST. Paroxetine did not modify the number of rearings and velocity of PSNL mice evaluated in the LAM ($P > 0.05$, Table 2). By contrast, the number of crossings and total distance traveled were significantly increased by both doses of paroxetine when compared to the PSNL group ($P < 0.05$, Table 2).

**Table 1**
Locomotor parameters of mice subjected to PSNL evaluated in the activity monitor after F-DPS acute treatment.

| | Number of crossings | Number of rearings | Velocity (mm/s) | Distance (dm) |
|---|---|---|---|---|
| Sham | 456.0 ± 34.9 | 15.8 ± 1.7 | 39.1 ± 3.3 | 67.1 ± 5.8 |
| PSNL | 467.0 ± 11.6 | 18.0 ± 1.4 | 35.2 ± 4.0 | 63.2 ± 2.5 |
| PSNL + F-DPS 0.1 mg/kg | 511.3 ± 48.9 | 15.7 ± 2.1 | 31.9 ± 3.0 | 67.5 ± 7.4 |
| PSNL + F-DPS 1 mg/kg | 437.1 ± 45.0 | 15.7 ± 0.9 | 30.7 ± 4.8 | 57.2 ± 4.3 |
| PSNL + F-DPS 10 mg/kg | 421.6 ± 76.6 | 17.7 ± 0.8 | 34.2 ± 4.4 | 52.2 ± 5.5 |

F-DPS was administered by intragastric (i.g.) route 30 min before evaluation in the locomotor activity monitor (LAM). Values are expressed as mean ± S.E.M of 9 animals/group. Data were analyzed by using a one-way analysis of variance (ANOVA), followed by Newman–Keuls test.

Response frequency of VFH stimulation was significantly increased by PSNL in ipsilateral paw when compared to the sham group [$t_{(12)} = 30.67$, $P < 0.001$; Fig. 5A]. In addition, the response frequency of contralateral paw was also increased by PSNL [$t_{(12)} = 2.30$, $P < 0.05$; Fig. 5B], demonstrating the presence of "mirror pain". However, F-DPS acute treatment neither modified ipsilateral [$t_{(12)} = 1.60$, $P > 0.05$; Fig. 5A] nor contralateral [$t_{(12)} = 0.52$, $P > 0.05$; Fig. 5B] response frequency when compared to the PSNL group. On the other hand, one-way ANOVA yielded a

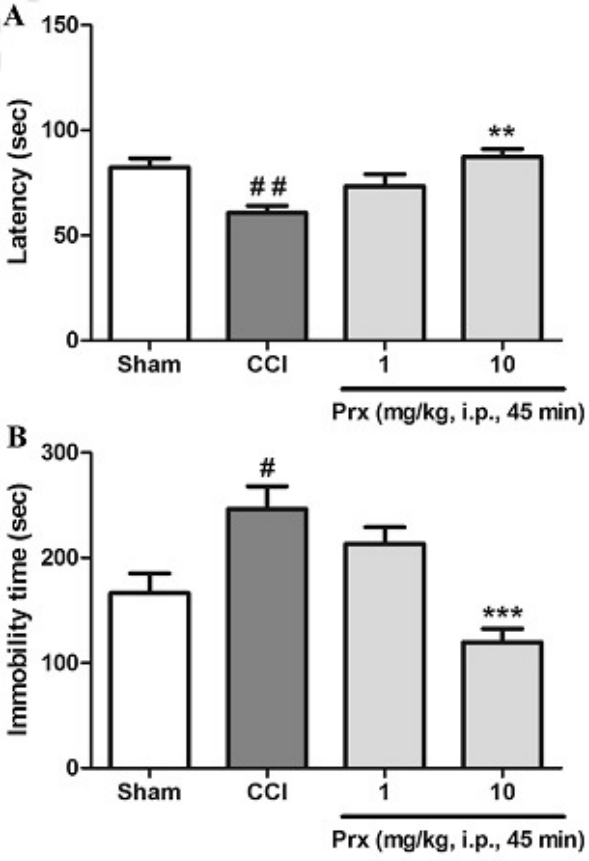


**Fig. 4.** Effect of acute treatment with paroxetine (1 and 10 mg/kg) on depression-like behavior induced by PSNL in the mouse FST. (**A**) represents latency to the first episode of immobility and (**B**) shows the total immobility time. Paroxetine was administered intraperitoneally (i.p.) 45 min before test. Values are expressed as mean ± S.E.M. of 7 animals. Hashtags denote the significance levels when compared to the sham group: (#) $P < 0.05$ and (##) $P < 0.01$ (unpaired *t*-test). Asterisks denote the significance levels when compared to the PSNL group: (**) $P < 0.01$ and (***) $P < 0.001$ (one-way ANOVA followed by the Newman–Keuls test). Prx means paroxetine.

**Table 2**
Locomotor parameters of mice subjected to PSNL evaluated in the activity monitor after paroxetine (Prx) acute treatment.

| | Number of crossings | Number of rearings | Velocity (mm/s) | Distance (dm) |
|---|---|---|---|---|
| Sham | 484.6 ± 45.1 | 16.4 ± 1.9 | 37.0 ± 3.9 | 63.3 ± 6.1 |
| PSNL | 495.6 ± 30.8 | 18.6 ± 1.2 | 34.6 ± 4.2 | 64.7 ± 2.9 |
| PSNL + Prx 1 mg/kg | 939.6 ± 98.3** | 21.0 ± 2.4 | 52.4 ± 6.9 | 119.1 ± 15.9* |
| PSNL + Prx 10 mg/kg | 956.4 ± 103.8** | 17.3 ± 3.4 | 47.9 ± 5.1 | 137.9 ± 24.5* |

Paroxetine was administered intraperitoneally (i.p.) 45 min before evaluation in the locomotor activity monitor (LAM). Values are expressed as mean ± S.E.M of 7 animals/group. Data were analyzed by using a one-way analysis of variance (ANOVA), followed by Newman–Keuls test. Asterisks denote the significance levels when compared to the CCl group: (*) $P < 0.05$ and (**) $P < 0.01$.

significant effect of acute paroxetine treatment on contra [$F_{(3,31)} = 5.33$, $P < 0.01$; Fig. 5B] and ispsilateral [$F_{(3,31)} = 73.23$, $P < 0.001$; Fig. 5A] response frequency. Administration of paroxetine at a dose of 10 mg/kg, forty-five minutes before mechanical allodynia test, was effective in reducing pain sensitivity of both contra ($P < 0.01$) and ipsilateral ($P < 0.001$) paws. The dose of 1 mg/kg was not effective in mechanical allodynia test ($P > 0.05$).

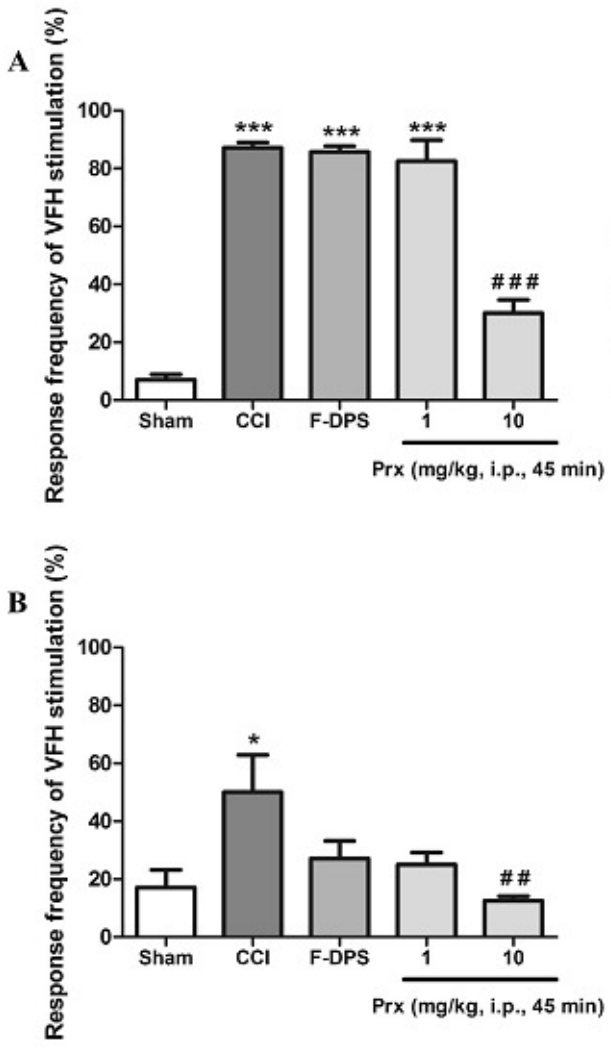


**Fig. 5.** Effect of acute treatment with F-DPS (10 mg/kg) on the response frequency to VFH stimulation in ipsilateral (**A**) and contralateral (**B**) paws in PSNL mice. F-DPS was administered intragastrically (i.g.) 30 min before test. Values are expressed as mean ± S.E.M. of 7 animals. Asterisks denote the significance levels when compared to the sham group: (*) $P < 0.05$ and (***) $P < 0.001$ (unpaired *t*-test). Hashtags denote the significance levels when compared to the PSNL group: (##) $P < 0.01$ and (###) $P < 0.001$ (one-way ANOVA followed by Newman–Keuls test).

### 3.2. Subchronic treatment with F-DPS produces antidepressant-like and anti-allodynic action in PSNL mice

Fig. 6 shows the effect of F-DPS treatment on the performance of Sham and PSNL mice in the TST. The two-way ANOVA revealed no significant effect of treatments on the latency for the first immobility episode [$F_{(2,54)} = 0.89$, $P > 0.05$; Fig. 6A]. On the other hand, it has been shown a significant main effect of both PSNL [$F_{(1,54)} = 29.59$, $P < 0.001$] and F-DPS [$F_{(2,54)} = 11.54$, $P < 0.001$] on the total immobility time in the TST (Fig. 6B). Post hoc analysis showed that PSNL increased the total immobility time when compared with the sham group (72.0%, $P < 0.001$) and treatment of mice with F-DPS at a dose of 0.1 mg/kg during 1 and 2 weeks decreased this parameter in PSNL mice when compared to the PSNL mice treated with vehicle ($P < 0.001$).

Effect of subchronic F-DPS treatment on depression-like behavior in the FST is shown in Fig. 7. The two-way ANOVA of latency for the first immobility episode yielded a significant F-DPS × PSNL interaction [$F_{(2,54)} = 6.68$, $P < 0.01$; Fig. 7A]. PSNL procedure significantly decrease the latency (39.8%, $P < 0.05$) when compared to the sham group, whilst both F-DPS treatments (1 and 2 weeks) were effective to increase it when compared to the PSNL group treated with vehicle. Further, post-hoc analysis revealed that daily administration of F-DPS at a dose of 0.1 mg/kg during 2 weeks caused a significant increase on the latency in the FST in sham-operated mice (62.9%, $P < 0.001$). Statistical analysis of total immobility time revealed a main effect of PSNL [$F_{(1,54)} = 4.51$, $P < 0.05$] and F-DPS treatment [$F_{(2,54)} = 25.30$, $P < 0.001$]. PSNL caused an increase on the mouse immobility time in the FST (50.5%, $P < 0.01$) and subchronic F-DPS treatments decreased this parameter in PSNL mice treated with vehicle (Fig. 7B). In Sham mice, F-DPS was effective to decrease the total immobility time after 2 weeks treatment (35.7%, $P < 0.01$).

Even though PSNL procedure did not produce any significant change in the LDT, statistical analysis yielded a significant main effect of F-DPS on the latency for the first transition [$F_{(2,54)} = 7.05$, $P < 0.01$], and total time spent in the light [$F_{(2,54)} = 13.43$, $P < 0.001$] and dark [$F_{(2,54)} = 13.43$, $P < 0.001$] compartments (Table 3). After treatment of mice during 2 weeks, F-DPS increased the latency of sham animals (138.9%, $P < 0.05$); however, it was not significant in PSNL-subjected mice (122.1%, $P > 0.05$). The time spent in the light compartment was increased by F-DPS treatment after 2 weeks in sham and also in PSNL mice (93.0%, $P < 0.01$ and 57.1%, $P < 0.05$), when compared to their respective vehicle-treated groups. Time in the dark compartment was decreased in both sham-operated (47.7%, $P < 0.01$) and PSNL mice (30.1%, $P < 0.05$) at the end of F-DPS treatment. The number of transitions between two compartments was not altered by treatments [$F_{(2,54)} = 12.21$, $P > 0.05$].

The two-way ANOVA revealed a significant F-DPS × PSNL interaction on the response frequency of VFH stimulation in both ipsilateral [$F_{(2,54)} = 33.16$, $P < 0.001$] and contralateral [$F_{(2,54)} = 10.16$, $P < 0.001$] paws (Fig. 8). Post-hoc analysis showed that PSNL injury increased in 12 times the response frequency in ipsilateral ($P < 0.001$; Fig. 8A) whilst pain sensitivity of contralateral paw was increased in 8 times ($P < 0.001$; Fig. 8B). Interestingly, F-DPS treatment during 2 weeks was effective in reducing both ipsi- and contralateral response frequency in PSNL mice ($P < 0.001$). However, treatment of PSNL mice with F-DPS during 1 week was not effective in ameliorating this parameter.

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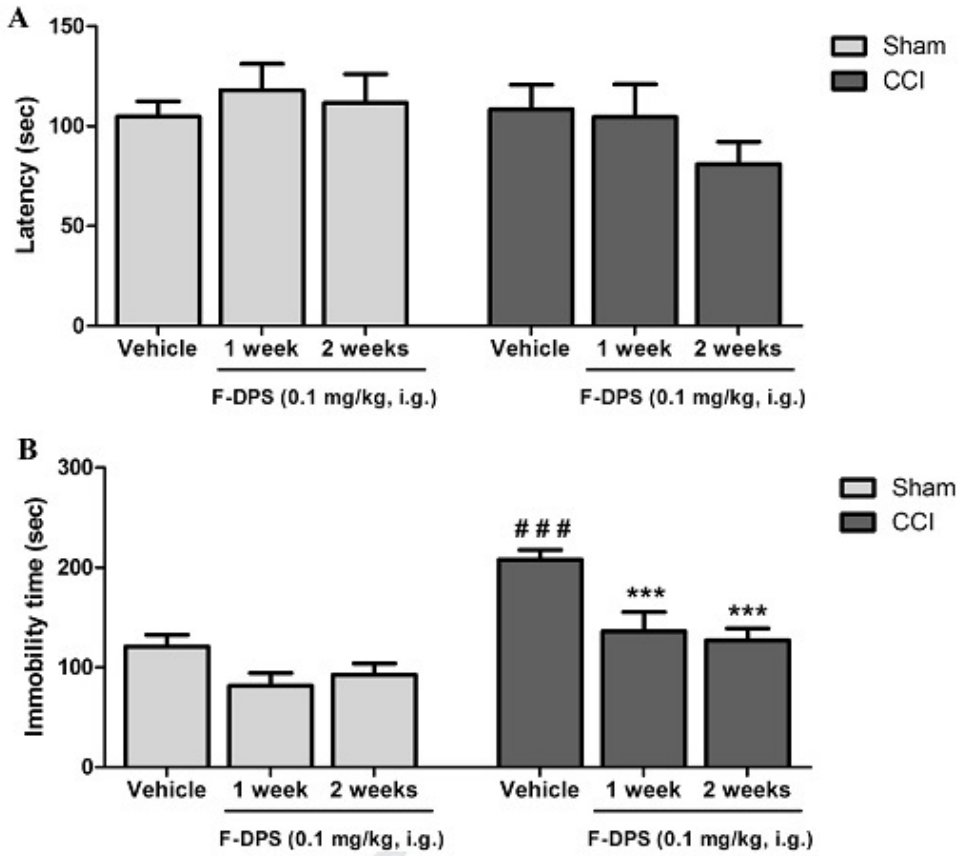


**Fig. 6.** Effect of subchronic treatment with F-DPS (0.1 mg/kg) on depression-like behavior induced by PSNL in the mouse TST. (**A**) represents latency to the first episode of immobility and (**B**) shows the total immobility time. F-DPS was daily administered by intragastric (i.g.) route during the 3rd and/or 4th weeks after PSNL surgical procedure. Values are expressed as mean ± S.E.M. of 10 animals. Hashtags denote the significance levels when compared to the sham group: (###) $P < 0.001$. Asterisks denote the significance levels when compared to the respective vehicle-treated group: (***) $P < 0.001$ (two-way ANOVA followed by the Newman–Keuls test).

Regarding locomotor activity, it was found that neither PSNL nor F-DPS affect the number of crossings and rearings, velocity and total distance traveled ($P > 0.05$) by mice in the LAM (Table 4).

## 4. Discussion

The aim of the present study was to evaluate the effect of treatment with F-DPS on behavioral consequences induced by neuropathic pain in the mouse PSNL model. The main pharmacological finding of this study was that acute and subchronic F-DPS treatments blocked depression-like behavior in PSNL mice. Interestingly, the acute antidepressant-like action of this selenophene was observed at lower doses than paroxetine. Despite acute treatment did not reduce pain sensitivity, the subchronic administration of F-DPS was effective in producing anti-allodynic action in PSNL mice. These findings permit further understanding the pharmacological properties of F-DPS as well as the mechanisms behind its antidepressant-like action.

The current study demonstrated that mice with partial ligation of the sciatic nerve developed depression-like behavior as reflected by a decrease in the latency for the first immobility episode and the increase in the total immobility time in the FST and TST. Since decreasing on immobility time was not accompanied by changes in the number of crossings, rearings, velocity and distance in the LAM, we can suggest that locomotion did not influence the depression-like behavior observed in PNSL animals. In addition, although immobility in the FST could be due to pain during movement – since the motor coordination for swimming is different from the ability to perform the monitor activity – a previous study from our research group has already excluded this possibility by using motivational tests (Jesse et al., 2010). Together, these data reinforce the hypothesis of a depression-like action induced by PNSL.

The fact that animals with neuropathic pain exhibited depression-like behavior is in agreement with clinical and preclinical evidence reporting a relationship between chronic pain and depression (Deshpande et al., 2006; Fukuhara et al., 2012; Jesse et al., 2010; Mico et al., 2006; Yalcin et al., 2011). Indeed, in this study, administration of paroxetine, a classical antidepressant drug, was effective in producing anti-immobility effect in PSNL mice when it was administered at a dose of 10 mg/kg. The effect of paroxetine in the FST was accompanied by a significant increase in the number of crossings and total distance traveled when the animals were evaluated in the LAM. In fact, it has been shown that the acute administration of paroxetine and other SRIs enhances spontaneous locomotor activity in mice, which seems to be related to the anxiolytic-like action (Brocco et al., 2002). Regarding the effects of F-DPS, the present results showed that acute treatment with this selenophene, at a dose of 1 mg/kg (10 times lower than the dose of paroxetine), administered 30 min before the FST, reduced the immobility time in PSNL mice without any change on the locomotor activity. Accordingly, a previous study has already shown the antidepressant-like effect of F-DPS in naïve mice, which was demonstrated when this compound was administered to mice at a dose of 50 mg/kg, at the same conditions used in this study (Gay

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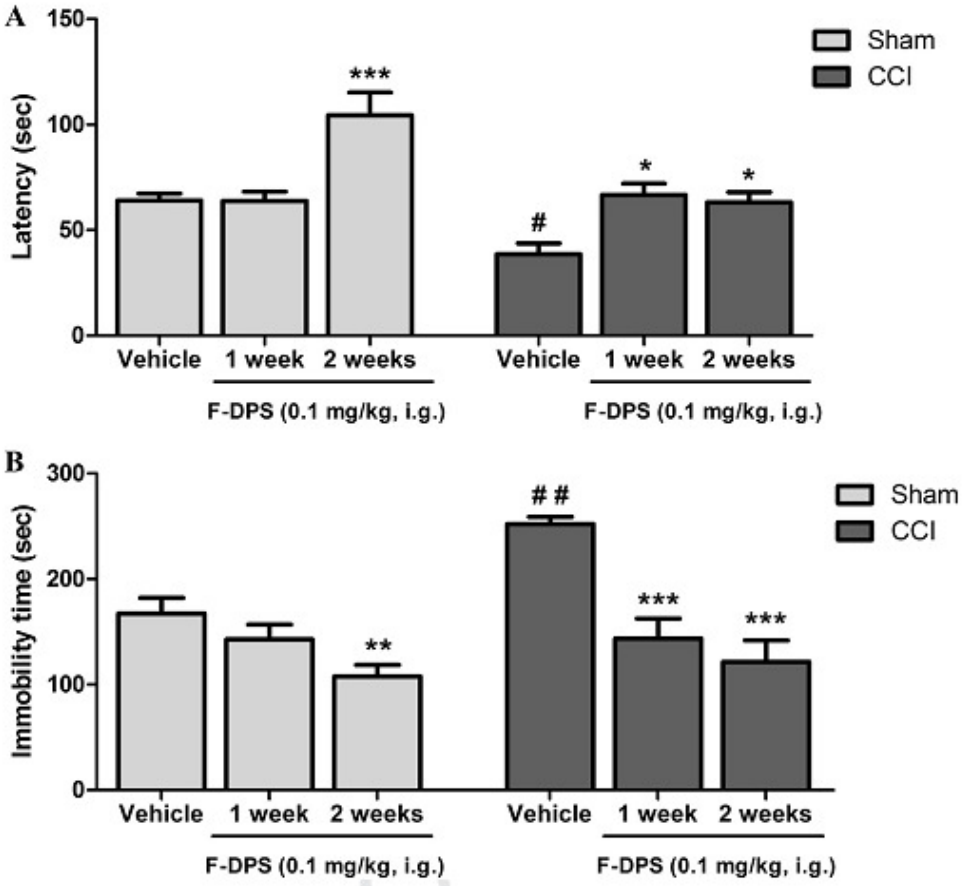


**Fig. 7.** Effect of subchronic treatment with F-DPS (0.1 mg/kg) on depression-like behavior induced by PSNL in the mouse FST. (**A**) represents latency to the first episode of immobility and (**B**) shows the total immobility time. F-DPS was daily administered by intragastric (i.g.) route during the 3rd and/or 4th weeks after PSNL surgical procedure. Values are expressed as mean ± S.E.M. of 10 animals. Hashtags denote the significance levels when compared to the sham group: (#) $P < 0.05$ and (##) $P < 0.01$. Asterisks denote the significance levels when compared to the respective vehicle-treated group: (*) $P < 0.05$, (**) $P < 0.01$ and (***) $P < 0.001$ (two-way ANOVA followed by the Newman–Keuls test).

et al., 2010). In addition to the antidepressant-like action of F-DPS at lower doses than paroxetine, it is possible to note that the action of F-DPS seems to be more pronounced when depression-like behavior was induced by chronic pain.

