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

Karine Roversi

INFLUÊNCIA BENÉFICA DA ESTIMULAÇÃO TÁTIL EM MODELOS EXPERIMENTAIS DE DEPRESSÃO: PARÂMETROS NEUROQUÍMICOS E COMPORTAMENTAIS EM RATOS

Santa Maria, RS
2020

Karine Roversi

INFLUÊNCIA BENÉFICA DA ESTIMULAÇÃO TÁTIL EM MODELOS EXPERIMENTAIS DE DEPRESSÃO: PARÂMETROS NEUROQUÍMICOS E COMPORTAMENTAIS EM RATOS

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

Orientadora: Profª Drª. Marilise Escobar Bürger
Coorientadora: Drª Caren de David Antoniazzi

Santa Maria, RS
2020

Roversi, Karine
INFLUÊNCIA BENÉFICA DA ESTIMULAÇÃO TÁTIL EM MODELOS EXPERIMENTAIS DE DEPRESSÃO: PARÂMETROS NEUROQUÍMICOS E COMPORTAMENTAIS EM RATOS / Karine Roversi.- 2020.
107 p.; 30 cm

Orientadora: Marilise Burger
Coorientadora: Caren Antoniazzi
Tese (doutorado) - Universidade Federal de Santa Maria, Centro de Ciências da Saúde, Programa de Pós Graduação em Farmacologia, RS, 2020

1. Depressão 2. Manuseio 3. Neurogênese 4. Serotonina 5. Eixo HPA I. Burger, Marilise II. Antoniazzi, Caren III. Titulo.

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.

Karine Roversi

INFLUÊNCIA BENÉFICA DA ESTIMULAÇÃO TÁTIL EM MODELOS EXPERIMENTAIS DE DEPRESSÃO: PARÂMETROS NEUROQUÍMICOS E COMPORTAMENTAIS EM RATOS

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

Aprovada em **20 de outubro de 2020**:

Marilise Escobar Bürger, Dra.
(Presidente/Orientadora)

Caren Tatiane de David Antoniazzi, Dra.
(Coorientadora)

César Augusto Bruning, Dr. (UFPEL)

Hecson Jesser Segat, Dr. (UFSM)

Lucielli Savegnago, Dra. (UFPEL)

Maribel Antonello Rubin, Dra. (UFSM)

Santa Maria, RS
2020

DEDICATÓRIA

Esta tese é dedicada ao meu pai Adecir.

AGRADECIMENTOS

Ao meu pai, por apoiar minhas decisões, por não medir esforços para me proporcionar a melhor educação e por sempre acreditar em mim. À minha madrasta e minhas irmãs, Marina, Mariana, Karoline, Francini por todo apoio e em especial à Katiane, que sempre esteve presente como uma mãe, me ajudando e me amparando em todos os momentos.

À minha eterna mãe, que já não está mais aqui presente, mas sei que estará sempre comigo, me guiando e protegendo.

À minha incrível orientadora Professora Marilise, por me acolher nestes quase 10 anos de laboratório FARMATOX. Pelas conversas, conselhos e exemplos, não só da vida profissional, mas também pessoal. Obrigada por ter acreditado em mim quando nem eu acreditava, e por não ter poupado nenhum esforço para eu ter chegado até aqui e ser quem eu sou hoje. Serei eternamente grata.

À minha amiga e coorientadora Caren, por aceitar embarcar nessa jornada comigo e estar sempre disponível para me aconselhar, me acalmar e clarear minha mente quando mais precisei. Agradeço por toda a tua dedicação e carinho em me ajudar a atingir meus objetivos e chegar até aqui.

Aos meus colegas do laboratório FARMATOX, em especial aos que não foram só colegas, mas sim grandes amigos, Vinícia e Hecson. Obrigada pelos momentos divertidos de trabalho, de descontração, e risadas que compartilhamos todos esses longos anos. Sentirei muitas saudades.

Aos meus amigos Daiane, Jade, Patrícia, Tamiris e Wilian que de alguma forma fizeram parte dessa jornada e conseguiram fazer todo esse caminho ser mais leve e tranquilo. A todos os meus amigos do “sanduíche”, Louise, Mariana, Nina, Renata, Rafael e Zsofi por fazerem meu ano de sanduíche incrivelmente especial e maravilhoso.

A professora Judith, pela oportunidade e por me receber com tanto carinho Donders Insitute for Brain, Cognition and Behaviour

Às agências de fomento FAPERGS, PROAP-UFSM, bem como CAPES CNPq pelas bolsas de estudos concedidas. À Universidade Federal de Santa Ma pela oportunidade da realização da pós-graduação.

Enfim, agradeço a todas as pessoas que de alguma forma me auxiliariam e me incentivaram para esta realização profissional.

RESUMO

INFLUÊNCIA BENÉFICA DA ESTIMULAÇÃO TÁTIL EM MODELOS EXPERIMENTAIS DE DEPRESSÃO: PARÂMETROS NEUROQUÍMICOS E COMPORTAMENTAIS EM RATOS

AUTORA: Karine Roversi
ORIENTADORA: Profª. Drª. Marilise Escobar Burger
COORIENTADORA: Drª Caren Tatiane de David Antoniazzi

A depressão é um transtorno mental que afeta milhões de pessoas em todo o mundo, e embora diferentes fármacos sejam utilizados para tratar a doença, a eficácia destes tratamentos ainda apresenta limitações. A estimulação tátil (ET) é uma terapia manual que pode ser aplicada desde o nascimento até a vida adulta, e evidências experimentais têm mostrado seus benefícios em diferentes modelos animais. O objetivo dos estudos que compõem esta tese foi avaliar a influência da ET aplicada durante o período neonatal ou na vida adulta de ratos expostos à diferentes modelos de depressão. O protocolo experimental 1 avaliou a influência da ET na idade adulta sobre comportamentos tipo depressivos em ratas. As fêmeas receberam administração de reserpina (1mg/kg/dia, s.c. 3 dias) para a indução de sinais de depressão. O fármaco antidepressivo imipramina (10mg/kg, i.p.) foi utilizado como controle positivo. Após a última administração de reserpina, o protocolo de ET foi realizado (15min/3xdia, 8 dias). Subsequentemente à eutanásia, biomarcadores de estresse foram quantificados no sangue, enquanto a expressão de BDNF, proBDNF, TrkB, GDNF, GFAP e GR foram quantificados através de eletroforese em gel em amostras do córtex pré-frontal (CPF). Os resultados mostraram que a ET reverteu os comportamentos tipo depressivo induzidos pela reserpina, reduzindo também os níveis plasmáticos de corticosterona e do hormônio adrenocorticotrófico, juntamente com menor peso das adrenais. No CPF, a ET aumentou a expressão do BDNF, TrkB, GFAP e GR, e reduziu os níveis de proBDNF. No segundo protocolo experimental avaliamos a influência da ET neonatal (10min/1xdia, 8 dias) aplicada em ratos heterozigotos para o transportador de serotonina ($SERT^{+/-}$) a fim de determinar a influência deste manuseio sobre o desenvolvimento de comportamentos social, ansiedade e anedonia. Após a eutanásia, a amígdala basolateral foi coletada e a expressão de BDNF e suas isoformas, transportador vesicular de glutamato (VGLUT) e do GABA (VGAT), enzima GAD67, GR e MR, assim como genes responsivos aos glicocorticoides foram mensurados por RT-PCR. Observamos que em animais $SERT^{+/-}$, a ET melhorou os comportamentos social e afetivos. Em nível molecular, somente a ET *per se* mostrou alterações na amígdala basolateral, na qual reduziu os níveis de BDNF e das isoformas IV e VI, a razão dos receptores GR/MR, dos genes responsivos aos glicocorticoides, e a razão VGLUT/VGAT, enquanto que aumentou os níveis da enzima GAD67. Por fim, uma revisão sistemática foi realizada para avaliar o impacto da ET em ratos. Após busca em um banco de dados, 55 estudos foram selecionados dentro dos critérios exigidos. De modo geral, observamos que a ET exerceu influências benéficas em diferentes comportamentos, modificando principalmente, comportamentos relacionados à cognição e à emoção. Além disto, alterações periféricas, observadas principalmente através dos níveis séricos de corticosterona como também sobre o SNC, sugerindo que a aplicação da ET constitui um protocolo de manipulação não invasivo e eficaz para melhorar funções neurobiológicas dos animais. A partir dos resultados obtidos nos protocolos experimentais descritos nesta tese, observavamos que a ET exerceu influência favorável sobre comportamentos do tipo depressivo e de ansiedade. Estes resultados confirmam nossa hipótese inicial sobre os benefícios da ET na redução da hiperatividade do eixo hipotalâmico-pituitária-adrenal, minimizando assim a liberação de corticosterona, e favorecendo a ativação de fatores neurotróficos e de outros sistemas relacionados à emocionalidade. Por fim, a revisão sistemática corroborou com os resultados experimentais apresentados, os quais em conjunto, confirmam os benefícios da ET em diferentes períodos da vida sobre aspectos neuroquímico-moleculares e comportamentais relacionados a diferentes transtornos psiquiátricos, tais como ansiedade e depressão.

Palavras-chave: manuseio, neurogênese, serotonina, eixo hipotálamo-pituitária-adrenal.

ABSTRACT

BENEFICIAL INFLUENCE OF TACTILE STIMULATION IN EXPERIMENTAL MODELS OF DEPRESSION: NEUROCHEMICAL AND BEHAVIORAL PARAMETERS IN RATS

AUTHOR: Karine Roversi
ADVISOR: Profª. Drª. Marilise Escobar Bürger
CO-ADVISOR: Drª Caren Tatiane de David Antoniazzi

Depression is a common disease worldwide and although there are several drugs for the treatment of depression, the effectiveness of this pharmacotherapy still presents limitations, possibly due to the difficulty of understanding its pathophysiology. Tactile stimulation (TS) is a manual therapy applied both in early and in adult life. Studies have shown the benefits of TS in rodents in models of psychiatric disorders, including anxiety and addiction. In this study, our objective was to evaluate the influence of TS applied in the neonatal period or adult life in different animal models of depression. The first experiment of this study evaluated the possible positive response of TS on the reversion of depression-like behaviors in adulthood after reserpine administration. Adult female *Wistar* rats received reserpine once daily for three consecutive days (1mg/kg s.c.). The antidepressant imipramine (10mg/kg i.p.) was used as a positive control. Immediately after the reserpine last administration, the TS protocol started (15min/3xday, 8 days). Afterward, depression behavioral tests were performed and then the animals were euthanized for blood collection, for analysis of corticosterone and adrenocorticotrophic hormone (ACTH) levels in plasma, besides of and prefrontal cortex removal for quantification of BDNF, proBDNF, TrkB, GDNF, GFAP and glucocorticoid receptor (GR) immunoreactivity analyzed by western blotting. We observed that TS reverse the depression-like behaviors induced by reserpine. Besides, our results showed that TS reduced plasma levels of corticosterone and ACTH, and reduced adrenal weight, parameters which were increased by reserpine administration. In the PFC, TS increased BDNF, TrkB, GFAP, and GR immunoreactivity and reduced proBDNF levels. The second experiment of this study aimed to evaluate the effect of neonatal TS (10min/1xday, 8 days) on heterozygous serotonin transporter rats ($SERT^{+/-}$) and evaluate anxiety and depression-like behaviors in adulthood. After the behavioral analyzes the animals were euthanized, and the basolateral amygdala was removed for the expression of BDNF and its isoforms, glutamatergic and gabaergic components as well as glucocorticoid (GR) and mineralocorticoid (MR) receptors and glucocorticoid responsive-genes. We observed that TS improved the anxiety, depressive-like and social behaviors in $SERT^{+/-}$ rats. In the molecular analyzes, only TS *per se* showed modifications in in the basolateral amygdala, which decreased BDNF and its isoforms IV and VI, the ratio of GR/MR, the glucocorticoid responsive-genes, as well as the ratio of VGLUT/VGAT, while TS increased the GAD67 levels. As a last section of the thesis, a systematic review was performed to evaluate the impact of TS in rats. A total of 55 studies were identified and we observed that TS in rats showed beneficial influences on behaviors, mainly on emotional behaviors. Also, the effects on the peripheric and central nervous system were observed, in which TS improved important stress markers, such as corticosterone levels and also improved neuroplasticity markers, hypothesizing that TS is an effective tool for the improvement of neurobiological and behavioral response in rats. Considering the results found in this thesis, we can conclude that TS exerts a positive influence on anxiety and depression-like behaviors, confirming the hypothesis that these beneficial effects are associated with HPA axis and neuroplasticity. Besides, the systematic review confirmed these hypotheses of the beneficial effects of TS in rats, especially those observed on neuropsychiatric disorders, such as depression and anxiety.

Keywords: handling, neurogenesis, serotonin, hypothalamic-pituitary-adrenal axis.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

5-HTT	Transportador de serotonina
5-HTTLPR	Polimorfismo do 5-HTT
ADT	Antidepressivo tricíclico
Akt	Proteína quinase B
AMPC	3´5´-adenosina-monofosfato-cíclico
BDNF	Fator neurotrófico derivado do encéfalo
ET	Estimulação tátil
FKBP5	Proteína ligante de FK506 de 51 kDa
GABA	Ácido gama amino-butírico
GADD45β	Growth arrest and DNA damage inducible beta
GDNF	Fator neurotrófico derivado da glia
GFAP	Proteína glial fibrilar ácida
GR	Receptor glicocorticoide
HPA	Hipotálamo-pituitária-adrenal
IMAO	Inibidores da MAO
ISRS	Inibidores Seletivos da Recaptação de Serotonina
MAO	Enzima monoaminaoxidase
MAPK	Proteína quinase ativada por mitógeno
MR	Receptor mineralocorticoide
NMDA	Receptor N-metil-D-aspartato
PKC	Proteína quinase C
SERT	Transportador de serotonina
SNC	Sistema Nervoso Central
TrkB	Receptor tropomiosina quinase B
VGAT	Transportador vesicular de GABA
VGLUT	Transportador vesicular de glutamato
VMAT	Transportador vesicular de monoaminas

SUMÁRIO

APRESENTAÇÃO

Esta tese está estruturada em seções dispostas da seguinte forma: Introdução, Desenvolvimento, Produção Científica, Discussão, Conclusões e Referências. Nos itens **INTRODUÇÃO** e **DESENVOLVIMENTO** encontram-se considerações iniciais sobre o tema desenvolvido e os objetivos desta tese.

Os itens Materiais e Métodos, Resultados, Discussão e Referências encontram-se inseridos como **ARTIGO CIENTÍFICO I, ARTIGO CIENTÍFICO II,** e **MANUSCRITO I** na seção **PRODUÇÃO CIENTÍFICA** e representam a íntegra deste estudo.

Ao fim, encontram-se os itens **DISCUSSÃO** e **CONCLUSÕES**, nos quais há interpretações e comentários gerais referentes aos resultados contidos neste estudo. As **REFERÊNCIAS** referem-se somente às citações apresentadas no item **INTRODUÇÃO**, **DESENVOLVIMENTO, DISCUSSÃO** e **CONCLUSÕES**.

