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

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INFLUÊNCIA BENÉFICA DA ESTIMULAÇÃO TÁTIL SOBRE DISTÚRBIOS DO MOVIMENTO E DANO OXIDATIVO CEREBRAL INDUZIDO POR HALOPERIDOL EM RATOS

Santa Maria, RS
2022

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Dissertação apresentada ao Curso do Programa de Pós-Graduação em Farmacologia, da Universidade Federal de Santa Maria (UFSM,RS), como requisito parcial para obtenção do título de Mestre em farmacologia.

Orientadora: Profª Drª Marilise Escobar Burger

Santa Maria, RS
20232

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

Rosa, Jéssica Leandra Oliveira da
INFLUÊNCIA BENÉFICA DA ESTIMULAÇÃO TÁTIL SOBRE DISTÚRBIOS DO MOVIMENTO E O DANO OXIDATIVO CEREBRAL INDUZIDO POR HALOPERIDOL EM RATOS / Jéssica Leandra Oliveira da Rosa.- 2023.
59 p.; 30 cm

Orientadora: Marilise Escobar Burger
Dissertação (mestrado) - Universidade Federal de Santa Maria, Centro de Ciências da Saúde, Programa de Pós Graduação em Farmacologia, RS, 2023

1. Discinesia tardia 2. Antipsicóticos clássicos 3. Tratamento não farmacológico 4. Manipulação sensorial I. Escobar Burger, Marilise II. Título.

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

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Jéssica Leandra Oliveira da Rosa

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Aprovado em 10 de fevereiro de 2023:

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Marilise Escobar Burger, Dra.
(Presidente/Orientador)

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Santa Maria, RS
2023

AGRADECIMENTOS

Agradeço primeiramente a Deus, pois sem sua influência em minha vida, certamente não teria chegado até aqui.

Agradeço aos meus pais, Alcindo Jader Costa da Rosa e Jussara Mendes de Oliveira, pois sempre me incentivaram e me apoiaram em tudo que precisei. A minha irmã Letícia Silva, pelo encorajamento para trilhar esse caminho.

Ao meu marido, Samuel Machado, por todo apoio e companheirismo, tanto nos momentos bons como nos difíceis. Obrigada por todo carinho e cuidado, sempre foi você que me manteve de pé e me fez ver que a vida tem muito a nos oferecer, agradeço pelo seu incansável apoio durante este período.

A minha orientadora Marilise Escobar Burger, pois me ajudou em tudo o que era necessário, pois este estudo se realizou graças ao seu empenho em ensinar e fazer. Obrigada pelos conhecimentos que me passaste, pelo apoio e paciência. Agradeço por me acolher em seu laboratório, sempre esteve disposta a ouvir e ensinar. Agradeço imensamente a oportunidade e apoio que me deste.

Aos colegas do grupo Farmatox, por todo conhecimento e experiência que passam para mim. E também em especial Hecson, Vínicia e Karine pela disposição em ajudar e contribuir com este trabalho.

E também a UFSM, por me proporcionar um ensino público e de qualidade.

RESUMO

INFLUÊNCIA BENÉFICA DA ESTIMULAÇÃO TÁTIL SOBRE DISTÚRBIOS DO MOVIMENTO E DANO OXIDATIVO CEREBRAL INDUZIDO POR HALOPERIDOL EM RATOS

AUTORA: Jéssica Leandra Oliveira da Rosa

ORIENTADORA: Profa Dra Marilise Escobar Burger

A esquizofrenia é uma doença que afeta cerca de 20 milhões de pessoas no mundo, constituindo um obstáculo à vida familiar, social e ao desempenho laboral, ocasionando assim, um sério problema de saúde pública, cuja fisiopatologia tem sido relacionada à hiperfunção dopaminérgica na região mesocórticolímbica. Fármacos antipsicóticos clássicos como o haloperidol (HAL), ainda fazem parte do arsenal terapêutico no tratamento da esquizofrenia, porém, o uso crônico destes fármacos promove sérios transtornos do movimento, podendo causar desde parkinsonismo e bradicinesia até uma condição irreversível e incapacitante, como a discinesia tardia (DT), os quais em conjunto constituem os conhecidos distúrbios extrapiramidais. Neste sentido, a prevenção e/ou a minimização dos distúrbios extrapiramidais consequentes ao tratamento com antipsicóticos clássicos são necessários. A estimulação tátil (ET) é uma forma de manipulação que têm mostrado respostas benéficas em diferentes situações neuropsiquiátricas, desde o período neonatal, como também na vida adulta. O objetivo deste estudo foi avaliar a possível influência benéfica da ET sobre os distúrbios extrapiramidais e marcadores de estresse oxidativo (EO) induzidos pelo HAL em ratos adultos. Os animais foram inicialmente designados em dois grupos, os quais receberam 4 doses de HAL (12 mg/kg/mL) ou solução veículo (controle) a cada 7 dias, durante 28 dias. A partir do 21º, imediatamente após a administração da última dose de HAL / veículo, metade dos animais de cada grupo foram submetidos ao protocolo de ET (15 min de estímulo, 3 vezes ao dia), durante os 9 dias subsequentes. Ao final do protocolo de ET, os animais foram submetidos a avaliações comportamentais de DO, catalepsia e teste locomoção em campo aberto. A ET minimizou o tempo de catalepsia e a frequência da DO, também revertendo à locomoção reduzida e o tempo de congelamento aumentado, induzidos pelo HAL. Ensaios bioquímicos realizados nas áreas extrapiramidais do encéfalo (estriado e substância negra) mostraram que a ET diminuiu marcadores de dano oxidativo em ambas as regiões. Esses achados indicam benefícios significativos da ET frente aos efeitos colaterais motores extrapiramidais consequentes ao tratamento neuroléptico.

Palavras-chave: Discinesia tardia. Catalepsia. Manipulação sensorial. Antipsicóticos clássicos. Tratamento não farmacológico.

RESUMO EM INGLÊS

BENEFICIAL INFLUENCE OF TACTILE STIMULATION ON MOVEMENT DISORDERS AND HALOPERIDOL-INDUCED BRAIN OXIDATIVE DAMAGE IN RATS

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ORIENTADORA: Profa Dra Marilise Escobar Burger

Schizophrenia is a disease that affects about 20 million people worldwide, constituting an obstacle to family and social life and to work performance, thus causing a serious public health problem, whose pathophysiology has been related to dopaminergic hyperfunction in the mesocorticolimbic region. Classic antipsychotic drugs such as haloperidol (HAL) are still part of the therapeutic arsenal in the treatment of schizophrenia, however, the use of drugs related to these drugs causes serious movement disorders, which can range from parkinsonism and bradykinesia to irreversible and disabling condition, such as tardive dyskinesia (TD), which together constitute extrapyramidal conflicts. In this sense, prevention and/or minimization of extrapyramidal disorders resulting from treatment with classic antipsychotics are necessary. Tactile stimulation (TS) is a form of therapy that has shown satisfactory responses in different neuropsychiatric situations, from the neonatal period, as well as in adult life. This study aimed to evaluate the possible tolerant influence of TS on extrapyramidal disorders and markers of oxidative stress (OS) induced by HAL in adult rats. The animals were initially allocated into two groups, which received 4 doses of HAL (12 mg/kg/mL) or vehicle solution (control) every 7 days, for 28 days. From day 21, immediately after administration of the last dose of HAL/vehicle, half of the animals in each group were submitted to the ET protocol (15 min of stimulation, 3 times a day), during the subsequent 9 days. At the end of the ET protocol, the animals were admitted to behavioral estimations of OD, catalepsy, and open field locomotion test. TS minimized catalepsy time and OD frequency, also reversing reduced locomotion and increased freezing time, induced by HAL. Biochemical assays carried out in the extrapyramidal areas of the brain (striatum and substantia nigra) found that TS absorbed markers of oxidative damage in both regions. These findings indicate prolonged effects of TS compared to extrapyramidal motor side effects resulting from neuroleptic treatment.

Palavras-chave: Tardive dyskinesia. Catalepsy. Sensory manipulation. Classic antipsychotics. Non-pharmacological treatment.