Results from experiment 2 showed that F-DPS subchronic treatment also produced antidepressant-like action. Daily administration of F-DPS at a dose of 0.1 mg/kg during 1 and 2 weeks was effective in blocking depression-like behavior induced by PSNL in the TST and the FST; F-DPS increased the latency for the first immobility episode in the FST and decreased the total immobility time of PSNL mice in both TST and FST. It is worth to note that even though a dose of 0.1 mg/kg was ineffective in the acute protocol, it produced pharmacological effect in PSNL mice when it was subchronically administered. In addition, whilst the first week of F-DPS treatment reduced the total immobility time of PSNL-subjected mice in the FST, only after 2 weeks of the F-DPS administration, it produced antidepressant-like action in sham mice, suggesting one more time that treatment with this selenophene is more effective when depression-like behavior is induced by PSNL model.

In spite of neuropathic pain did not produce anxiogenic-like behavior, the present results showed that treatment with F-DPS at a dose of 0.1 mg/kg administered during 2 weeks to mice increased the total time spent in the light compartment of both sham and PSNL mice evaluated in the LDT, demonstrating for the first time the anxiolytic-like property of this organoselenium compound. In fact, some studies failed to show any association between neuropathic pain and anxiety-related behaviors (Kontinen et al., 1999) whereas other have reported anxiogenic-like phenotypes only after 4 (Matsuzawa-Yanagida et al., 2008) and 8 weeks (Suzuki et al., 2007). These differences are mainly attributed to the model used for inducing chronic pain, time after surgery and paradigm of anxiety-related behavior (Yalcin et al., 2011).

**Table 3**
Effect of subchronic treatment with F-DPS on anxiety-like behavior in the LDT in PSNL-mice.

| | Latency (sec) | Time in the light compartment (sec) | Time in the dark compartment (sec) | Number of transitions |
|---|---|---|---|---|
| Sham | 21.1 ± 6.5 | 102.5 ± 17.3 | 197.5 ± 17.3 | 11.9 ± 1.9 |
| F-DPS 1 week | 26.2 ± 5.3 | 138.0 ± 14.3 | 162.0 ± 14.4 | 10.3 ± 1.2 |
| F-DPS 2 weeks | 50.4 ± 9.6* | 197.8 ± 22.0** | 102.2 ± 22.0** | 11.6 ± 3.2 |
| PSNL | 14.9 ± 2.2 | 115.1 ± 10.9 | 184.9 ± 10.9 | 14.4 ± 1.4 |
| PSNL + F-DPS 1 week | 19.7 ± 4.3 | 120.0 ± 13.6 | 180.0 ± 13.6 | 14.7 ± 2.0 |
| PSNL + F-DPS 2 weeks | 33.1 ± 8.9 | 180.8 ± 16.5* | 129.2 ± 18.9* | 12.9 ± 2.3 |

F-DPS was daily administered at a dose of 0.1 mg/kg during 1 or 2 weeks, by the intragastric (i.g.) route. Values are expressed as mean ± S.E.M of 10 animals/group. Data were analyzed by using a two-way analysis of variance (ANOVA), followed by the Newman–Keuls test. Asterisks denote the significance levels when compared to the vehicle-treated group: (*) $P < 0.05$ and (**) $P < 0.01$.

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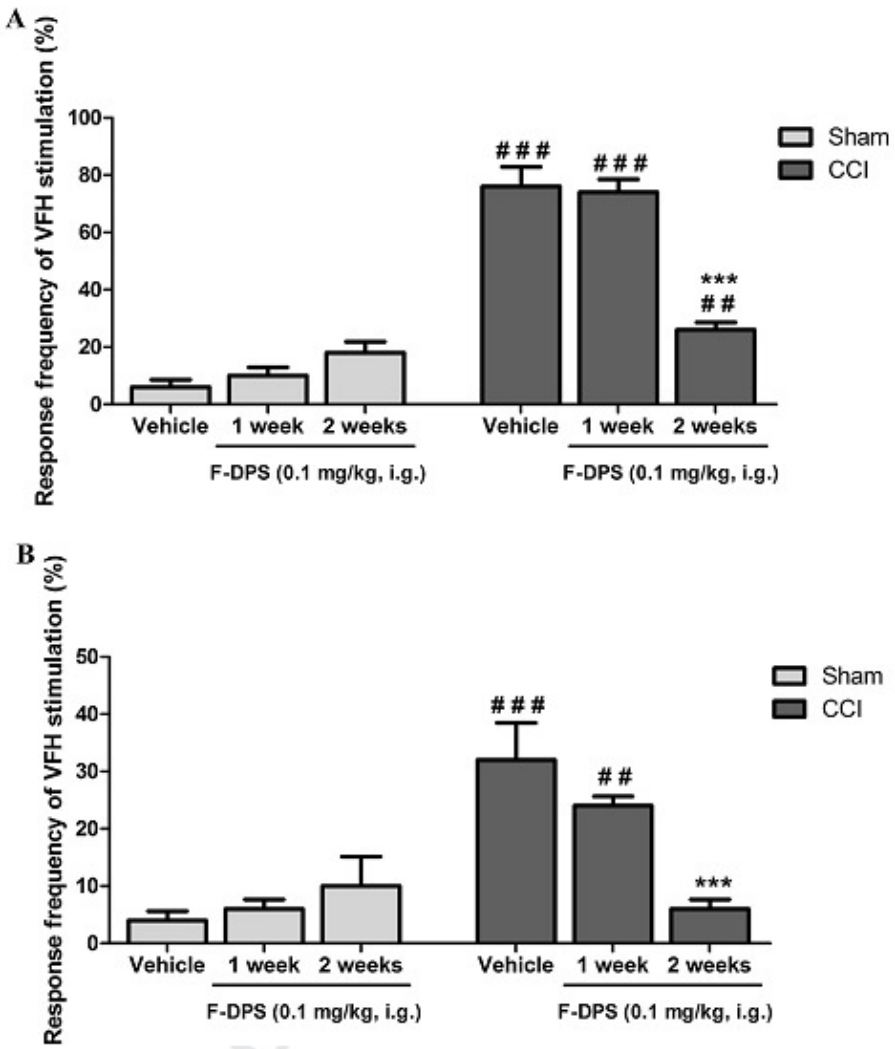


**Fig. 8.** Effect of subchronic treatment with F-DPS (0.1 mg/kg) on the response frequency to VFH stimulation in ipsilateral (**A**) and contralateral (**B**) paws in PSNL mice. F-DPS was daily administered by intragastric (i.g.) route during the 3rd and/or 4th weeks after PSNL surgical procedure. Hashtags denote the significance levels when compared to the sham group: (##) $P < 0.01$ and (###) $P < 0.001$. Asterisks denote the significance levels when compared to the respective vehicle-treated group: (***) $P < 0.001$ (two-way ANOVA followed by the Newman–Keuls test).

Regarding pain sensitivity, the results showed that acute F-DPS treatment at a dose of 10 mg/kg did not reduce mechanical allodynia in PSNL mice, suggesting the antidepressant-like action of F-DPS is not related to an anti-allodynic effect. Different result was obtained after acute treatment with the classical antidepressant paroxetine; we showed that the administration of paroxetine at an effective dose in the depression test (10 mg/kg, i.p., 45 min before) reduced pain sensitivity in sciatic nerve-ligated mice, demonstrating an association between antidepressant-like and anti-allodynic actions of paroxetine.

**Table 4**
Locomotor parameters of mice subjected to PSNL after subchronic F-DPS treatment.

| | Number of crossings | Number of rearings | Velocity (mm/s) | Distance (dm) |
|---|---|---|---|---|
| Sham | 493.9 ± 51.8 | 17.8 ± 2.4 | 34.4 ± 3.3 | 77.6 ± 8.6 |
| F-DPS 1 week | 475.8 ± 62.2 | 15.8 ± 2.5 | 28.7 ± 3.2 | 69.4 ± 7.8 |
| F-DPS 2 weeks | 432.2 ± 63.6 | 17.2 ± 1.9 | 32.4 ± 5.6 | 70.7 ± 13.1 |
| PSNL | 545.2 ± 75.9 | 20.0 ± 2.6 | 35.4 ± 4.8 | 84.2 ± 10.1 |
| PSNL + F-DPS 1 week | 500.4 ± 58.2 | 17.6 ± 2.4 | 31.1 ± 3.8 | 72.1 ± 9.4 |
| PSNL + F-DPS 2 weeks | 414.5 ± 58.8 | 15.3 ± 1.8 | 26.9 ± 3.9 | 65.6 ± 10.0 |

F-DPS was daily administered at the dose of 0.1 mg/kg during 1 or 2 weeks, by intragastric (i.g.) route. Values are expressed as mean ± S.E.M of 10 animals/group. Data were analyzed by using a two-way analysis of variance (ANOVA), followed by Newman–Keuls test.

On the other hand, when F-DPS was administered during 2 weeks, at a dose that is subeffective in depression tests (0.1 mg/kg), the response frequency to the VFH stimulation in both ipsilateral and contralateral paws was significantly decreased, demonstrating an anti-allodynic action of this organoselenium compound. These data are in agreement with previously published studies, which demonstrate that the analgesic effect of some antidepressant drugs, including paroxetine, was only seen after 10–14 days of treatment (Benbouzid et al., 2008; Matsuzawa-Yanagida et al., 2008). Similar to our results, a previous study has already shown that the anti-allodynic action of subchronic treatment with paroxetine in sciatic nerve-ligated mice (4 mg/kg, s.c., during 3 weeks) started only after the 2nd week (Matsuzawa-Yanagida

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et al., 2008). However, although it would seem intuitive that reduction of nociception intensity should improve depression-related behavior, the current results demonstrated that anti-allodynic effect of F-DPS occurred after the starting of its antidepressant-like action. These data might reinforce dissociation between anti-allodynic and antidepressant-like actions of F-DPS in the model of neuropathic pain induced by PSNL. However, it remains a myth why the anti-allodynic effects of F-DPS are only seen after at least two weeks of daily treatment with F-DPS. We believe that, in addition to the serotonergic system, additional mechanisms can be activated at the 2nd week of subchronic treatment with this selenophene compound, which could be responsible for reduction of pain sensitivity.

Taking account the results described here and recent studies demonstrating the effect of F-DPS on blocking depression-related behavior, our research group have been devoted to explore the mechanisms on the antidepressant-like action of this selenophene. Recently, we showed the involvement of 5-$HT_{1A}$, 5-$HT_{2A/2C}$ and 5-$HT_3$ receptors on the acute anti-immobility effect of F-DPS in the mouse FST (Gay et al., 2010). Additionally, F-DPS has been shown to inhibit synaptosomal 5-HT uptake after a single i.g. administration, which suggests that F-DPS interacts with 5-HT receptors by increasing cerebral serotonergic neurotransmission (Gay et al., 2010). In fact, it is already well known that SRIs, in addition to inhibit 5-HT uptake also interact with 5-HT receptors at neuronal membrane, which activates its own signal transduction pathway inside the postsynaptic neuron (Blier and Abbott, 2001; Kroeze et al., 2012). It is postulated that 5-HT receptors targeted by SRIs are linked to cAMP-PKA and PLC-PKC pathways converging on CREB (Kroeze et al., 2012). CREB is a transcription factor that mediates many of the actions of the cAMP cascade on gene expression and is up-regulated by antidepressant treatment (Blendy, 2006; Kroeze et al., 2012). Thus, based on our data and recent studies of our research group, we suggest that up-regulation of CREB could be involved on the antidepressant-like effect of F-DPS in the PSNL model. We are already developing a research project in order to investigate the effect of F-DPS treatment on CREB activation pathways.

## 5. Conclusion

In this study, depression-related behavior induced by neuropathic pain was reversed by both acute and subchronic treatments with F-DPS. The subchronic administration of this selenophene was effective in producing anti-allodynic and anxiolytic-like action in mice subjected to PSNL. These results suggest that F-DPS might be an attractive therapeutic tool in the development of novel therapies for pain-emotion diseases.

## Conflict of interest statement

The authors declare no conflict of interests in the present study.

## Uncited reference

Q1 Tanis and Duman, 2007.

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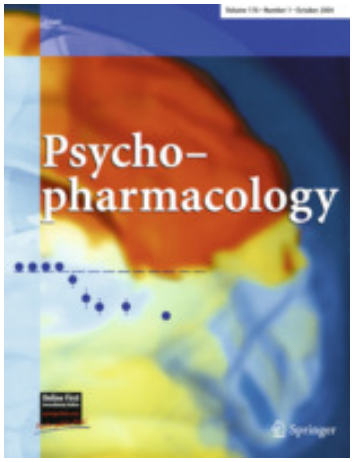
## ARTIGO 2:

**An organoselenium compound improves behavioral, endocrinal and neurochemical changes induced by corticosterone in mice**

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ORIGINAL INVESTIGATION

# An organoselenium compound improves behavioral, endocrinal and neurochemical changes induced by corticosterone in mice

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**Abstract**
*Rationale* 3-(4-Fluorophenylselenyl)-2,5-diphenylselenophene (F-DPS) is a promising organoselenium compound that shows antidepressant-like properties related to interaction with the serotonergic system.
*Objectives* In this study, a mouse model of anxiety/depressant-like behavior induced by long-term corticosterone treatment was used to evaluate behavioral, endocrinal, and neurochemical changes in mice and their possible modulation of F-DPS treatment.
*Methods* Swiss mice were subjected to 4 weeks of corticosterone administration (20 μg/ml in drinking water) and a new therapeutic approach with F-DPS (0.1 mg/kg/day, intragastric route, during 1 week) was employed to modulate changes induced by corticosterone exposure.
*Results* Treatment with corticosterone caused a significant depressant-like behavior in the forced swimming test and tail suspension test, which was accompanied by anxiety-like condition in the light–dark test and novelty suppressed-feeding; similarly to the classical antidepressant drug paroxetine, F-DPS treatment was effective in reversing these behavioral changes. Further, F-DPS normalized serum levels of corticosterone and adrenocorticotropic hormone, which were increased after corticosterone exposure. Corticosterone also significantly inhibited glutamate uptake in the prefrontal cortex of mice, whereas glutamate release was not modified. Besides normalizing glutamate uptake in the corticosterone-exposed mice, F-DPS promoted an inhibition of 5-HT uptake in the prefrontal cortex and hippocampus. In addition, hippocampal monoamine oxidase-A activity was also inhibited by F-DPS treatment.
*Conclusions* These findings suggest a modulation of both serotonergic and glutamatergic systems by F-DPS after a long-term corticosterone exposure in mice, which may be involved in the antidepressant- and anxiolytic-like actions of this organoselenium compound.

**Keywords** Selenium · Selenophene · Mice · Corticosterone · Stress · Anxiety · Depression · Serotonin · Glutamate

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## Introduction

Stress represents a major etiological factor in the development of emotional disorders, including depression and anxiety (Bosch et al. 2012; Cirulli et al. 2009; Danese and McEwen 2012). Physiological changes induced by stress comprise a cascade of neuroendocrine events mediated by stress systems such as the sympathetic nervous system and the hypothalamic–pituitary–adrenal (HPA) axis. Activation of the HPA axis results in the release of hypothalamic corticotropin-releasing hormone (CRH) that in turn releases pituitary adrenocorticotropin-releasing hormone (ACTH), which triggers the synthesis and secretion of adrenal glucocorticoids (cortisol in humans and corticosterone in rodents) into the circulatory system. Glucocorticoids released interact with their receptors in multiple target tissues, including the HPA axis, where they are responsible for a feedback inhibition on pituitary, hypothalamus, and extra-hypothalamic brain sites (Antoni 1993). Although stress is a necessary mechanism for survival, severe and/or long-term stress disrupts normal brain structure and function, increasing the risk for mental illness (Bosch et al. 2012; Cirulli et al. 2009;

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De Kloet et al. 1998). In fact, the relationship between the onset of psychiatric diseases and the HPA axis dysregulation and/or impaired glucocorticoid receptor negative feedback is already known (Bosch et al. 2012; De Kloet et al. 1998; Pariante and Lightman 2008). In rodents, chronic exposure to the stress hormone corticosterone is frequently used as an experimental model of emotional disease. The long-term corticosterone exposure alters anxiety and depression-associated behaviors and promotes neurochemistry and brain morphology changes, which have already been observed in humans (David et al. 2009; Gourley et al. 2008a, b, c; Murray et al. 2008). In addition, both behavioral and cerebral changes induced by corticosterone exposure seem to be normalized by antidepressant drugs currently used in the therapy of mood disorders (Gourley et al. 2008a, b, c; Olausson et al. 2013; Rainer et al. 2011).

Despite the improved tolerability of novel antidepressants, adverse events associated with their use can influence adherence rates. Therefore, there is an unmet need for a better understanding of the mechanisms and therapeutic management of depression. In this way, organoselenium compounds have emerged as new therapeutic approach for many disorders (Nogueira and Rocha 2011). We highlight selenophenes, a large class of heterocyclic aromatic compounds, which have pharmacological properties mainly related to central nervous system (Gai et al. 2012; Gay et al. 2010; Wilhelm et al. 2012a, b). In particular, 3-(4-fluorophenylselenyl)-2,5-diphenylselenophene (F-DPS) produces an antidepressant-like action in the mouse forced swimming test (FST) and tail suspension test (TST) that seems most likely to be mediated through and interaction with serotonergic system (Gay et al. 2010).

Thus, considering an already assessed model of corticosterone-induced anxiety and depression and the promising therapeutic properties of F-DPS, this study focused on the effect of this organoselenium compound on both the behavioral and neurochemical changes induced by long-term corticosterone exposure in mice. The aim of the study was to investigate whether F-DPS is effective in abolishing anxiogenic- and depressant-like actions of corticosterone as well as to observe its effects on serotonergic and glutamatergic neurotransmission systems in prefrontal cortex and hippocampus of mouse.

## Materials and methods

### Animals

The experiments were conducted using male Swiss mice (25–35 g) maintained at 22–25 °C with free access to corticosterone/vehicle solution and food, under a 12:12 h light/dark cycle, with lights on at 7:00 A.M. All manipulations were carried out between 08:00 A.M. and 04:00 P.M. and mice were acclimated to the behavioral room at least 2 h before the test. The experiments were performed according to a randomized schedule and each animal was used only once in each test. The animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal Resources of the Federal University of Santa Maria, Brazil (#124/2010). The procedures in this study were performed in accordance with the NIH Guide for the Care and Use of Laboratory Animals. All efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

### Drugs

3-(4-Fluorophenylselenyl)-2,5-diphenylselenophene (F-DPS, Fig. 1) was prepared and characterized in our laboratory according to a previously described method (Stein et al. 2008). 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 studied compound (99.9 %) was determined by gas chromatography–mass spectrometry. Corticosterone and paroxetine were purchased from Sigma-Aldrich (St. Louis, MO, USA). [$^{3}$H]5-Hydroxytryptamine (5-HT) creatinine sulfate (specific activity 23 Ci/mmol) and L-[$^{3}$H]glutamate (specific activity 50 Ci/mmol) were purchased from LabEx Inc. (Hinsdale, IL, USA). All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Corticosterone was dissolved in a minimum volume of ethanol (1 %) plus distilled water. F-DPS was dissolved in canola oil and given to mice in a constant volume of 10 ml/kg of body weight. Paroxetine was dissolved in saline plus 1 % DMSO (10 ml/kg).