1 INTRODUÇÃO

Os transtornos de humor e de ansiedade estão entre as situações mentais mais comuns que ocorrem em todo o mundo (WORLD HEALTH ORGANIZATION, 2017). Experiências traumáticas como estresse, abusos, violência estão associadas com um maior risco de desenvolvimento de psicopatologias, incluindo depressão e ansiedade, tanto nos períodos iniciais da vida quanto na fase adulta (KINDERMAN et al., 2013). A depressão é a principal causa de incapacidade, contribuindo para a carga global de doenças (WORLD HEALTH ORGANIZATION, 2020), cujos sintomas são caracterizados por alteração do humor, perda de interesse ou prazer, sentimento de culpa e fracasso, desesperança e incapacidade de concentração (AMERICAN PSYCHIATRIC ASSOCIATION, 2017; WORLD HEALTH ORGANIZATION, 2020), com maior vulnerabilidade para mulheres desenvolverem a doença (WORLD HEALTH ORGANIZATION, 2018). Existem diversas hipóteses acerca da etiologia da depressão, sendo que a mais aceita relaciona-se ao desequilíbrio de aminas biogênicas, em que a disfunção nas vias da serotonina e noradrenalina seriam a principal causa dos sintomas depressivos (SCHILDKRAUT, 1965). Outros estudos ainda sugerem que o desenvolvimento da depressão pode estar associado à diminuição da neurogênese (DUMAN; HENINGER; NESTLER, 1997) e a hiperatividade do eixo hipotalâmico-pituitária-adrenal (HPA) (BLACKBURN-MUNRO; BLACKBURN-MUNRO, 2001), quando os elevados níveis de glicocorticoides, principalmente no córtex e hipocampo, levariam a uma maior predisposição ao distúrbio (PARIANTE; LIGHTMAN, 2008). O tratamento convencional mais utilizado para tratar esses sintomas de depressão e ansiedade envolve o uso de antidepressivos da classe dos Inibidores Seletivos da Recaptação de Serotonina (ISRS), antidepressivos tricíclicos e inibidores da monoamina oxidase A e B, entre outros (BRUNTON, 2012). Contudo, o uso dessas classes de medicamentos não tem se mostrado totalmente eficaz e a busca por novas terapias torna-se de extrema importância para o tratamento desta enfermidade.

Condições ambientais são capazes de alterar tanto a estrutura cerebral quanto comportamentos associados a transtornos neuropsiquiátricos, como medo, ansiedade e depressão. Em modelos animais, a estimulação tátil (ET) é uma forma positiva de manipulação, que independentemente do período de aplicação, já demonstrou ser capaz de exercer influências benéficas em roedores (ANTONIAZZI

et al., 2017). Diversos estudos mostram os efeitos da ET neonatal, onde se observou melhora nas funções cognitivas (DASKALALASKIS et al., 2009; RAZA et al., 2015), desenvolvimento neuronal mais acelerado (SCHAPIRO; VUKOVICH, 1970) e diminuição dos níveis de corticosterona, associado ao aumento de receptores glicocorticoides (GR, do inglês glucocorticoid receptor) no hipocampo em resposta ao estresse (LIU et al., 1997; MEANEY et al., 1991; PANAGIOTARAPOULOS et al., 2004). Além disso, não somente efeitos da ET neonatal, mas também os efeitos da ET realizada em animais na idade adulta já foram observados, tais como recuperação de dano cerebral, aumento de níveis de neurotrofinas e de dopamina em um modelo de doença de Parkinson (GIBB et al., 2010; EFFENBERG et al., 2014). O possível mecanismo da ET estaria relacionado a uma alteração na regulação do eixo HPA (JUTAPAKDEEGUL et al., 2003) e aumento na expressão de neurotrofinas, como o fator neurotrófico derivado do encéfalo (BDNF, do inglês brain-derived neurotrophic factor), o qual aumenta o comprimento dos dendritos cerebrais, levando a uma melhora da plasticidade neural (GIBB et al., 2010).

A seguir, está apresentado um breve referencial teórico sobre as hipóteses relacionadas à depressão, além de modelos animais desenvolvidos para o estudo destes transtornos neuropsiquiátricos, investigados nesta tese. Será também considerada a influência da ET em roedores, e a importância da utilização deste manuseio na composição desta tese.

2 DESENVOLVIMENTO

2.1 A DEPRESSÃO

A depressão é uma doença psiquiátrica muito comum em todo o mundo, e está entre as doenças mais debilitantes, cujo desenvolvimento afeta o indivíduo como um todo, apresentando efeitos psicobiológicos, sociais e econômicos (WITTCHEN et al., 2011; WORLD HEALTH ORGANIZATION, 2020). Geralmente caracterizada por alteração do humor e perda de interesse ou prazer, a depressão também pode levar a sentimento de culpa e fracasso, desesperança, incapacidade de concentração e muitas vezes acompanhada de sintomas de ansiedade (AMERICAN PSYCHIATRIC ASSOCIATION, 2017; WORLD HEALTH ORGANIZATION, 2020). Dados epidemiológicos apontam que a depressão afeta mais de 264 milhões de pessoas em todo o mundo, tornando-se a longo prazo, um sério problema de saúde (WORLD HEALTH ORGANIZATION, 2020). Considera-se ainda que a depressão seja o principal contribuinte para o desenvolvimento de outras doenças, entre elas doenças cardíacas e acidente vascular cerebral (WHOOLEY; WONG, 2013), além de aumentar o risco de suicídio, visto que cerca de 800 mil pessoas por ano cometem suicídio em decorrência da depressão.

Com índices progressivos no número de pacientes apresentando a doença, interessantemente a depressão é duas vezes mais frequente em mulheres do que em homens, com prevalência anual global de 5,5% e 3,2%, respectivamente (ALBERT, 2015; WORLD HEALTH ORGANIZATION, 2018). Evidências sugerem que essa diferença seja principalmente devido a fatores biológicos, incluindo hormônios, maior vulnerabilidade ao estresse ambiental e o próprio estilo de vida das mulheres poderiam favorecer essa suscetibilidade à depressão (KRISHNAN et al., 2010; ALBERT, 2015). O tratamento para este transtorno abrange terapia não-farmacológica, que inclui terapia cognitivo-comportamental e exercício físico (PARK et al., 2013), mas geralmente estão associadas à terapia farmacológica. Este tratamento consiste na utilização de fármacos que favorecem a melhora dos sintomas, porém evidências apontam que apenas cerca de um terço dos pacientes não respondem ao tratamento (GARTLEHNER et al., 2011; AL-HARBI, 2012). A discrepância nas respostas ao tratamento pode ser resultado direto da

heterogeneidade etiológica da depressão (KEERS, UHER, 2012) já que a base fisiopatológica ainda não é compreendida totalmente.

2.2 A FISIOPATOLOGIA DA DEPRESSÃO

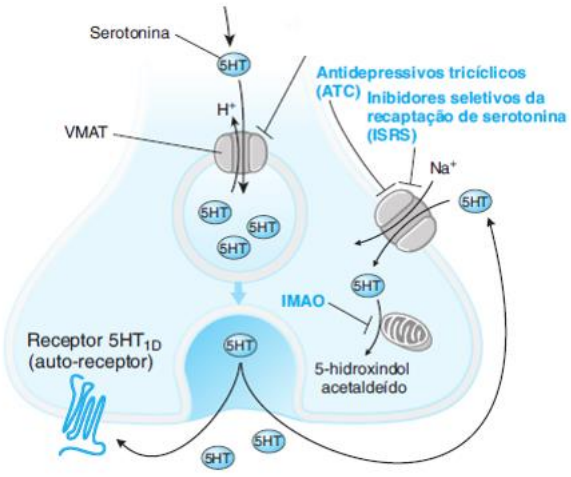
Devido a heterogeneidade tanto clínica quanto etiológica da depressão, tem sido difícil elucidar sua fisiopatologia. Embora existam diferentes teorias que explicam os contextos da doença, nenhuma delas são totalmente abrangentes e conclusivas. Atualmente, os mecanismos reconhecidos que ajudam a explicar a fisiopatologia da depressão incluem, a hipótese monoaminérgica, a desregulação do eixo HPA e a neurogênese. Outros possíveis contribuintes são alterações no sistema glutamatérgico, gabaérgico, aumento da secreção de citocinas inflamatórias e anormalidades da glia. Compreender a fisiopatologia da depressão se torna muito desafiador já que os sinais e sintomas desta doença envolvem múltiplos e interligados mecanismos e não conseguem ser explicados em uma única hipótese.

2.2.1 A hipótese monoaminérgica da depressão

A teoria monoaminérgica foi a primeira teoria que tentou explicar a fisiopatologia da depressão e sugere que este distúrbio poderia ser causado por uma deficiência funcional das monoaminas, dopamina, noradrenalina e serotonina, no sistema nervoso central (SNC) (SCHILDKRAUT, 1965). Esses neurotransmissores encontram-se distribuídos em diversas regiões do SNC envolvidas na regulação de uma ampla gama de funções, incluindo humor, atenção, sono, apetite, entre outras (BELMAKER; AGAM, 2008). Essa teoria surgiu na década de 50 após um aumento na procura por tratamentos psiquiátricos devido ao fim da segunda guerra mundial (QUEVEDO; SILVA, 2013). Acidentalmente, o psiquiatra Ronald Kuhn descobriu a ação antidepressiva da imipramina, molécula com propriedades anti-histamínicas, anticolinérgicas e sedativas. Kuhn observou que pacientes em tratamento com reserpina, medicamento utilizado na época por seus efeitos anti-hipertensivos, apresentavam sintomas semelhantes a depressão (GARDNER; BOLES, 2011) e o tratamento com imipramina revertia esse quadro de sintomas, possivelmente por inibir a recaptação de noradrenalina, aumentando os níveis cerebrais desse neurotransmissor (HERTING; AXELROD; WHITBY, 1961).

Assim, desenvolveu-se a classe dos antidepressivos tricíclicos (ADTs), a qual logo incluiu novas moléculas, como amitriptilina e desipramina, que apresentavam diferentes propriedades farmacológicas (BOSSONG, 2008). Também na década de 50, o pesquisador Ernst Abert Zeller analisou os efeitos antidepressivos da iproniazida, um fármaco tuberculostático. Após observar que esse fármaco inibia a atividade da enzima monoaminoxidase (MAO) e com isso apresentava capacidade de aumentar os níveis cerebrais de serotonina e melhorar o humor, surgiu o primeiro grupo medicamentoso de ação antidepressiva, os inibidores da MAO (IMAO). Assim como a rápida expansão para o tratamento de transtornos do humor, essa classe teve seu declínio muito rápido também, principalmente por seus efeitos colaterais, o que acabou resultando na ascensão da classe dos ADTs (LOPEZ; ALAMOS, 2009). Entre as décadas de 60 e 70, outros pesquisadores postularam a hipótese de que dentre as monoaminas, a desregulação da serotonina na fenda sináptica seria a principal causa bioquímica dos sintomas da depressão (Figura 1) (LAPIN; OXENKRUG, 1969).

Figura 1- Local de ação dos antidepressivos tricíclicos e inibidores seletivos da recaptação de serotonina no neurônio serotonérgico



Fonte: (GOLAN, 2009, p. 194).

Essa hipótese originou uma crescente busca por moléculas com ação seletiva para a serotonina e em 1974, com o auxílio de indústrias farmacêuticas,

desenvolveu-se a fluoxetina, o primeiro fármaco da classe dos inibidores seletivos da recaptação da serotonina (ISRS). Essa classe revolucionou o tratamento dos distúrbios do humor, uma vez que apresentam menos efeitos colaterais e melhor tolerabilidade quando comparado com os IMAOs e ADTs. Logo a classe dos ISRSs tornou-se a mais prescrita em todo o mundo (STAHL, 2014).

Evidências que apoiam essa teoria baseiam-se em estudos demonstrando que pacientes com dieta restrita de triptofano, precursor da serotonina, e tirosina, precursor da noradrenalina, apresentam humor deprimido (MILLER et al., 1996). Também, uma menor disponibilidade do receptor serotoninérgico 5-HT_{1A}, responsável pela regulação da função da serotonina, e maior disponibilidade da MAO, que metaboliza a serotonina, tem sido encontrado em diferentes regiões cerebrais de pacientes depressivos (MEYER et al., 2006; HASLER, 2010).

Porém, apesar de quase todos os antidepressivos estabelecidos clinicamente serem direcionados aos sistemas monoaminérgicos, com o passar dos anos, percebeu-se que não era diretamente o aumento nos níveis dessas catecolaminas que melhoravam os sintomas depressivos. Isso foi observado visto que a utilização desses fármacos apresentava início de ação tardio (três semanas ou mais após o início do tratamento), baixas taxas de respostas e que a depleção de monoaminas endógenas não produzia alteração de humor em indivíduos saudáveis, mas apenas em pacientes depressivos (NEMEROFF, 2007; PENN; TRACY, 2012; RUHÉ; MASON; SCHENE, 2007). Além do mais, diversos estudos sugerem que não existem evidências claras de que somente a deficiência de monoaminas seja a causa da depressão (STAHL, 2014; LIU et al., 2017). Portanto, novas teorias surgiram na tentativa de explicar a fisiopatologia da depressão e como esses medicamentos já estabelecidos clinicamente exerceriam seus efeitos na melhora clínica dos sintomas.