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LISTA DE ABREVIATURAS E SIGLAS

BDNF	Fator neurotrófico derivado do encéfalo
D1R	Receptor dopaminérgico do tipo 1
D2R	Receptor dopaminérgico do tipo 2
DAT	Transportador de dopamina
DO	Discinesia orofacial
DT	Discinesia tardia
EO	Estresse oxidativo
ER	Espécies reativas
ERN’s	Espécies reativas de nitrogênio
ERO’s	Espécies reativas de oxigênio
ET	Estimulação tátil
HAL	Haloperidol
MMV	Movimentos de mascar no vazio
PC	Proteína Carbonil
SN	Substância negra
TBARS	Substâncias reativas ao ácido tiobarbitúrico

SUMÁRIO

1. INTRODUÇÃO

A esquizofrenia é uma doença incapacitante que atinge cerca de 1% da população mundial (STĘPNICKI, KONDEJ, KACZO, 2018). A hipótese da hiperatividade dopaminérgica durante a doença levou ao desenvolvimento da primeira classe terapeutica de antipsicóticos (HILAL-DANDAN e BRUNTON, 2015). O uso de drogas antipsicóticas desta primeira geração, como o Haloperidol (HAL) está relacionado com o desenvolvimento de alguns efeitos adversos extrapiramidais como a discinesia tardia (DT), parkinsonismo, tremor, rigidez e comprometimento do equilíbrio postural (BEACH et al., 2020). Esses sintomas extrapiramidais podem ser incapacitantes, enquanto a prevenção ou reversão continua limitada (KRONBAUER et al., 2015; WIDSCHWENDTER, HOFER, 2019). Além disso, esses efeitos têm sido relacionados com a não aderência ao tratamento farmacológico (KRONBAUER et al., 2017; MORITZ et al., 2013).

A DT é considerada a manifestação mais grave desses sintomas, sendo caracterizada por movimentos involuntários e repetitivos, ocorrendo principalmente na região orofacial, incluindo os movimentos de mastigação, protusão de língua e atos de franzir o rosto e piscar os olhos (BASHIR, JANKOVIC, 2020; NOVICK et al., 2010). Uma das hipóteses que explicam seu desenvolvimento e irreversibilidade mais aceita é a da hipersensibilidade dopaminérgica, onde há um aumento na expressão dos receptores e também na sua sensibilidade a resposta do neurotransmissor (CADET et al., 1994; FREI, 2018), promovendo um aumento exacerbado na síntese e metabolismo da dopamina, levando a sua auto-oxidação, formando radicais livres que contribuem para o estresse oxidativo e resultando nos distúrbios extrapiramidais (BARCELOS et al., 2010; ALKADI, 2020; TREVIZOL et al., 2011).

Dessa forma, pesquisas em busca de novos avanços terapêuticos são muito importantes para a clínica. Alguns estudos de modelos animais já demonstraram prevenção e reversão parcial desses efeitos extrapiramidais com substancias naturais (ARTUKOGLU et al., 2020; BARCELOS et al., 2010, 2011; MACÊDO et al., 2011; PEROZA et al., 2013; TREVIZOL et al., 2011). Em vista disso, a estimulação tátil (ET) é uma forma positiva de manipulação, e já demonstrou ser capaz de prevenir os danos oxidativos gerados por administração de anfetamina e o estresse relacionado à sua abstinência (ANTONIAZZI et al., 2014). Além disso, traz benefícios ao cérebro como o aumento do fator neurotrófico derivado do encéfalo (BDNF), que está relacionado com

o refinamento das sinapses e com a plasticidade neuronal (KOWIAŃSKI et al., 2018; KUCZEWSKI, N. et al., 2009). Nesse sentido, é importante avaliar se a ET poderia reverter os efeitos adversos extrapiramidais induzidos pela administração de HAL, uma vez que o haloperidol é um fármaco muito utilizado na clínica (BEACH et al., 2020;).

1.2 OBJETIVO

1.2.1 **Objetivo Geral**

Avaliar a possível infuência benéfica da estimulação tátil sobre parâmetros comportamentais de discinesia orofacial (DO), catalepsia e atividade locomotora, acompanhados do perfil oxidativo bioquímico das áreas cerebrais envolvidas, em modelo animal de discinesia tardia induzida pelo haloperidol (HAL).

1.2.2 Objetivos Especificos

- Expor animais adultos ao protocolo de administração de HAL a fim de estabelecer os distúrbios extrapiramidais (DO e catalepsia);
-Submeter os animais ao protocolo da ET, monitorando os movimentos extrapiramidais durante esta exposição;
-Avaliar a possível influência da ET sobre os distúrbios extrapiramidais (DO e catalepsia), bem como sobre distúrbios do movimento (atividade locomotora e tempo de congelamento);
- Avaliar parâmetros oxidativos bioquímicos (peroxidação lipídica e carbonilação de proteínas) em áreas extrapiramidais (substância negra e estriado);

1.3 JUSTIFICATIVA

O haloperidol (HAL) é um fármaco muito utilizado no controle sintomático da esquizofrenia e outras psicoses, especialmente devido à sua alta eficácia, ampla disponibilidade e baixo custo (BEACH et al., 2020; PONTO, 2010). De fato, este fármaco destaca-se por sua alta potência antipsicótica (FROTA, 2001), apresentando também uma boa biodisponilidade oral, descrita em aproxidamente 75% (JAVAID,

1994). No entanto, seu uso crônico relaciona-se ao desenvolvimento de efeitos adversos de diferentes categorias, sendo os distúrbios do movimento, tais como as distonias, acatisia, parkinsonismo, rigidez muscular e a discinesia tardia, considerados os problemas mais graves da clínica psiquiátrica (BURGER et al. 2005; KRONBAUER, 2019). Dessa forma, é importante investigar novas estratégias não invasivas e de baixo custo, que possam contribuir para a minimização desses efeitos extrapiramidais associados ao tratamento neuroléptico. Contudo, a Estimulação Tátil (ET) já demonstrou ser benéfica frente à distúbios neuropsiquiátricos (ROVERSI, 2019), também prevenindo danos oxidativos em diferentes áreas cerebrais (ANTONIAZZI et al., 2014). Devido a isso, buscamos verificar se a ET é eficaz para atenuar ou reverter os efeitos nocivos motores induzidos pelo HAL.

2. REVISÃO BIBLIOGRÁFICA

2.1 ESQUIZOFRENIA

Esquizofrenia: Definição, Epidemiologia, Diagnóstico, Tratamento

A esquizofrenia é uma doença ou um transtorno mental crônico e grave, caracterizada por uma alteração entre as funções do pensamento, da afetividade e do comportamento (KRONBAUER, M. 2019). Afeta cerca de 24 milhões de pessoas no mundo, com incidência de 1 a cada 222 adultos (OMS, 2022). E está frequentemente associada a prejuízo em áreas pessoais, familiares, sociais, educacionais, ocupacionais e outras áreas importantes da vida, tornando-se um problema de saúde pública. A esquizofrenia é uma doença que possui sintomas divididos em positivos e negativos. Os primeiros são mais comuns no início da doença (LIEBERMAN, 1999), manifestados por alucionações, delírios, pensamento desorganizado, agitação e alterações psicomotoras, já os sintomas negativos são observados como pobreza de expressão, anedonia, falta de motivação, apatia e embotamento social (GONZALEZ-BURGOS; FISH; LEWIS, 2011). Ainda, esta doença possui sintomas cognitivos como déficits de memória operacional, velocidade de processamento e cognição social (HILAL-DANDAN e BRUNTON, 2015). O diagnóstico é feito seguindo o Manual Diagnóstico e Estatístico de Transtornos Mentais (DSM-IV-TR), no qual o profissional capacitado avalia a presença dos sintomas apresentados pelo paciente, duração, se apresenta disfunção social/ocupacional, entre outros fatores (TANDON et al., 2013).

O fenótipo clínico varia muito, dessa forma é difícil determinar a escolha do tratamento inicial, busca-se evitar os efeitos adversos com base nas características do fármaco (sedação, etc.) e do paciente (PELUSO et al., 2012). Todos os fármacos antipsicóticos comercialmente disponíveis reduzem a neurotransmissão dopaminérgica, no entanto, os medicamentos conhecidos como típicos ou fármacos de primeira geração/ clássicos possuem sua principal ação farmacológica bloqueando receptores de dopamina do tipo dois com alta taxa de ligação (STĘPNICKI; KONDEJ; KACZOR, 2018). Devido a sua forte ligação aos receptores dopaminérgicos e inespecifidade, podem provocar sintomas extrapiramidais, como tais como distonia, sintomas parkinsonianos (rigidez, bradicinesia, tremor), acatisia e também a síndrome da discinesia tardia (DT) (KRONBAUER et al., 2019).

A fim de reduzir os efeitos adversos provocados pelos antipsicóticos de primeira

geração, foram desenvolvidos os antipsicóticos de segunda geração, também chamados de atípicos. Estes possuem a capacidade de bloquear tanto os receptores da serotonina (5-HT) quanto os dopaminérgicos (HILAL-DANDAN e BRUNTON, 2015). Apesar de essa classe mostrar resultados mais significativos e satisfatórios em pacientes acometidos com os sintomas negativos da doença e menores efeitos extrapiramidais, são considerados desreguladores metabólicos, implicados com o desenvolvimento de diabetes melittus, pancreatite, ganho de peso, além de desencadear discrasias sanguíneas graves (CARBON et al., 2017). Esses efeitos adversos ocorrem devido a capacidade de ligação dessa classe farmacológica em outros receptores, como receptor tipo um de histamina (H1), receptor muscarínico da acetilcolina (mACh) e receptor alfa adrenérgico (α adr) (CARBON et al., 2018). Dessa forma, a escolha do fármaco pode ser um desafio, além disso, pelo fato de os efeitos do fármaco anticolinérgico poder agravar o delirium e a demência gerados pela esquizofrenia, os antipsicóticos típicos de alta potência (p. ex., haloperidol) ou agentes antipsicóticos atípicos com propriedades antimuscarínicas limitadas (p. ex., risperidona) são muitas vezes os fármacos de escolha (HILAL-DANDAN e BRUNTON, 2015).