Pilot experiments were previously conducted in order to select the appropriate corticosterone concentration as well as the treatment duration. F-DPS treatment was based on unpublished results from our research group in which a 7-day treatment produced a significant antidepressant-like action in a model of depression-like behavior induced by chronic pain.

### Experimental design

The experimental design of this study is depicted in Fig. 1. Mice were randomized into four groups ($n=12$ animals/group). The first group was treated with vehicle (canola oil 10 ml/kg/day during 1 week and distilled water plus ethanol 1 % during 4 weeks) and was considered as control. The second group was given an intragastric (i.g.) administration of F-DPS at a dose of 0.1 mg/kg body weight for 1 week and distilled water plus ethanol 1 % during 4 weeks. The third group was given corticosterone (20 μg/ml) available ad libitum in the drinking water in opaque bottles to protect it from light for 4 weeks and canola oil during 1 week. The fourth group was treated with corticosterone, as in the third group, for 4 weeks and then given a daily i.g. administration of F-DPS for 1 week.

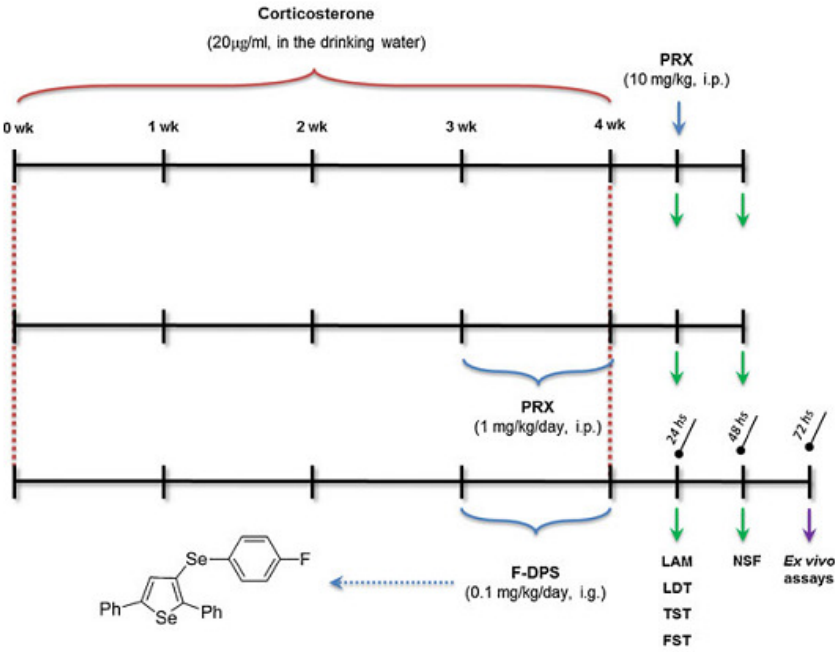


**Fig. 1** Schematic representation of the experimental design of this study. Corticosterone (20 μg/ml) was diluted in the drinking water and given to mice during 4 weeks. During the last week, mice received a daily i.g. or i.p. administration of F-DPS (0.1 mg/kg) or paroxetine (1 mg/kg), respectively. Paroxetine (10 mg/kg) was also administered acutely 24 h after the finishing of corticosterone exposure. Behavioral tests started at 24 h after the last dose of F-DPS or paroxetine (subchronic treatment) or at 2 h after paroxetine (acute treatment). Ex vivo analyses were performed at 72 h after the last F-DPS administration (*PRX* paroxetine, *LAM* locomotor activity monitor, *LDT* light–dark test, *TST* tail suspension test, *FST* forced swimming test, *NSF* novelty suppressed-feeding)

Drinking and food consumption and body weight were monitored during the treatment.

At the end of fourth week, corticosterone solution was replaced by distilled water. Behavioral assays ($n=12$) started 24 h after the last F-DPS administration. Novelty suppressed-feeding (NSF) was performed 48 h after the last F-DPS dose.

Twenty-four hours after NSF, mice were anaesthetized ($n=7$) and blood samples were collected by cardiac puncture. Blood was then centrifuged at 4,000×*g* for 10 min, and serum was collected and stored at −20 °C for the determination of serum adrenocorticotropic hormone (ACTH) and corticosterone levels.

A separate group of animals, which was not subjected to the behavioral tests, was killed by cervical dislocation, brains were removed, and prefrontal cortex and hippocampus were dissected for monoamine oxidase (MAO) activity ($n=10$), and [$^3$H]5-HT uptake ($n=5$) and [$^3$H]glutamate uptake ($n=5$) and release ($n=3$) assays.

In the first part of this study (behavioral evaluation), the classical antidepressant drug paroxetine was used as positive control. Animals that received vehicle (water plus 1 % ethanol) or corticosterone (20 μg/ml in the drinking water) were subchronically (1 mg/kg/day, i.p., during 1 week) or acutely (10 mg/kg, i.p.) treated with paroxetine ($n=9$ mice/group). At 24 h after the last injection of paroxetine (subchronic treatment), mice were evaluated in the locomotor activity monitor (LAM), light–dark test (LDT), tail suspension test (TST) and FST; NSF was performed 48 h after the end of treatment. Behavioral assessment of paroxetine acute treatment started at 2 h after the i.p. injection; NSF was performed 24 h after the acute administration (Fig. 1). Since saline i.p. injection did not modify behavior of animals when compared to oil administration, animals were combined into the same vehicle-treated group.

## Behavioral testing

### *Spontaneous locomotor activity*

The LAM is a clear acrylic plastic box (45×45×45 cm) with a removable plastic lid perforated with holes for ventilation. The monitor contains photocell beams and detectors that are mounted on opposite walls (2 cm above the chamber floor). General locomotor activity and the mouse's position in the chamber are detected by breaks of the photocell beams, which are recorded by a computer. Animals were placed in the center of the apparatus and allowed to freely explore the arena. Number of crossings and rearings, average velocity (mm/s), and total distance traveled (dm) were recorded for a 4-min period.

### *Light–dark test*

The LDT is a sensitive model to detect activity in disorders related to anxiety (Bourin and Hascoet 2003). The apparatus

was an acrylic box (46×27×30 cm) divided into light and dark chambers. The light chamber (27×27 cm) was painted white and was connected via an opening (7.5×7.5 cm) at floor level to the dark chamber (18×27 cm), which was painted black. A lamp with a 60-W white light was placed 40 cm above the light chamber. Mice were placed in the light chamber facing the opening into the dark chamber, and the following measures were recorded during a 5-min trial: latency to the first transition, number of zone transitions, and time spent in the light and dark compartments.

*Tail suspension test*

Briefly, animals both acoustically and visually isolated were suspended upside down by their tails with adhesive tape to a horizontal bar 30 cm above the table. The total duration of immobility induced by tail suspension was recorded during a 6-min trial measured according to a method previously reported (Steru et al. 1985). Animals were considered immobile only when they hung passively and were completely motionless.

*Forced swimming test*

The procedure was based on a previous study (Porsolt et al. 1979). In this test, mice were individually forced to swim in an open cylindrical container (10×25 cm), containing 19 cm of water at 25±1 °C. Each mouse was gently placed in the cylinder and the total duration of floating was recorded during a 6-min 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.

*Novelty suppressed-feeding*

The testing apparatus consisted of a square wooden arena (45×45×45 cm); the floor was covered with wooden bedding. The test was carried out during a 5-min period according to a previous study (Santarelli et al. 2003). Twenty-four hours before behavioral testing, food was removed from the home cage. At the time of testing, a single pellet of food (regular chow) was placed in the center of the box. An animal was placed in a corner of the box, and a stopwatch was immediately started. Latency to begin eating (defined as the mouse sitting on its haunches and biting the pellet with the use of forepaws) was used as an index of anxiety-like behavior. Then, the animal was transferred to its home cage, and the amount of food consumed by the mouse in 5 min was measured.

Ex vivo assays

*Determination of serum ACTH and corticosterone levels*

Hormone levels in the serum of mice were carried out using a radioimmunoassay test kit (Coat-A-Count; Diagnostic Products Corporation, USA) according to the manufacturer's instructions. Assay sensitivity was <1–50 pg/ml for corticosterone and 5–50 pg/ml for ACTH.

*Monoamine oxidase activity*

A preparation of mitochondria was used for MAO assay as previously described (Soto-Otero et al. 2001). Activity of MAO isoforms was determined in prefrontal cortex and hippocampus based on a previous study (Krajl 1965) with some modifications (Matsumoto et al. 1984). MAO-A and MAO-B activities were expressed as nanomoles of 4-hydroxyquinoline formed per milligram of protein per minute.

*Synaptosomal [$^3$H]5-HT uptake*

Crude synaptosomes from prefrontal cortices and hippocampi were obtained as previously described (Gray and Whittaker 1962). [$^3$H]5-HT uptake into synaptosomes was carried out as previously reported (Rocha et al. 2007). Results were expressed as picomoles of [$^3$H]5-HT uptake per milligram of protein per minute.

*Slice [$^3$H]glutamate uptake*

Glutamate uptake of prefrontal cortex and hippocampus slices was performed according to a previous study (Thomazi et al. 2004). Results were expressed as picomoles of [$^3$H]glutamate uptake per milligram of protein per minute.

*Synaptosomal [$^3$H]glutamate release*

A synaptosomal suspension from prefrontal cortices and hippocampi (Gray and Whittaker 1962) was washed three times in a non-depolarizing medium (low potassium), containing (in millimolar) HEPES 27, NaCl 133, KCl 2.4, $MgSO_4$ 1.2, $KH_2PO_4$ 1.2, glucose 12, and $CaCl_2$ 1.0, by centrifugation at 12,000×*g* for 15 min (at 4 °C). Determination of [$^3$H]glutamate release was accomplished based on a previous study (Migues et al. 1999).

*Protein determination*

Protein concentration was measured according to (Bradford 1976), using bovine serum albumin (1 mg/ml) as the standard. Sensitivity was 0.06–0.30 mg protein/ml.

### Statistical analysis

All experimental results are given as the mean±SEM. First, we evaluated the normality of data using the D'Agostino and Pearson omnibus normality test. Comparisons between experimental and control groups were performed by two-way analysis of variance (ANOVA) followed by Newman–Keuls test for post hoc comparison when appropriate. Main effects of first-order interactions are presented only when interaction was not significant. Pearson's correlation coefficient was used for correlation analysis between the total immobility time of mice in the FST and their serum corticosterone levels. All analyses were performed using STATISTICA for Windows software version 7 (StatSoft, Tulsa, OK, USA). Probability values less than 0.05 ($P<0.05$) were considered to be significant.

## Results

### Body weight and drinking and food consumption after corticosterone exposure and subchronic treatment with F-DPS

Body weight, drinking, and food consumption were neither altered by corticosterone exposure nor by F-DPS treatment (data not shown).

### F-DPS blocks anxiogenic- and depressant-like behavior induced by corticosterone

Two-way ANOVA revealed a significant main effect of paroxetine treatment on number of crossings [$F_{(2,54)}=17.15$, $P<0.001$] and rearings [$F_{(2,54)}=9.11$, $P<0.001$], velocity [$F_{(2,54)}=21.50$, $P<0.001$], and total distance traveled [$F_{(2,54)}=23.67$, $P<0.001$] in the LAM. Paroxetine acute treatment significantly increased number of crossings ($P<0.001$) and rearings ($P<0.05$), velocity ($P<0.05$), and total distance traveled ($P<0.01$) when compared to the control group. Neither corticosterone nor F-DPS changed spontaneous locomotor activity in mice (Table 1).

Figure 2 shows the latency to the first transition to the dark zone and the total time spent in the light compartment in the LDT. The two-way ANOVA showed a significant main effect of corticosterone exposure [$F_{(1,44)}=18.49$, $P<0.001$] and paroxetine treatment [$F_{(2,54)}=12.31$, $P<0.001$] on latency to the first transition. Corticosterone decreased latency in the LDT (63.2 %) while subchronic treatment with paroxetine increased this parameter when compared to the control group (193.8 %) (Fig. 2a). Statistical analysis of the total time spent in the light compartment yielded significant corticosterone×F-DPS [$F_{(1,44)}=5.29$, $P<0.05$] and corticosterone×paroxetine [$F_{(2,54)}=6.70$, $P<0.01$] interactions. Post hoc comparisons demonstrated that F-DPS and paroxetine treatments were effective to avoid the decrease in the time spent in the light compartment induced by corticosterone exposure (45.8 %, $P<0.05$, Fig. 2b). The two-way ANOVA of number of transitions between dark and light compartments and total time spent in the dark compartment in the LDT revealed no significant differences (Table 2).

The effects of long-term corticosterone exposure and F-DPS or paroxetine treatment on the total immobility time in the TST and FST are shown in Fig. 3. The two-way ANOVA of TST [$F_{(1,44)}=9.62$, $P<0.01$] and FST [$F_{(1,44)}=7.61$, $P<0.01$] data showed a significant corticosterone×F-DPS interaction. In a similar way to F-DPS, a significant corticosterone×paroxetine interaction was also yielded in the TST [$F_{(2,54)}=14.89$, $P<0.001$] and FST [$F_{(2,54)}=5.69$, $P<0.01$]. Corticosterone treatment significantly increased immobility time in the TST (160.4 %, $P<0.01$) and FST (154.7 %, $P<0.001$) when compared to the control group. F-DPS and paroxetine treatments protected against the increase on immobility time caused by corticosterone in both TST (Fig. 3a) and FST (Fig. 3b).

**Table 1** Locomotor parameters of mice evaluated in the activity monitor after a long-term exposure to corticosterone and F-DPS or paroxetine treatments

| | Number of crossings | Number of rearings | Velocity (mm/s) | Distance (dm) |
|---|---|---|---|---|
| Vehicle | 303.1±23.0 | 12.0±1.8 | 24.1±1.8 | 55.3±1.2 |
| F-DPS | 296.3±40.9 | 12.4±1.5 | 25.4±3.6 | 53.8±6.0 |
| Prx (subchronic) | 235.6±35.98 | 9.5±1.2 | 29.6±6.5 | 49.7±6.8 |
| Prx (acute) | 582.9±59.69*** | 16.9±2.1** | 75.9±14.2*** | 108.2±12.4** |
| Corticosterone | 325.7±37.8 | 11.9±1.5 | 23.2±2.8 | 53.5±6.8 |
| Cort+F-DPS | 318.3±20.4 | 11.3±1.8 | 24.9±2.0 | 55.1±3.9 |
| Cort+Prx (subchronic) | 366.1±56.16 | 11.6±1.2 | 37.9±3.9 | 82.5±6.4 |
| Cort+Prx (acute) | 608.7±52.68*** | 17.6±2.3** | 53.7±3.0* | 130.5±19.6*** |

Values are expressed as mean±SEM of 9–12 animals/group. Asterisks denote the significance levels when compared to the vehicle group: *$P<0.05$, **$P<0.01$, ***$P<0.001$

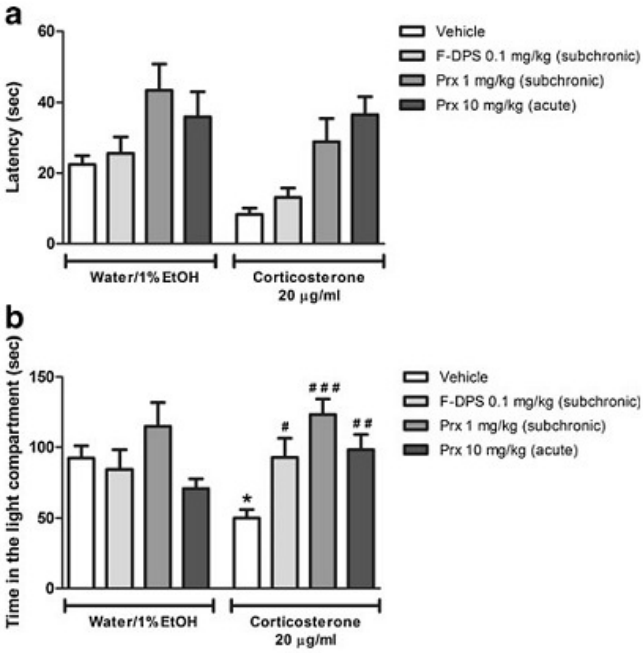


**Fig. 2** Effect of treatment with F-DPS or paroxetine on anxiety-like behavior in the LDT induced by long-term exposure of mice to corticosterone (20 μg/ml, during 4 weeks). (**a**) Latency to the first enter in the dark compartment and (**b**) time spent in the light compartment. F-DPS was administered at the dose of 0.1 mg/kg, i.g., during 1 week (subchronic treatment). Paroxetine was administered at the dose of 1 mg/kg, i.p., during 1 week (subchronic treatment) or 10 mg/kg, i.p. (single injection, acute treatment). LDT was performed 24 h after the last injection of F-DPS or paroxetine (subchronic treatment) or 2 h after the acute treatment with paroxetine. Values are expressed as mean±SEM of 9–12 animals. *Asterisk* denotes the significance level when compared to the vehicle group: $*P<0.05$. *Hashtags* denote the significance levels when compared to the corticosterone-exposed group: $\#P<0.05$, $\#\#P<0.01$, and $\#\#\#P<0.001$

The two-way ANOVA of NSF data demonstrated significant corticosterone×F-DPS [$F_{(1,44)}=10.12$, $P<0.01$] and corticosterone×paroxetine [$F_{(1,54)}=4.03$, $P<0.05$] interactions.

**Table 2** Food consumption in the NSF and parameters of anxiolytic-like behavior in the LDT in mice after a long-term exposure to corticosterone and F-DPS or paroxetine treatments

| | LDT | | NSF |
|---|---|---|---|
| | Time in the dark compartment (s) | Number of transitions | Food consumption (mg) |
| Vehicle | 207.7±8.7 | 10.3±0.8 | 227.8±21.9 |
| F-DPS | 217.1±13.7 | 10.4±1.3 | 235.4±25.7 |
| Prx (subchronic) | 185.2±16.9 | 9.1±1.5 | 208.3±66.5 |
| Prx (acute) | 229.0±6.9 | 9.7±1.6 | 236.8±32.8 |
| Corticosterone | 239.9±10.6 | 9.6±1.6 | 219.8±22.5 |
| F-DPS+cortic | 207.2±13.6 | 10.7±1.4 | 213.7±12.4 |
| Cort+Prx (subchronic) | 176.7±11.0 | 12.9±1.2 | 234.6±22.6 |
| Cort+Prx (acute) | 201.7±10.1 | 11.1±1.2 | 225.8±20.0 |

Values are expressed as mean±SEM of 9–12 animals/group

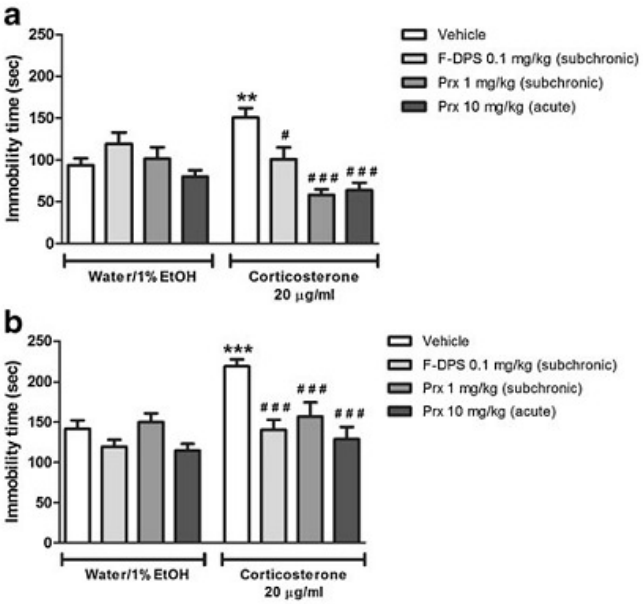


**Fig. 3** Effect of treatment with F-DPS or paroxetine on depressant-like behavior in the TST (**a**) and the FST (**b**) induced by long-term exposure of mice to corticosterone (20 μg/ml, during 4 weeks). F-DPS was administered at the dose of 0.1 mg/kg, i.g., during 1 week (subchronic treatment). Paroxetine was administered at the dose of 1 mg/kg, i.p., during 1 week (subchronic treatment) or 10 mg/kg, i.p. (single injection, acute treatment). Behavioral tests were performed 24 h after the last injection of F-DPS or paroxetine (subchronic treatment) or 2 h after the acute treatment with paroxetine. Values are expressed as mean±SEM of 9–12 animals. *Asterisks* denote the significance levels when compared to the vehicle group: $**P<0.01$ and $***P<0.001$. *Hashtags* denote the significance levels when compared to the corticosterone-exposed group: $\#P<0.05$ and $\#\#\#P<0.001$

Post hoc comparisons showed that corticosterone exposure significantly increased the latency to begin eating (201.3 %, $P<0.01$). F-DPS ($P<0.01$) and acute paroxetine ($P<0.05$) treatments protected against the increase caused by corticosterone (Fig. 4). Paroxetine subchronic treatment was not effective ($P>0.05$). Food consumption in the NSF did not change significantly among different groups (Table 2).