2.2.2 Hipótese neurotrófica

A ideia de que a fisiopatologia da depressão vai muito além da desregulação do nível das monoaminas iniciou-se na década de 90. Evidências demostravam que o aumento das catecolaminas na fenda sináptica e a ativação dos receptores monoaminérgicos desencadeavam uma série de alterações neuroquímicas intracelulares capazes de provocar modificações na expressão gênica, o que se

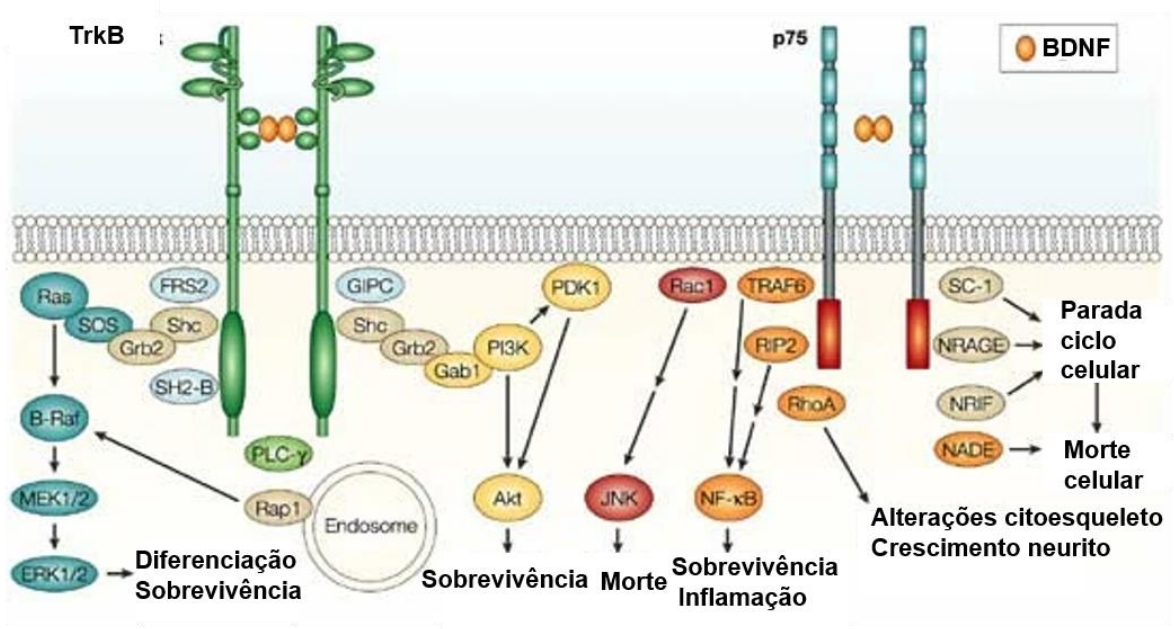
traduziria em um aumento da síntese de fatores neurotróficos ou neurotrofinas (DUMAN; HENINGER; NESTLER, 1997). As neurotrofinas são um grupo de moléculas que desempenham papéis fundamentais em vários processos biológicos, como o desenvolvimento, manutenção e sobrevivência dos neurônios, tanto no sistema nervoso em desenvolvimento quanto no adulto (FOLTRAN; DIAZ, 2016). Dentre elas, o fator neurotrófico derivado do encéfalo (BDNF) tem sido amplamente associado com a depressão (PHILLIPS, 2017). A expressão do gene do BDNF é controlada por múltiplos promotores dependente de atividade e específicos de tecidos. Em ratos, o gene do BDNF é composto por 8 éxons-5', que são associados com promotores distintos e 1 éxon-3', que codifica a proteína madura (AID et al., 2007). De particular interesse, os éxons IV e VI, são duas transcrições que contém promotores direta ou indiretamente regulados pela atividade neuronal e, a desregulação de ambos, está associada com a depressão (SAKATA et al., 2009). O éxon IV, por estar localizado na soma, apresenta indicativo de alteração neuronal, enquanto que o éxon VI está direcionado para os dendritos (PATTABIRAMAN et al., 2005; CHIARUTTINI et al., 2008).

Assim como todas as neurotrofinas, o BDNF se liga ao receptor $p75^{NGFR}$, e com maior afinidade ao receptor tropomiosina quinase B (TrkB) onde diversas cascatas são acionadas, como mostrado na figura 2. Por exemplo, o BDNF induz uma fosforilação no receptor TrkB e ativa vias da proteína quinase C (PKC), associada às ações rápidas do BDNF e às vias da proteína quinase B (Akt) e da proteína quinase ativada por mitógeno (MAPK), que relacionam os efeitos do BDNF à sobrevivência neuronal (HUANG; REICHARDT, 2001; CHAO et al., 2003; BLUM; KONNERTH, 2005). Clinicamente, uma diminuição nos níveis de BDNF no sangue e no fluido cerebroespinal está relacionada com o aparecimento de sintomas depressivos (KAREGE et al., 2002; ZHOU et al., 2011).

Também, neuroimagens mostram redução do hipocampo nestes pacientes, sendo o estresse um fator conhecido por causar atrofia dessa região cerebral, bem como redução nos níveis de BDNF (DUMAN; HENINGER; NESTLER, 1997; LARSEN et al., 2010; QIAO et al., 2017). Em modelos animais, a deficiência de BDNF está associada ao desenvolvimento de comportamentos do tipo depressivos (ZHANG; PARDRIDGE, 2006; BARANOVA; RYBNIKOVA; SAMOLOIV, 2015), maior vulnerabilidade a transtornos de ansiedade (JANKE et al., 2015) e alguns estudos evidenciaram que a utilização de antidepressivos aumenta a expressão de BDNF

nesses animais (NIBUYA; NESTLER; DUMAN, 1996; BJORKHOLM; MONTEGGIA, 2016), mais uma vez sugerindo que a sinalização da cascata BDNF-TrkB no hipocampo e córtex, regiões com alta concentração de BDNF, teria papel fundamental para a melhora dos sintomas tipo-depressivos. Essa ação dos antidepressivos aumentarem a neurogênese via BDNF, se dá pela ação dos antidepressivos estimularem o sistema 3´5´-adenosina-monofosfato-cíclico-proteína de ligação responsiva ao AMPc (AMPc-CREB), alterando a expressão gênica do BDNF e aumentando a sobrevivência neuronal (GUR et al., 2007; WATANABE et al., 2010).

Figura 2- Via de sinalização do BDNF e seus receptores



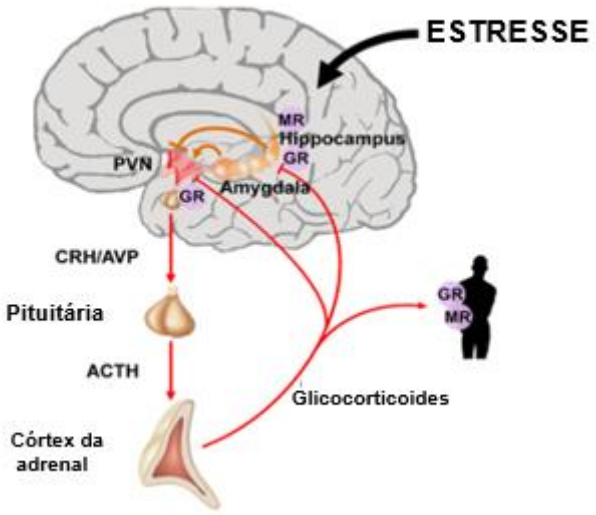
Fonte: Adaptado de Chao, 2003 (CHAO, 2003, p. 301).

Entretanto, assim como a teoria monoaminérgica, a teoria neurotrófica apresenta suas limitações, dentre elas, a principal é que a dosagem sérica de BDNF ainda não pode ser realizada na prática clínica por limitações de detecção da técnica e também devido a essa neurotrofina encontrar-se alterada em outros transtornos neuropsiquiátricos, e não somente a depressão (GASS; HELLWEG, 2010; SKILLETER et al., 2015).

2.2.3 Hipótese neuroendócrina

A hipótese neuroendócrina surgiu a partir da ideia de que modelos de estresse crônico levariam ao desenvolvimento da depressão pelo aumento da liberação de glicocorticoides (NEMEROFF, 1996; WILLNER, 2005). O eixo HPA é responsável pela liberação de corticoides no organismo. Ele é controlado inicialmente pela secreção do hormônio liberador de corticotrofina (CRH) no hipotálamo, que leva à ativação da secreção do hormônio adrenocorticotrófico (ACTH) na pituitária e que por fim estimula a secreção dos glicocorticoides (cortisol em humanos e corticosterona em roedores) no córtex da adrenal (Figura 3) (PARIANTE; LIGHTMAN, 2008). Os glicocorticoides agem através da ligação aos seus receptores, os receptores mineralocorticoides (MR) e GR, que estão distribuídos em regiões cerebrais especificamente envolvidas na regulação do estresse e da retroalimentação negativa do eixo HPA, portanto, reduzindo a liberação de glicocorticoides. Os glicocorticoides apresentam papel fundamental na regulação da neurogênese, sobrevivência celular e função estrutural do córtex, hipocampo e amígdala, regiões cerebrais fundamentais para o aprendizado, memória, emoções e medo (MCEWEN; NASCA; GRAY, 2016).

Figura 3- Ativação do eixo HPA e regulação pelos receptores glicocorticoides.

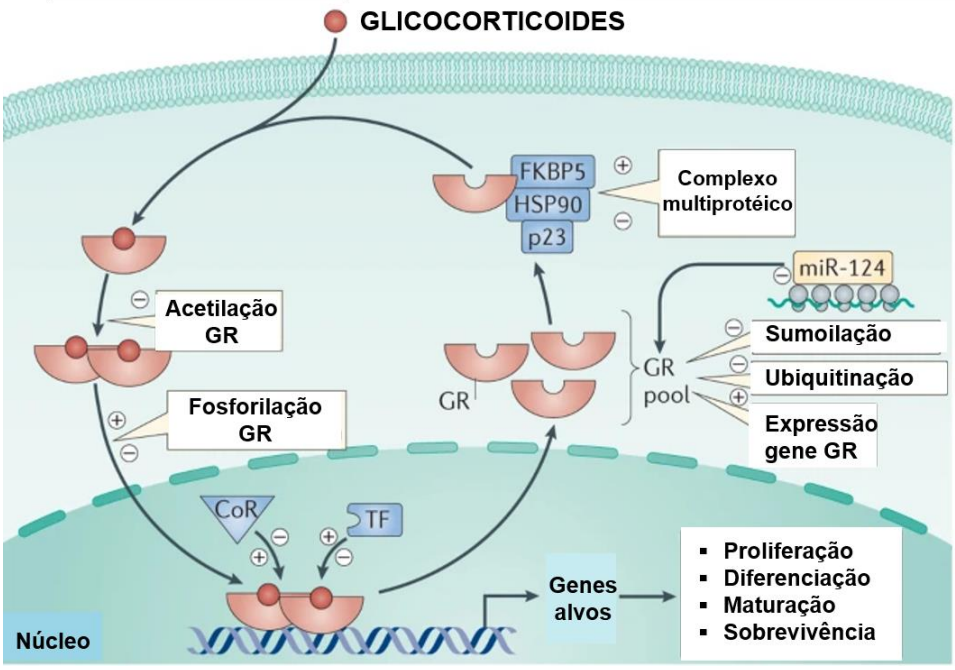


Fonte: Adaptado de Raabe, Spengler, 2013 (RAABE, SPENGLER, 2013).

O estresse é conhecido por causa hiperatividade do eixo HPA, e essa hiperatividade é um dos achados biológicos mais consistentes em transtornos relacionados a depressão e ansiedade (HOLSBOER, ISING, 2010), visto que a inibição da retroalimentação negativa mediada pelo GR parece estar prejudicada nesses transtornos neuropsiquiátricos, não ocorrendo esta inibição e portanto, aumentando a liberação de glicocorticoides no organismo, que pode ser clinicamente observado no plasma, saliva, urina e por hipertrofia da glândula adrenal (Figura 3) (NEMEROFF; VALE, 2005). Dessa maneira, esta desregulação presente na depressão e ansiedade influencia diretamente na ação cerebral dos glicocorticoides.

Os GRs são receptores nucleares e estão normalmente localizados no citosol das células. Quando inativos ou com baixa afinidade aos ligantes, o GR está associado a um complexo multiproteico, que contém as chaperonas de choque térmico e as imunofilinas, que inclui a proteína ligante de FK506 de 51 kDa (FKBP5, do inglês FK506 binding protein 5), formando o chamado “complexo de GR”, como mostrado na figura 4.

Figura 4- Sinalização dos glicocorticoides e receptores de glicocorticoides



Fonte: Adaptado de Egland et al., 2005 (EGLAND et al., 2005, p. 192).

Quando ativado, ocorre uma alteração conformacional, e o GR se desliga do complexo e transloca-se para o núcleo celular (GRAD; PICARD, 2007; BINDER, 2009). Então, neste local o GR interage com os elementos responsivos aos glicocorticoides (GREs), que são encontrados nas regiões promotoras de genes responsivos aos glicocorticoides e a outras proteínas celulares, a fim de controlar a expressão gênica (NICOLAIDES et al., 2014). Dentre os genes responsivos aos glicocorticoides, o fator de transcrição Nr4a1 e a proteína Gadd45β (do inglês, growth arrest and DNA damage inducible beta) apresentaram modificações a nível de expressão hipocampal após exposição ao estresse (KEMBER et al., 2012; BRIVIO et al., 2019), mostrando importante relação na modulação funcional em resposta a estímulos externos (SIMONS, 2010). Além do mais, polimorfismos do gene FKBP5 parecem estar diretamente relacionados com o desenvolvimento de transtornos psiquiátricos (MINELLI et al., 2013), corroborando com as hipóteses das alterações do eixo HPA com o desenvolvimento de sintomas de depressão e ansiedade.

Sabe-se que o eixo HPA recebe projeções serotonérgicas, apresentando efeito direto na serotonina (CHMIELARZ et al., 2015) e sua hiperatividade está associada tanto a uma redução da regulação do BDNF, via redução na fosforilação da CREB e portanto, diminuindo a transcrição do BDNF, o que prejudica a neurogênese e a sobrevivência neuronal, inclusive de neurônios serotonérgicos (WANG et al., 2013). Modelos de estresse mostram que animais apresentam maiores níveis de corticosterona plasmática associado diretamente ao aparecimento de comportamentos do tipo depressivos e alterações estruturais no córtex e hipocampo (SNYDER et al., 2011; DUMAN; AGHAJANIAN, 2012; SUN et al., 2016). Além disso, a administração de glicocorticoides em roedores reduz a produção de BDNF, levando a atrofia dendrítica e a morte celular (McEWEN, 2007; WILLNER; SCHEEL-KRÜGER; BELZUNG, 2013). Evidências sugerem que o tratamento com antidepressivos pode aumentar a expressão de GR e com isso reduzir a hiperatividade do eixo HPA, favorecendo a melhora dos sintomas depressivos e de ansiedade (NIKKHESLAT et al., 2017) e protegendo os neurotransmissores monoaminérgicos do possível dano causado pela hiperatividade do eixo HPA (WANG et al., 2018).

2.2.4 Outras hipóteses

O envolvimento do sistema glutamatérgico surgiu a partir de estudos que mostraram o efeito antidepressivo da quetamina, fármaco que age como um antagonista dos receptores N-metil-D-aspartato (NMDA). Uma única dose de quetamina diminuiu os sintomas depressivos em pacientes resistentes ao tratamento com fármacos convencionais (ZARATE et al., 2006). O possível efeito antidepressivo de baixas doses da quetamina está associado à sua capacidade de reverter processos neurodegenerativos observados na depressão, como a redução do número de sinapses e a dearborização dendrítica (HENDERSON et al., 2016). Também, evidências sugerem que essa ação da quetamina seja dependente de BDNF, uma vez que esses efeitos não ocorrem em animais nocaute para o BDNF (LIU, et al., 2012). Alterações nos níveis cerebrais de glutamato e de GABA (ácido gama-aminobutírico) tem sido encontrado tanto em pacientes depressivos quanto em animais em modelos de estudo de depressão, além de que a eficácia de substâncias que interagem diretamente nestes sistemas, auxilia as hipóteses alternativas para explicar a neurobiologia da depressão (LENER et al. 2017). Também, a literatura aborda que em transtornos psiquiátricos pode ocorrer uma alteração na enzima GAD67, enzima que converte glutamato em GABA (FATEMI et al., 2005) confirmando a hipótese que o desequilíbrio desses dois sistemas está conectado com o desenvolvimento dos sintomas de depressão e ansiedade.