Esquizofrenia: Fisiopatologia

A esquizofrenia é ainda uma doença com etiologia desconhecida e várias são as hipóteses que tentam explicar a gênese da doença (LIEBERMAN et al., 1998). Neste sentido, uma das hipóteses mais aceita e pesquisada é a que trata da hiperfunção dopaminérgica na região mesolímbica, mediada por receptores de dopamina do tipo D2, e uma hipofunção dopaminérgica na região cortical, mediada por receptores D1, levando aos sintomas positivos e negativos, respectivamente (DAVIS et al., 1991; KAPUR, 2009). Para fundamentar essa hipótese, foram utilizados antagonistas dopaminérgicos, os quais foram capazes de diminuir os sintomas da doença (CREESE; BURT; SNYDER, 1996). Howes (2013) demonstrou que a enzima que regula a taxa de síntese da dopamina, a tirosina hidroxilase, é aumentada de forma significativa na substancia negra (SN) de pacientes com esquizofrenia em comparação com pacientes saudáveis (HOWES et al., 2013), confirmando que há uma hiperprodução de dopamina no cérebro nos corpos neuronais dopaminérgicos durante a esquizofrenia.

Outra hipótese que vem sendo pesquisada atualmente é a do envolvimento do sistema glutamatérgico. Foi observado que indivíduos que tomaram as drogas

anestésicas fenciclidina e cetamina recreativamente eram propensos a desenvolver surtos psicóticos comuns na esquizofrenia como alucinações, delírios e os sintomas negativos da doença (JAUHAR, JOHNSTONE, MCKENNA, 2022; KRYSTAL et al., 1994). Ambos os fármacos possuem sua principal ação farmacológica bloqueando o receptor N-metil-D-aspartato (NMDA), uma das duas principais classes de receptores pós-sinápticos glutamatérgicos, levando ao conceito de uma hipofunção do glutamato na esquizofrenia (STONE et al., 2007).

Ainda, há outras hipóteses que vem sendo estudadas, como a hipótese da serotonina que propõe que uma hiperfunção serotoninérgica e de seu receptor 5-HT2A no córtex resulta em psicose (STAHL, 2016). Como exemplo disso, drogas psicodélicas como a dietilamida do ácido lisérgico (LSD) e psilocibina resultam em alucinações visuais e delírios e tem sua principal ação como agonistas do receptor de serotonina 5-HT2A (HOLLAND et al., 2014). Além disso, os antipsicóticos de segunda geração como a clozapina, considerados muito eficazes no tratamento da esquizofrenia, possuem sua principal ação como antagonistas dos receptores de sertonina, principalmente o 5-HT2A e uma ação farmacologica mais fraca nos receptores de dopamina (HILAL-DANDAN e BRUNTON, 2015). No entanto, é importante ressaltar que todas as teorias possuem vias interconectadas e é provável que mais de uma dessas vias estejam envolvidas na esquizofrenia (STAHL, 2018).

2.2 ESTRESSE OXIDATIVO E DEFESAS ANTIOXIDANTES

A oxidação é fundamental para o metabolismo celular do nosso organismo, no entanto, desencadeia a produção de radicais livres, podendo ocorrer de forma natural ou por uma disfunção biológica (BARREIROS et al., 2006). A mitocôndria é a principal fonte de produção de radicais livres, por meio da cadeia transportadora de elétrons, durante a produção de energia a partir de substratos metabólitos oxidáveis (SCHNEIDER e OLIVEIRA, 2004). Esses radicais livres possuem um elétron desemparelhado em sua órbita de valência (HALLIWEL e GUTERRIDGE, 1999).

Os principais produtos do metabolismo do oxigênio são os radicais superóxido ($O2^{-}$), hidroxila (OH^{-}) e, ainda, peróxido de hidrogênio (H_2O_2), esse processo acontece devido a reações específicas, catalisadas por enzimas e com a participação dos íons ferro e de cobre (ALA e MATSUBARA, 1997). Além disso, o oxigênio pode reagir com o óxido nítrico (NO) e gerar espécies reativas de nitrogênio (ERN) como o

peroxinitrito ($ONOO^-$), óxido nitroso (N_2O_3), ácido nitroso (HNO_2), nitritos (NO_2^-), nitratos ($NO3^-$) (SCHNEIDER e OLIVEIRA, 2004; BARREIROS et al., 2006). Esses produtos desencadeiam processos maléficos ao organismo como peroxidação lipídica de membrana, agressão às proteínas e DNA (BARREIROS et al., 2006). Bem como, estão relacionados com desordens neurológicas como o Alzheimer, esquizofrenia, esclerose lateral amiotrófica e discinesia tardia (MARKESBERY, 1997; COYLE e PUTFARCKEN, 1993; TSAI et al., 1998), entre outras.

Normalmente, esses radicais livres são combatidos pelo sistema antioxidante produzido pelo nosso organismo ou absorvidos na dieta (BARREIROS et al., 2006). O sistema antioxidante é dividido em enzimático e não- enzimático e a principal função dos dois é regenerar o substrato ou prevenir significativamente a sua oxidação (HALLIWELL et al., 2000). O sistema de defesa enzimático é composto pelas enzimas Superóxido Dismutase (SOD), Catalase (CAT) e Glutationa Peroxidase (GPx) e são capazes de impedir o acúmulo de espécies reativas (ALA e MATSUBARA, 1997). E o sistema de defesa não-enzimático é composto por antioxidantes de origem dietética 7 incluindo principalmente as vitaminas, minerais e compostos fenólicos (BIANCHI E ANTUNES, 1999).

Quando ocorre desequilíbrio entre o sistema oxidativo e a capacidade de defesa antioxidante do organismo é denominado de estresse oxidativo (EO) (JENNER e OLNAW, 1996). Neste sentido, conforme já mencionado anteriormente, o HAL é um fármaco com ação sobretudo nos sintomas positivos da esquizofrenia, mas seu uso pode levar à massiva geração de espécies reativas e consequentemente ao desenvolvimento de EO. Dessa maneira, estudos com tratamentos que visam reduzir o EO (principalmente cerebral), são desenvolvidos na tentativa de reduzir os efeitos da síndrome extrapiramidal induzida por antipsicóticos, como o HAL (KRONBAUER et al., 2017; BURGER et al., 2005; BARCELOS et al., 2010; BENVEGNÚ et al., 2012).

2.3 HALOPERIDOL (HAL)

O Haloperidol (HAL) é um fármaco antipsicótico e neuroléptico pertencente à classe das butirofenonas e dos antipsicóticos de primeira geração ou típicos, e se destaca por sua potência, especificidade e longa duração (fig 1) (FROTA, 2001; BENVEGNÚ, et al., 2012). É um fármaco muito utilizado na clínica no tratamento da esquizofrenia e em pacientes hospitalizados que possuem agitação e delírios, sendo eficaz na indução da

sedação (PONTO et al., 2010; TILLEMANS et al., 2021). O HAL possui sua principal ação farmacológica envolvendo o bloqueio dos receptores dopaminérgicos D2 na via mesolímbica, sendo um medicamento muito eficaz no controle dos sintomas positivos da esquizofrenia (fig 2) (CREESE et al., 1976). Porém, seu uso acaba comprometido por estar relacionado com o desenvolvimento de sintomas extrapiramidais, pois pode antagonizar outras vias dopaminérgicas conferindo um risco aumentado de efeitos colaterais extrapiramidais, tais como distonia, sintomas parkinsonianos (rigidez, bradicinesia, tremor), acatisia e também a síndrome da discinesia tardia (DT) (HEIMER, 2003).