## Treatment with F-DPS normalizes stress-related hormone levels in the serum of mice exposed to corticosterone

Serum ACTH and corticosterone levels after corticosterone and F-DPS treatments are depicted in Fig. 5. The two-way ANOVA of ACTH levels showed a significant corticosterone×F-DPS interaction [$F_{(1,24)}=293.03$, $P<0.001$]. Figure 5a shows that exposure to corticosterone resulted in a significant decrease in the serum ACTH levels when compared with control (47.1 %, $P<0.001$) and F-DPS treatment was effective in normalizing these levels ($P<0.001$). In addition, F-DPS subchronic treatment reduced significantly the ACTH levels in control mice (21.6 %, $P<0.01$).

Regarding corticosterone levels, two-way ANOVA showed a significant main effect of corticosterone exposure

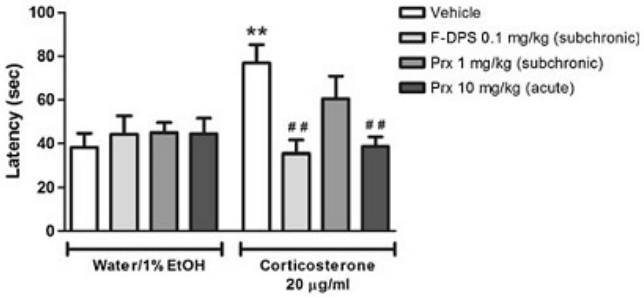


**Fig. 4** Effect of treatment with F-DPS or paroxetine on anxiolytic-like behavior in the NSF induced by long-term exposure of mice to corticosterone (20 µg/ml, during 4 weeks). F-DPS was administered at the dose of 0.1 mg/kg, i.g., during 1 week (subchronic treatment). Paroxetine was administered at the dose of 1 mg/kg, i.p., during 1 week (subchronic treatment) or 10 mg/kg, i.p. (single injection, acute treatment). NSF was performed 24 h after the last injection of F-DPS or paroxetine (subchronic treatment) or 2 h after the acute treatment with paroxetine. Values are expressed as mean±SEM of 9–12 animals. *Asterisks* denote the significance levels when compared to the vehicle group: $^{**}P<0.01$. *Hashtags* denote the significance levels when compared to the corticosterone-exposed group: $\#\#P<0.01$

$[F_{(1,24)}=56.50,\ P<0.001]$ and F-DPS treatment $[F_{(1,24)}=85.43,\ P<0.001]$ (Fig. 5b). Mice exposed to long-term corticosterone administration showed high levels of this hormone in the serum (121.9 %). Further, subchronic treatment with F-DPS reduced serum corticosterone levels of mice when compared to the vehicle-treated group (21.6 %) (Fig. 5b).

Moreover, correlation analysis (Pearson's correlation coefficient) revealed a positive correlation between total immobility time in the FST and serum corticosterone levels ($r=0.5743$, $P<0.01$, Fig. 6). Corticosterone levels also correlates positively with the total immobility time of mice in the TST ($r=0.4517$, $P<0.05$, data not shown).

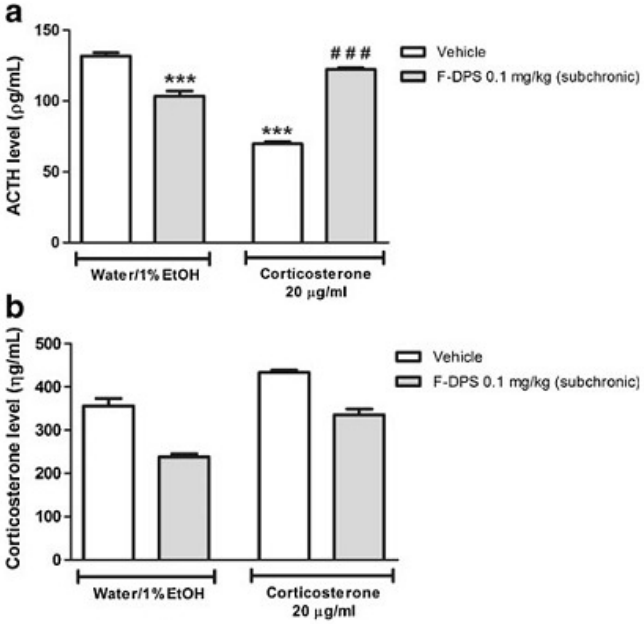


**Fig. 5** Effect of subchronic treatment with F-DPS (0.1 mg/kg, i.g., during 1 week) on serum ACTH (**a**) and corticosterone (**b**) levels after a long-term exposure of mice to corticosterone (20 µg/ml, during 4 weeks). Values are expressed as mean±SEM of seven animals. *Asterisks* denote the significance level when compared to the vehicle group: $^{***}P<0.001$. *Hashtags* denote the significance level when compared to the corticosterone-exposed group: $\#\#\#P<0.001$

## F-DPS treatment inhibits MAO-A activity from hippocampus of mice

MAO-A and MAO-B activities in the frontal cortex and hippocampus of mice after corticosterone and F-DPS treatments are shown in Table 3. Statistical analysis revealed a significant main effect of F-DPS treatment in hippocampal MAO-A activity $[F_{(1,36)}=17.88,\ P<0.001]$. F-DPS was effective in inhibiting MAO-A activity in both vehicle- and corticosterone-treated groups (25.9 % and 23.8 %, respectively, $P<0.05$). The two-way ANOVA of prefrontal cortex MAO-A activity revealed no significant differences $[F_{(1,36)}=0.5613,\ P>0.05]$. MAO-B activity was also not changed by treatments $[F_{(1,36)}=3.09,\ P>0.05$ for prefrontal cortex and $F_{(1,36)}=0.4730,\ P>0.05$ for hippocampus].

## Synaptosomal 5-HT uptake from prefrontal cortex and hippocampus is inhibited by subchronic F-DPS administration

The statistical analysis revealed that corticosterone exposure did not have significant effect on synaptosomal 5-HT uptake from prefrontal cortex and hippocampus of mice $[F_{(1,16)}=0.2606,\ P>0.05$ and $F_{(1,16)}=0.1660,\ P>0.05$, respectively]. By contrast, F-DPS treatment produced a significant main effect on both prefrontal cortex $[F_{(1,16)}=19.58,\ P<0.001]$ and hippocampal $[F_{(1,16)}=20.10,\ P<0.001]$ 5-HT uptake. Post hoc comparison means showed that treatment of mice with F-DPS during 1 week inhibited significantly $[^3H]$5-HT uptake in prefrontal cortex (27.9 %, $P<0.05$, Fig. 7a) and hippocampus (20.4 %, $P<0.05$, Fig. 7b) when compared to the control group.

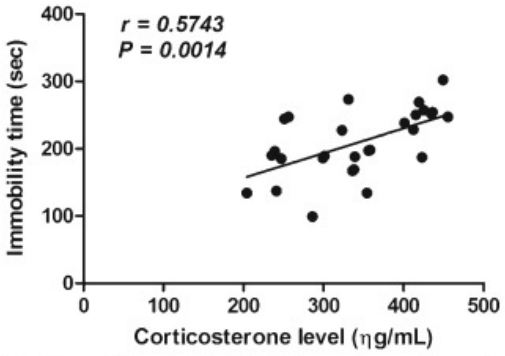


**Fig. 6** Total immobility time in the FST correlates with corticosterone levels in the serum of mice. Data are individual values for each mice of all groups ($n=7$)

**Table 3** MAO-A and MAO-B activities (nmol 4-hydroxyquinoline formed/mg protein $min^{-1}$) in prefrontal cortex and hippocampus from mice subjected to a long-term exposure to corticosterone and subchronically treated with F-DPS

| | MAO-A | | MAO-B | |
|---|---|---|---|---|
| | Prefrontal cortex | Hippocampus | Prefrontal cortex | Hippocampus |
| Vehicle | 25.0±1.9 | 16.86±0.4 | 124.8±6.2 | 29.7±2.6 |
| F-DPS | 23.3±2.1 | 12.5±1.4* | 106.2±10.5 | 33.9±1.7 |
| Corticosterone | 27.1±1.5 | 16.8±0.8 | 118.8±7.5 | 27.3±2.0 |
| Cortic+F-DPS | 22.6±1.9 | 12.8±1.0* | 123.5±9.5 | 28.2±3.2 |

The results are expressed as mean±SEM of 10 animals/group. Asterisks denote the significance levels when compared to the control group: *$P<0.05$

### Subchronic F-DPS treatment reverses the inhibition of glutamate uptake from prefrontal cortex slices induced by corticosterone

The two-way ANOVA of glutamate uptake data from prefrontal cortex demonstrated a significant corticosterone and F-DPS interaction [$F_{(1,16)}=5.40$, $P<0.05$]. Post hoc analysis showed that corticosterone exposure inhibited [$^3$H]glutamate uptake in prefrontal cortex (55.3 %, $P<0.001$). Furthermore, subchronic F-DPS treatment protected against the inhibition produced by corticosterone ($P<0.01$ when compared to the corticosterone-treated group) (Fig. 8a). Treatments did not affect glutamate uptake from hippocampus of mice [$F_{(1,16)}=0.2237$, $P>0.05$] (Fig. 8b).

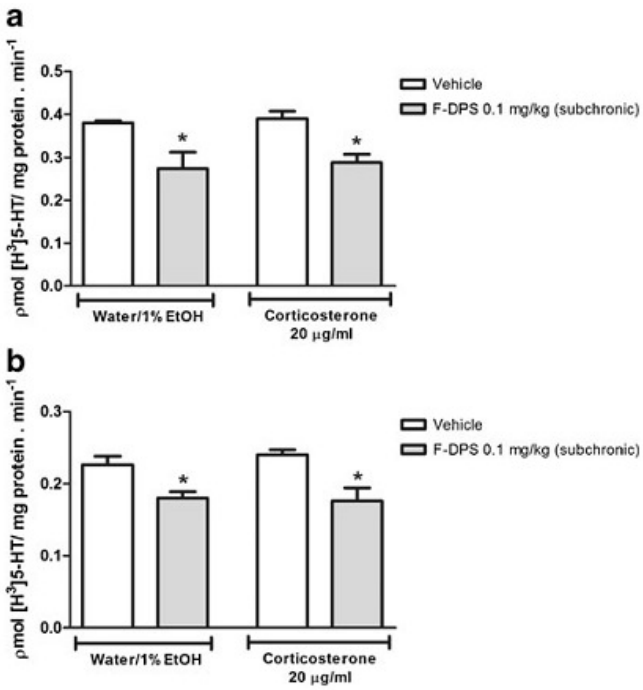


**Fig. 7** Effect of subchronic treatment with F-DPS (0.1 mg/kg, i.g., during 1 week) on synaptosomal [$^3$H]5-HT uptake in prefrontal cortex (**a**) and hippocampus (**b**) from mice after a long-term exposure to corticosterone (20 µg/ml, during 4 weeks). Values are expressed as mean±SEM of five animals. *Asterisks* denote the significance level when compared to the vehicle group: *$P<0.05$

### F-DPS treatment and corticosterone exposure do not alter glutamate release from mice synaptosomes

Basal and evoked glutamate release from prefrontal cortex and hippocampus of mice after corticosterone and F-DPS treatments are shown in Table 4. The two-way ANOVA of basal glutamate release data for prefrontal cortex [$F_{(1,8)}=3.92$, $P>0.05$] and hippocampus [$F_{(1,8)}=0.0621$, $P>0.05$] revealed no significant differences. Evoked glutamate release was not affected by treatments [$F_{(1,8)}=0.0450$, $P>0.05$, for prefrontal cortex and $F_{(1,8)}=0.3051$, $P>0.05$, for hippocampus].

## Discussion

The aim of the present study was to evaluate the effect of subchronic treatment with F-DPS on behavioral and

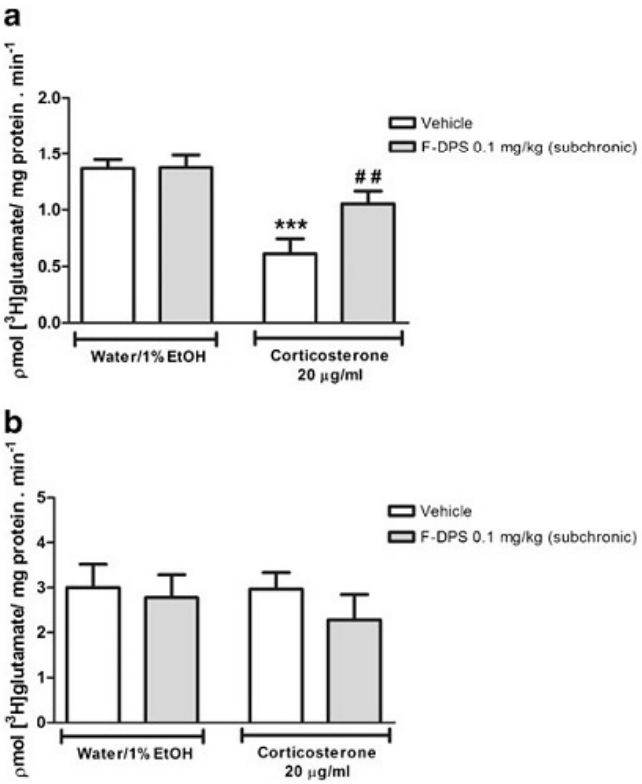


**Fig. 8** Effect of subchronic treatment with F-DPS (0.1 mg/kg, i.g., during 1 week) on slice [$^3$H]glutamate uptake in prefrontal cortex (**a**) and hippocampus (**b**) from mice subject to a long-term exposure to corticosterone (20 µg/ml, during 4 weeks). Values are expressed as mean±SEM of five animals. *Asterisks* denote the significance level when compared to the vehicle group: ***$P<0.001$. *Hashtags* denote the significance level when compared to the corticosterone-exposed group: ##$P<0.01$

**Table 4** Basal and evoked glutamate release in prefrontal cortex and hippocampus from mice subjected to a long-term exposure to corticosterone and subchronically treated with F-DPS

| | Basal glutamate release (%) | | Evoked glutamate release (%) | |
|---|---|---|---|---|
| | Prefrontal cortex | Hippocampus | Prefrontal cortex | Hippocampus |
| Vehicle | 22.9±0.7 | 21.6±3.7 | 39.5±2.9 | 36.4±2.6 |
| F-DPS | 19.6±2.5 | 24.3±4.4 | 37.1±1.4 | 36.8±4.5 |
| Corticosterone | 20.5±1.9 | 22.8±2.1 | 35.5±1.6 | 37.7±3.3 |
| Cortic+F-DPS | 24.2±1.3 | 23.6±4.5 | 33.9±0.3 | 32.8±7.4 |

The results are expressed as mean±SEM of 3 animals/group

neurochemical consequences induced by a long-term corticosterone exposure in mice. Our main findings were that, similar to the classical antidepressant drug paroxetine, F-DPS suppressed anxiogenic- and depressant-like behaviors induced by corticosterone in the LDT, NSF, TST, and FST, without any change in the behavioral performance of animals when evaluated in the LAM. In addition, subchronic F-DPS treatment reduced synaptosomal 5-HT uptake from prefrontal cortex and hippocampus, and inhibited the MAO-A activity in the hippocampus of mice. Furthermore, F-DPS treatment reversed the glutamate uptake inhibition in prefrontal cortex after corticosterone exposure. These results suggest that the mechanisms behind anxiolytic- and antidepressant-like actions of F-DPS may involve the modulation of both monoaminergic and glutamatergic systems.

The long-term exposure to corticosterone, originally designed by Gourley et al. (2008a), b), was used as a model to induce anxiogenic- and depressant-like symptoms as is often reported in stress-precipitated major depression (Cryan and Holmes 2005). In the present study, the anxiogenic-like action induced by corticosterone in mice was evaluated using LDT and NSF tasks. At the end of the fourth week, mice exposed to corticosterone showed anxiety-related behavior elicited by decrease in the latency for the first transition as well as in the total time spent in the light compartment in the LDT. Latency for biting the pellet of food in the NSF was increased by corticosterone treatment, which also suggests an anxious behavior. Interestingly, although F-DPS subchronic treatment (0.1 mg/kg, i.g., during 1 week) did not produce any anxiolytic-like action in control mice, it protected against behavioral changes in the LDT and NSF induced by corticosterone, demonstrating for the first time the anxiolytic-like action of this organoselenium compound in mice. Moreover, our results showed that subchronic and acute treatments with the classical antidepressant drug paroxetine, which is a well-recognized anxiolytic drug (Andrisano et al. 2013), were also effective in blocking the anxiety behavior induced by corticosterone.

Regarding depression-like behavior, FST and TST, two well-recognized models of antidepressant efficacy, were used to demonstrate the antidepressant-like action of F-DPS in corticosterone-exposed mice. Consistent with previous findings, corticosterone-treated group presented high total immobility time in both tasks (Ago et al. 2013; Gourley et al. 2008a, b; Murray et al. 2008). In fact, we demonstrated a positive correlation between serum corticosterone levels and depressant-like behavior, i.e., mice with higher concentrations of this hormone in the serum showed higher immobility time in the FST and TST. We also demonstrated the efficacy of both acute and subchronic paroxetine treatments in reversing the depressant-like behavior induced by corticosterone. In addition, our results showed that subchronic F-DPS treatment reduced total immobility time in the FST and TST in mice exposed to corticosterone. It is worthy to note that even though the subchronic administration of F-DPS and paroxetine in naive mice was ineffective, they produced pharmacological effect on corticosterone-exposed mice. These results suggest that treatments with these drugs are more effective when the depression- and anxious-like behaviors are induced by using a pharmacological tool.

The antidepressant-like action produced by F-DPS administration has already been shown by our research group (Gai et al. 2012; Gay et al. 2010). This action has been associated to an interaction with serotonergic system since the acute anti-immobility effect of this selenophene compound (at a dose of 50 mg/kg, intragastrically administered) was blocked by the administration of 5-$HT_{1A}$, 5-$HT_{2A/2C}$, and 5-$HT_3$ receptor antagonists (Gay et al. 2010). Thus, in spite of differences between doses and treatment duration, we suggest that serotonergic receptors could be involved in the antidepressant-like property of F-DPS in mice subjected to corticosterone exposure. Further, since some serotonergic drugs are commonly used in the anxiety therapy (Bandelow et al. 2008), we can speculate that its anxiolytic-like action might also be due to a modulation of 5-HT receptors.

Besides behavioral changes, corticosterone exposure also induced a decrease in serum ACTH levels. This decrease occurs in response to the high serum levels of corticosterone, which lead to glucocorticoid receptors activation starting a negative feedback mechanism in order to down-regulate HPA axis and decrease corticosterone release (Antoni 1993; Gourley et al. 2008a). In regard to this, F-DPS treatment in animals exposed to corticosterone regularized functional status of HPA axis by normalizing the stress-related hormone levels to the levels of those in the control group. In fact, it has been reported that therapeutic actions of antidepressants are produced through the intermediacy of HPA axis in depressive patients and the restoration of a normal status of HPA axis may be critically involved in the therapeutic intervention of mood disorders (Bosch et al. 2012; Himmerich et al. 2007; O'Dwyer et al. 1995; Pan et al. 2006; Schule et al. 2006). Moreover, the successful management of post-traumatic stress disorders with selective serotonin reuptake inhibitors (SSRIs)

has increased attention to the role of 5-HT in the neurobiology and treatment of stress-related diseases. The actions of these drugs at $5HT_{1A}$ and $5HT_{2A}$ receptors in critical limbic regions appear central to their anxiolytic and antidepressant actions, playing a key role in influencing HPA-axis activity (Harvey 1997; Millan 2006; Stein et al. 2000). Thus, based on the present findings and previous studies concerning the role of 5-HT receptors in F-DPS effects, we can suggest that modulation of HPA axis is, at least in part, involved in the anxiolytic- and antidepressant-like actions of F-DPS in mice exposed to corticosterone. Interestingly, subchronic F-DPS treatment induced a HPA axis down-regulation elicited by decreasing on both serum ACTH and corticosterone levels that were not accompanied by behavioral changes. This result demonstrates that F-DPS not only dampen HPA axis changes but also affected it directly. Taken together, these behavioral and endocrine changes provide a backdrop for analyzing the neurochemical changes following corticosterone exposure and F-DPS treatment.

Over the past decades, the studies concerning psychiatric disorders have focused on brain monoaminergic systems, and SSRIs have been commonly used in the therapy of mood disorders (Bandelow et al. 2008; Bosch et al. 2012; Millan 2006). In the post-traumatic stress disorder, the effect of SSRI is associated with a reduction in stress-induced HPA axis hyperactivity (Bandelow et al. 2008), suggesting an interaction between serotonergic system and HPA axis regulation. According with this, our findings showed that animals subjected to 1 week of F-DPS treatment had a significant decrease in synaptosomal 5-HT uptake either in naive or corticosterone-exposed mice. In addition, F-DPS treatment was also effective in inhibiting hippocampal MAO-A activity. It is widely known that 5-HT uptake process and MAO-A are two well-described mechanisms responsible for regulating 5-HT levels at synaptic cleft, and they are involved on the action mechanism of the most antidepressant drugs (Millan 2006). Further, these results are in agreement with the F-DPS reduction in ACTH and corticosterone levels observed in this study and suggest a serotonergic system×HPA axis interaction after subchronic treatment with this organoselenium compound. The serotonergic modulation and HPA axis down-regulation are not enough for inducing an antidepressant-like action in the naive mice, although it can represent an important mechanism of endocrine normalization when the animals are exposed to corticosterone.