Similarmente, a neuroinflamação ganhou importância na etiologia da depressão, uma vez que a inflamação gera a liberação de mediadores inflamatórios, incluindo citocinas, interleucinas pró inflamatórias, fatores de necrose, entre outros. Esses mediadores podem atravessar a barreira hemato-encefálica e produzir alterações comportamentais relacionadas a sintomas depressivos (DANTZER et al., 2008), visto que as citocinas podem modificar a sinalização da serotonina, depletar níveis de fatores neurotróficos assim como a ativação de micróglia e prejudicar o funcionamento cerebral (CHO; TSUNODA; SHARMA, 1999; JANGRA et al., 2017).

A disfunção da glia, principalmente dos astrócitos, também ganhou interesse nos últimos anos como papel chave na patogênese da depressão (MÉNARD; HODES; RUSSO, 2016; RIAL et al., 2016). Essa teoria foi inicialmente suportada pela observação de dados clínicos onde pacientes apresentaram redução na densidade e número de células gliais em regiões límbicas (RAJKOWSKA;

STOCKMEIER, 2013). O fator neurotrófico derivado da glia (GDNF) apresenta função principalmente relacionada à sobrevivência de neurônios dopaminérgicos (LIN et al., 1993) porém recentes evidências mostraram sua importância no desenvolvimento e manutenção de neurônios serotonérgicos e noradrenérgicos (ZAMAN et al., 2003; HUANG et al., 2005; DUCRAY et al., 2006). A hipotrofia astrocitária está diretamente associada a diminuição da proteína glial fibrilar ácida (GFAP) no córtex pré-frontal, hipocampo e amígdala (MIGUEL-HIDALGO et al., 2000; GOS et al., 2013). O GFAP é um fator importante para a sustentação da morfologia dos astrócitos, para o auxílio na interação astrócito-neurônio e também promovendo a neurogênese hipocampal (MIDDELDORP; HOL, 2011). Portanto, a desregulação desse fator também implica em um favorecimento do desenvolvimento da depressão. Pelo contrário, a administração de fármacos antidepressivos modifica a morfologia e função dos astrócitos, como a síntese e regulação da liberação de neurotransmissores, favorecendo a melhora dos sintomas depressivos (TSYBKO; ILCHIBAEVA; POPOVA, 2017; ZHAO et al., 2018).

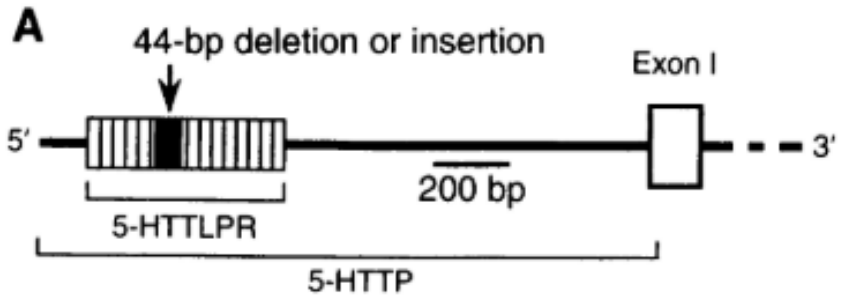
2.3 MODELOS ANIMAIS PARA O ESTUDO DA DEPRESSÃO

Os modelos animais são ferramentas que auxiliam na investigação do desenvolvimento e progressão de doenças bem como para testar novos tratamentos. Existe a dificuldade de quantificar os sintomas da depressão em animais, visto que é uma doença de origem psicológica limitada a humanos, porém existem diversos modelos que auxiliam na avaliação dos comportamentos do tipo depressivo. Modelos utilizados para induzir esses comportamentos tipo depressivos variam na característica fenotípica que o animal apresentará ou variam na forma de indução, podendo ser via farmacológica ou não. O modelo farmacológico mais clássico é a indução a partir da administração da reserpina. A reserpina é uma droga que atua bloqueando irreversivelmente o transportador de monoaminas vesiculares, depletando os níveis de serotonina, noradrenalina e dopamina (LEITH; BARRET, 1980; SCHULDINER; SHIRVAN; LINIAL, 1995) e essa diminuição está associada ao desenvolvimento de comportamentos tipo depressivos (IRITANI et al., 2006; NAGAKURA et al., 2009; ARORA et al., 2011). Além do mais, um outro modelo que mimetiza a anedonia comportamental em ratos é o paradigma de estresse crônico imprevisível. Este paradigma é um modelo animal que transpõe pequenas e

imprevistas irritações ao longo da vida, o qual causa impactos neurofisiológicos e comportamentais relevantes para os transtornos do humor (WILNNER et al., 1987; KOMPAGNE et al., 2008).

Diversas alterações no sistema serotonérgico têm sido associadas com muitos transtornos neuropsquiátricos (COPPEN et al., 1973; COWEN; PARRY-BILLING; NEWSHOLME, 1989), e, mais especificamente, alterações genéticas no transportador de serotonina (5-HTT em humanos; SERT em roedores) (OLIVIER et al., 2008). Em humanos, a transcrição do gene 5-HTT é modulada por um polimorfismo chamado de região polimórfica ligada ao gene 5-HTT (5-HTTLPR, do inglês 5-HTT gene-linked polymorphic region). Este polimorfismo é caracterizado por uma inserção ou deleção de 44 pares de bases repetidos, podendo gerar um alelo curto (HTTLPR-s, do inglês short) ou um alelo longo (HTTPLPR-l, do inglês long), como representado na figura 5 (LESCH, 1996).

Figura 5- Mapa do gene promotor do transportador de serotonina.



Fonte: (Lesch, 1996, p. 1528).

5-HTTLPR: Polimorfismo do transportador de serotonina; bp: pares de base.

Esta variante alélica do tipo HTTLPR-s seria responsável pelas características de maior sensibilidade às influencias ambientais, sejam positivas ou negativas, que os indivíduos portadores desse polimorfismo apresentam (LICHT; MORTENSEN; KNUDSEN, 2011; MOORE; DEPUE, 2015). Estudos apontam que indivíduos com pelo menos uma cópia do alelo HTTLPR-s apresentam maior vulnerabilidade a desenvolver sintomas depressivos quando em contato com situações consideradas estressantes ao longo da vida, principalmente aquelas no início da vida, do que

indivíduos com genes homozigotos para o alelo HTTLPR-s (CASPI et al., 2003). Além disto, a variante alélica-s também tem sido associada a um maior potencial de agressividade e a maiores tendências suicidas (PLUESS et al., 2010; MITCHELL et al., 2011).

Em animais, este polimorfismo é simulado por uma inativação genética que origina animais nocaute homozigotos ($SERT^{-/-}$) e heterozigotos ($SERT^{+/-}$) para o SERT. As características fenotípicas dos animais nocautes $SERT^{-/-}$ estão associadas a uma maior propensão aos comportamentos de ansiedade quando expostos a ambientes novos, imobilidade prolongada em resposta a uma ameaça, aumento da inibição de pré-pulso, refletindo a sensibilidade às sutilezas ambientais, além de apresentar maior tendência ao isolamento social, maior tempo de imobilidade no teste de nado forçado e menor consumo de sacarose, caracterizando assim um modelo animal viável para o estudo da depressão (OLIVIER et al., 2008; NONKES; de POOTER; HOMBERG, 2012; NONKES; HOMBERG, 2013; KROEZE et al., 2016; HOMBERG et al, 2007, 2016). Também, semelhantemente ao que ocorre em indivíduos portadores do alelo-s, que são apenas heterozigóticos para essa alteração, muitos dos comportamentos observados nos animais heterozigotos $SERT^{+/-}$ apresentam diferenças quando comparados aos animais com o genótipo selvagem, porém comportamentos de agressividade, alteração no comportamento sexual e na atividade locomotora muitas vezes não são observados (HOLMES, et al., 2003; HOMBERG et al., 2007; CHAN et al., 2011). Portanto, devido a estas similaridades, animais $SERT^{+/-}$ podem ter maior valor translacional para a situação humana do que animais homozigotos $SERT^{-/-}$.

A nível neuronal, foi demonstrado que a falta de SERT resulta em um aumento dos níveis extracelulares de serotonina, tanto em ratos $SERT^{-/-}$ quanto $SERT^{+/-}$ (HOMBERG et al., 2007). Este aumento extracelular nos níveis de serotonina causa um excesso de atividade nos receptores serotonérgicos pós-sinápticos, que por sua vez poderiam estar subjacentes ao aumento dos comportamentos do tipo depressivo e de ansiedade (GRAEFF, 1996; OLIVIER et al., 2008). Sabe-se que os antidepressivos ISRSs apesentam eficácia no tratamento da depressão em adultos pela inibição seletiva do transportador da serotonina, porém o tratamento neonatal com ISRS (BOULLE et al., 2006) ou a inativação genética do SERT nos animais $SERT^{-/-}$ e $SERT^{+/-}$ induz sintomas tipo depressivos e de ansiedade. Acredita-se que tanto uma subatividade ou superatividade do sistema

serotonérgico produza estes distúrbios neuropsiquiátricos, sugerindo que a relação entre a serotonina e distúrbios na serotonina operam de acordo com uma função em “U invertido”, uma vez que altos níveis de serotonina em ratos e baixos níveis em humanos mostram sintomas semelhantes (CALABRESE; BALDWIN, 2001; OLIVIER et al., 2008).

2.4 A ESTIMULAÇÃO TÁTIL

Diversos estudos apontam a influência de intervenções externas sobre a organização cerebral e a melhora de resultados comportamentais em roedores, tanto na idade adulta como no período pós-natal (GOES et al., 2015; SHILPA et al., 2017; BIRCH et al., 2013). Os períodos iniciais da vida são extremamente críticos para o desenvolvimento cerebral, pois é nesse período que ocorre uma série de etapas do desenvolvimento, como proliferação, migração, diferenciação celular, sinaptogênese, entre outros (JANSSEN et al., 2015), e todos esses processos podem ser influenciados por fatores ambientais externos. Experiências pós-natais negativas, como o isolamento neonatal por longo período, ou estresse materno, são vistas como um grande potencial para aumentar a vulnerabilidade a doenças psiquiátricas (SALOMONS et al., 2010; TAKAHASHI, 2015) enquanto que intervenções enriquecedoras, como a estimulação tátil (ET), podem ocasionar mudanças positivas na estrutura e função do SNC, melhorando o comportamento em diversos transtornos neuronais.

A ET em ratos é o modelo utilizado como forma de mimetizar a massagem terapêutica realizada em humanos, cuja terapia proporciona estimulação sensorial ou cinestésica ao paciente. A massagem terapêutica aplicada em bebês prematuros ou em crianças, tem se mostrado um procedimento interessante, já que muitos benefícios são observados. Em bebês prematuros, a massagem melhorou o desenvolvimento dos neonatos (MATHAI et al., 2001), aumentou o ganho de peso e os bebês se mostraram mais ativos (SOLKOFF, 1969), reduzindo também o tempo de hospitalização (MENDES; PROCIANOY, 2008). Já, em crianças autistas, a massagem terapêutica mostrou influências positivas em comportamentos estereotipados, melhora do comportamento social e menor aversão ao toque (CULLEN et al., 2005; ESCALONA et al., 2001).

Em roedores, a ET neonatal é uma forma de manipulação sensorial aplicada sobre a pele, e que, em certo nível, imita o comportamento materno de lambedura e limpeza dos filhotes. Estudos mostram que a ET neonatal tem efeitos importantes sobre o desenvolvimento normal de roedores, a qual melhorou a conectividade sináptica no córtex pré-frontal, via aumento das ramificações e comprimento dos dendritos (RICHARDS et al., 2012), alterou a organização estrutural e favoreceu a mielinização do nervo óptico (HORIQUINI-BARBOSA; LACHAT, 2016). Além do mais, a ET aplicada após modelos de separação materna ou estresse neonatal, facilitou a neurogênese no giro denteado do hipocampo (de LOS ANGELES et al., 2016), melhorou funções cognitivas de memória e aprendizado (DASKALAKIS et al., 2009; de LOS ANGELES et al., 2016), diminuiu a ansiedade (RÍO-ALAMOS et al., 2015) e a sensibilidade à dor (IMANAKA et al., 2008; STEPHAN et al., 2002). Ainda, o manuseio neonatal aumentou a expressão de receptores de serotonina no córtex e hipocampo de ratos, fator importante para um desenvolvimento neuronal ideal, já que alteração desfavorável da sinalização da serotonina pode induzir transtornos do desenvolvimento neurológico e neuropsquiátricos (STAMATAKIS et al.,2006; LESCH; WAIDER, 2012; AKATSU et al., 2015).

Estudos realizados pelo nosso grupo, também mostraram os benefícios da ET neonatal na redução da preferência por cocaína e anfetamina (ANTONIAZZI et al., 2014a, 2014b), diminuição dos níveis de corticosterona plasmática (FREITAS et al., 2015; ANTONIAZZI et al., 2017), aumento da imunoreatividade do BDNF e GR no hipocampo, favorecendo comportamentos de memória (ANTONIAZZI et al., 2017), alteração na resposta de drogas ansiolíticas (BOUFLEUR et al., 2012, 2013) e antidepressivas (FREITAS et al., 2015) melhorando a anedonia e prevenindo o desenvolvimento do comportamento de ansiedade e do tipo depressivo. Os principais efeitos benéficos da ET neonatal podem ser explicados devido ao fato que as vias neurais da pele para o SNC amadurecem mais cedo em relação aos outros sistemas. Além disso, evidências sugerem que a aplicação da ET nos primeiros 14 dias após o nascimento parece ser mais efetiva, mais especificamente, entre os dias pós-natal 8 a 14 (MEANEY, et al., 1992; FREITAS et al., 2015; ANTONIAZZI et al., 2017), uma vez que é nesse período que pode ocorrer alteração da sensibilidade do eixo HPA, onde o animal se encontra em seu maior período hiporresponsivo ao estresse (JUTAPAKDEEGUL et al., 2003; VAZQUEZ et al., 2006). Também é neste período que a serotonina apresenta papel semelhante a fatores neurotróficos,

auxiliando na regulação da formação do circuito neural e é o período no qual ocorre o maior pico de BDNF, favorecendo a neurogênese e plasticidade sináptica (AID et al., 2007).

Por outro lado, dados na literatura demonstraram que a neurogênese pode ocorrer também na idade adulta (CAMERON; MCKAY, 2001; MU; GAGE, 2011; KATSIMPARDI; LLEDO, 2018) e por isso a investigação da ET aplicada em outros períodos do desenvolvimento pode ser de grande interesse. Gibb e colaboradores demonstraram pela primeira vez o efeito da ET aplicada na idade adulta, onde a ET recuperou danos motores causados por lesão cortical (GIBB et al., 2010). Além disso, outros estudos mostraram que a ET pós-desmame foi capaz de diminuir parâmetros de ansiedade (SOARES et al., 2014), aumentando os níveis de BDNF e do fator de crescimento de fibroblastos do tipo 2 (FGF-2) no hipocampo e estriado e diminuindo níveis de GFAP, além de aumentar o comprimento dendrítico no estriado após lesão por 6-hidroxidopamina (EFFENBERG et al., 2014). Isto confirma que a ET tem efeito não só na fase inicial do desenvolvimento, mas também quando aplicada em outros períodos ao longo da vida.