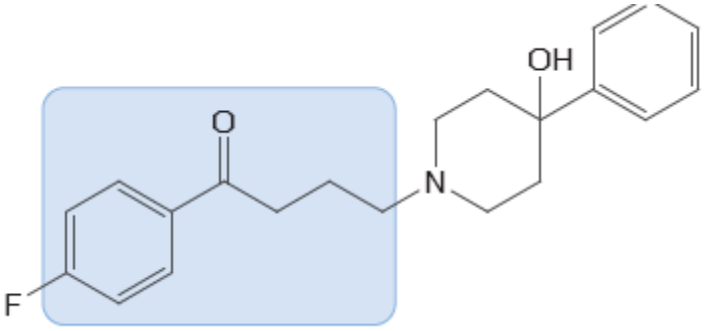

Figura 1 (Golan, 2012)

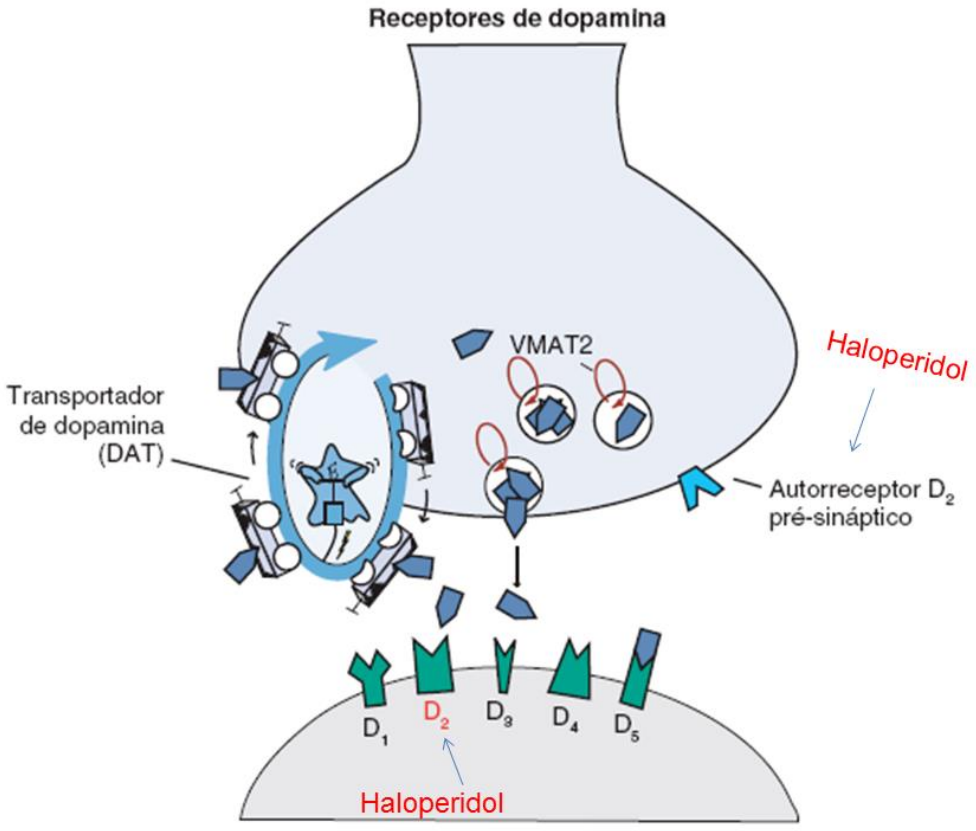

Figura 2: Mecanismo de ação do haloperidol (adaptado de Stahl, 2019), haloperidol

bloqueia o autoreceptor de dopamina D2 pré-sináptico que regula a liberação de dopamina pelo neurônio pré-sináptico e pós sináptico. O transportador de dopamina (DAT) é responsável pela eliminação do excesso de dopamina na sinapse, encontrado na região pré-sináptica. O transportador vesicular de monoaminas (VMAT2) capta a dopamina em vesículas sinápticas para neurotransmissão futura.

2.4 DISCINESIA TARDIA

A síndrome de discinesia tardia (DT) é caracterizada por movimentos involuntários e repetitivos da região orofacial, pescoço e tronco, podendo ser incapacitante e irreversível (CUNHA et al., 2016; JESTE; CALIGIURI, 2010; MARSÁLEK, 2000). É considerada a manifestação mais grave dos sintomas extrapiramidais, induzida pelo uso prolongado de medicamentos que atuam no sistema dopaminérgico (CUNHA et al., 2016) como os antipsicóticos de primeira geração, incluindo o HAL. A discinesia tardia tem sido estudada através de modelos animais de discinesia orofacial induzida por fármacos em roedores, observada através do desenvolvimento de movimentos de mascar no vazio (MMV), protrusão da língua e tremores faciais. A discinesia orofacial induzida por HAL é um modelo validado por diversas evidencias cientificas como uma representação adequada para o desenvolvimento de DT humana (BURGER et al., 2005; BARCELOS et al., 2010; KRONBAUER et al., 2019;2017; TREVIZOL et al., 2011 AN, HUI et al., 2016). Além disso, os animais deste modelo de estudo apresentam catalepsia, imobilidade provocada pela diminuição de dopamina na região nigro-estriatal.

Um dos mecanismos que leva ao desenvolvimento da DT pelo uso de fármacos antipsicóticos, como o HAL é o desenvolvimento de estresse oxidativo, levando à degeneração neuronal (CADET; KAHLER, 1994). A instalação de um quadro de EO, de acordo com a literatura, ocorre devido à administração de antipsicóticos de primeira geração, que promovem aumento na síntese de dopamina e em resposta disso, a monoamino-oxidase (MAO) aumenta o seu metabolismo (ZHU, 2004), formando o ácido 3,4-dihidroxifenilacetico (DOPAC), produção de peróxido de hidrogênio (H2O2) e dopamino-quinonas (NAPOLITANO; MANINI; D'ISCHIA, 2011) produzindo radicais livres. Os radicais livres formados e o aumento dos produtos de lipoperoxidação resultam em EO em diferentes regiões cerebrais (BARCELOS et al., 2010; TREVIZOL et al., 2011). Além disso, o bloqueio dopaminérgico, pelo HAL, leva ao aumento da liberação de glutamato, podendo causar excitotoxicidade e aumentar os

danos oxidativos (BURGER et al. 2005; TSAI et al. 1998). Ainda, o HAL sofre intensa metabolização pelo fígado (FORSMAN et al., 1977), e seu principal metabólito tóxico é o haloperidol piridinium (HPP+), o qual promove uma hepato e neurotoxicidade (BLOOMQUIST et al., 1993;1994; PARIKH et al., 2003).

2.5 ESTIMULAÇÃO TÁTIL (ET)

A ET é representada por uma forma de manipulação sensorial aplicada sobre a pele, e que imita o comportamento materno de lambedura e limpeza dos filhotes quando aplicado na fase neonatal (ROVERSI et al., 2020). Na fase adulta pode ser considerado como um contato de enriquecimento social, onde em humanos poderia ser mimetizado através da massagem, podendo promover o bem-estar e melhora de distúrbios psiquiátricos (LINDGREN et al., 2012; WEZE et al., 2007), dessa forma é uma importante ferramenta aplicada, realizada na direção céfalo-caudal do animal (Fig 3).

No desenvolvimento neonatal já demonstrou prevenir danos oxidativos no hipocampo, striatum e córtex cerebral gerados por administração de anfetamina (ANTONIAZZI et al., 2014), reduziu sintomas de ansiedade em modelo de estresse crônico e alterou respostas a drogas ansiolíticas (BOUFLEUR et al., 2012;2013) apresentou melhora em comportamentos depressivos e de ansiedade (FREITAS et al., 2015; RÍO-ÀLAMOS et al., 2015), melhora das funções cognitivas (DASKALAKIS et al., 2009; STAMATAKIS et al., 2008) da neurogênese hipocampal (DE LOS ANGELES et al., 2016), assim como melhora a neuroplasticidade (RICHARDS et al., 2012).

Em animais adultos foi capaz de prevenir lesão cortical (GIBB et al., 2010) e de aumentar os níveis de fator neurotrófico derivado do encéfalo (BDNF) e fator de crescimento fibroblástico básico (FGF_2) em modelo de doença de Parkinson (EFFENBERG et al., 2014; ROVERSI et al., 2019), além disso, previniu a recaída por anfetamina em ratos expostos ao paradigma de preferência de lugar condicionada (PLC) e reduziu parâmetros dopaminergicos elevados pela exposição a anfetamina (Rossato et al., 2022). A ET foi capaz de exercer um papel benéfico dos distúrbios psiquiatricos da vida adulta e um efeito positivo sobre a sinalização do eixo hipotálamo-pituitáriaadrenal (HPA) (ROVERSI et al., 2019).

Figura 3 (Rossato, 2022).

Devido a isso, a ET é considerada uma intervenção enriquecedora (DASKALAKIS et al., 2009). No entanto, seu mecanismo de ação ainda não está bem esclarecido, porém já foi demonstrado que devido ao seu envolvimento com o eixo HPA, a ET reduz a liberação de hormônio adrenocorticotrófico e corticosterona (ANTONIAZZI et al., 2017; FREITAS et al., 2015), e aumenta os níveis de receptores glicocorticoides cerebrais (ANTONIAZZI et al., 2017) gerando a uma boa adaptação do animal frente a situações estressantes ao longo da vida (LEVINE, 1957; 2001; 2002).

3. DESENVOLVIMENTO

3.1 MANUSCRITO CIENTÍFICO

Os resultados inseridos nessa dissertação apresentam-se sob a forma de manuscrito científico, o qual se encontra aqui estruturado. Os itens Materiais e Métodos, Resultados, Discussão e Referências encontram-se no mesmo formato em que o manuscrito foi submetido para publicação.