In addition to the serotonergic transmission, many findings have shown that increased excitatory amino acid transmission in areas of the forebrain can also be associated with stress response (Bagley and Moghaddam 1997; Lowy et al. 1995; Reznikov et al. 2007). Behavioral changes induced by corticosterone (after stress or endogenous exposure) are generally attributed to its interaction with glucocorticoid receptors, which increases glutamate levels in different cerebral areas (de Kloet et al. 2008; Groeneweg et al. 2012; Karst et al. 2005). Banasr et al. (2010) reported a decrease on glutamate turnover in the prefrontal cortex of rats exposed to chronic unpredictable stress, and a previous study have also shown that stress up-regulates depolarization-evoked exocytotic glutamate release by increasing the circulating levels of corticosterone, stimulating glucocorticoid receptors and facilitating neurotransmitter release from presynaptic membranes (Tardito et al. 2010). Here, even though corticosterone exposure did not affect glutamate release, glutamate uptake in prefrontal cortex of mice was significantly inhibited, demonstrating a clear disrupt on glutamate neurotransmission. Accordingly, a previous study from Gourley and colleagues has already demonstrated that chronic corticosterone exposure diminishes the glial glutamate transporter 1 (GLT-1) expression in prefrontal cortex of mice (Gourley et al. 2012b). Furthermore, treatment of animals with antidepressant drugs seems to decrease glutamate levels in the synaptic cleft after treatment with anxiogenic drugs or stress protocols (Bagley and Moghaddam 1997; Gourley et al. 2012a; Lowy et al. 1995; Reznikov et al. 2007). In addition, both basic research and clinical studies indicate that modulation of glutamate metabolism, uptake, and/or release represents viable targets for antidepressant drugs (Ago et al. 2013; Autry et al. 2011; Banasr et al. 2010; Gourley et al. 2012a; Li et al. 2010; Paul and Skolnick 2003). In fact, clinical studies consistently demonstrate that the administration of ketamine, a glutamatergic NMDA receptor antagonist, produces antidepressant responses in patients suffering from depression (Berman et al. 2000; Zarate et al. 2006). Also, the glutamate-modulating drug riluzole has been shown antidepressant-like action in different depression models in rodents, including the corticosterone model (Banasr et al. 2010; Gourley et al. 2012a). Interestingly, subchronic F-DPS treatment was effective to prevent inhibition in glutamate uptake induced by corticosterone treatment without any effect in vehicle-treated mice. This result indicates a possible modulation of glutamate neurotransmission after chronic treatment with F-DPS in animals exposed to corticosterone. Moreover, given that F-DPS normalizes HPA axis, reducing circulating serum glucocorticoid hormones and glucocorticoid receptors activation, and since activation of these receptors is strongly linked to glutamate neurotransmission, it can constitute a link between HPA axis and glutamate uptake normalization after F-DPS treatment.

## Conclusion

In conclusion, F-DPS produced antidepressant- and anxiolytic-like actions in mice exposed to corticosterone, accompanied by down-regulation of HPA axis, stimulation of serotonergic and normalization of glutamatergic neurotransmission. Thus, F-DPS may represent a potential therapeutic

agent in the treatment of stress-related disorders including depression and anxiety. Nevertheless, further studies are needed to clarify the exact F-DPS mechanism of action and the contribution of other neurotransmission systems to its antidepressant- and anxiolytic-like properties.

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**Conflict of interest** The authors declare no conflicts of interest in the present study.

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# DISCUSSÃO

Os selenofenos são uma importante classe de compostos heterocíclicos contendo selênio. São intermediários muito utilizados em química orgânica, consistindo uma ferramenta sintética bastante útil (Rhoden and Zeni, 2011). Além disso, apresentam importantes atividades biológicas, dentre as quais se destacam as propriedades antimicrobianas (Abdel-Hafez, 2008), antitumorais (Juang et al., 2007; Shiah et al., 2007; Xiao and Parkin, 2006), anticonvulsivantes (Wilhelm et al., 2012a; Wilhelm et al., 2009a; Wilhelm et al., 2012b) e do tipo antidepressivas (Gai et al., 2012).

Em um trabalho recentemente publicado, foi demonstrada pela primeira vez a ação do tipo antidepressiva de vários compostos pertences a esta classe (Gai et al., 2012). Este estudo consistiu no tratamento de camundongos com uma única dose de diferentes 3-(organosseleno)-2,5-difenil-selenofenos e posterior avaliação dos animais no teste do nado forçado (TNF) após um intervalo de 30 minutos entre a administração das drogas e o teste. Embora nem todos os compostos tenham sido efetivos, o estudo da ação farmacológica das moléculas **H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** e **$CF_3$-DPS** (Figura 6) pareceu ser promissor. Observou-se uma estreita relação entre a estrutura molecular destes selenofenos e sua ação do tipo antidepressiva no TNF. Além do mais, a molécula **F-DPS** destacou-se por apresentar uma efetividade comparável ao efeito produzido pela droga antidepressiva clássica paroxetina. O efeito apresentado pelo **F-DPS** foi atribuído, em parte, à presença do grupamento fluorofenila, que é comum a fármacos amplamente utilizados na terapia da depressão como os inibidores seletivos da recaptação de serotonina (ISRSs) paroxetina, citalopram e escitalopram. De fato, um estudo prévio já havia demonstrado a ação do tipo antidepressiva do **F-DPS** em camundongos, que envolve uma modulação do sistema serotoninérgico, incluindo a inibição no processo de recaptação de serotonina. No entanto, como a avaliação dos selenofenos foi realizada levando em consideração apenas uma dose e um tempo de pré-tratamento, não estava claro se diferentes propriedades farmacocinéticas poderiam influenciar ou não os efeitos observados no TNF. Além disso, como o estudo constituiu-se de uma mera seleção para a avaliação da ação do tipo antidepressiva dos 3-(organosseleno)-2,5-difenil-selenofenos, a interferência de efeitos locomotores, os quais poderiam influenciar os resultados do TNF, não foi estudada (Gai et al., 2012).

Desse modo, com o objetivo de continuar os estudos acerca das propriedades farmacológicas de 3-(organosseleno)-2,5-difenil-selenofenos, os trabalhos que fazem parte

desta tese de doutorado foram realizados. Primeiramente, na parte inicial deste estudo, avaliou-se o perfil farmacológico da ação do tipo antidepressiva dos cinco selenofenos mais promissores **H-DPS**, **$CH_3$-DPS**, **Cl-DPS**, **F-DPS** e **$CF_3$-DPS** em camundongos (Manuscrito 1). Os compostos foram administrados nas doses de 10, 25 e 50 mg/kg e os tempos de pré-tratamento variaram de 15 a 240 minutos. A investigação da ação do tipo antidepressiva foi realizada pelo uso no TNF enquanto que os parâmetros locomotores e exploratórios foram acompanhados pelo uso de um monitor de atividades.

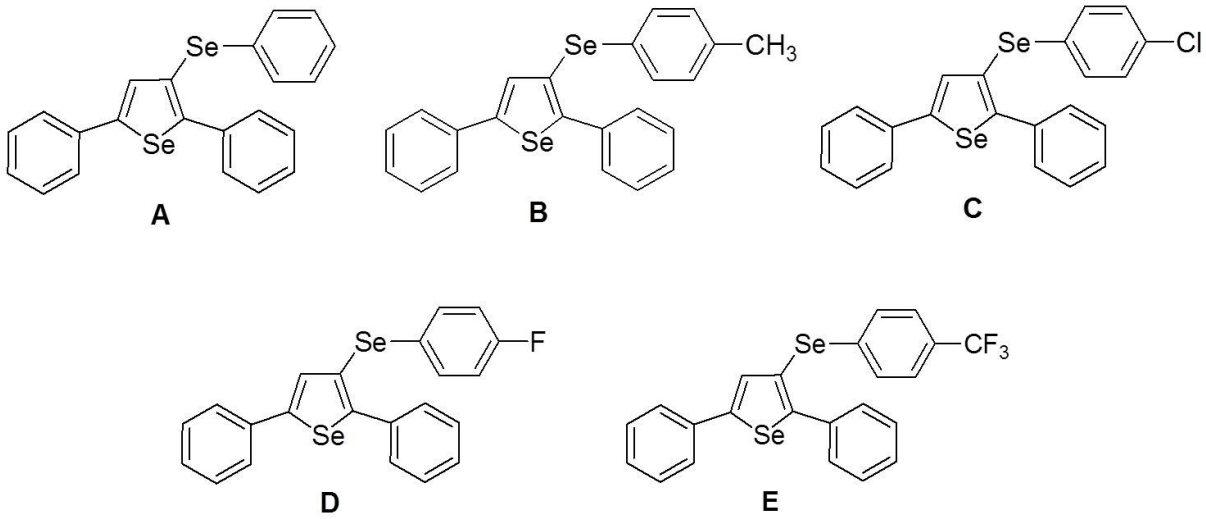


**Figura 6.** Estrutura química dos 3-(organosseleno)-2,5-difenil-selenofenos **H-DPS** (**A**), **$CH_3$-DPS** (**B**), **Cl-DPS** (**C**), **F-DPS** (**D**) e **$CF_3$-DPS** (**E**).

Os resultados demonstraram que todos os compostos foram efetivos em reduzir o tempo total de imobilidade dos camundongos no TNF, sem causar qualquer alteração na atividade locomotora e exploratória. Estes dados confirmam a ação do tipo antidepressiva destes selenofenos, que não parece ser devido a um efeito psicoestimulante. Por outro lado, o perfil farmacológico apresentado por estes selenofenos foi distinto, de modo que a ação antidepressiva foi observada em tempos de pré-administração e doses que foram diferentes para cada composto. Enquanto o composto **Cl-DPS** apresentou uma ação mais prolongada, que se manteve até 180 minutos após a administração, o efeito anti-imobilidade do **F-DPS** foi observado em menores doses, embora somente no tempo de 30 minutos. Estes resultados confirmam, assim, uma relação entre estrutura e atividade farmacológica dos 3-(organosseleno)-2,5-difenil-selenofenos testados neste trabalho. Sabe-se que a halogenação de moléculas é uma alternativa bastante usada no desenvolvimento de fármacos com características farmacocinéticas previamente desfavoráveis; a presença de um átomo de cloro

ou flúor em posições específicas da molécula pode, por exemplo, alterar suas propriedades físico-químicas, como acidez, basicidade e lipofilicidade, podendo reduzir seu metabolismo, aumentando sua meia vida no organismo (Banks, 2000; Silverman, 2004). No caso dos selenofenos testados neste estudo, parece evidente que a inserção do grupo clorofenil em **Cl-DPS** tenha prolongado seu tempo de ação do tipo antidepressiva observado no TNF em comparação aos selenofenos **H-DPS** e **$CH_3$-DPS**, o que confirma a hipótese de que a halogenação poderia ampliar o tempo de meia-vida destas moléculas. Tal modificação, no entanto, reduziu a eficácia antidepressiva da molécula. Quando a halogenação foi realizada pela inserção de átomos de flúor (**F-DPS**), embora um prolongamento do efeito anti-imobilidade não tenha sido observado, a eficácia deste composto foi maior e sua ação do tipo antidepressiva ocorreu em doses menores quando comparado aos outros selenofenos. De fato sabe-se que a presença da porção fluorofenil na estrutura química dos ISRSs está intimamente relacionada à potência com que estes fármacos inibem a proteína pré-sináptica que faz o transporte de serotonina (Banks, 2000). É provável que futuras modificações na estrutura destes selenofenos sejam capazes de conferir um tempo de ação mais prolongado, como o observado para o **Cl-DPS**, e uma boa eficácia, como a produzida pelo **F-DPS**. Desse modo, tendo em vista a ação mais potente do **F-DPS** em comparação aos outros selenofenos testados, cuja eficácia foi comparada, em estudos prévios, àquela produzida pela paroxetina, no primeiro manuscrito que faz parte deste estudo, este selenofeno foi escolhido como o composto mais promissor dentre os cinco testados. Embora tenha sido demonstrado que o **F-DPS** modula o sistema de neurotransmissão serotoninérgica, outros mecanismos responsáveis pela ação do tipo antidepressiva deste selenofeno ainda não eram conhecidos e foram investigados nos experimentos subsequentes.

A via de sinalização da ERK representa umas das vias mais estudadas na depressão. A modulação desta via parece estar envolvida nos efeitos comportamentais e moleculares/celulares tanto de terapias farmacológicas como não-farmacológicas que produzem um efeito antidepressivo (Carreno and Frazer, 2014). Além disso, a análise *post-mortem* do cérebro de pacientes depressivos que cometeram suicídio revelou uma significante redução na expressão da ERK, que foi mais evidente no córtex pré-frontal e no hipocampo (Dwivedi et al., 2001). Paralelamente a estes achados, tem sido demonstrado que modelos experimentais de depressão em roedores modulam negativamente a via de fosforilação da ERK; recentes estudos demonstraram que modelos de estresse crônico diminuem os níveis das isoformas fosforiladas da ERK tanto em córtex pré-frontal e hipocampo, ação esta que

pode ser revertida pela terapia com fármacos antidepressivos (Marsden, 2013). Por outro lado, as isoformas 1 e 2 desta proteína desempenham um importante papel na plasticidade sináptica, regulando a diferenciação, transmissão e conectividade entre os circuitos neuronais. De fato, um grande número de evidências demonstrando que a depressão altera a estrutura cerebral, bem como a transmissão e a excitabilidade neuronal (Marsden, 2013; Popoli et al., 2012), tem sido acumuladas, o que reforça a ligação entre um prejuízo na sinalização da ERK e o aparecimento do comportamento depressivo. Nesse sentido, o próximo passo do Manuscrito 1 teve como objetivo investigar a participação da via de sinalização da ERK na ação do tipo antidepressiva do **F-DPS** em camundongos. Os resultados demostraram claramente que o tratamento dos animais com este selenofeno causou um aumento significativo nos níveis das proteínas fosforiladas ERK1 e ERK2, tanto em córtex pré-frontal como hipocampo, e que a inibição da ativação desta proteína pelo uso de ferramentas farmacológicas foi capaz de bloquear completamente a ação do tipo antidepressiva do **F-DPS**. Tais dados demonstraram que a via de sinalização da ERK está envolvida no efeito farmacológico do **F-DPS**. Isto sugere, portanto, que uma melhora na plasticidade sináptica em decorrência deste efeito, influenciando a transmissão sináptica de diferentes mediadores, poderia influenciar na ação do tipo antidepressiva deste selenofeno. No entanto, o fato de que o **F-DPS** desempenha efeitos positivos sobre a neuroplasticidade, produzindo uma ação do tipo antidepressiva aguda, não significa, necessariamente, que ele seja efetivo em reverter as alterações comportamentais e neuroquímicas induzidas pela depressão, que se instala cronicamente. A fim de responder a esta questão, fez-se necessário testar o efeito do **F-DPS** em modelos animais que mimetizassem os sintomas e distúrbios crônicos encontrados nesta doença.

Sabendo-se que existe uma forte associação entre os processos que levam à dor crônica e o aparecimento de depressão em humanos e tendo em vista uma série de estudos na literatura que se utiliza de modelos animais de dor neuropática para induzir um processo de comportamento do tipo depressivo (Jesse et al., 2010; Liu and Chen, 2014; Matsuzawa-Yanagida et al., 2008; Suzuki et al., 2007), escolheu-se o modelo de ligação parcial do nervo ciático (LPNC) em camundongos com o objetivo de investigar as ações farmacológicas do **F-DPS**. A LPNC é um modelo bem reconhecido de dor neuropática que simula as alterações dolorosas presentes em humanos após uma injúria dos nervos periféricos. Neste modelo os animais apresentam uma alta sensibilidade decorrente de estímulos nociceptivos mecânicos e térmicos (Otsubo et al., 2012). Além disso, estudos têm sugerido que a plasticidade sináptica

hipocampal, a exemplo do que parece ocorrer em humanos que sofrem de dor crônica, pode ser influenciada negativamente pela condição crônica induzida pela LPNC (Kodama et al., 2007). Tal alteração em nível de sinapse neuronal pode ser responsável pelas alterações comportamentais presentes nos casos em que a dor instala-se cronicamente, como em co-morbidades entre doenças afetivas e dor (Marsden, 2013). De acordo, modelos experimentais de dor crônica induzem comportamentos do tipo depressivo e ansiogênico tanto em ratos como camundongos, os quais podem ser revertidos pela terapia com drogas antidepressivas (Liu and Chen, 2014; Matsuzawa-Yanagida et al., 2008). Por outro lado, os fármacos antidepressivos são eficazes clinicamente e vem sendo amplamente usadas como alternativa farmacológica no tratamento da dor (Blier and Abbott, 2001; Cipriani et al., 2012). Em diferentes modelos experimentais de nocicepção, o tratamento dos animais com drogas antidepressivas, que vão desde ADTS até ISRSs, também melhoram a sensibilidade à dor, incluindo a dor induzida por neuropatias periféricas (Benbouzid et al., 2008; Cegielska-Perun et al., 2013; Jesse et al., 2010; Matsuzawa-Yanagida et al., 2008). Assim, o conjunto destes fatores tornou o modelo de dor crônica induzida pela LPNC atrativo para o estudo dos efeitos comportamentais do **F-DPS**, possibilitando a investigação tanto de parâmetros relacionados ao comportamento do tipo depressivo, como o seu possível efeito na atenuação da sensibilidade a dor.

Os resultados apresentados no Artigo 1 demonstraram que tanto o tratamento agudo como subcrônico com **F-DPS** reverteram o comportamento do tipo depressivo induzido pela injúria crônica do nervo ciático em camundongos. Interessantemente, a ação do tipo antidepressiva aguda deste selenofeno foi observada em uma dose cerca de 10 vezes menor que o antidepressivo clássico paroxetina. Por outro lado, mesmo que o tratamento agudo com **F-DPS** não tenha produzido uma ação antialodínico, sua administração durante duas semanas – em uma dose que foi subefetiva nos testes de depressão quando administrada de forma aguda – reduziu a sensibilidade à dor frente a um estímulo mecânico. Embora os resultados presentes no Artigo 1 tenham demonstrado que o tratamento agudo com paroxetina reverteu a alodinia induzida pela LPNC, os estudos envolvendo a ação antialodínica dos antidepressivos é controverso no que diz respeito à duração dos tratamentos. Jesse e colaboradores (2010) observaram a ação antialodínica dos antidepressivos amitriptilina, fluoxetina e bupropiona em um modelo de dor neuropática em camundongos cerca de 1 hora após a administração, que foi concomitante ao efeito anti-imobilidade induzido por estas substâncias no TNF. Por outro lado, segundo um estudo publicado por Benbouzid e

colaboradores (2008), o tratamento crônico, mas não o agudo, com os fármacos antidepressivos tricíclicos, amitriptilina e nortriptilina, aliviou a dor neuropática induzida pela injúria crônica do nervo ciático. Ainda, um resultado similar foi observado por Matsuzawa-Yanagida e colaboradores (2008) que demonstraram que o tratamento agudo com imipramina, milnacipram e paroxetina não produziu efeito antinociceptivo em camundongos sujeitos a LPNC, enquanto que a administração subcrônica destes compostos – no geral a partir da segunda semana – reduziu significativamente a sensibilidade à dor. Apesar das divergências, o fato é que a utilização de fármacos antidepressivos na terapia da dor crônica vem crescendo desde os últimos anos, particularmente, em casos onde a comorbidade dor-depressão está presente (Blier and Abbott, 2001; Liu and Chen, 2014). Desse modo, com base nos dados apresentados no Artigo 1, sugere-se que o composto **F-DPS** pode constituir uma promissora terapia no tratamento das doenças afetivas relacionadas à dor crônica. No entanto, com base nos resultados apresentados pelo Manuscrito 1 e pelo Artigo 1, não se pode concluir com certeza de que maneira este selenofeno desempenha sua ação do tipo antidepressiva após um tratamento prolongado. Além disso, uma vez que o Artigo 1 foi baseado apenas em evidências comportamentais, experimentos adicionais tornaram-se necessários a fim de desvendar quais sistemas de neurotransmissão e/ou possíveis mecanismos poderiam estar envolvidos nos efeitos farmacológicos do **F-DPS**. Assim, tendo em vista que a dose e o protocolo de administração do composto já haviam sido determinados no estudo anterior, os experimentos realizados no Artigo 2 tiveram como objetivo a investigação dos efeitos deste selenofeno frente às alterações comportamentais, neuroquímicas e endócrinas induzidas por um modelo de depressão em camundongos envolvendo a administração crônica do hormônio glicocorticóide, corticosterona.