Os possíveis mecanismos protetores da ET independente do período de aplicação sugerem a associação dos efeitos no eixo HPA. Estudos mostram que a ET causa alterações na funcionalidade do eixo HPA, reduzindo a liberação de ACTH e corticosterona (FREITAS et al., 2015; ANTONIAZZI et al., 2017), bem como aumentando os níveis de GR (ANTONIAZZI et al., 2017) levando a uma melhor adaptação do animal frente a situações estressantes ao longo da vida (LEVINE, 2001, 2002; MEANEY, 1991; BOUFLEUR et al., 2013). Também, o mecanismo pode estar envolvido com o aumento de fatores de crescimento neuronais, incluindo o BDNF, o fator de crescimento neural (NGF), o FGF-2 ou até mesmo a serotonina. Esses fatores estão associados à promoção da proliferação celular, diferenciação neuronal e sobrevivência de novas células, favorecendo o aumento da arborização dendrítica, alteração da anatomia do cérebro e melhorando a plasticidade cerebral (DASKALAKIS et al., 2009; GIBB et al., 2010; RICHARDS et al., 2012). Portanto, a soma desses elementos pode sugerir um mecanismo pelo qual a ET possa estar exercendo seus efeitos, porém, uma investigação mais aprofundada da via de sinalização é necessária a fim de estabelecer o exato mecanismo desta intervenção em roedores.

2.5 JUSTIFICATIVA

Considerando que a depressão é responsável por diferentes alterações no SNC e nenhuma terapia mostra-se totalmente efetiva, e que a ET tem mostrado benefícios sobre diferentes vias neuronais em roedores, o estudo da ET sobre modelos de depressão torna-se de grande importância. Desta forma, o presente estudo visou determinar se a ET aplicada em diferentes períodos do desenvolvimento, pode alterar parâmetros neuroquímicos e comportamentais relacionados à depressão e ansiedade em ratos adultos. Além do mais, buscou-se investigar através de revisão sistemática, o que a literatura mostra sobre os efeitos comportamentais, fisiológicos e neuronais que ET exerce em ratos.

3 OBJETIVOS

3.1 OBJETIVO GERAL

Avaliar a influência da estimulação tátil aplicada em diferentes períodos do desenvolvimento sobre comportamentos de depressão e ansiedade e possíveis alterações cerebrais em ratos, bem como investigar o impacto da estimulação tátil em nível comportamental, fisiológico e cerebral em ratos através de uma revisão da literatura.

3.2 OBJETIVOS ESPECÍFICOS

- Realizar a ET em ratas na idade adulta após a indução de um modelo de depressão com reserpina e avaliar a influência da ET sobre:
  - a reversão de parâmetros comportamentais relacionados à depressão;
  - a alteração de parâmetros moleculares relacionados a sobrevivência e plasticidade sináptica como a expressão do pró-fator neurotrófico derivado do encéfalo (pró-BDNF), do fator neurotrófico derivado do encéfalo (BDNF), receptor tirosina quinase B (TrkB), fator neurotrófico derivado de células da glia (GDNF) e a proteína glial fibrilar ácida (GFAP) no córtex pré-frontal;
  - parâmetros indicadores de alteração do eixo hipotalâmico-pituitária-adrenal (HPA), como dosagem plasmática de corticosterona e hormônio adrenocorticotrófico (ACTH) e densidade de receptores glicocorticoides no córtex pré-frontal.
- Realizar a estimulação tátil em ratos heterozigotos para o transportador de serotonina durante o período neonatal, e avaliar na idade adulta:
  - Comportamentos de ansiedade, do tipo depressivo, e comportamento social;
  - Parâmetros moleculares relacionados a sobrevivência e plasticidade neuronal como a expressão do BDNF e suas isoformas;
  - Expressão dos receptores mineralocorticoides (MR) e glicocorticoides (GR), e de genes responsivos aos glicocorticoides, bem como expressão de marcadores moleculares relacionados ao sistema glutamatérgico e gabaérgico.

- Realizar uma revisão sistemática da literatura a fim de determinar a influência da ET sobre comportamento, função neurofisiológica e neuroendócrina em ratos.

4 PRODUÇÃO CIENTÍFICA

Os resultados inseridos nesta tese apresentam-se sob a forma de Artigo científico I, Artigo científico II e manuscrito I, os quais se encontram aqui estruturados. Os itens Materiais e Métodos, Resultados, Discussão e Referências, encontram-se nos próprios Artigos Científicos I e II, publicados nos periódicos científicos Molecular Neurobiology e Frontiers in Behavioral Neuroscience, respectivamente. O manuscrito I aqui apresentado contém uma revisão sistemática e encontra-se em fase de redação.

4.1 ARTIGO CIENTÍFICO I

Tactile Stimulation on Adulthood Modifies the HPA Axis, Neurotrophic Factors, and GFAP Signaling Reverting Depression-Like Behavior in Female Rats

Karine Roversi, Caren T.D Antoniazzi, Laura H. Milanesi, Higor Z. Rosa, Maikel Kronbauer, Domenika Rossato, Marta M. Duarte, Thiago Duarte, Marilise E. Burger

Molecular Neurobiology
DOI: 10.1007/s12035-019-1522-5

SPRINGER NATURE

Thank you for your order!

Dear Ms. Karine Roversi,

Thank you for placing your order through Copyright Clearance Center's RightsLink® service.

Order Summary

Licensee:	Ms. Karine Roversi
Order Date:	Aug 21, 2020
Order Number:	4893911264815
Publication:	Molecular Neurobiology
Title:	Tactile Stimulation on Adulthood Modifies the HPA Axis, Neurotrophic Factors, and GFAP Signaling Reverting Depression-Like Behavior in Female Rats
Type of Use:	Thesis/Dissertation
Order Total:	0.00 USD

View or print complete details of your order and the publisher's terms and conditions.

Sincerely,

Copyright Clearance Center

Tel: +1-855-239-3415 / +1-978-646-2777
customercare@copyright.com
https://myaccount.copyright.com

Copyright Clearance Center RightsLink®

Molecular Neurobiology (2019) 56:6239–6250
https://doi.org/10.1007/s12035-019-1522-5

Tactile Stimulation on Adulthood Modifies the HPA Axis, Neurotrophic Factors, and GFAP Signaling Reverting Depression-Like Behavior in Female Rats

Kr. Roversi[1] · Caren Tatiane de David Antoniazzi[1] · L. H. Milanesi[1] · H. Z. Rosa[1] · M. Kronbauer[1] · D. R. Rossato[2] · T. Duarte[1] · M. M. Duarte[3] · Marilise E. Burger[1]

Received: 9 October 2018 / Accepted: 30 January 2019 / Published online: 11 February 2019

Abstract
Depression is a common psychiatric disease which pharmacological treatment relieves symptoms, but still far from ideal. Tactile stimulation (TS) has shown beneficial influences in neuropsychiatric disorders, but the mechanism of action is not clear. Here, we evaluated the TS influence when applied on adult female rats previously exposed to a reserpine-induced depression-like animal model. Immediately after reserpine model (1 mg/kg/mL, 1×/day, for 3 days), female *Wistar* rats were submitted to TS (15 min, 3×/day, for 8 days) or not (unhandled). Imipramine (10 mg/kg/mL) was used as positive control. After behavioral assessments, animals were euthanized to collect plasma and prefrontal cortex (PFC). Behavioral observations in the forced swimming test, splash test, and sucrose preference confirmed the reserpine-induced depression-like behavior, which was reversed by TS. Our findings showed that reserpine increased plasma levels of adrenocorticotropic hormone and corticosterone, decreased brain-derived neurotrophic factor (BDNF) and tropomyosin receptor kinase B, and increased proBDNF immunoreactivity in the PFC, which were also reversed by TS. Moreover, TS reestablished glial fibrillary acidic protein and glucocorticoid receptor levels, decreased by reserpine in PFC, while glial cell line–derived neurotrophic factor was increased by TS per se. Our outcomes are showing that TS applied in adulthood exerts a beneficial influence in depression-like behaviors, modulating the HPA axis and regulating neurotrophic factors more effectively than imipramine. Based on this, our proposal is that TS, in the long term, could be considered a new therapeutic strategy for neuropsychiatric disorders improvement in adult life, which may represent an interesting contribution to conventional pharmacological treatment.

Keywords Reserpine · Handling · BDNF · Hypothalamus-pituitary-adrenal axis

Introduction

Depression is the most common and serious mental disorder, characterized by depressed mood, persistent sadness, loss of interest, and cognitive impairments [1, 2]. According to World Health Organization, it is estimated that 300 million people are affected, and women are more likely to experience depression than men, considering that one-third of women will experience a major depressive episode in their lifetime [2]. The pathophysiology of depression had been involved with impairments in the monoaminergic system, and the pharmacological treatment is mainly based on drugs that can increase the brain levels of monoamines, as occurs with imipramine, a tricyclic antidepressant, whose mechanism of action consists in inhibit the brain neuronal transporter of both noradrenaline and serotonin [3]. However, these pharmacotherapies have been related to slow onset or low response rates which limit their application [4–6]. Based on this, recent hypotheses have proposed that interactions among monoaminergic systems, neurotrophins [7, 8], and glucocorticoid (GC) signaling dysregulation can lead towards symptoms of depression [9].

While the monoaminergic system, especially serotonin, is closely related to neurotrophic factors [10, 11], brain-derived neurotrophic factor (BDNF) is the main and most abundant

✉ Caren Tatiane de David Antoniazzi
carenantoniazzi@yahoo.com.br

✉ Marilise E. Burger
marilise.burger@ufsm.br

[1] Centro de Ciências da Saúde Programa de Pós-Graduação em Farmacologia, Universidade Federal de Santa Maria (UFSM), Santa Maria, RS 97105-900, Brazil

[2] Departamento de Fisiologia e Farmacologia, UFSM, Santa Maria, RS, Brazil

[3] Universidade Luterana do Brasil (ULBRA), Santa Maria, RS, Brazil

neurotrophin in the brain and is critically involved in the maintenance of the neurogenesis, neuronal survival, and plasticity. Inadequate regulation of BDNF may be related to depression-like behaviors [12] since BDNF can regulate serotonin signaling and reuptake, because its receptor, TrkB, is also present on serotonergic neurons [13]. As a precursor of BDNF form, proBDNF is known to play opposing influences, since it acts on the neuronal death regulation via its receptor $p75^{NTR}$. In this sense, an upregulation on this factor has been related to the mood disorders development in rodents [14]. On the other hand, glial cell line–derived neurotrophic factor (GDNF) promotes differentiation and protection to dopaminergic and serotonergic neurons [15, 16], facilitating the neurites growth to different neuronal types [17, 18]. Decreased brain and plasma levels of GDNF in patients and rodents have been related to mood disorders [19, 20], and also the glial fibrillary acidic protein (GFAP) levels were found to be reduced in the brain of rodents submitted to depression models [21, 22]. Moreover, it was recently described that decreasing levels of GFAP could cause an astrocyte hypotrophy and the astrocytes are known as nutrient suppliers to neurons and are involved in the synaptic development and signalization processes [23].

Depression has also been related to a dysfunction of the hypothalamus-pituitary-adrenal (HPA) axis. GC is synthesized and secreted by adrenal glands in response to HPA axis. Following the HPA axis activation, the corticotrophin-release-hormone (CRH) is activated in the hypothalamus, increasing the adrenocorticotrophic hormone (ACTH) release, which results in the adrenal glands secreting GC, such as corticosterone in rodents [24]. The glucocorticoid receptor (GR) plays a key role in negative feedback to the HPA axis, and alterations in this receptor in cortico-limbic areas, such as prefrontal cortex (PFC), amygdala, and hippocampus, may be related to depressive disorders due to altering the GC secretion [25, 26]. Furthermore, GR is closely involved in the biosynthesis regulation of both serotonin [27] and noradrenaline [28], since it is present on noradrenergic and serotonergic neurons [29]. Additionally, increased secretion of GC negatively regulates the BDNF pathways, impairing the brain synaptic plasticity by spine density and neurogenesis downregulation [30], which may be a possible mechanism to explain the GC involvement on depression.

Growing evidence from experimental studies has suggested that non-pharmacological treatments, including physical exercise and environmental enrichment, can exert neuroprotective effects and improve depression-like symptoms by changing the BDNF and the glucocorticoid signaling [31, 32]. In line with this, tactile stimulation (TS) constitutes a simple procedure that can modify the brain organization by increasing neurogenesis [33] and neuroplasticity [34] in the hippocampus, improving anxiety-like behaviors [35], and preventing preference to addictive drugs [36, 37] and depression-like behaviors [38] when applied during initial periods of development. Experimental studies also showed TS beneficial influence on the brain function, when applied in adult rats, preventing cortical lesion [39], and increasing neurotrophins and dendritic length [40].

In view of this, we evaluate the possible beneficial influence of TS on depression-like behaviors induced by reserpine, a promoter of monoaminergic dysfunction that acts by blocking irreversibly the vesicular monoamine transporter type 2, thus mimicking depression-like behaviors [41], thus assessing behavioral and HPA axis changes, and its reflexes on the molecular neurotrophic factors in the PFC.

Material and Methods

Animals and Experimental Procedures

Thirty-five female *Wistar* rats (60 days old) from the breeding facility of Universidade Federal de Santa Maria (UFSM), RS, Brazil, were kept in Plexiglas cages with free access to water and food in a room with controlled temperature (22 ± 1 °C) and on a 12-h light/dark cycle with lights on at 7:00 a.m. All procedures were performed in accordance with the Animal Ethics Committee (#2359150517) guidelines, affiliated to the National Council for the Control of Animal Experimentations (CONCEA), following international norms of care and animal maintenance.

Considering that female rats show increased susceptibility to different neuropsychiatric disorders, such as depression [6], we preferred to use female rats in the current study. First, female rats were randomly distributed in two experimental groups: vehicle ($n = 14$) and reserpine ($n = 21$). The animals received vehicle or reserpine (1 mg/kg/mL), subcutaneously (s.c.) once a day, for 3 consecutive days [42]. Immediately after the last vehicle/reserpine administration, animals were subdivided into five experimental groups ($n = 7$), to start the TS procedure (for 8 days) or not (unhandled; UH) as follows: group (I) vehicle-UH; group (II) vehicle-TS; group (III) reserpine-UH; group (IV) reserpine-TS; and group (V) reserpine-UH-imipramine (positive control). Following 8 days of TS, all the animals were submitted to behavioral evaluations; 30 min before of each test, the positive control group received one administration of imipramine, while all other experimental groups received NaCl 0.9% (imipramine vehicle) instead (Fig. 1).