Tactile stimulation reverses haloperidol-induced movement disturbances, reducing oxidative damages in the nigrostriatal brain area of adult rats: a pilot study

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Abstract

Movement disorders such as parkinsonism, akathisia, and tardive dyskinesia are commonly related to typical antipsychotics treatment, such as haloperidol (HAL), whose mechanism involves excitotoxicity and oxidative damage in extrapyramidal brain areas. Tactile stimulation (TS) has been helpful in neonatal neuropsychiatric conditions, awakening our interest in evaluating its possible beneficits on movement disturbances HAL-induced in adult rats. Subsequently to sub-chronic HAL administration (12mg/kg, i.m) and orofacial dyskinesia (OD) development, male rats were exposed to TS protocol (15-min session, 3 times a day, for 9 days). TS reversed both OD and catalepsy time HAL-induced, reestablishing partially crossing- and rearing-number, as also freezing time. Furthermore, TS exposed rats did not show oxidative damages in striatum or substantia nigra. Given these outcomes, we can consider TS as a promissory procedure to control and/or reduce movement disorders consequent to antipsychotic treatment, which can be serious and extremely disabling. Based on our current and previous studies about the beneficial influence of the TS on different neuropsychiatric conditions, and subsequently to additional clinical studies, in an innovative form, we propose the inclusion of the TS as a complementary and integrative practice in health, which could constitute a valuable contribution together with the conventional medicine.

Keywords: Tardive dyskinesia; Extrapyramidal side effects; Orofacial dyskinesia; Handling; Typical antipsychotic.

Highlights

- Haloperidol (HAL) treatment induced orofacial dyskinesia and motor disorders;
- HAL increased oxidative damages in both striatum and *substantia nigra;*
- Tactile stimulation (TS) reversed movement disturbances in adult rats;
- TS exposed rats did not show oxidative damages in extrapyramidal brain areas;

Introduction

Dystonias, akathisia, parkinsonism, and tardive dyskinesia (TD) are serious side effects consequent to typical antipsychotic treatment, often compromising adherence to treatment (DIBONAVENTURA et al., 2012; WIDSCHWENDTER, HOFER, 2019). The most serious situation is TD, which is characterized by involuntary, stereotyped, and repetitive movements in different body parts, frequently irreversible and disabling (AMERICAN PSYCHIATRIC ASSOCIATION, 2013; KRONBAUER et al., 2019). Although second-generation antipsychotics present a lower risk for the development of TD, their use is not excused from extrapyramidal syndrome (EPS), which encourages the continuity of studies in search of prevention and treatment for this serious neuropsychiatric condition (DIVAC et al., 2014; CORPONI et al., 2019). Despite being poorly understood, the pathophysiology of TD has been related to the oversensitivity of striatal postsynaptic dopamine receptors (TURRONE et al., 2003; FREI, 2018). Furthermore, prolonged blockade of dopamine D2 receptors in the basal ganglia favors dopamine metabolism, thus increasing the generation of reactive species and consequently leading to neurodegeneration (CREED; NOBREGA, 2013; BURGER et al., 2005a). In recent years, our research group has explored innovative tools to elucidate the pathophysiology of the TD (BURGER et. al., 2005a; 2005b; TEIXEIRA et al., 2009; BARCELOS et al., 2011; KRONBAUER et al., 2015) as also prevent and/or alleviate movement disturbances following typical antipsychotic treatment (TEIXEIRA et. al., 2011; BARCELOS et al., 2010; BENVEGNÚ et al., 2012; KRONBAUER et al., 2017; 2019;). In this sense, new approaches also include integrative therapies, which constitute alternative treatments widely recommended by the World Health Organization (WHO). Generally, such therapies present low cost and elevated efficacy, especially if they are introduced together with conventional medicines to prevent or

reduce different diseases, including the chronic extrapyramidal symptoms resulting from antipsychotic treatment. In this context, neonatal tactile stimulation (TS) has shown benefits in different animal models involving HPA-induced behavioral and neurological sensitization (Lovic et al., 2006). In this sense, TS is a craniosacral procedure that has shown interesting benefits in pre-clinical studies carried out in neonates (Antoniazzi et. al., 2014a, 2014b) by our research group. More recently, our studies were also disseminated to adulthood, when we observed the beneficial influence of the TS on different neuropsychiatric disorders, including depression (Roversi et al., 2019) and prevention of relapse in amphetamine drug addiction (Rossato et al., 2022). Considering these economic and no invasive benefits conferred by TS, we decided to evaluate the possible beneficial influences of the TS on the neurobiochemical and behavioral modifications related to extrapyramidal side effects haloperidol-induced in adult animals.

Methods

Animals

For this experimental design, we used male Wistar rats about 3 months old (weighing 250-320g), which were maintained in Plexiglas cages (groups of three animals) with free access to food (standard chow) and water in a room with controlled temperature (22-23°C) and 12h-light/dark cycle with lights on at 7:00 a.m. Animals received standard chow ad libitum (PuroTrato®, RS, Brazil) with adequate levels of nutrients, following recommendations from the National Research Council (NRC, 1995), during all experiments. The experimental protocol was approved by the Animal Ethics Committee (Universidade Federal de Santa Maria – UFSM, under the number 2359150517), which is affiliated with the Council of Animal Experiments (CONCEA), following international norms of animal care and maintenance.

Drugs

Haloperidol decanoate (Haloperidol decanoate - Janssen-Cilag) was dissolved in Tween 80® and diluted to a final concentration of 1% Tween 80® with distilled water. The vehicle was a 1% Tween 80® suspension.

Experimental procedure

Twenty-four rats were randomly designated to two groups of twelve animals each (n=12) and injected with haloperidol solution (H group- 12 mg/Kg/mL; i.m.) or vehicle (C group), once a week, for 4 weeks (days 1, 7, 14 and 21). Twenty-one days after the first H/ vehicle administration, orofacial dyskinesia (OD) development was quantified (basal). Subsequently, H and C groups received the last haloperidol/vehicle administration. On this same day, one-half of each experimental group was submitted to TS protocol (15-min session, 3 times a day, for 9 days) or not (unhandled; UH),

resulting in four experimental groups: i) vehicle/UH (n=6); ii) vehicle/TS (n=6); iii) H/UH (n=6); iv) H/TS (n=6). On the subsequent day (30 days after the first H/vehicle administration), OD, catalepsy time, and locomotor performance were quantified. On the next day, 24h after behavioral evaluations, all animals were anesthetized and euthanized. Brains were immediately removed and cut coronally at the caudal border of the olfactory tubercle. Striatum and SN were dissected according to Paxinos and Watson (WATSON; PAXINOS, 2012), and homogenized in 10 volumes (w/v) of 10mM Tris–HCl buffer (pH7.4) to determine protein carbonyl (PC) and lipid peroxidation (LP) levels (Figure 1).

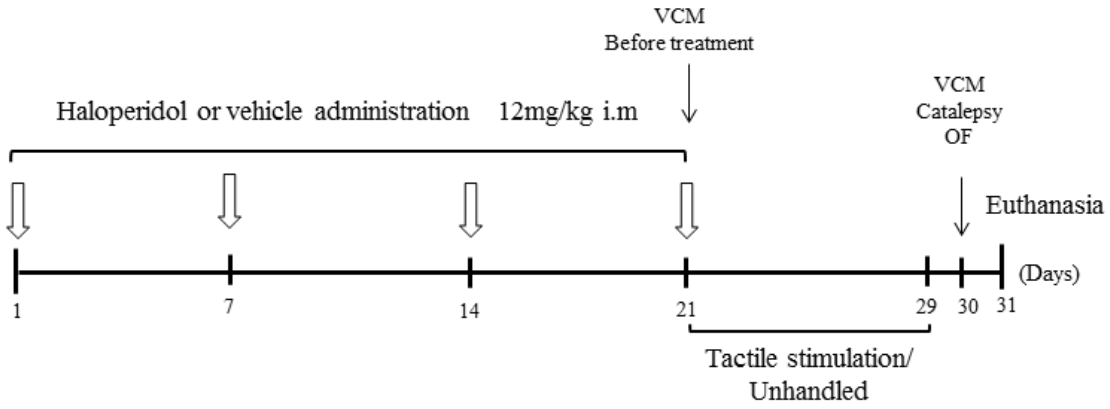

Figure 1. Experimental design. Rats were injected with HAL (12 mg/Kg/mL; i.m) or vehicle once a week, for 4 weeks (days 1, 7, 14, and 21). For basal behavioral evaluation, OD was quantified before TS protocol (15 min, 3 times a day, for 9 days). Subsequently, animals were evaluated in OD again, when catalepsy time and locomotor performance were also evaluated. Animals were euthanized for biochemical assessments in both striatum and SN.
Abbreviations: Haloperidol, HAL; orofacial dyskinesia, OD; tactile stimulation, TS; *substantia nigra* (SN); vacuos movement chewing, VCM; open field, OF.