Esse modelo tem sido amplamente usado no estudo da patofisiologia da depressão, bem como na pesquisa por novas terapias para o tratamento desta doença (Ago et al., 2013; Crupi et al., 2013; Liu et al., 2013b; Tse et al., 2011; Wu et al., 2013; Wuwongse et al., 2013). A administração crônica de corticosterona em roedores mimetiza em muitos aspectos o comportamento e as modificações cerebrais encontrados em pacientes que sofrem de doenças afetivas. Em pacientes acometidos pela depressão, acredita-se que o fator desencadeante da doença estaria relacionado a uma disfunção do eixo hipotálamo-pituitária-adrenal (HPA), que por sua vez pode comprometer a resposta do indivíduo a eventos estressantes. Tal distúrbio aumenta os níveis de hormônios glicocorticoides circulantes, os quais, através de mecanismos ainda não muito bem conhecidos, são os responsáveis por

causar, pelo menos em parte, os sintomas da depressão (Levy and Tasker, 2012; Popoli et al., 2012). De acordo, animais tratados cronicamente com a corticosterona, o principal hormônio glicocorticoide dos roedores, apresentam um comportamento do tipo depressivo característico, que é muitas vezes acompanhado por um comportamento relacionado à ansiedade (Ago et al., 2013; Crupi et al., 2013; Liu et al., 2013b; Wu et al., 2013). Além disso, modificações sinápticas importantes, como se acredita ocorrer no cérebro de pacientes depressivos, também são observadas em animais expostos cronicamente à corticosterona (Tse et al., 2011; Wuwongse et al., 2013). Desse modo, uma vez que a maioria destas alterações pode ser revertida pela terapia com compostos antidepressivos, a hipótese de que o tratamento com **F-DPS** também poderia ser efetivo neste modelo experimental de depressão foi levantada. De fato, os resultados do Artigo 2 apontaram interessantes evidências no que diz respeito tanto aos efeitos neurocomportamentais como endócrinos produzidos por este selenofeno.

Os dados presentes nesta tese demonstraram que, de acordo com os relatos de estudos previamente publicados, as alterações comportamentais, endócrinas e bioquímicas induzidas pela administração de corticosterona foram reproduzidas pelos experimentos que fazem parte do Artigo 2. O significante aumento dos níveis séricos de corticosterona, decorrente da sua administração endógena, foi acompanhado pelo desenvolvimento de comportamentos do tipo depressivo – avaliado nos TNF e TSC – e ansiolítico – observado pelo uso do teste do claro-escuro (TCE). Paralelamente, o mecanismo de recaptação de glutamato apresentou-se reduzido no córtex pré-frontal dos animais expostos à corticosterona, sugerindo que um distúrbio da neurotransmissão glutamatérgica instalou-se após o tratamento. Confirmando este resultado, Fontella e colaboradores (2004) demonstraram previamente que a exposição crônica de ratos a um evento estressante causou uma significante redução na captação de glutamato no córtex pré-frontal. De acordo, tem sido demonstrado que a exposição prolongada dos roedores aos hormônios liberados durante situações estressantes diminuem a expressão do transportador de glutamato do tipo 1 (GLT-1) e do RNA mensageiro que codifica para esta proteína (Gourley et al., 2012; Sanacora and Banasr, 2013). Juntamente aos estudos citados, uma ampla variedade de estudos também aponta para uma alteração na transmissão glutamatérgica em diferentes regiões cerebrais após situações de estresse que induzem a liberação de hormônios glicocorticóides (Bagley and Moghaddam, 1997; Fontella et al., 2004; Lowy et al., 1995; Musazzi et al., 2010b; Musazzi et al., 2011; Musazzi et al., 2013; Musazzi et al., 2012; Reznikov et al., 2007). Além dos mecanismos de

recaptação, o processo de liberação de glutamato também parece ser alterado durante eventos estressantes (Fontella et al., 2004; Musazzi et al., 2010b; Musazzi et al., 2011; Musazzi et al., 2013). Embora não se tenha observado nenhuma alteração significativa na liberação deste neurotransmissor após a exposição dos animais ao hormônio glicocorticoide, corticosterona, durante quatro semanas, Musazzi e colaboradores (2010) sugerem que o estresse, particularmente o agudo, através do aumento rápido dos níveis de hormônios glicocorticoides circulantes, induz a liberação de glutamato pelo neurônio pré-sináptico e, portanto, facilitando a transmissão glutamatérgica. Juntos, estes dois fatores (aumento da liberação e inibição da recaptação de glutamato) podem gerar um desequilíbrio na transmissão sináptica excitatória pelo aumento dos níveis de glutamato na fenda sináptica, produzindo alterações na neuroplasticidade que podem ser responsáveis pelos efeitos comportamentais observados em modelos experimentais de comportamento do tipo depressivo e que, com base em sólidas evidências, tem sido apontada como um dos fatores envolvidos na patofisiologia da depressão em humanos (Musazzi et al., 2011; Musazzi et al., 2012; Sanacora et al., 2012). Um resultado importante deste estudo foi que o tratamento com **F-DPS** durante a última semana de exposição à corticosterona reverteu a inibição na captação de glutamato induzida pela exposição repetida a este hormônio. Apesar de não ficar claro se a ação deste selenofeno sobre o sistema glutamatérgico ocorra de uma maneira direta ou se ocorre secundariamente devido a modulação de outras vias de transmissão, sabe-se que outras drogas antidepressivas também interferem na neurotransmissão excitatória. Um estudo recente, que investigou o efeito de diferentes antidepressivos sobre o aumento na transmissão sináptica glutamatérgica induzida por eventos estressantes, demonstrou que o tratamento crônico dos animais com fluoxetina, desipramina e venlafaxina atenuou a liberação de glutamato em córtex pré-frontal de ratos após um evento estressante agudo (Musazzi et al., 2010b). Além disso, o tratamento crônico com fluoxetina, um ISRSs amplamente usado clinicamente, induz um aumento na expressão do GLT-1 em hipocampo e córtex pré-frontal de ratos, enquanto que os efeitos observados para o antidepressivo tricíclico, desipramina, e o inibidor da MAO, triancinolona, foram menos pronunciados, embora presentes (Zink et al., 2011). Estas evidências corroboram, portanto, com os resultados obtidos com relação aos efeitos moduladores do **F-DPS** sobre o sistema glutamatérico.

Por outro lado, os ISRSs, principal terapia utilizada em casos de depressão, também constituem os fármacos de escolha para o tratamento do estresse pós-traumático, uma séria condição psiquiátrica que está relacionada com uma alteração na função do eixo HPA e,

provavelmente, com um desequilíbrio no sistema de neurotransmissão glutamatérgica (Difede et al., 2014; Gola et al., 2014; Jones and Moller, 2011). As pesquisas envolvendo este assunto apontam que mecanismos serotoninérgicos estão envolvidos na regulação do eixo HPA e que o uso de medicamentos antidepressivos parece desempenhar um efeito benéfico no controle da liberação excessiva de glicocorticoides (Bremne and Vermetten, 2001; Difede et al., 2014; Jones and Moller, 2011). Essa evidência fortalece a hipótese de que existe uma forte ligação entre o sistema serotoninérgico e distúrbios do eixo HPA (Difede et al., 2014). Interessantemente, os resultados do Artigo 2 demonstraram que o tratamento subcrônico com **F-DPS**, a exemplo dos fármacos monoaminérgicos, além de modular a transmissão glutamatérgica e os níveis séricos dos hormônios relacionados ao estresse, também inibiu a recaptação de serotonina em hipocampo e córtex pré-frontal de camundongos expostos ou não à corticosterona. Esse dado, além de confirmar o envolvimento do sistema serotoninérgico no mecanismo de ação do tipo antidepressiva deste selenofeno, já demonstrado em estudos anteriores (Gay et al., 2010), levanta questões acerca do papel deste sistema na regulação negativa do eixo HPA observada nos animais tratados cronicamente com este selenofeno. Como demonstrado pelos resultados presentes nesta tese, a administração de **F-DPS** a camundongos que não foram expostos à corticosterona causou uma significante redução na atividade do eixo HPA, caracterizado por uma diminuição dos níveis séricos de corticosterona e do hormônio adrenocorticotrófico (ACTH). O ACTH atua no córtex da glândula adrenal, estimulando a liberação de hormônios glicocorticoides, incluindo o cortisol, nos humanos, e a corticosterona, nos roedores. A liberação do ACTH é realizada pela hipófise anterior (ou adenohipófise), pela ação do hormônio liberador de corticotrofina (CFR). O CFR é o principal coordenador da resposta endócrina ao estresse e é produzido pelo hipotálamo. Sabe-se que a liberação deste hormônio a partir dos núcleos hipotalâmicos sofre influência de diversas regiões cerebrais e que é claramente sujeita a um controle serotoninérgico (Owens et al., 1990). Recentemente, demonstrou-se que a ativação de receptores serotoninérgicos do tipo 1A, possa estar envolvida no controle da liberação tanto de CFR como ACTH e, portanto, na regulação da atividade do eixo HPA (Medeiros et al., 2013). Desse modo, tendo em vista o envolvimento dos receptores serotoninérgicos, inclusive 5-$HT_{1A}$, na ação do tipo antidepressiva do **F-DPS** (Gay et al., 2010), pode-se sugerir que o controle da atividade do eixo HPA em decorrência do aumento da transmissão serotoninérgica podem estar, em parte, ligados aos efeitos farmacológicos deste composto (Figura 2).

Os processos que envolvem a dor crônica e os eventos estressantes, embora com causas diversas, aparentemente possuem características neurobiológicas em comum (Hung et al., 2014; Liu and Chen, 2014; Musazzi et al., 2012; Tang, 2013). Fator que confirma isso é o uso de medicamentos antidepressivos tanto para a terapia de doenças relacionadas ao estresse, como depressão maior e estresse pós-traumático, bem como no tratamento de dores crônicas causadas, por exemplo, por fibromialgia e enxaqueca. Além disso, como já mencionado acima, é bem conhecido o fato de que eventos estressantes, atuando via elevação nos níveis de hormônios glicocorticoides, causam uma desregulação na transmissão sináptica excitatória provocada pelo aumento dos níveis cerebrais de glutamato (Musazzi et al., 2010b; Musazzi et al., 2011; Musazzi et al., 2013; Musazzi et al., 2012). De modo similar, têm surgido evidências de que os processos que levam à dor crônica também induzem alterações neuroquímicas importantes na transmissão glutamatérgica e na ativação do eixo HPA (Benedetti et al., 2012; Hung et al., 2014; Liu et al., 2013a; Victoria et al., 2013). Em um estudo publicado recentemente por Hung e colaboradores (2014), foi demonstrado que, a exemplo do que ocorre em modelos de depressão relacionados ao estresse, a injúria crônica do nervo ciático em ratos induziu um aumento na liberação de glutamato em sinaptossomas de córtex pré-frontal. Segundo os autores, essa indução foi provocada pelo aumento na expressão de proteínas responsáveis pela exocitose das vesículas sinápticas. De acordo, Liu e colaboradores (2013a) demonstraram que, além do aumento das proteínas responsáveis pela liberação de glutamato, ocorre uma importante redução nos níveis do transportador pré-sináptico deste neurotransmissor (GLT-1) e que a reversão deste processo é diminuiu a sensibilidade dos animais à dor. Somado a isso, sabe-se que diferentes modelos de indução de dor, incluindo injúrias neuropáticas crônicas, ativam o eixo HPA e aumentam os níveis séricos de corticosterona (Benedetti et al., 2012), fechando, assim, o círculo que liga os processos patológicos comuns envolvidos no desenvolvimento da dor e da depressão.

É importante notar que a hiperatividade glutamatérgica ou hiperexcitabilidade gerada pela potenciação dos sinais pós-sinápticos neuronais após a ligação do glutamato aos seus receptores, pode causar alterações nos circuitos neurais e de plasticidade. Embora também seja descrito que os receptores metabotrópicos podem mediar as modificações sinápticas induzidas pelo estresse (Wagner et al., 2013), a ativação contínua dos receptores glutamatérgicos ionotrópicos do tipo AMPA e NMDA é principalmente descrita. A ativação destes receptores, embora importante na manutenção de processos fisiológicos, quando ocorre de forma exacerbada e contínua, reduz a capacidade de rearranjo das redes neuronais e

dificulta a resposta adaptativa do indivíduo frente a novas experiências, podendo prejudicar o controle cognitivo frente a emoções negativas (Marsden, 2013; Timmermans et al., 2013). De fato, um grande número de estudos já demonstrou que prejuízos na plasticidade sináptica são induzidos tanto por modelos experimentais de dor (Kodama et al., 2007; Kohno et al., 2008; Min et al., 2011; Rahn et al., 2013) como de comportamento do tipo depressivo (Musazzi et al., 2010a; Ryan et al., 2009; Sebastian et al., 2013; Timmermans et al., 2013; Walker et al., 2013). Além disso, também já foi demonstrado que o tratamento com drogas antidepressivas produz efeitos farmacológicos que são mediados por uma melhora nos mecanismos que regulam a plasticidade sináptica (Musazzi et al., 2009; Musazzi et al., 2010a; Tardito et al., 2009; Tardito et al., 2006). Tais mecanismos permitem que uma lesão ao nível da transmissão de informação neuronal seja recuperada através da criação de outras redes neuronais que possam substituir os danos causados pela lesão. De modo geral, diversas vias estão envolvidas nos mecanismos de neuroplasticidade, entre elas a via de sinalização da ERK (Marsden, 2013), já discutida acima e que, a exemplo de outras terapias antidepressivas, também parece ser afetada pelo tratamento com **F-DPS** (Figura 7).

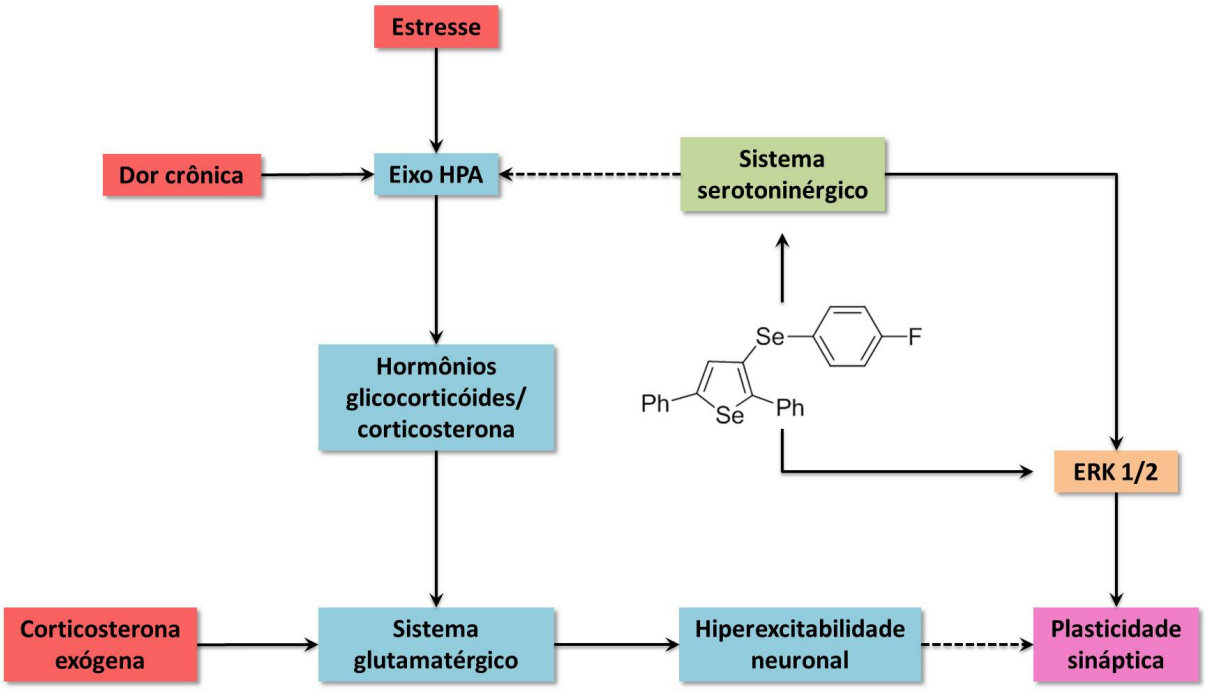


**Figura 7.** Esquema geral dos mecanismos envolvidos na ação farmacológica do **F-DPS**. Os traços pontilhados indicam uma modulação negativa enquanto que os traços cheios indicam uma modulação positiva/ativação.

Assim, com base nos dados explanados até aqui, pode-se inferir que os efeitos endócrinos e neuroquímicos observados após o tratamento dos animais com **F-DPS** sejam responsáveis pelos efeitos farmacológicos deste composto tanto após a administração crônica de corticosterona quanto no modelo de depressão induzida pela LPNC. Como demonstrado na Figura 7, acredita-se que estes efeitos poderiam, inclusive, ser extrapolados para um modelo experimental que mimetizasse as alterações neurocomportamentais induzidas pelo estresse. A ação farmacológica deste selenofeno pode ser atribuída, em parte, por seu efeito sobre o sistema serotoninérgico, cuja ativação regula negativamente a atividade do eixo HPA e, portanto a liberação dos hormônios glicocorticoides. Embora os mecanismos de plasticidade sináptica não tenham sido avaliados, tendo em vista a modulação da fosforilação da ERK e a redução na transmissão glutamatérgica por este selenofeno, é possível que uma melhora na transmissão sináptica e nos circuitos neuronais também ocorra após o tratamento com **F-DPS**.

# CONCLUSÃO

Os selenofenos são uma importante classe de compostos heterocíclicos contendo selênio e muitos estudos têm demonstrado o efeito biológico e o potencial farmacológico de diferentes moléculas pertencentes a essa classe. No entanto, poucos estudos envolvendo a ação antidepressiva destes compostos têm sido desenvolvidos. Neste estudo, foi demonstrado que 3-(organosseleno)-2,5-difenil-selenofenos são promissoras drogas no tratamento do comportamento relacionado à depressão e que a ação do tipo antidepressiva destes compostos parece ter uma relação entre estrutura química e atividade biológica.

Em particular, o composto 3-(4-fluorofenilselenil)-2,5-difenil-selenofeno (**F-DPS**), cujo efeito farmacológico agudo envolve a ativação da ERK, cuja via está relacionada à plasticidade sináptica, foi efetivo em diferentes modelos animais de depressão, revertendo as alterações comportamentais induzidas tanto pela dor crônica quanto pela administração de corticosterona em camundongos.

Embora os mecanismos envolvidos na ação do tipo antidepressiva do **F-DPS** ainda necessitem ser investigados, os dados aqui apresentados demonstraram que a fosforilação da proteína quinase regulada por sinal extracelular (ERK), a regulação do eixo HPA e a modulação dos sistemas de neurotransmissão serotoninérgico e glutamatérgico são importantes alvos farmacológicos deste selenofeno.

Finalmente, os resultados presentes neste estudo reforçam a hipótese de que o **F-DPS** pode representar uma alternativa importante no estudo e desenvolvimento de futuras drogas para a terapia da depressão.

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# APÊNDICE A

## Mecanismos farmacológicos envolvidos na ação do tipo antidepressiva do 3-(4-fluorofenilselenil)-2,5 difenilselenofeno em um modelo de dor crônica induzida pela ligação parcial do nervo ciático em camundongos

### 1 Objetivo

O objetivo deste experimento foi investigar se as vias de sinalização da proteína quinase A (PKA), proteína quinase C (PKC) e da proteína quinase regulada por sinal extracelular (ERK) estão envolvidas na ação do tipo antidepressiva aguda do **F-DPS** no modelo de dor crônica induzida pela ligação parcial do nervo ciático em camundongos.

### 2 Materiais e Métodos

### 2.1 Animais

Foram utilizados camundongos adultos machos da raça Swiss, pesando entre 25 e 30g, provenientes do Biotério Central da Universidade Federal de Santa Maria (UFSM). Os animais foram acondicionados sob condições de temperatura de 22 ± 2ºC e mantidos em um ciclo de 12h luz/12h escuro. A dieta foi constituída de ração comercial (GUABI, RS, Brasil) e água fresca *ad libitum*.

Os testes foram realizados com grupos separados de animais e cada animal foi usado apenas uma vez em cada teste. Todos os procedimentos foram realizados a fim de minimizar o sofrimento e reduzir o número de animais usados nos experimentos. Os protocolos experimentais descritos neste estudo foram realizados de acordo com o projeto número nº 124/2010, aprovado pelo Comitê de Ética e Bem-Estar Animal da UFSM.

**2.2 Drogas**

O composto 3-(4-p-fluorofenilselenil)-2,5-difenilselenofeno (**F-DPS**) foi sintetizado e caracterizado em nosso laboratório pelo método previamente descrito por Stein et al. (2008). O **F-DPS** foi dissolvido em óleo de canola e administrado pela via intragástrica (i.g.) num volume constante de 10 mL/kg de peso corporal.

Cloreto de queleritrina, H-89 e PD98,059 foram obtidos da Sigma-Aldrich (St. Louis, MO, EUA), dissolvidos em salina e administrados pela via intracerebroventricular (i.c.v.).