Drugs and Drug Administration

Reserpine was obtained from Sigma (St. Louis, MO, USA). Reserpine was dissolved in glacial acetic acid (vehicle) and diluted to a final concentration of 0.5% acetic acid with distilled water. On postnatal day 60 (PND 60), the animals received a dose of 1 mg/kg/mL s.c. of reserpine or acetic acid

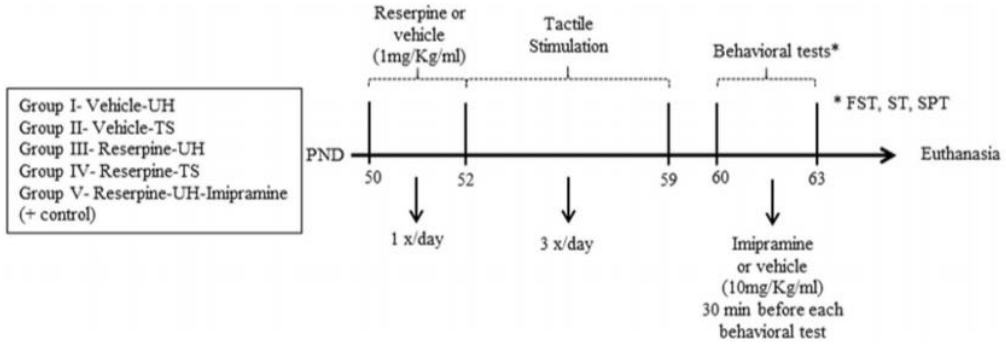


Fig. 1 Schematic representation of the experimental procedure for reserpine-induced depression and tactile stimulation treatment in female rats. Rats received reserpine (1 mg/kg/mL) or vehicle for 3 days, and then, tactile stimulation was performed for 8 days. Imipramine was used as positive control and only was administered 30 min before each behavioral test to the group reserpine-UH-imipramine. The remaining groups received NaCl 0.9% (imipramine vehicle) before behavioral testing. UH, unhandled; TS, tactile stimulation; PND, postnatal day; FST, forced swimming test; ST, splash test; SPT, sucrose preference test

0.5% once a day, for 3 consecutive days. This protocol of reserpine administration was used as the animal model to induce the depression-like symptoms [42]. Administrations were performed between 09:00 and 12:00 a.m. Imipramine (10 mg/kg/mL i.p.) was obtained from a local drug store and administered 30 min before performing each behavioral test. Imipramine vehicle (NaCl 0.9%) was administered to the other groups.

Tactile Stimulation

Immediately after the last administration of reserpine, TS was initiated. The procedure was based on Effenberg et al., with some modifications and consisted of removing the animals from home cage and petting them individually on the experimenter's lap with one hand for 15 min. The TS was applied 3 times per day between 09:00 a.m. and 04:00 p.m., for 8 days. After the procedures, the animals were returned to their home cages [40].

Behavioral Testing

Forced Swimming Test

Behavioral responses related to depression-like symptoms are experimentally assessed in the forced swimming test (FST) [43, 44]. On the first session, rats were forced to swim for a 15-min period (pretest session), and dried before returning to their home cages. Twenty-four hours following the pretest session, the animals were submitted again to the FST for 5 min (test session). Trained raters blinded to the experiment quantified immobility, climbing, and swimming time. The immobility was considered as no additional activity other than the required to keep the head above water. Climbing is defined as upward struggling movements of the forepaws at the side of the cylinder while movements around the cylinder are indicative of swimming time [43]. A decrease in the duration of immobility is indicative of an antidepressant-like effect.

Splash Test

The splash test consists of squirting a 10% sucrose solution on the rat's dorsal coat in its home cage. The sucrose solution dirties the coat and animals initiate grooming behavior. After the sucrose solution application, the time spent grooming (nose/face grooming, head washing, and body grooming) was recorded for 5 min as an index of self-care and motivational behavior [45, 46].

Sucrose Preference Test

In this test, the animals were allowed to consume 10% (*w*/*v*) sucrose/water solution or tap water in their home cage, individually. They were deprived of food and water for 12 h and then presented with two bottles containing either tap water or sweet solution. One-hour intake was measured by weighing bottles before and after the test [47]. The sucrose preference test (SPT) was calculated according to the following equation:

$$\mathrm{SPT} = \left(\frac{\text{Sucrose intake}}{\text{Sucrose intake} + \text{Water intake}}\right) \times 100$$

Tissue Preparations

Following 24 h of the last behavioral assessment, all animals were anesthetized (isoflurane, the dose to the effect) and euthanized by exsanguination. The blood (collected by cardiac puncture in heparinized tubes) was centrifuged at 3000*g*/15 min to obtain the plasma. Brains were removed and cut coronally at the caudal border of the olfactory tubercle to

remove the prefrontal cortex (PFC) [48] and stored in a freezer at − 80 °C for subsequent analysis.

Adrenocorticotropic Hormone and Corticosterone Assay

Quantification of adrenocorticotropic hormone (ACTH) and corticosterone levels were assessed in the plasma samples by ELISA using commercial kits (Sigma-Aldrich®, St. Louis, MO, USA, for ACTH and LDN® immunoassays and services, Nordhorn, Germany, for corticosterone), according to the manufacturer's instructions.

Molecular Assessments

Western Blotting

The PFC tissue was homogenized in a lysis buffer [49], and total protein concentration was determined according to Bicinchoninic Acid (BCA) Protein Assay Kit (Pierce, IL) using bovine serum albumin as standard. After, protein samples were separated by electrophoresis on a 10% or 12.5% polyacrylamide gel and electrotransferred to a polyvinylidene difluoride (PVDF) membrane (Millipore, MA, USA). Non-specific binding sites were blocked and membranes were rinsed in buffer and incubated with primary antibodies: anti-β-actin (1:50000; Sigma-Aldrich, St. Louis, USA), anti-BDNF (1:1000; Abcam, Cambridge, UK), anti-proBDNF (1:500; Santa Cruz Biotechnology, CA, USA), anti-TrkB (1:500; Santa Cruz Biotechnology, CA, USA), anti-GDNF (1:1000; Santa Cruz Biotechnology, CA, USA), anti-GFAP (1:1000 Santa Cruz Biotechnology, CA, USA), and anti-GR (1:500; Santa Cruz Biotechnology, CA, USA), followed by anti-goat (1:10000; Santa Cruz Biotechnology, CA, USA) or anti-rabbit (1:20000; Santa Cruz Biotechnology, CA, USA) IgG horseradish peroxidase conjugate. Immunocomplexes were visualized using Luminata (Millipore, USA) according to the manufacturer's instructions. Film signals were digitally scanned (Chemidoc™ Imaging Systems) and then quantified using ImageJ software. β-actin was used as an internal control, so that data were standardized according to actin values.

Statistical Analysis

Levene's test was performed to verify the homogeneity of data. Two-way ANOVA (2 treatments (vehicle/reserpine) × 2 procedures (UH/TS)) followed by the Newman-Keuls post hoc test was used for all analysis, when appropriate. One-way ANOVA (1 treatment (reserpine) × 3 procedures (UH/TS/Imipramine)) followed by the Newman-Keuls post hoc test was used to compare the positive control and TS (only reserpine-treated groups). All data are expressed as means ± SEM. $p < 0.05$ was considered statistically significant for all comparisons made.

Results

Depression-Like Behavior Observed in the Forced Swimming Test

Two-way ANOVA revealed a significant influence of handling, reserpine administration, and an interaction of handling × reserpine on immobility ($F(1,24) = 223.93$, $p = 0.000$; $F(1,24) = 247.49$, $p = 0.000$; $F(1,24) = 19.93$, $p = 0.000$, respectively), swimming time ($F(1,24) = 18.59$, $p = 0.000$; $F(1,24) = 24.19$, $p = 0.000$; $F(1,24) = 12.83$, $p = 0.001$, respectively), and climbing time ($F(1,24) = 10.85$, $p = 0.000$; $F(1,24) = 18.50$, $p = 0.000$; $F(1,24) = 5.52$, $p = 0.000$, respectively).

The Newman-Keuls test showed that reserpine increased immobility and climbing time and reduced swimming time compared to vehicle-UH, while reserpine-TS animals showed reduced immobility and climbing time and increased swimming time when compared to the reserpine-UH group.

The post hoc test of one-way analysis showed that imipramine administration reduced immobility time when compared to reserpine-UH animals and increased immobility and climbing time when compared to reserpine-TS animals. Moreover, imipramine administration reduced the swimming time compared to reserpine-TS animals and increased this parameter when compared to reserpine-UH group (Fig. 2).

Anhedonia Behavior Was Observed in the Splash Test and Sucrose Consumption

In the splash test, two-way ANOVA revealed a significant influence of handling on latency time to grooming ($F(1,24) = 37.06$, $p = 0.000$, and grooming time $F(1,24) = 21.76$, $p = 0.000$). The post hoc of Newman-Keuls showed that TS per se reduced the latency to grooming when compared to the vehicle-UH group, while reserpine-UH animals increased latency to grooming when compared to the vehicle-UH and reserpine-TS groups. In grooming time, TS per se and reserpine-TS animals increased this behavior when compared to the vehicle-UH and reserpine-UH groups, respectively.

One-way analysis followed by the post hoc test showed imipramine administration reduced the latency to grooming, compared to reserpine-UH animals. Also, imipramine administration decreased the grooming time when compared to reserpine-TS group (Fig. 3a, b).

In the sucrose consumption, two-way ANOVA revealed a significant influence of handling, reserpine, and an interaction of handling × reserpine on sucrose consumption ($F(1,24) = 4.84$, $p = 0.003$; $F(1,24) = 4.15$, $p = 0.054$; and $F(1,24) = 7.41$,

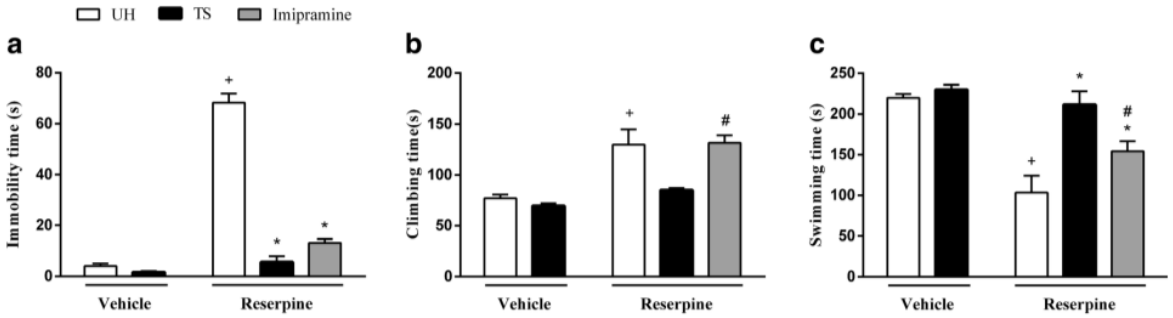


Fig. 2 Influence of reserpine and TS on immobility (**a**), climbing (**b**), and swimming time (**c**) measured in the forced swimming test (FST). Data are expressed as mean ± SEM ($n = 7$). Asterisk indicates significant difference from UH to TS or imipramine groups ($p < 0.05$). Plus sign indicates significant difference from vehicle to reserpine groups ($p < 0.05$). Number sign indicates significant difference from reserpine-TS to imipramine group ($p < 0.05$). UH, unhandled; TS, tactile stimulation

$p = 0.013$, respectively). The Newman-Keuls test showed that reserpine-UH animals reduced sucrose consumption compared to vehicle-UH and reserpine-TS group, while reserpine-TS reversed this parameter. The post hoc test of one-way analysis showed that imipramine administration increased sucrose consumption, compared to reserpine-UH animals, and had no difference in reserpine-TS animals (Fig. 3c).

Adrenal Weight, Body Weight, and Adrenal Weight/Body Weight Ratio

Two-way ANOVA revealed a significant main effect of handling in adrenal weight ($F(1,24) = 16.88$, $p = 0.000$) and adrenal weight/body weight ratio (AW/BW) ($F(1,24) = 17.96$, $p = 0.000$) while in the body weight was shown a significant main effect of handling × reserpine ($F(1,24) = 5.808$, $p = 0.025$).

The Newman-Keuls post hoc test showed that TS per se and reserpine-TS animals showed a decrease in adrenal weight and adrenal weight/body weight ratio when compared to vehicle-UH and reserpine-UH animals. The post hoc test of one-way analysis of imipramine administration showed higher adrenal weight/body weight ratio than reserpine-TS animals and had no difference when compared to reserpine-UH group (Table 1).

Adrenocorticotropic Hormone and Corticosterone Levels

Two-way ANOVA of ACTH revealed a significant main effect of handling ($F(1,24) = 52.84$, $p = 0.000$) and reserpine ($F(1,24) = 761.30$, $p = 0.000$). The Newman-Keuls post hoc test showed that reserpine-UH animals increased ACTH levels compared to vehicle-UH, while TS per se and reserpine-TS animals decreased these levels when compared to vehicle-UH and reserpine-UH animals, respectively. Also, reserpine-TS animals showed lower ACTH levels, compared to TS per se. The post hoc test of one-way analysis showed that imipramine administration decreased ACTH levels when compared to reserpine-UH and reserpine-TS animals (Fig. 4a).

Two-way ANOVA of corticosterone revealed a significant main effect of handling ($F(1,24) = 22.48$, $p = 0.000$) and reserpine ($F(1,24) = 531.32$, $p = 0.000$). The Newman-Keuls post hoc test showed that reserpine-UH animals increased

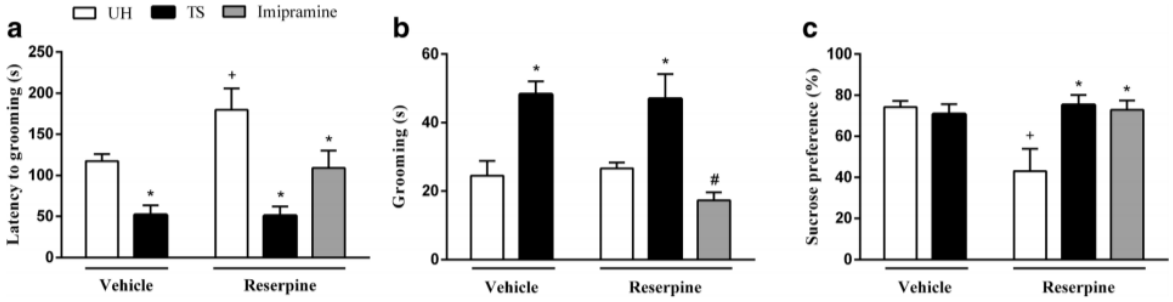


Fig. 3 Influence of reserpine and TS on anhedonia behavior. Latency to grooming (**a**) and grooming time (**b**) was measured in the splash test (ST), and sucrose preference percentage (**c**) was measured in the sucrose preference test (SPT). Data are expressed as mean ± SEM ($n = 7$). Asterisk indicates significant difference from UH to TS or imipramine groups ($p < 0.05$). Plus sign indicates significant difference from vehicle to reserpine groups ($p < 0.05$). Number sign indicates significant difference from reserpine-TS to imipramine group ($p < 0.05$). UH, unhandled; TS, tactile stimulation

Table 1 Effects of reserpine and TS on adrenal weight (AW), final body weight (BW), and adrenal weight/body weight ratio (AW/BW) in female rats

Treatment	Adrenal weight (g)	Body weight (g)	AW/BD (mg)
Vehicle-UH	0.1076 ± 0.005	237.66 ± 4.44	0.45 ± 0.020
Vehicle-TS	0.0908 ± 0.003*	262.16 ± 6.52	0.34 ± 0.012*
Reserpine-UH	0.1131 ± 0.001	251.83 ± 6.69	0.44 ± 0.007
Reserpine-TS	0.0959 ± 0.004*	242.16 ± 9.70	0.39 ± 0.020*
Imipramine	0.1050 ± 0.006	227.40 ± 8.28	0.46 ± 0.020*

UH, unhandled; *TS*, tactile stimulation; data are expressed as mean ± SEM; *indicates significant difference from UH to TS or imipramine groups ($p < 0.05$)

corticosterone levels when compared to vehicle-UH, while TS per se and reserpine-TS animals decreased these levels. Also, reserpine-TS animals had lower corticosterone levels when compared to TS per se. The post hoc test of one-way analysis showed that imipramine administration decreased corticosterone levels, compared to the reserpine-UH group, and had no difference when compared to reserpine-TS animals (Fig. 4b).