Tactile Stimulation (TS)

TS protocol was started on day 21 after the first haloperidol injection, subsequently to OD behavioral observation. The adult rats (vehicle/TS and H/TS groups) were removed from the home cage and stimulated manually from the head to the beginning of the tail

individually on the experimenter's lap for 15 min. Animals were exposed to this procedure 3 times per day between 09:00 a.m. and 05:00 p.m., returning to their home cages after each protocol, which was performed for 9 days. This procedure was based on previous TS protocols, also performed in adult rats (Roversi et al., 2019; Rossato et al., 2022).

Behavioral assessments

Orofacial dyskinesia (OD)

For this behavioral observation, rats were placed individually in cages (20x20x19 cm) containing one mirror under the floor and one behind the back wall of the cage to allow the quantification when the animal was facing away from the observer. To quantify the occurrence of OD, the frequency of vacuous chewing movements (VCM) was recorded for two sets of 5 min with intervals of 5 min, totaling 10 min of observation. VCM were referred to as single mouth opening in the vertical plane not directed towards physical material (BURGER et al., 2005a). Observers were blind to the drug treatment. In a preliminary study (using 5 control and 10 haloperidol-injected rats) of inter-rater reliability, we found that the use of this method of observation and parameter definition usually results in >91% agreement between the three different observers. This task was adapted from previous studies (KRONBAUER et al., 2019; 2017; BARCELOS et al., 2010).

Catalepsy time

Catalepsy was measured subsequently after each OD observation on haloperidol-injected rats (H-unhandled rats (UH) and H-TS), using a wire grid (25 X 30 cm2) inclined 45° relative to the bench top. Each rat was individually placed on the inclined grid and observed for 60 s, with its forepaws near the edge of the grid and the amount

of time spent in this atypical position (motionless) was recorded three times, with a 5-min interval between them. At the end of the three replications, the mean time spent by the rat without moving was calculated for each test (adapted from ROCHA et al., 1997).

Open-field (OF) task

Animals' locomotor, exploratory and freezing performances were quantified in the OF paradigm. In this test, rats were individually placed in the center of an area (40 × 40 × 30 cm) divided into nine quadrants (KERR et al., 2005). The number of square crossings (horizontal squares crossed), rearings (vertical movements), and freezing time were quantified for 5 min (KABUKI et al., 2009). The apparatus was cleaned with a 5% alcohol solution after each animal was tested.

Neurobiochemical assays

Protein carbonyl (PC) levels quantification

PC levels were quantified by the method of Levine (LEVINE et al., 1990), with some modifications. Soluble protein was mixed with 2,4-dinitrophenylhydrazine (DNPH; 10 mM in 2 M HCl) or HCl (2 M) and incubated at room temperature for 1 h. Denaturing buffer (150 mM sodium phosphate buffer, pH 6.8, with 3% sodium dodecyl sulfate), ethanol (99.8%), and hexane (99.5%) were added, mixed by shaking, and centrifuged. The protein isolated from the interface was washed twice with ethyl acetate/ethanol 1:1 (v/v) and suspended in denaturing buffer. Each DNPH sample was read at 370 nm in a spectrophotometer against the corresponding HCl sample (blank). The data were expressed as % of control.

Lipid peroxidation levels estimation by thiobarbituric acid reactive species (TBARS)

TBARS assay measures lipid peroxidation (LP) which occurs by excessive ROS

generation. LP was estimated through the pink chromogen produced by the reaction of thiobarbituric acid (TBA) with malondialdehyde (MDA) at 100 ◦C, measured spectrophotometrically at 535 nm. In plasma, TBARS was estimated by the method described by Lapenna et al. (2001). Results were expressed as % of control.

Statistical analysis

Student's T-test was used in the basal assessment of OD (vehicle and H groups), whose behavioral observation was performed on the 21st day after the first haloperidol administration. From this day, when the TS protocol was already initiated, two-way ANOVA was applied (2 (vehicle/H) × 2 (UH/TS)) on the behavioral (OD, crossing, rearing, and freezing) obtained on the 30th day after the first haloperidol administration (following 9 days of TS protocol), as also on the neurobiochemical findings (PC and LP), which were followed by Duncan's post hoc test. Catalepsy time was analyzed by Student's T-test on the 30th experimental day (only H groups; one independent variable (UH/TS)).

Results

Influence of tactile stimulation (TS) on the reversal of sub-chronic haloperidol (HAL)-induced orofacial dyskinesia (OD) and catalepsy development

Following 21 days after the first HAL administration, basal OD was quantified. Student's T-test showed that HAL-injected rats presented increased VCM (P<0,0000), so indicating OD development, as expected (Fig 2A).

Two-way ANOVA of VCM revealed a significant main effect of HAL [F(1,20)=104.21; P<0.000], ET [F(1,20)=8.60; P=0.008], and a significant interaction between HAL and ET [F(1,20)=8.90; P=0.007]. Considering unhandled (UH) animals, Duncan's test showed that sub-chronic administration of haloperidol (HAL) increased VCM frequency in relation to the control group. Between HAL-injected groups, TS exposure decreased VCM frequency in relation to UH group. This decrease is

considered partial since the VCM frequency observed in HAL-TS was higher than those values quantified in the control-TS group (Fig. 2B).

Comparisons between HAL-injected groups, Student's T-tests showed that the TS was able to decrease 56% of the catalepsy time in relation to UH group (Fig 2C).

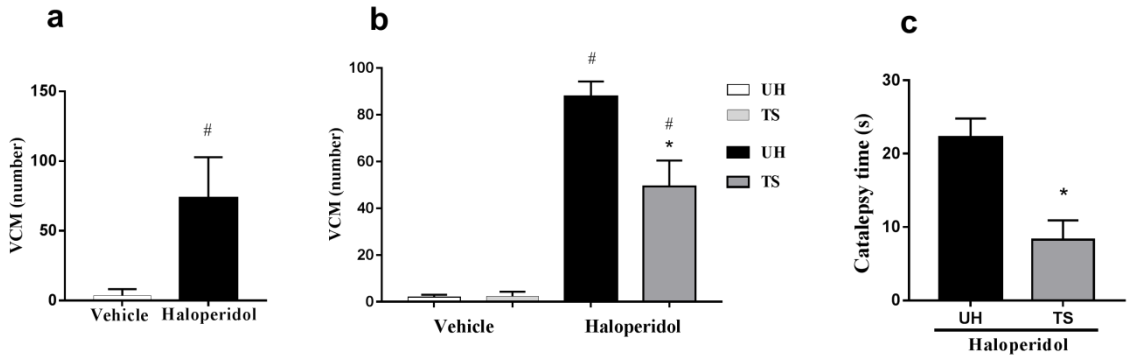

Figure 2: Orofacial dyskinesia (OD) HAL induced (12mg/kg/mL, i.m, once a week, for four weeks) observed by the VCM number before (basal) TS exposure (A). OD quantification following three doses of HAL, when TS protocol (15 min, 3 times a day for 9 days) was started (B). Catalepsy time following four doses of HAL together with TS exposure (15 min, 3 times a day for 9 days) (C). Data are expressed as mean±S.E.M. Statistical differences were considered when $p<0.05$.* Indicates significant diference from the HAL-UH group. # Indicates significant diference from its respective control group.

Abbreviations: Orofacial dyskinesia, OD; haloperidol, HAL; VCM, vacuous chewing movements; TS, tactile stimulation; UH, unhandled.

Influence of tactile stimulation (TS) on the reversal of sub-chronic haloperidol (HAL)-induced decreased crossing number and rearing frequency and increased freezing time development in the open-field (OF) task

Two-way ANOVA of open-field task revealed a significant main effect of HAL [$F(1,20)=162.58$ and 96.05; $P<0.000$] for crossing number and rearing frequency, respectively, as also a significant main effect of HAL, TS, and a significant HAL x TS interaction ([$F(1,20)=33.47$; $P<0.000$], 6.51; $P=0.018$ and 9,23; $P=0.006$, respectively]

for freezing time.

Considering unhandled animals, the post-hoc test showed that sub-chronic HAL decreased crossing number, rearing frequency, and freezing time in relation to the control group (Fig. 3). Comparisons between HAL-injected animals showed that TS exposure partially reversed the decreasing of crossing number (Fig. 3A), recovering the increasing of freezing time (Fig.3C). TS exposure was not able to recover the increased frequency of rearing HAL-induced, whose values were comparable (Fig.3B).