**2.3 Procedimento cirúrgico**

A cirurgia de ligação parcial do nervo ciático foi realizada de acordo com Narita et al. (2005), sob as condições descritas no Artigo 1 da presente tese.

**2.4 Injeção intracerebroventricular (i.c.v.)**

Cloreto de queleritrina, H-89 e PD98,059 foram administrados pela via i.c.v. de acordo com o método previamente descrito por Haley e McCormick (1957), levando em consideração a fissura do bregma como ponto de referência para a injeção.

**2.5 Testes comportamentais**

O teste do nado forçado (TNF), realizado de acordo com o método descrito por Porsolt et al. (1979), foi utilizado na investigação do comportamento do tipo depressivo dos animais. O tempo total de imobilidade apresentado pelos animais foi observado durante um período de 6 minutos.

O efeito dos tratamentos sobre as atividades locomotora e exploratória dos animais foi analisado por meio do monitor de atividades. Parâmetros como número de cruzamentos e elevações, velocidade média e distância percorrida foram observados.

Ambos os testes estão descritos no Manuscrito 1 e no Artigo 1 da presente tese.

## 2.6 Procedimento experimental

A investigação dos mecanismos envolvidos na ação do tipo antidepressiva aguda do **F-DPS** deu-se ao término da quarta semana após a cirurgia de ligação parcial do nervo ciático. O procedimento experimental está representado na Figura 1. Os animais receberam uma injeção i.c.v de veículo ou dos inibidores das vias de sinalização H-89 (1 e 5 µg/sítio; inibidor da PKA), queleritrina (1 e 5 µg/sítio; inibidor da PKC) ou PD98,059 (5 e 7,5 µg/sítio; inibidor da ativação da ERK), quinze minutos antes do tratamento com **F-DPS** (1 mg/kg, i.g.). Trinta minutos após a administração do **F-DPS**, os animais foram avaliados no TNF. A fim de descartar que a alteração na locomoção dos animais poderia interferir no teste de depressão, imediatamente antes do TNF, os animais foram avaliados no monitor de atividades.

As doses e os tempos de tratamento dos inibidores das vias de sinalização utilizados neste experimento foram selecionados com base em dados da literatura.

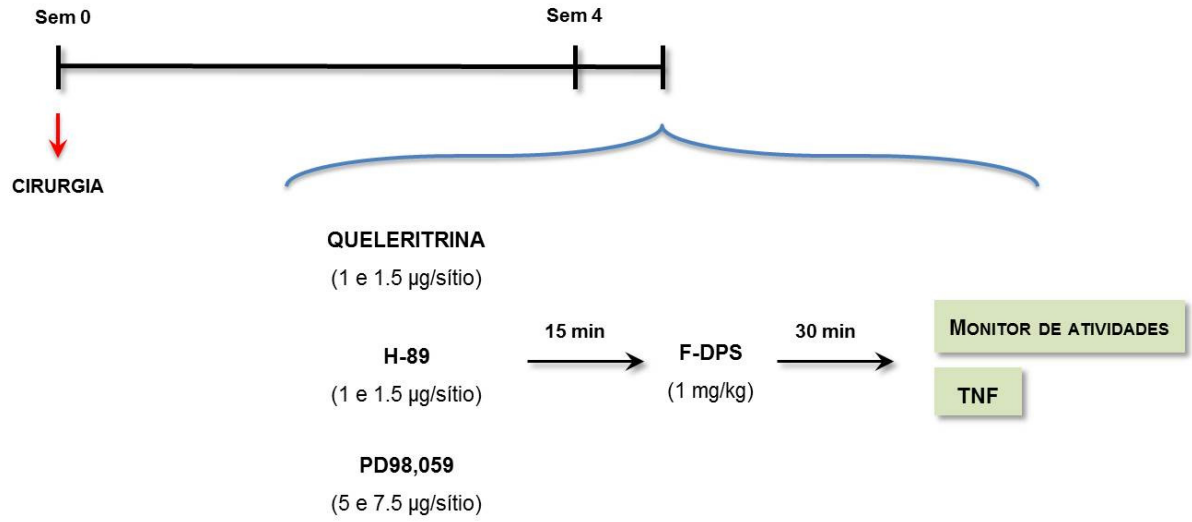


**Figura 1.** Procedimento experimental: investigação dos mecanismos envolvidos na ação do tipo antidepressiva aguda do **F-DPS**. Os testes iniciaram no início da quinta semana após a cirurgia de ligação parcial do nervo ciático. Os inibidores foram administrados pela via intracerebroventricular (i.c.v.). O **F-DPS** foi administrado pela via intragástrica (i.g.). TNF: Teste do nado forçado.

## 2.7 Análise estatística

Os dados obtidos foram expressos como média ± erro padrão. A distribuição normal dos dados foi testada de acordo com o teste de normalidade de D'Agostino e Person. Os dados foram apresentados usando análise de variância (ANOVA) de duas vias seguida pelo teste *post-hoc* de Newman-Keuls.

**3 Resultados**

A Figura 2 demonstra o efeito do pré-tratamento com os inibidores das vias de sinalização da PKA, PKC e MAPK/ERK sobre a redução no tempo de imobilidade induzida pelo tratamento agudo com **F-DPS** (1 mg/kg, i.g.). A análise estatística revelou uma significante interação entre a injeção de H-89 e o tratamento agudo com F-DPS [$F_{(2,42)}$ = 11.08, $P < 0.001$]. Embora a concentração mais baixa de H-89 (um inibidor da PKA) não tenha modificado o efeito do **F-DPS**, o tratamento dos animais com a maior concentração deste inibidor (1,5 µg/sítio, i.c.v) foi capaz de bloquear significantemente a ação do tipo antidepressiva do selenofeno no TNF ($P < 0.001$, Figura 2A).

Os resultados também demonstraram uma significante interação entre o tratamento com o inibidor da PKC, queleritrina, e a administração de **F-DPS** [$F_{(2,42)} = 10.17$, $P < 0.001$]. O teste *post-hoc* revelou que a ação do tipo antidepressiva do **F-DPS** foi parcialmente influenciada pela injeção de queleritrina na menor concentração (1,0 µg/sítio, i.c.v). Por outro lado, a injeção de queleritrina na concentração de 1,5 µg/sítio foi capaz de bloquear completamente o efeito do **F-DPS** no TNF ($P < 0.001$, Figura 2B).

Do mesmo modo, observou-se uma significante interação entre PD 98,059 × **F-DPS** [$F_{(2,42)} = 12.00$, $P < 0.001$]. O inibidor da fosforilação da ERK, PD98,059, em ambas as concentrações injetadas, apresentou um significante efeito em bloquear a ação do tipo antidepressiva do **F-DPS** no TNF em camundongos ($P < 0.001$, Figura 2C).

Finalmente, nenhum dos tratamentos alterou os parâmetros locomotores e exploratórios dos animais quando os mesmos foram avaliados no monitor de atividades (Tabela 1). O número de cruzamentos, de elevações, a velocidade média e a distância percorrida não foram modificados.

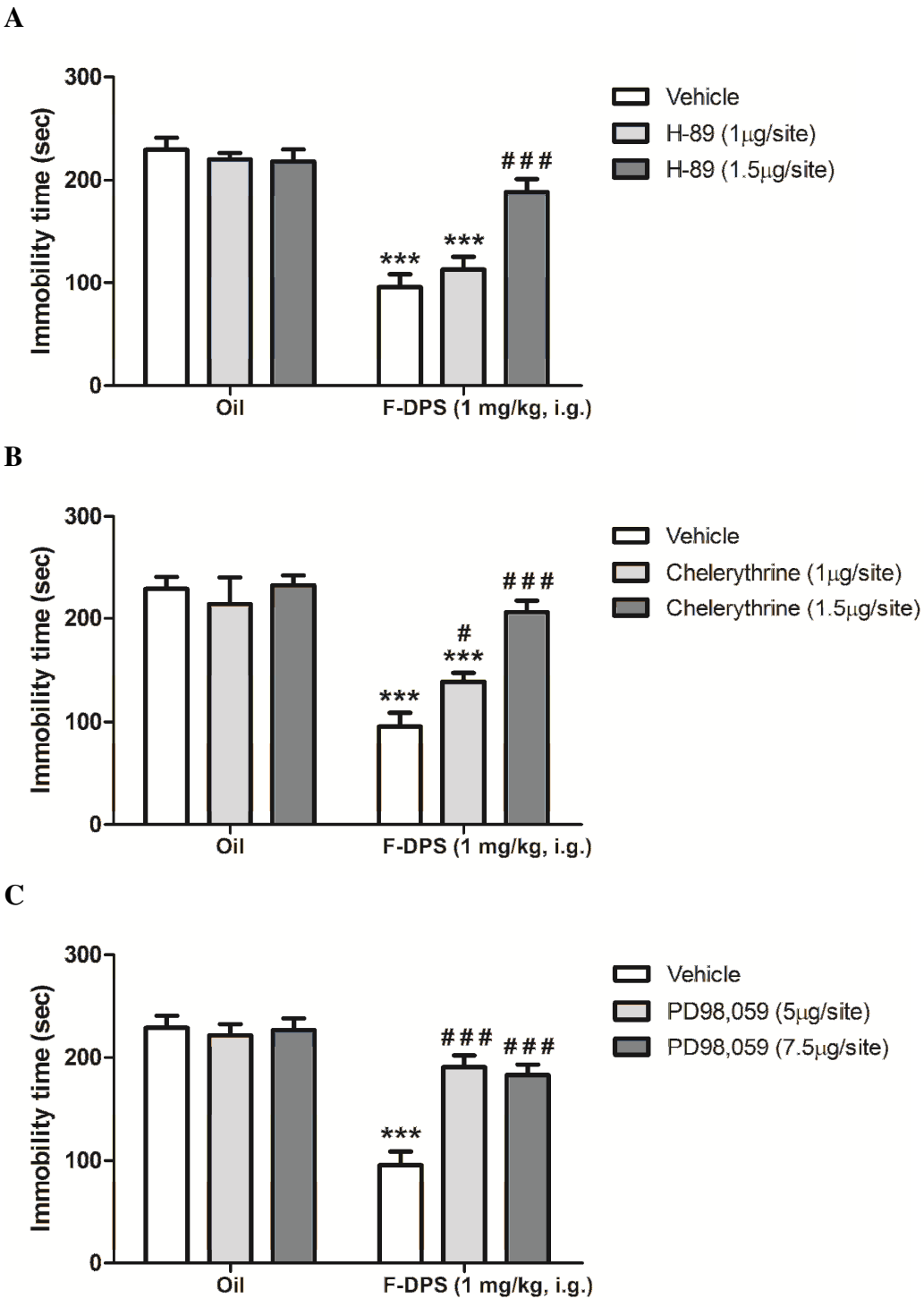


**Figura 2.** Efeito do tratamento com (**A**) H-89 (1 e 1,5 µg/sítio), (**B**) queleritrina (1 and 1,5 µg/sítio) e (**C**) PD98,059 (5 e 7,5 µg/sítio) sobre o comportamento do tipo antidepressivo induzido pela administração de F-DPS (1 mg/kg) no TNF em camundongos. O selenofeno foi administrado pela via i.g. 15 minutos depois dos inibidores e 30 minutos antes do teste. Os inibidores foram administrados pela via i.c.v. Os valores estão expressos como média ± erro padrão de 8 animais por grupo. Os asteriscos denotam o nível de significância em comparação ao grupo tratado com óleo: (***) $P < 0.001$. Os sustenidos denotam o nível de significância em comparação ao grupo tratado com veículo: (###) $P < 0.001$ (ANOVA de duas vias seguida pelo teste de Newman-Keuls).

**Tabela 1.** Efeito do tratamento agudo com **F-DPS** e seu tratamento combinado com H-89, queleritrina e PD98,059 sobre as atividades locomotora e exploratória de camundongos sujeitos à ligação parcial do nervo ciático.

| | **Número de cruzamentos** | **Número de elevações** | **Velocidade (mm/s)** | **Distância (dm)** |
|---|---|---|---|---|
| CCI | 447.1 ± 69.9 | 13.1 ± 2.4 | 32.1 ± 4.9 | 69.1 ± 13.4 |
| CCI + H-89 (1µg/sítio) | 501.6 ± 98.0 | 8.6 ± 2.7 | 32.8 ± 10.5 | 47.5 ± 11.9 |
| CCI + H-89 (1.5µg/ sítio) | 317.6 ± 68.5 | 10.5 ± 3.0 | 38.8 ± 5.5 | 80.7 ± 15.5 |
| CCI + F-DPS | 499.5 ± 70.8 | 14.2 ± 2.3 | 33.0 ± 3.9 | 68.8 ± 9.5 |
| CCI + H-89 (1µg/sítio) + F-DPS | 367.9 ± 58.7 | 9.3 ± 2.4 | 26.1 ± 4.4 | 51.1 ± 8.6 |
| CCI + H-89 (1.5µg/sítio) + F-DPS | 421.5 ± 60.0 | 9.3 ± 2.1 | 34.6 ± 4.2 | 72.8 ± 12.7 |
| | | | | |
| CCI | 396.9 ± 65.6 | 10.0 ± 1.8 | 28.3 ± 4.9 | 59.9 ± 13.5 |
| CCI + Chelerythrine (1µg/sítio) | 317.6 ± 68.5 | 14.2 ± 1.4 | 31.3 ± 4.9 | 60.7 ± 9.9 |
| CCI + Chelerythrine (1.5µg/sítio) | 333.5 ± 54.5 | 8.1 ± 2.0 | 40.9 ± 6.6 | 51.4 ± 9.2 |
| CCI + F-DPS | 449.5 ± 51.5 | 13.0 ± 1.8 | 31.5 ± 4.6 | 73.8 ± 8.4 |
| CCI + Chelerythrine (1µg/sítio) + F-DPS | 405.4 ± 35.7 | 10.9 ± 1.7 | 37.2 ± 6.6 | 74.7 ± 15.4 |
| CCI + Chelerythrine (1.5µg/sítio) + F-DPS | 459.1 ± 56.9 | 11.5 ± 1.7 | 33.8 ± 3.9 | 85.2 ± 16.7 |
| | | | | |
| CCI | 362.3 ± 71.9 | 9.6 ± 1.5 | 28.8 ± 3.2 | 55.5 ± 14.4 |
| CCI + PD 98,059 (5µg/sítio) | 438.1 ± 50.8 | 8.9 ± 1.2 | 29.5 ± 1.9 | 62.8 ± 5.2 |
| CCI + PD 98,059 (7.5µg/sítio) | 391.0 ± 81.3 | 8.7 ± 2.0 | 29.5 ± 5.5 | 64.3 ± 14.0 |
| CCI + F-DPS | 438.4 ± 69.5 | 14.0 ± 2.1 | 35.1 ± 3.3 | 62.7 ± 9.3 |
| CCI + PD 98,059 (5µg/sítio) + F-DPS | 407.4 ± 41.8 | 11.6 ± 2.2 | 30.6 ± 3.0 | 57.1 ± 6.9 |
| CCI + PD 98,059 (7.5µg/sítio) + F-DPS | 411.6 ± 31.2 | 6.7 ± 1.1 | 30.9 ± 3.1 | 68.3 ± 7.8 |

O selenofeno foi administrado pela via intragástrica (i.g.), na dose de 1 mg/kg 15 minutos após a injeção intracerebroventricular (i.c.v) de H-89, queleritrina e PD98,059. Os parâmetros locomotores foram observados 30 minutos após a administração de **F-DPS**. Os valores estão expressos como média ± erro padrão de 8 animais por grupo. Os dados foram analisados por ANOVA de duas vias seguida pelo teste de Newman-Keuls.

## 4 Considerações finais

Os resultados deste experimento sugerem que a ativação das vias de sinalização da PKA, PKC e MAPK/ERK estão envolvidas na ação do tipo antidepressiva do **F-DPS** no modelo de dor crônica induzida pela ligação parcial do nervo ciático em camundongos.

# APÊNDICE B

## Ação do 3-(4-fluorofenilselenil)-2,5 difenilselenofeno sobre o comportamento do tipo depressivo induzido pelo estresse crônico em camundongos

### 1 Objetivos

O objetivo deste experimento foi investigar se o tratamento subcrônico com **F-DPS** é capaz de reverter as alterações comportamentais induzidas pelo estresse crônico imprevisível moderado (ECIM) em camundongos.

### 2 Materiais e Métodos

#### 2.1 Animais

Foram utilizados camundongos adultos machos da raça Swiss, pesando entre 25 e 30g, provenientes do Biotério Central da Universidade Federal de Santa Maria (UFSM). Os animais foram acondicionados sob condições de temperatura de 22 ± 2ºC e mantidos em um ciclo de 12h luz/12h escuro. A dieta foi constituída de ração comercial (GUABI, RS, Brasil) e água fresca *ad libitum*.

Todos os procedimentos foram realizados a fim de minimizar o sofrimento e reduzir o número de animais usados nos experimentos. Os protocolos experimentais descritos neste estudo foram realizados de acordo com o projeto número nº 124/2010, aprovado pelo Comitê de Ética e Bem-Estar Animal da UFSM.

#### 2.2 Drogas

O composto 3-(4-p-fluorofenilselenil)-2,5-difenilselenofeno (**F-DPS**) foi sintetizado e caracterizado em nosso laboratório pelo método previamente descrito por Stein et al. (2008). O **F-DPS** foi dissolvido em óleo de canola e administrado pela via intragástrica (i.g.).

O fármaco antidepressivo amitriptilina, usada como controle positivo neste experimento, foi obtida de Sigma-Aldrich (St. Louis, MO, EUA), dissolvida em salina e administrada aos animais pela via intraperitoneal (i.p.).

Tanto **F-DPS** como amitriptilina foram administrados num volume constante de 10 mL/kg de peso corporal.

### 2.3 Protocolo de estresse crônico imprevisível moderado

Os animais foram submetidos a um protocolo de ECIM, realizada conforme Monleon et al. (1995), com algumas modificações. Enquanto um grupo controle de animais não foi submetido ao protocolo de estresse crônico, tendo livre acesso a água e comida, outro grupo de animais foi submetido a diferentes tipos de agentes estressores durante um período de 4 semanas. As situações de estresse incluíram: restrição de movimentos (2 horas, 2 vezes na semana), inclinação da caixa a 45º (6 horas, 1 vezes na semana), maravalha úmida (10 horas, 1 vez na semana), retirada da maravalha da caixa (10 horas, 1 vez na semana), substituição da maravalha por água (cerca de 3 cm de altura; 8 horas, 1 vez na semana), restrição de água e ração (24 horas, 1 vez na semana), nado forçado em água a 4 ºC (3 minutos, 1 vez na semana) e inversão do ciclo claro-escuro.

Os animais receberam um ou dois desses estressores por dia, sendo que o horário de início do estresse e a ordem dos agentes estressores no decorrer das semanas foram alterados a fim de evitar a habituação dos animais.

### 2.4 Testes comportamentais

O teste de preferência à sacarose foi realizado como medida de comportamento do tipo anedônico. Num primeiro momento, os animais foram ambientados, em caixas individuais, com duas garrafas contendo uma solução de sacarose a 2% durante 24 horas. No segundo dia, uma das garrafas com sacarose foi substituída por outra contendo água. Após 24 horas podendo escolher entre uma garrafa com sacarose e outra com água, os animais foram privados de qualquer tipo de líquido e comida por mais 24 horas. O teste aconteceu no quarto dia, onde uma garrafa com água e outra com sacarose foram oferecidas aos camundongos durante 1 hora. A preferência à sacarose foi determinada pela medida do volume de sacarose consumida com relação ao volume total de líquido ingerido.

O teste da suspensão da cauda (TSC), realizado de acordo com o método descrito por Steru et al., (1985), foi utilizado na observação do comportamento do tipo depressivo dos camundongos. O tempo total de imobilidade foi observado durante um período de 6 minutos.

O teste do claro-escuro (TCE) foi utilizado como medida de comportamento relacionado à ansiedade. O teste foi realizado de acordo com o método descrito por Bourin e Hascoet (2003). Parâmetros como latência para entrada no compartimento escuro, tempo total de permanência no compartimento claro e número de transições entre os dois compartimentos foram observados durante um período de teste de 5 minutos.

O teste da placa perfurada (*hole board*) foi utilizado na medida de parâmetros locomotores e exploratórios. O número de cruzamentos que o animal faz entre as áreas demarcadas do equipamento durante cinco minutos representa a atividade locomotora. Por sua fez, a investigação dos orifícios presentes na placa é indicativa de atividade exploratória. Os animais foram observados durante um período de 5 minutos.