Molecular Parameters Analysis in Western Blotting

Two-way ANOVA of BDNF revealed a significant interaction between handling × reserpine ($F(1,24) = 6.97$, $p = 0.020$). The Newman-Keuls post hoc test showed that reserpine-UH animals decreased BDNF levels when compared to vehicle-UH and reserpine-TS animals. One-way analysis followed by the post hoc test showed that imipramine had no difference when compared to the other groups (Fig. 5a).

Two-way ANOVA of proBDNF revealed a significant main effect of handling ($F(1,24) = 8.39$, $p = 0.01$) and handling × reserpine ($F(1,24) = 5.37$, $p = 0.03$). The Newman-Keuls post hoc test showed that reserpine-UH animals increased proBDNF levels when compared to vehicle-UH and reserpine-TS animals. The post hoc test of one-way analysis showed that imipramine administration decreased proBDNF levels, compared to reserpine-UH, and had no difference when compared to reserpine-TS animals (Fig. 5b).

Two-way ANOVA of TrkB revealed a significant main effect of handling ($F(1,24) = 202.11$, $p = 0.000$), reserpine ($F(1,24) = 8.12$, $p = 0.014$), and handling × reserpine ($F(1,24) = 45.66$, $p = 0.000$). The Newman-Keuls post hoc test showed that reserpine-UH animals decreased TrkB levels when compared to vehicle-UH and reserpine-TS. TS per se increased TrkB levels when compared to the vehicle-UH group, while reserpine-TS animals showed TrkB higher levels, compared to TS per se and reserpine-UH animals. The post hoc test of one-way analysis showed that imipramine administration had no difference when compared to reserpine-UH animals, but decreased TrkB levels when compared to reserpine-TS animals (Fig. 5c).

Two-way ANOVA of GDNF revealed a significant main effect of handling ($F(1,24) = 22.69$, $p = 0.000$). The Newman-Keuls post hoc test showed that TS per se and reserpine-TS animals increased GDNF levels when compared with vehicle-UH and reserpine-UH animals. The post hoc test of one-way analysis showed that imipramine administration increased GDNF levels when compared to reserpine-UH and decreased these levels when compared to reserpine-TS animals (Fig. 5d).

Two-way ANOVA of GFAP revealed a significant main effect of reserpine ($F(1,24) = 5.56$, $p = 0.03$) and handling × reserpine ($F(1,24) = 8.90$, $p = 0.01$). The Newman-Keuls post hoc test showed that reserpine-UH animals decreased GFAP levels when compared with vehicle-UH and reserpine-TS animals. The post hoc test of one-way analysis showed that imipramine administration had no difference when compared to the other groups (Fig. 5e).

Two-way ANOVA of GR revealed a significant main effect of handling ($F(1,24) = 24.65$, $p = 0.001$) and handling × reserpine ($F(1,24) = 7.34$, $p = 0.02$). The Newman-Keuls post hoc test revealed that TS per se showed higher levels of GR when

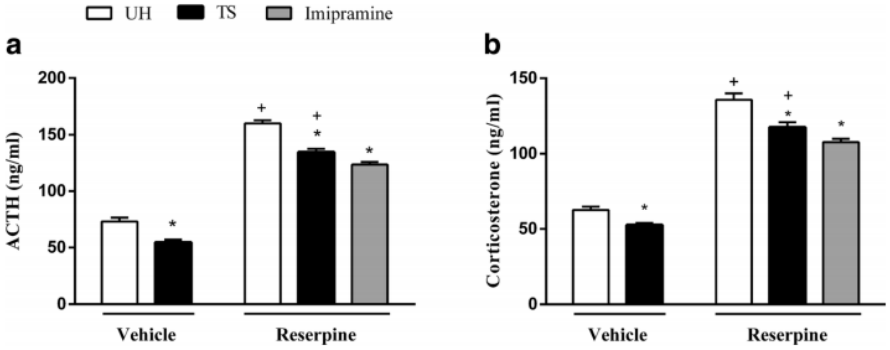


Fig. 4 Influence of reserpine and TS on ACTH and corticosterone in plasma. Data are expressed as mean ± SEM ($n = 7$). Asterisk indicates significant difference from UH to TS or imipramine groups ($p < 0.05$). Plus sign indicates significant difference from vehicle to reserpine groups ($p < 0.05$). Number sign indicates significant difference from reserpine-TS to imipramine group ($p < 0.05$). UH, unhandled; TS, tactile stimulation; ACTH, adrenocorticotrophic hormone

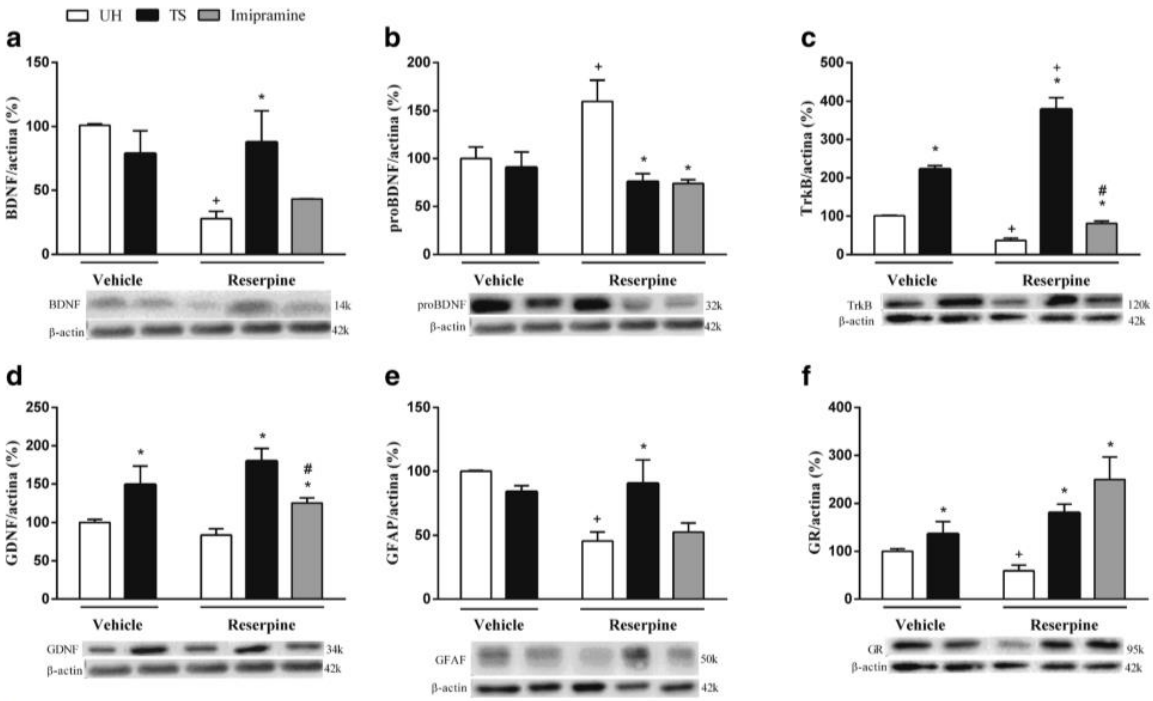


Fig. 5 Influence of reserpine and TS on BDNF (**a**), proBDNF (**b**), TrkB (**c**), GNDF (**d**), GFAP (**e**), and GR immunoreactivity (**f**) in the prefrontal cortex. Data are expressed as mean ± SEM ($n = 7$). Asterisk indicates significant difference from UH to TS or imipramine groups ($p < 0.05$). Plus sign indicates significant difference from vehicle to reserpine groups ($p < 0.05$). Number sign indicates significant difference from reserpine-TS to imipramine group ($p < 0.05$). UH, unhandled; TS, tactile stimulation; BDNF, brain-derived neurotrophic factor; TrkB, tropomyosin receptor kinase B; GDNF, glial-derived neurotrophic factor; GFAP, glial fibrillar acidic protein; GR, glucocorticoid receptor

compared to vehicle-UH animals, while reserpine-UH animals decreased GR levels when compared to vehicle-UH and reserpine-TS animals. The post hoc test of one-way analysis showed that imipramine increased these levels, compared to reserpine-UH, and had no difference when compared to reserpine-TS (Fig. 5f).

Discussion

Our study is showing that the tactile stimulation (TS) applied in adult animals reversed reserpine-induced depression-like behaviors, which were evidenced in the forced swim test (FST), splash test, and sucrose preference test. Furthermore, TS decreased adrenal weight, as well as the plasma levels of ACTH and corticosterone, thus reversing the decreased immunoreactivity of GR, GFAP, TrkB, proBDNF, and BDNF induced by reserpine administration. These findings are indicative that TS could reverse depressive behaviors by different pathways.

The FST is an experimental paradigm originally proposed to investigate beneficial effects of antidepressant drugs [43], in which reduced passive behaviors (immobility) and increased active behaviors (climbing and swimming) display the drug effect on depressive symptoms improvement [50], possibly through noradrenaline and serotonin system activation [51]. Here, TS and imipramine showed a reduction in the immobility time, thus increasing the swimming time in the FST. More precisely, imipramine also increased the climbing behavior. As this drug is a tricyclic antidepressant, it can reverse the depressive-like behavior, since the increase in the release of neurotransmitters such as noradrenaline and serotonin is directly associated with the climbing behavior enhancement [52]. Meanwhile, TS treatment increased only the swimming time, suggesting that the main effect of TS could be more dependent on serotoninergic system modulation. Despite that, more investigations about the monoaminergic system are needed. Besides FST, anhedonia behaviors (or hyposensitivity to pleasure) also have been employed to access the effectiveness of the antidepressant treatment [46, 53]. Here, both TS and imipramine administration reversed the increased latency time to grooming in the splash test, and the reduced sucrose consumption induced by reserpine.

Previous studies from our laboratory have shown the beneficial influence of TS applied during the neonatal period, which was able to reduce anhedonia in adult rats, preventing anxiety after stress exposure [54], improving benzodiazepine drugs responsiveness [55]. Moreover, TS presented itself as an

efficient tool against cocaine and amphetamine addiction [36, 37], also showing its effectiveness to increase the sertraline responsiveness in an animal model of depression [38]. Regarding the different objectives, our current findings primarily differ from these previous studies in two fundamental aspects: (i) TS was applied in adult animals; (ii) the beneficial influence of TS was effective to reverse reserpine-induced depression-like behaviors, while in previous studies the TS benefits were exclusively preventive. Although reserpine showed no reduction in grooming time in the splash test, TS increased this parameter per se and in the reserpine-TS group, confirming the hypothesis that TS exerts a beneficial approach to improve emotionality.

It is well known that women are more susceptible to develop depression symptoms [2]. Thus, we performed our research with female rats in order to mimic the human clinic. Although literature demonstrates that the estrous cycle has an important effect on depressive-like behaviors, since the estradiol can show antidepressant effects [56], female rodents are known to be more vulnerable to neuropsychiatric disorders models [6] and more responsive to the impacts upon modulation of the BDNF signaling and HPA axis [57, 58]. In the present study, TS treatment reduced ACTH and corticosterone levels in plasma, decreased adrenal glands weight, and increased GR immunoreactivity in PFC. Our findings are in accordance with previous studies showing that the glucocorticoid (GC) levels increase, deregulation in GR and the HPA axis hyperactivity were associated with depression-like behaviors [59, 60]. On the other hand, GR activation may improve the impaired HPA axis negative feedback and consequently, inhibit GC release. Such enhancement of GR levels in the brain, including PFC, is associated with decreased ACTH and corticosterone release and is considered pivotal to reduce depression-like behaviors since antidepressant treatment can reduce HPA axis activity by increasing GR expression, translocation, binding, and function [61, 62]. Our outcomes are in agreement with this evidence, given that TS applied during animals' adulthood showed the ability to decrease the HPA axis activity, minimizing the corticosteroid cascade, inciting the GR expression. Also, higher GR levels in the PFC after reserpine could explain the animals' depression-like behavior, whereas GR levels could be increased by TS or imipramine to regulate the GC release reversing the depression-like behavior. Together these findings may be linked to a better biological adaptation of the animal and a possible antidepressant effect, considering antidepressants can modify GR expression in brain areas such as the PFC [63].

Also, the GR changes in the PFC are directly associated with plasma GC release, and evidence demonstrated that GC exerts different influences on the PFC. Meanwhile, the increase of this hormone can impair the PFC neuronal architecture, thus facilitating the development of neuropsychiatric disorders such as depression [64, 65]. Similar to TS, the imipramine-induced reversion of depressive-like behaviors could be, at least partly, consequent to its influence on the hormones and GR levels, which is in accordance with previous findings, suggesting the antidepressant-like effects could be glucocorticoid-dependent [66, 67]. It is important to notice that HPA axis receives serotonergic input, acting as a regulator of behavior and emotion and the hyperactivity of the HPA axis can impair monoaminergic neurons, leading to a BDNF downregulation in the brain [68, 69]. Thus, an increase in glucocorticoid release could exert detrimental effects on CNS, reducing dendritic complexity, altering synaptic plasticity, and suppressing GDNF and BDNF expression, leading to atrophy and disruption of connectivity in cortex and hippocampus [70, 71].