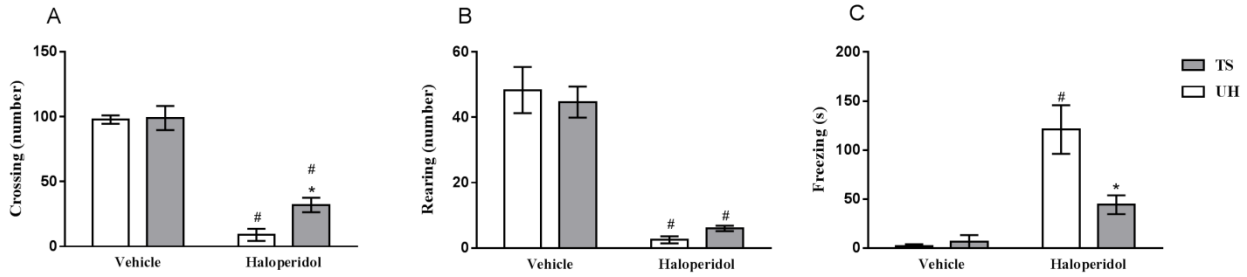

Figure 3. Quantification of crossing- (A) and rearing-frequency (B) and freezing time (C) in adult animals, which were exposed to HAL administration (12mg/kg/mL, i.m, once a week, for four weeks) and submitted to TS (15 min, 3 times a day for 9 days). Data are expressed as mean±S.E.M. Statistical differences were considered when $p<0.05$.* Indicates significant diference from the HAL-UH group. # Indicates significant diference from its respective control group.

Abbreviations: Haloperidol, HAL; tactile stimulation, TS; UH, unhandled.

Influence of tactile stimulation (TS) on increased levels of protein carbonyl (PC) and lipid peroxidation (LP) haloperidol (HAL)-induced in both striatum and *substantia nigra* (SN)

Two-way ANOVA of biochemical measurements in the striatum revealed a significant main effect of HAL [$F(1,20)=19.44$, $P<0.000$ and 5.17; $P<0.05$] for PC and LP levels, respectively, a significant main effect of TS [$F(1,20)= 31.73$ and 25.44; $P<0.000$) for PC and LP, respectively, and a significant HAL x TS interaction ([$F(1,20)=53.62$, $P<0.000$] for PC levels. Two-way ANOVA of biochemical measurements in the SN

revealed a significant main effect of HAL [F(1,20)=58,24; P<0.000] for PC levels, as also a significant main effect of TS [F(1,20)=47,65; P<0.000) for LP and a significant HAL x TS interaction ([F(1,20)=9.88; P=0.0059 and 7.50; P=0.012] for PC and LP, respectively.

Haloperidol sub-chronic administration increased PC and LP levels in both striatum and SN (Fig 4). When animals were exposed to TS following HAL administrations, PC and LP levels were completely reversed in the striatum (Fig 4A and 4B). In SN, the TS was able to reverse LP levels in part, while the levels of PC were completely recovered, whose value was comparable to the control group (Fig. 4C and 4D).

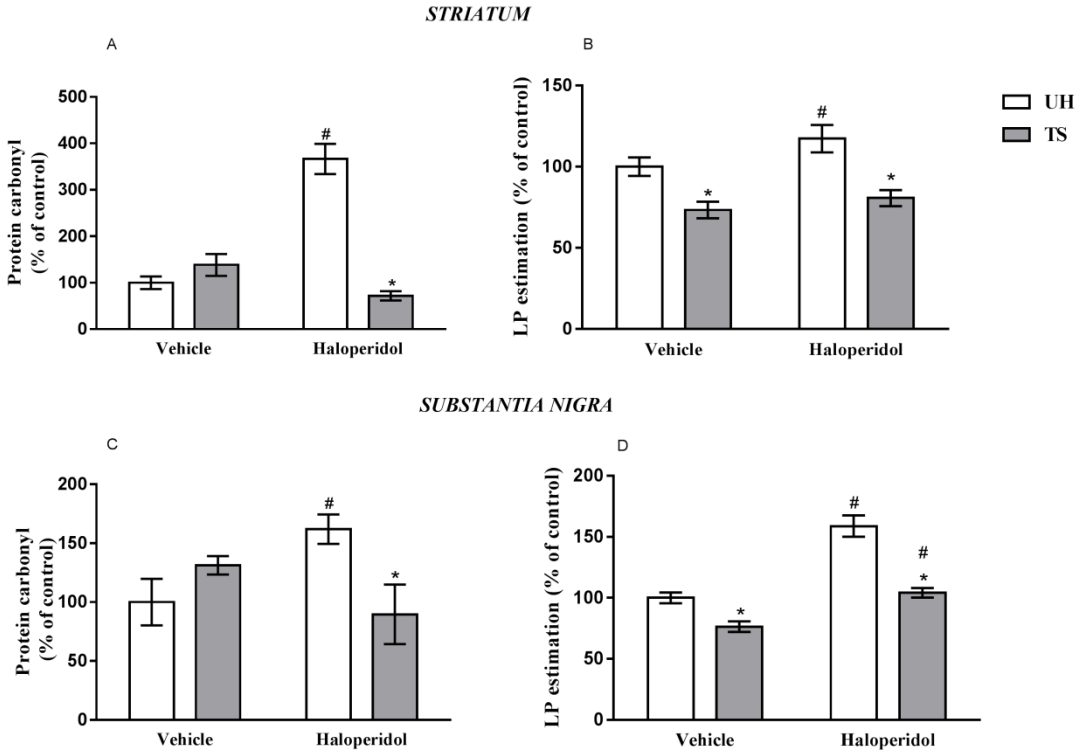

Figure 4. Quantification of PC (A) and LP (B) in the *striatum* and in *substantia nigra* (SN) (C and D, respectively) of adult rats previously exposed to HAL (12mg/kg/mL, i.m, once a week, for four weeks) and S (15 min, 3 times a day for 9 days). Data are expressed as mean±S.E.M. Statistical differences were considered when p<0.05.* Indicates significant diference from UH group. # Indicates significant diference from its respective control group.

Abbreviations: Haloperidol, HAL; UH, unhandled; TS, tactile stimulation. PC, protein carbonyl; LP, lipid peroxidation.

Linear correlations among behavioral modifications and oxidative damage biochemical markers, considering both haloperidol chronic administration and TS exposure

In the striatum, PC and LP levels showed a positive correlation with OD (r^2=0.36, p=0.0025 and r^2=0.32, p=0.0036, respectively) and with freezing time (r^2=0.52, p=0.0000 and r^2=0.20, p=0.027, respectively); as also a negative correlation with crossing number (r^2=0.36, p=0.0023 and r^2=0.17, p=0.045, respectively) (Table 1).

In the SN, LP levels showed a positive correlation with OD and freezing time (r^2=0.61, p=0.0000 and r^2=0.52, p=0.0000, respectively); as also a negative correlation with crossing and rearing number (r^2=0.54, p=0.0000 and r^2=0.37, p=0.0014, respectively) (Table 1).

Table 1. Multiple regression between OD, freezing, and crossing behavior and biochemical parameters, which were estimated in both striatum and *substantia nigra* of rats injected with HAL and subsequently exposed to TS.

	Analysis	OD		Freezing		Crossing	
		r^2	P value	r^2	P value	r^2	P value
Striatum	LP	0.32	0.0036	0.20	0,0278	0.16	0.045
	PC	0.36	0.0025	0.52	0,00009	0.36	0.00239
SN	LP	0.61	0.000006	0.51	0.00007	0.54	0.000035
	PC	NC		NC		NC	

NC: no significant correlation; LP: Lipid peroxidation; PC: Protein carbonil; OD: orofacial dyskinesia.

Discussion

Onward of the current study we intended to evaluate the influence of adulthood TS on the reversion of haloperidol-induced movement disturbances, also assessing the protective properties of this handling on the oxidative status of extrapyramidal brain

areas. Extrapyramidal side-effects are a common consequence of the typical antipsychotic treatment, whose severity may include tardive dyskinesia (TD) that presents as irreversible and bad prognosis (ANDREASSEN; JØRGENSEN, 2000; MORITZ et al., 2013). The pathophysiology of the TD is poorly understood, however, events such as excitotoxicity and oxidative stress have been deeply correlated (FREI, 2018; PEROZA et al., 2013; TEIXEIRA et al., 2011; TREVIZOL et al., 2011).

Our motivation for the current study was the observation that despite the innovation and evolution of atypical antipsychotics, the classical or typical ones remain under medical prescription, especially due to their high efficacy and low cost (BEACH et al., 2020). Furthermore, atypical antipsychotics also have been related to extrapyramidal syndrome development, even if to a lesser extent (CARPONI et al 2019). Whereas typical antipsychotics continue to be widely used in psychiatric practice with a high frequency of severe motor disorders, the current study was developed, presenting the following findings: i) whereas haloperidol increased VCM frequency as expected, TS exposure was able to partially reverse this OD; ii) TS exposure decreased haloperidol-induced catalepsy; iii) haloperidol decreased crossing and rearing number and increased freezing time, TS partially restored these movement disturbances. In this sense, DO, catalepsy, and movement disturbances according to our previous studies (KRONBAUER et al., 2019), whose development is primarily related to haloperidol-induced extrapyramidal disorders development (ABDEL-SALAM et al., 2012), given that in this brain area occurs an imbalance in the production of reactive species that can lead to oxidative damages (BASHIR, JANKOVIC, 2020) of difficult-to-resolve.