**2.5 Procedimento experimental**

Os camundongos foram submetidos ao protocolo de ECIM durante 4 semanas. Durante a última semana de estresse, um grupo foi tratado diariamente com **F-DPS** (0,1 mg/kg, i.g) e um segundo grupo recebeu amitriptilina (10 mg/kg, i.p), totalizando um total de sete administrações (Figura 1). Enquanto que os animais tratados com **F-DPS** receberam uma injeção i.p. de salina, o grupo amitriptilina foi tratado com uma administração i.g. de óleo. O terceiro grupo foi tratado com os veículos destas drogas, ou seja, uma administração i.p. de salina e uma i.g. de óleo de canola por dia. Um grupo controle, que não foi submetido ao procedimento de estresse também recebeu as injeções diárias de salina e óleo de canola. Os grupos experimentais foram constituídos por 6 animais cada e estão descritos abaixo:

Grupo I: Não estressado (veículos i.g. e i.p. durante a última semana)
Grupo II: ECIM (veículos i.g. e i.p. durante a última semana)
Grupo III: ECIM + **F-DPS** (0,1 mg/kg, i.g., durante a última semana)
Grupo IV: ECIM + amitriptilina (10 mg/kg, i.p., durante a última semana)

Durante todo o período de estresse, o ganho de peso corporal e o consumo diário de água e comida foram acompanhados. Vinte quatro horas após a última administração de **F-DPS** ou amitriptilina, os animais foram avaliados no teste de preferência à sacarose, teste da placa perfurada, TCE e TSC, nesta ordem.

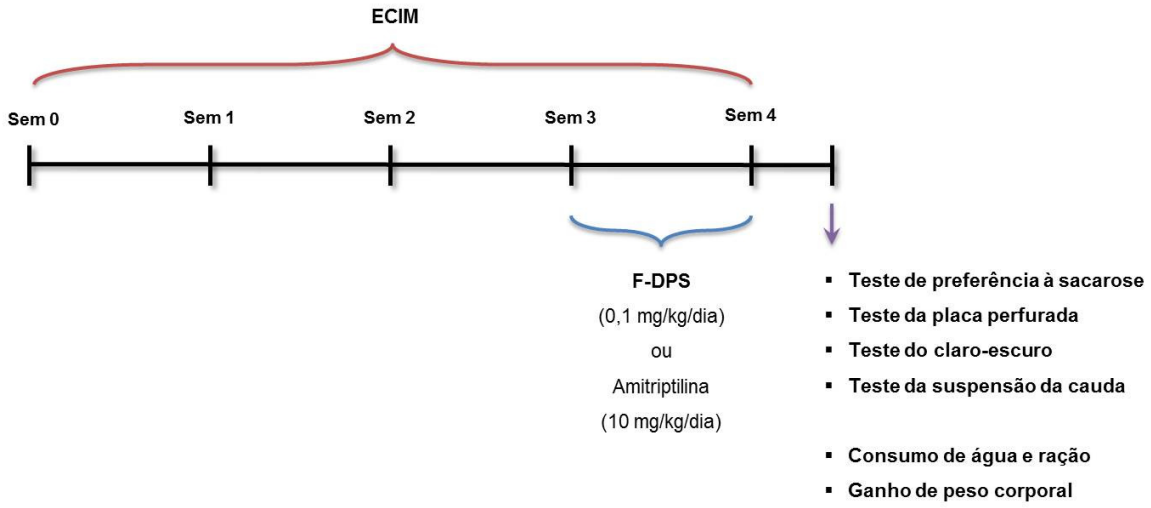


**Figura 1.** Procedimento experimental: tratamento com **F-DPS** e amitriptilina em animais sujeitos ao protocolo de Estresse Crônico Imprevisível Moderado (ECIM). O selenofeno foi administrado pela via intragástrica (i.g.) enquanto que a amitriptilina foi injetada intraperitonealmente (i.p.). Os testes comportamentais foram realizados 24 horas após a última administração das drogas.

## 2.6 Análise estatística

Os dados obtidos foram expressos como média ± erro padrão. A distribuição normal dos dados foi testada de acordo com o teste de normalidade de D'Agostino e Person. Os dados foram analisados usando análise de variância (ANOVA) de uma via seguida pelo teste *post-hoc* de Newman-Keuls.

## 3 Resultados

Os resultados obtidos no teste de preferência à sacarose não demonstraram nenhum efeito significativo [$F_{(3,23)} = 1{,}24$, $P > 0{,}05$; Figura 2]. No entanto, observou-se uma tendência do ECIM em reduzir a preferência à sacarose nos animais tratados com o veículo em comparação ao grupo controle não estressado. Em uma análise mais detalhada, observou-se que o comportamento do tipo anedônico apareceu em 50% dos animais. Essa tendência parece ser revertida pelo tratamento dos animais com **F-DPS** e amitriptilina.

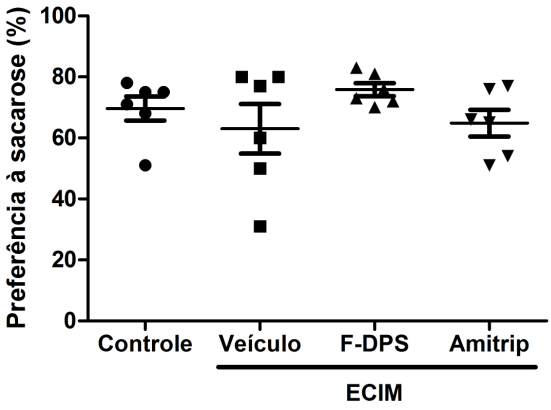


**Figura 2.** Efeito do tratamento subcrônico com **F-DPS** e amitriptilina sobre o comportamento do tipo anedônico induzido pelo estresse crônico imprevisível moderado (ECIM) em camundongos. O selenofeno foi administrado pela via i.g. na dose de 0,1 mg/kg durante 1 semana, enquanto que a amitriptilina foi administrada na dose de 10 mg/kg. Os valores estão expressos como média ± erro padrão de 6 animais por grupo. Os dados foram analisados por ANOVA de uma via seguida pelo teste de Newman-Keuls.

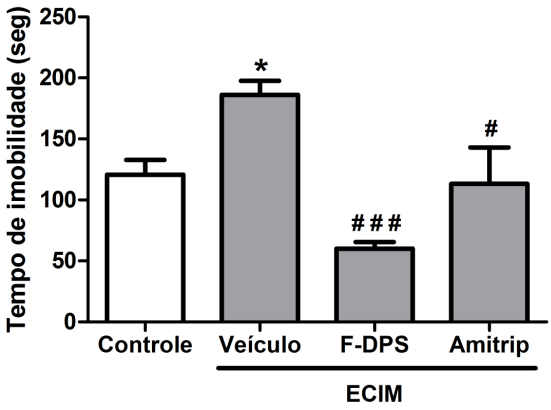


**Figura 3.** Efeito do tratamento subcrônico com **F-DPS** e amitriptilina sobre o comportamento do tipo depressivo induzido pelo estresse crônico imprevisível moderado (ECIM) no teste da suspensão da cauda em camundongos. O selenofeno foi administrado pela via i.g. na dose de 0,1 mg/kg durante 1 semana, enquanto que a amitriptilina foi administrada na dose de 10 mg/kg. Os valores estão expressos como média ± erro padrão de 6 animais por grupo. Os asteriscos indicam o nível de significância em comparação ao grupo controle: (*) $P < 0.05$. Os sustenidos indicam o nível de significância em comparação ao grupo ECIM tratado com veículo: (#) $P < 0.05$ e (###) $P < 0.001$ (ANOVA de uma via seguida pelo teste de Newman-Keuls).

A análise estatística demonstrou um significante efeito do tratamento sobre o tempo total de imobilidade dos animais no TSC [$F_{(3,23)} = 0,65$, $P < 0,001$; Figura 3]. Os animais sujeitos ao protocolo de ECIM durante 4 semanas apresentaram um aumento significativo no tempo de imobilidade, demonstrando o aparecimento de um comportamento do tipo depressivo. O tratamento com **F-DPS**, administrado na dose de 0,1 mg/kg durante a última semana de estresse, reverteu este aumento ($P < 0,001$), demonstrando a ação do tipo antidepressiva deste comporto orgânico de selênio. De modo semelhante, a administração de

amitriptilina também produziu um efeito anti-imobilidade nos animais estressados, embora este efeito tenha sido menos significativo ($P < 0,05$).

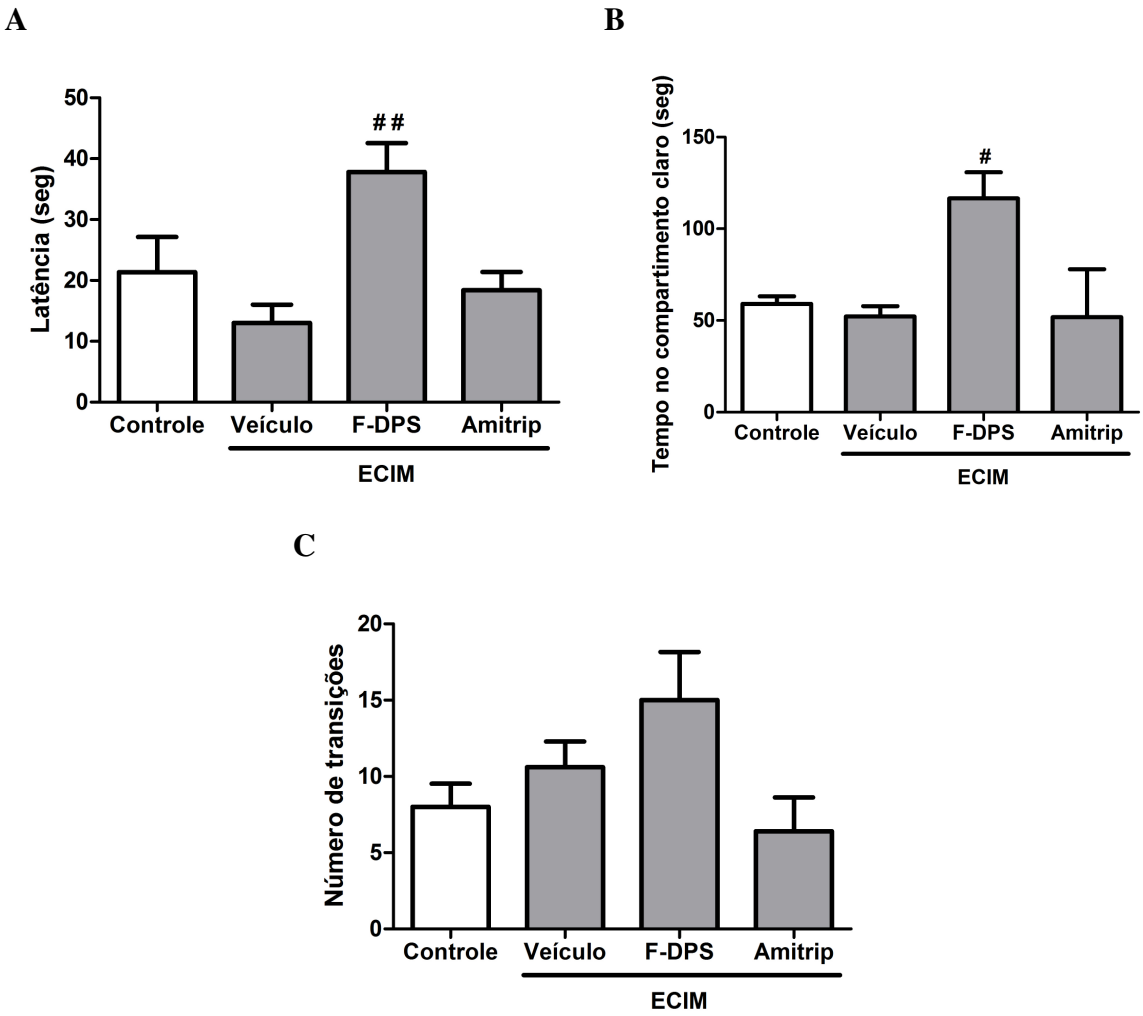


**Figura 4.** Efeito do estresse crônico imprevisível moderado (ECIM) e do tratamento subcrônico com **F-DPS** e amitriptilina sobre (**A**) a latência para a primeira entrada no compartimento escuro, (**B**) tempo total de permanência no compartimento claro e (**C**) número total de transições no teste do claro-escuro em camundongos. O selenofeno foi administrado pela via i.g. na dose de 0,1 mg/kg durante 1 semana, enquanto que a amitriptilina foi administrada na dose de 10 mg/kg. Os valores estão expressos como média ± erro padrão de 6 animais por grupo. Os sustenidos indicam o nível de significância em comparação ao grupo ECIM tratado com veículo: (#) $P < 0.05$ e (##) $P < 0.01$ (ANOVA de uma via seguida pelo teste de Newman-Keuls).

Os resultados demonstraram um significante efeito do tratamento sobre a latência para a primeira entrada no compartimento escuro [$F_{(3,23)} = 7,83$, $P < 0,001$; Figura 4A] e sobre o tempo total de permanência no compartimento claro [$F_{(3,23)} = 4,19$, $P < 0,05$; Figura 4B] no TCE. Embora nenhum efeito significante do ECIM e do tratamento com amitriptilina tenha sido observado, os animais tratados subcronicamente com **F-DPS** demonstraram um significante aumento nestes parâmetros quando comparados aos animais tratados com óleo (*P*

< 0,01 e $P < 0,05$, respectivamente), indicando que este composto induz um comportamento do tipo ansiolítico. Nenhum efeito significante foi observado no número total de transições entre os dois compartimentos [$F_{(3,23)} = 2,55$, $P > 0,05$; Figura 4C].

Com relação ao teste da placa perfurada, nenhum dos tratamentos alterou as atividades locomotora [$F_{(3,23)} = 0,76$, $P > 0,05$; Figura 5A] e exploratória [$F_{(3,23)} = 1,12$, $P > 0,05$; Figura 5B] dos camundongos.

O ganho de peso corporal e o consumo de água e ração não foram alterados no decorrer das 4 semanas em nenhum dos grupos ($P > 0,05$).

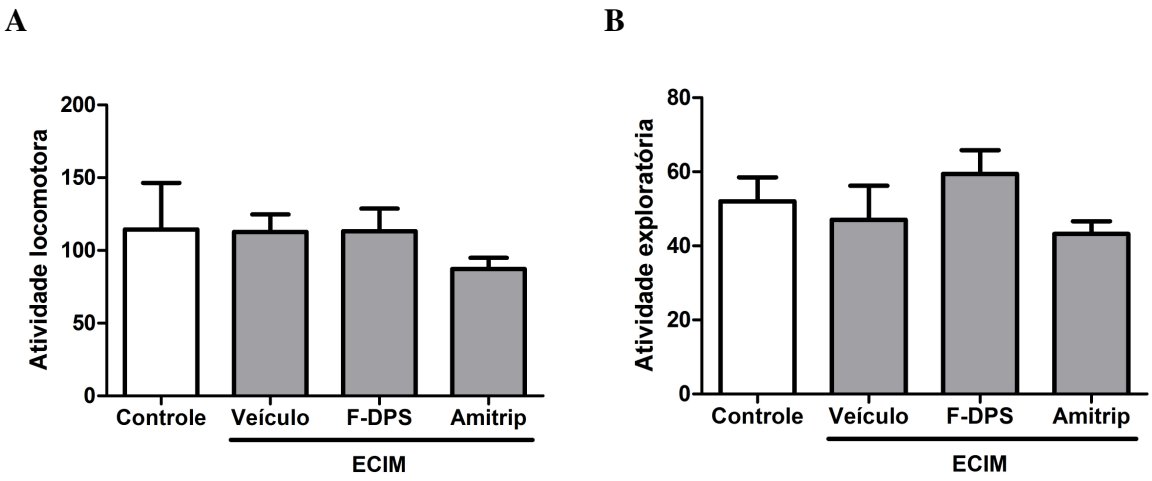


**Figura 5.** Efeito do estresse crônico imprevisível moderado (ECIM) e do tratamento subcrônico com **F-DPS** e amitriptilina sobre (**A**) a atividade locomotora e (**C**) a atividade exploratória no teste da placa perfurada em camundongos. O selenofeno foi administrado pela via i.g. na dose de 0,1 mg/kg durante 1 semana, enquanto que a amitriptilina foi administrada na dose de 10 mg/kg. Os valores estão expressos como média ± erro padrão de 6 animais por grupo. Os dados foram analisados por ANOVA de uma via seguida pelo teste de Newman-Keuls.

## 4 Consideração final

Os resultados obtidos neste experimento nos permitem sugerir que o tratamento subcrônico com **F-DPS** apresenta ação do tipo antidepressiva em animais submetidos ao ECIM. Esta ação é, ainda, acompanhada por uma significativa ação do tipo ansiolítica, que não é observada pela terapia com amitriptilina. Estes dados estão de acordo e complementam os resultados descritos no Artigo 2 desta tese.

# APÊNDICE C

## Efeito do tratamento subcrônico com 3-(4-fluorofenilselenil)-2,5 difenilselenofeno sobre parâmetros de toxicidade hepática e renal em camundongos

### 1 Objetivo

O objetivo deste experimento foi determinar se o esquema de administração de **F-DPS** que foi utilizado nos Artigos 1 e 2 desta tese produz alterações em parâmetros básicos de toxicidade hepática e renal em camundongos.

### 2 Materiais e Métodos

### 2.1 Animais

Foram utilizados camundongos adultos machos da raça Swiss, pesando entre 25 e 30g, provenientes do Biotério Central da Universidade Federal de Santa Maria (UFSM). Os animais foram acondicionados sob condições de temperatura de 22 ± 2ºC e mantidos em um ciclo de 12h luz/12h escuro. A dieta foi constituída de ração comercial (GUABI, RS, Brasil) e água fresca ad libitum.

Os protocolos experimentais descritos neste estudo foram realizados de acordo com o projeto número nº 124/2010, aprovado pelo Comitê de Ética e Bem-Estar Animal da UFSM.

### 2.2 Drogas

O composto 3-(4-p-fluorofenilselenil)-2,5-difenilselenofeno (**F-DPS**) foi sintetizado e caracterizado em nosso laboratório pelo método previamente descrito por Stein et al. (2008). O **F-DPS** foi dissolvido em óleo de canola e administrado pela via intragástrica (i.g.).

**2.3 Procedimento experimental**

Os animais foram divididos em três grupos experimentais de 8 animais cada. Os animais receberam uma administração intragástrica diária de **F-DPS** na dose de 0,1 mg/kg durante 1 ou duas semanas, de acordo com o descrito abaixo.

Grupo I: Óleo de canola durante 2 semanas.

Grupo II: Óleo de canola durante a 1ª semana e **F-DPS** durante a 2ª semana.

Grupo III: **F-DPS** durante 2 semanas.

Vinte e quatro horas após a última administração, os animais foram anestesiados e as amostras de sangue foram removidas por punção cardíaca. O sangue foi então centrifugado a 4000 g durante 10 minutos e o soro foi separado e estocado a -20ºC para a determinação de parâmetros de toxicidade plasmática.

**2.4 Análises bioquímicas**

A atividade das enzimas aspartato aminotransferase (AST), alanina aminotransferase (ALT) e lactato desidrogenase (LDH) e os níveis séricos de ácido úrico e ureia foram determinados usando kits comerciais (LABTEST, Diagnostica S.A., Minas Gerais, Brasil). As atividades enzimáticas foram expressas como U/L, enquanto que os níveis de ureia e ácido úrico foram expressos como mg/dl.

**2.5 Análise estatística**

Os dados obtidos foram expressos como média ± erro padrão. A distribuição normal dos dados foi testada de acordo com o teste de normalidade de D'Agostino e Person. Os dados foram analisados usando análise de variância (ANOVA) de uma via seguida pelo teste *post-hoc* de Newman-Keuls.

**3 Resultados**

Como apresentado na Tabela 1, os resultados deste experimento demonstraram que a administração subcrônica de **F-DPS** (0,1 mg/kg) a camundongos não produz qualquer

alteração nas atividades da AST, ALT e LDH e nos níveis séricos de ureia e acido úrico ($P > 0,05$).

**Tabela 1.** Parâmetros de toxicidade plasmática de camundongos tratados subcronicamente com **F-DPS**.

| | **AST (U/l)** | **ALT (U/l)** | **LDH (U/l)** | **Ureia (mg/dl)** | **Ácido Úrico (mg/dl)** |
|---|---|---|---|---|---|
| Veículo | 142.8 ± 26.8 | 52.4 ± 5.2 | 2262.3 ± 329.7 | 56.5 ± 5.7 | 6.5 ± 0.4 |
| **F-DPS** (1 Sem) | 154.0 ± 22.7 | 46.8 ± 3.0 | 2449.1 ± 279.4 | 42.5 ± 3.1 | 5.3 ± 0.5 |
| **F-DPS** (2 Sem) | 123.1 ± 14.1 | 50.5 ± 4.7 | 2115.3 ± 300.5 | 50.0 ± 5.1 | 5.7 ± 0.3 |

O composto foi diariamente administrado pela via intragástrica (i.g.) na dose de 0,1 mg/kg durante 1 ou 2 semanas. Os valores estão expressos como média ± erro padrão de 8 animais por grupo. Os dados foram analisados por ANOVA de uma via seguida pelo teste de Newman-Keuls.

## 4 Consideração final

Os dados deste experimento complementam os resultados apresentados nos Artigos 1 e 2 desta tese e permitem sugerir que o composto **F-DPS** apresenta ação do tipo antidepressiva em diferentes modelos de depressão em camundongos sem produzir efeitos tóxicos aparentes.