It's well known that haloperidol blocks pre-synaptic D2R, in opposition to the negative feedback of DA release, increasing DA synthesis and metabolism (CREESE et

al., 1996). Literature data have shown that an increased DA metabolism is a precursor of DA-quinones, resulting in reactive species (RS) generation (BUSANELLO et al., 2017). Furthermore, haloperidol also blocks post-synaptic striatal D2R, affecting the inhibitory control of DA. This blockade activates glutamate neurotransmission in NMDA receptors, generating neurotoxicity in the nigrostriatal system (NAIDU; KULKARNI, 2001; BURGER et al., 2005). These mechanisms may be related to the development of movement disturbances that were observed in our current findings.

Considering the neurobiochemical markers of oxidative damage, our outcomes showed that sub-chronic haloperidol was related to oxidative damage in the extrapyramidal brain areas, as observed through increasing protein carbonyl (PC) and lipid peroxidation (LP) levels in both striatum and SN. Interestingly, only nine consecutive days of TS exposure were able to restore the physiological levels of PC and LP in the striatum. TS also was able to restore the normal levels of PC, partially reversing LP levels in the SN. Considering previous studious from our research group, neonatal TS increased plasma levels of vitamin C and catalase activity (ANTONIAZZI et al., 2014a; 2014b), indicating being able to exert an important protective role on the antioxidant defense system against oxidative damage, which is related to the development of different pathologies, besides dyskinesias and movement disturbances haloperidol-induced.

Considering the limitations of the present study, it is important to admit that initially, we believed that the TS applied in adulthood animals would not be able to reverse haloperidol-induced movement disorders, since the imbalance of the nigrostriatal system is, in fact, extremely deep, difficult to revert, even with muscarinic blockers such as biperiden or trihexyphenidyl (BERGMAN; SOARES-WEISE, 2018). Thus, we began a pilot study, with no intention of publishing the findings. Surprisingly,

we realized the potency of TS protocol, even when it was applied to already adult animals with developed movement disorders. In this sense, no biochemical or molecular analysis could be programmed and executed, and therefore, any mechanistic hypothesis for the beneficial actions of TS can only be speculatively proposed, based on previous studies by us and other laboratories, as follows: i) TS was able to increase brain derived neurotrophic factor (BDNF) and glial cell line-derived neurotrophic factor (GDNF) (EFFENBERG et al., 2014; ROVERSI et al., 2019); in fact, these proteins are important for neuron survival and neurogenesis which contribute to a higher neural plasticity (RICHARDS et al., 2012); ii) TS exerted a positive influence on the HPA axis signaling by increasing glucocorticoid receptor (GR) (ROVERSI et al., 2019), thus affording a better response against stressful situations (DE LOS ANGELES et al., 2016); iii) TS was able to reverse the expression of dopaminergic receptor D1 and dopamine transporter (DAT) increase by amphetamine exposure (ROSSATO et al., 2022); showing that TS as well as environmental stimulation may be useful for treatment of brain disorders associated with dopaminergic imbalances (THOMAS et al., 2015).

Interestingly, even without presenting biochemical outcomes related to antioxidant defenses and/or molecular markers involving the DA cascade and neurotrophins, our preliminary findings showed interesting correlations with behavioral observations. So, LP levels in both *striatum* and SN showed a positive correlation with OD and freezing time indicating an interesting causal relationship. Furthermore, negative correlations between LP and PC levels in the striatum, as well as between PC levels in SN, were observed with crossing numbers in the open field task. In the same brain area, PC levels also showed a positive correlation with freezing time and OD. Taken together, our findings reinforce that the increased levels of LP and PC haloperidol-induced were directly related to movement damages observed in the adult

animals, whose frequency and intensity was significantly assuaged by TS exposure, so important and meaningful for extrapyramidal side effects including the tardive dyskinesia.

Based on the present study, we have a strong intention to continue studies involving the molecular mechanism of TS, in this and other animal models of movement disorders, including an animal model of Parkinson's disease.

Conclusion

In conclusion, here we are showing for the first time the influence of TS on haloperidol-induced OD, catalepsy, and locomotor behavior, as well, TS was able to attenuate haloperidol-induced oxidative damage through LP and PC levels. Studies with TS showed its protective effect against drug addiction in neonatal animals and adulthood (ANTONIAZZI et al., 2014; ROVERSI et al., 2020; ROSSATO et al., 2022) and against anxiety-like behavior (BOUFLEUR et al., 2012), this study is innovative and contributes to the beginning of new preventative approaches.

Funding Information

This study was supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001. The funding had no further role in study design; in the collection, analysis, and data interpretation; in the report’s writing, or in the decision to submit the paper for publication. The authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil) and Universidade Federal de Santa Maria (UFSM/PRPGP/PROAP).

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Neuroscience Research

Tactile stimulation reverses haloperidol-induced movement disturbances, reducing oxidative damages in the nigrostriatal brain area of adult rats: a pilot study

--Manuscript Draft--

Manuscript Number:	
Article Type:	Research Paper
Section/Category:	Sensory and Motor Systems
Keywords:	Tardive dyskinesia; Extrapyramidal side effects; Orofacial dyskinesia; Handling; Typical antipsychotic.
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4. CONSIDERAÇÕES FINAIS

A partir dos resultados apresentados no manuscrito científico, o qual compõe a presente dissertação, demonstramos a influência benéfica da estimulação tátil aplicada nos animais adultos, sobre distúrbios do movimento, exercendo também um papel protetor frente a danos oxidativos provocados pela exposição ao haloperidol.

Através dos resultados experimentais obtidos, chegamos às seguintes conclusões:

- A ET reverteu parâmetros de discinesia orofacial induzida pelo HAL;
- A ET reverteu a menor a atividade locomotora e o aumentado tempo de congelamento, induzidos pelo HAL;
- A ET reduziu os danos oxidativos na SN e estriado, induzidos pelo HAL

ANEXO I – CERTIFICADO DE APROVAÇÃO DA COMISSÃO DE ÉTICA NO USO DE ANIMAIS DA UNIVERSIDADE FEDERAL DE SANTA MARIA

Universidade Federal de Santa Maria

Comissão de Ética no Uso de Animais

CERTIFICADO

Certificamos que a proposta intitulada "A INFLUÊNCIA DA ESTIMULAÇÃO TÁTIL EM DIFERENTES PERÍODOS DE DESENVOLVIMENTO SOBRE MODELOANIMAL DE FIBROMIALGIA", protocolada sob o CEUA nº 2359150517 (ID 001545), sob a responsabilidade de **Marilise Escobar Bürger** *e equipe; Karine Roversi; Hecson Jesser Segat; Vinícia Garzella Metz; Domenika Rubert Rossato; Luisa Pagliarini Weber* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **APROVADA** pela Comissão de Ética no Uso de Animais da Universidade Federal de Santa Maria (CEUA/UFSM) na reunião de 12/07/2017.

We certify that the proposal "INFLUENCE OF TACTILE STIMULATION ON DIFFERENT PERIODS OF DEVELOPMENT ON ANIMAL FIBROMYALGIA MODEL", utilizing 162 Heterogenics rats (64 males and 98 females), protocol number CEUA 2359150517 (ID 001545), under the responsibility of **Marilise Escobar Bürger** *and team; Karine Roversi; Hecson Jesser Segat; Vinícia Garzella Metz; Domenika Rubert Rossato; Luisa Pagliarini Weber* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **APPROVED** by the Ethic Committee on Animal Use of the Federal University of Santa Maria (CEUA/UFSM) in the meeting of 07/12/2017.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 07/2017 a 12/2020 Área: Farmacologia

Origem:	Biotério Central UFSM			
Espécie:	Ratos heterogênicos	sexo: Fêmeas	idade: 60 a 90 dias	Quantidade: 34
Linhagem:	Wistar		Peso: 300 a 400 g	
Origem:	Biotério Central UFSM			
Espécie:	Ratos heterogênicos	sexo: Machos	idade: 35 a 45 dias	Quantidade: 64
Linhagem:	Wistar		Peso: 150 a 190 g	
Origem:	Biotério Central UFSM			
Espécie:	Ratos heterogênicos	sexo: Fêmeas	idade: 35 a 45 dias	Quantidade: 64
Linhagem:	Wistar		Peso: 140 a 180 g	

Santa Maria, 17 de novembro de 2022

Dra. Patrícia Bräunig
Presidente da Comissão de Ética no Uso de Animais
Universidade Federal de Santa Maria

Profa. Dra. Vania Lucia Loro
Vice-Presidente da Comissão de Ética no Uso de Animais
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