Recent reports have shown that TS applied during the neonatal period and adulthood was able to increase trophic factor levels, including BDNF and fibroblast growth factor 2 in different brain areas, also increasing dendritic lengths after brain injury and enhancing memory performance of rodents [40, 72]. In the current study, TS applied in adult animals reversed the decreased reserpine-induced BDNF immunoreactivity, thus increasing TrkB and reducing proBDNF in the PFC, a brain area closely implicated in depression development [73, 74]. Indeed, BDNF signaling exerts an essential role on the maturation and survival of neurons, regulating neuropsychiatric-like behavioral phenotypes in adulthood [75]. In contrast, its precursor, proBDNF, can bind on $p75^{NTR}$ receptor inducing apoptosis, decreasing spine density and dendritic arbor [76, 77], thus facilitating depression-like behaviors. Moreover, BDNF-TrkB signaling also has demonstrated antidepressant activity similarly to our results, since the increase of TrkB levels in the brain tissue has been associated with depression improvement [78, 79]. Besides, BDNF signaling is crucial to the normal functioning of 5-HT_{2A} receptors, which are involved in the antidepressant activities [10]. In this sense, alterations in the conversion of proBDNF to BDNF together with reduced TrkB immunoreactivity, as observed in the reserpine-UH group, could explain the depression-like behaviors observed in this experimental group, while TS, and in minor proportion, imipramine, improved this signaling cascade and decreased the depression-like behavior.

Regarding imipramine, it is known that antidepressant drugs can increase BDNF/TrkB levels, thus helping to recover depression-like behaviors. However, our findings are showing that although imipramine has reduced the immobility time in TS-exposed animals, changes in BDNF/TrkB cascade were not observed in this experimental group. We believe this contradiction between the current outcomes and literature data can be only apparent considering the doses of imipramine and treatment time were in fact, different [80, 81].

Of particular importance, our findings also showed that reserpine exposure did not decrease GDNF levels in the PFC,

while TS and imipramine treatment increased the immunoreactivity of this molecular marker. GDNF is known to be an important factor for the survival and maintenance of dopaminergic and serotonergic neurons [82]. According to recent findings, all types of antidepressants, including imipramine, can modulate GDNF expression, possibly due to the increased extracellular level of 5-HT caused by antidepressant treatment, leading to increased ERK/MAPK activation and consequently stimulating the GDNF release [82, 83]. TS procedure increased this neurotrophic factor, suggesting the monoamines levels maintenance, which can be related to the depression-like behavior improvement. Studies have demonstrated that TS applied for 8 days can alter trophic factors in brain regions after a dopaminergic lesion, and that might have a direct relation between TS and dopaminergic release in the brain, and maybe other monoamines, which could contribute to improving psychiatry conditions such as depression [40, 84]. It is important to note that a limitation of our study was the absence of monoamines quantification in the PFC. Such an analysis could show the real monoamines reduction in the animals' brain and the possible recovery promoted by TS. This handling procedure showed similar, but subtly superior influences than those observed after imipramine administration. Thus, the continuity of studies approaching the TS as a therapeutic resource is still needed to better understand the relation among TS in adult animals, brain neurotrophic factors, and monoamines levels.

With similar functions to the neurotrophic factors, astrocytes are a type of glial cell responsible for providing energy, morphological, and metabolic support to neurons, and are also involved in differentiation, proliferation, and survival of neurons [85]. Reduced numbers of astrocytes have been related to different CNS diseases, including depression [22]. Likewise, GFAP, which is an essential marker of astrocytes and the major component of intermediate cytoskeletal filaments, presented reduced levels in both human and rodent depressed brain [86]. In our findings, reserpine reduced GFAP immunoreactivity in the PFC, while TS reestablished these levels. Decreased levels of GFAP also indicate a reduced number of astrocytes, and their hypofunction was recently associated with lower neuron survival and disruption in synaptic plasticity, which may be closely related to depression-like behavior [24] and literature data have shown that some antidepressant drugs can recover the astrocytes levels, ameliorating depression symptoms [24]. In our current findings, imipramine did not recover GFAP levels, but TS increased GFAP levels, enhancing reserpine-induced depression-like behaviors. In this sense, we suggest that TS can affect astrocytes proliferation and morphology, increasing synaptic plasticity and improving depression-like behaviors. Therefore, we hypothesized that the improvement in the HPA axis signaling caused by TS could enhance the function of neurotrophic factors and GFAP and consequently improve depression-like behavior in adult rats.

In summary, TS exerts a positive effect on the HPA axis signaling, which was reflected in the adrenal weight of animals, corticosterone release, and changes in GR expression in PFC. The protective cascade triggered by TS in adult female rats recovered the cortical levels of neurotrophic factors, such as BDNF, TrkB, GNDF, and GFAP, which were impaired by the reserpine-induced animal model of depression. Thus, to the best of our knowledge, the current study is demonstrating for the first time that TS can reverse depression-like behaviors in adult rats, through its influence on gene transcription, enhancing the neurogenesis. Also, the beneficial influence of TS was similar but subtly higher to those observed with the imipramine, a classical antidepressant drug. Our findings suggest that TS exerts a beneficial role on the psychological disorders of adult life and could be a practical approach in the human clinic, in the form of massage therapy, since this therapy consists in kinesthetic or sensory stimulation [87], contributing to the treatment of depression. Despite that, performing additional studies would be imperative to deepen the knowledge related to a possible involvement of the monoaminergic system succeeding the TS procedure.

Funding Information This study was financially supported by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brazil (CAPES) - Finance Code 001. Authors are grateful to Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil); Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS, Brazil) and PRPGP-UFSM (PROAP).

Compliance with Ethical Standards

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

Research Involving Animals All procedures were approved by the Animal Ethics Committee of the Federal University of Santa Maria and were carried out according to the Guidelines for Animal Experiments.

References

1. American Psychiatric Association (APA) (2017) What is depression? https://www.psychiatry.org/patients-families/depression/what-is-depression. Accessed May 2018
2. World Health Organization (WHO) (2017) Depression: let's talk. http://www.who.int/mental_health/management/depression/en/. Accessed May 2018
3. Haenisch B, Bönisch H (2011) Depression and antidepressant: insights from knockout of dopamine, serotonin or noradrenaline reuptake transporters. Pharmacol Ther 129(3):352–368. https://doi.org/10.1016/j.pharmthera.2010.12.002
4. Nemeroff CB (2007) The burden of severe depression: a review of diagnostic challenges and treatment alternatives. J Psychiatr Res 41:189–206. https://doi.org/10.1016/j.jpsychires.2006.05.008
5. Penn E, Tracy DK (2012) The drugs don't work? Antidepressants and the current and future pharmacological management of

depression. Ther Adv Psychopharmacol 2:179–188. https://doi.org/10.1177/2045125312445469
6. Connolly KR, Thase ME (2012) Emerging drugs for major depressive disorder. Expert Opin Emerg Drugs 17(1):105–126. https://doi.org/10.1517/14728214.2012.660146
7. Chmielarz P, Kreiner G, Kot M, Zelek-Molik A, Kowalska M, Bagińska M, Daniel WA, Nalepa I (2015) Disruption of glucocorticoid receptors in the noradrenergic system leads to BDNF up-regulation and altered serotonergic transmission associated with a depressive-like phenotype in female GRDBHCre mice. Pharmacol Biochem Be 137:69–77. https://doi.org/10.1016/j.pbb.2015.08.001
8. Kraus C, Castrén E, Kasper S, Lanzenberger R (2017) Serotonin and neuroplasticity-links between molecular, functional and structural pathophysiology in depression. Neurosci Biobehav Rev 77:317–326. https://doi.org/10.1016/j.neubiorev.2017.03.007
9. Vincent MY, Hussain RJ, Zampi ME, Sheeran K, Solomon MB, Herman JP, Khan A, Jacobson L (2013) Sensitivity of depression-like behavior to glucocorticoids and antidepressants is independent of forebrain glucocorticoid receptors. Brain Res 1525:1–15. https://doi.org/10.1016/j.brainres.2013.05.031
10. Tsybko AS, Il'chibaeva TV, Kondaurova EM, Bazovkina DV, Naumenko VS (2014) Effect of central administration of the neurotrophic factors BDNF and GDNF on the functional activity and expression of 5-HT2A serotonin receptors in mice genetically predisposed to depressive-like behavior. Mol Biol 48:864–869. https://doi.org/10.1134/S0026893314060181
11. Diniz BS, Teixeira AL, Miranda AS, Talib LL, Gattaz WF, Forlenza OV (2012) Circulating glial-derived neurotrophic factor is reduced in late-life depression. J Psychiatr Res 46:135–139. https://doi.org/10.1016/j.jpsychires.2011.09.007
12. Lindholm JS, Autio H, Vesa L et al (2012) The antidepressant-like effects of glutamatergic drugs ketamine and AMPA receptor potentiator LY 451646 are preserved in bdnf+/− heterozygous null mice. Neuropharmacology 62:391397–391397. https://doi.org/10.1016/j.neuropharm.2011.08.015
13. Madhav TR, Pei Q, Zetterstrom TS (2001) Serotonergic cells of the rat raphe nuclei express mRNA of tyrosine kinase B (TrkB), the high-affinity receptor for brain-derived neurotrophic factor (BDNF). Brain Res Mol Brain Res 93:56–63. https://doi.org/10.1016/S0169-328X(01)00183-8
14. Bai YY, Ruan SH, Yang CR, Li JI, Kang ZI, Zhou L, Liu D, Zeng YQ et al (2016) ProBDNF signalling regulates depression-like behaviours in rodents under chronic stress. Neuropsychopharmacology 41(12):2882–2892. https://doi.org/10.1038/npp.2016.100
15. Ducray A, Krebs SH, Schaller B, Seiler RW, Meyer M, Widmer HR (2006) GDNF family ligands display distinct action profiles on cultured GABAergic and serotonergic neurons of rat ventral mesencephalon. Brain Res 1069:104–112. https://doi.org/10.1016/j.brainres.2005.11.056
16. Jaumotte JD, Zigmond MJ (2014) Comparison of GDF5 and GDNF as neuroprotective factors for postnatal dopamine neurons in ventral mesencephalic cultures. J Neurosci Res 92:1425–1433. https://doi.org/10.1002/jnr.23425
17. Takaku S, Yanagisawa H, Watabe K, Horie H, Kadoya T, Sakumi K, Nakabeppu Y, Poirier F et al (2013) GDNF promotes neurite outgrowth and upregulates galectin-1 through the RET/PI3K signaling in cultured adult rat dorsal root ganglion neurons. Neurochem Int 62:330–339. https://doi.org/10.1016/j.neuint.2013.01.008
18. Wakeman DR, Redmond DE Jr, Dodiya HB, Sladek JR Jr, Leranth C, Teng YD, Samulski RJ, Snyder EY (2014) Human neural stem cells survive long term in the midbrain of dopamine depleted monkeys after GDNF overexpression and project neurites toward an appropriate target. Stem Cells Transl Med 3:692–701. https://doi.org/10.5966/sctm.2013-0208
19. Liu Q, Zhu HY, Li B, Wang YQ, Yu J, Wu GC (2012) Chronic clomipramine treatment restores hippocampal expression of glial cell line-derived neurotrophic factor in a rat model of depression. J Affect Disord 141:367–372. https://doi.org/10.1016/j.jad.2012.03.018
20. Tseng PT, Lee Y, Lin PY (2013) Age-associated decrease in serum glial cell line-derived neurotrophic factor levels in patients with major depressive disorder. Prog Neurobiol 40:334–339. https://doi.org/10.1016/j.pnpbp.2012.09.009
21. Zhang X, Ru B, Sha W, Xin W, Zhou H, Zhang Y (2014) Performance on the Wisconsin card-sorting test and serum levels of glial cell line-derived neurotrophic factor in patients with major depressive disorder. Asia Pac Psychiatry 6:302–307. https://doi.org/10.1111/appy.12120
22. Ding XF, Liu Y, Yan Z et al (2017) Involvement of normalized glial fibrillary acidic protein expression in the hippocampi in antidepressant-like effects of xiaoyaosan on chronically stressed mice. Evid-Based Compl Alt Med 2017:13. https://doi.org/10.1155/2017/1960584
23. Zhao Y, Lin Z, Chen L, Ouyang L, Gu L, Chen F, Zhang Q (2018) Hippocampal astrocyte atrophy in a mouse depression model induced by corticosterone is reversed by fluoxetine instead of benzodiazepine diazepam. Prog Neurobiol 83:99–109. https://doi.org/10.1016/j.pnpbp.2018.01.011
24. Rial D, Lemos C, Pinheiro H, Duarte JM, Gonçalves FQ, Real JI, Prediger RD, Gonçalves N et al (2016) Depression as a glial-based synaptic dysfunction. Front Cell Neurosci 9(521):1-11. https://doi.org/10.3389/fncel.2015.00521
25. Pariante CM (2006) The glucocorticoid receptor: part of the solution or part of the problem? J Psychopharmacol 20:79–84. https://doi.org/10.1177/1359786806066063
26. Tyrka AR, Parade SH, Welch ES, Ridout KK, Price LH, Marsit C, Philip NS, Carpenter LL (2016) Methylation of the leukocyte glucocorticoid receptor gene promoter in adults: associations with early adversity and depressive, anxiety and substance-use disorders. Transl Psychiatry 6(7):e848. https://doi.org/10.1038/tp.2016.112
27. Clark JA, Flick RB, Pai LY, Szalayova I, Key S, Conley RK, Deutch AY, Hutson PH et al (2008) Glucocorticoid modulation of tryptophan hydroxylase-2 protein in raphe nuclei and 5-hydroxytryptophan concentrations in frontal cortex of C57/Bl6 mice. Mol Psychiatry 13:498–506. https://doi.org/10.1038/sj.mp.4002041
28. Makino S, Smith MA, Gold PW (2002) Regulatory role of glucocorticoids and glucocorticoid receptor mRNA levels on tyrosine hydroxylase gene expression in the locus coeruleus during repeated immobilization stress. Brain Res 943:216–223. https://doi.org/10.1016/S0006-8993(02)02647-1
29. Czyrak A, Chocyk A (2001) Search for the presence of glucocorticoid receptors in dopaminergic neurons of rat ventral tegmental area and substantia nigra. Pol J Pharmacol 53:681–684
30. Gulyaeva NV (2017) Interplay between brain BDNF and glutamatergic systems: a brief state of the evidence and association with the pathogenesis of depression. Biochemistry 82(3):301–307. https://doi.org/10.1134/S0006297917030087
31. Hong YP, Lee HC, Kim HT (2015) Treadmill exercise after social isolation increases the levels of NGF, BDNF, and synapsin I to induce survival of neurons in the hippocampus and improves depression-like behavior. J Exerc Nutr Biochem 19:11–18. https://doi.org/10.5717/jenb.2015.19.1.11
32. Shilpa BM, Bhagya V, Harish G, Srinivas Bharath MM, Shankaranarayana Rao BS (2017) Environmental enrichment ameliorates chronic immobilisation stress-induced spatial learning deficits and restores the expression of BDNF, VEGF, GFAP and glucocorticoid receptors. Prog Neuro-Psycho 76:88–100. https://doi.org/10.1016/j.pnpbp.2017.02.025
33. de Los Angeles GAM, Del Carmen RO, Wendy PM, Socorro RM (2016) Tactile stimulation effects on hippocampal neurogenesis and spatial learning and memory in prenatally stressed rats. Brain Res Bull 124:1–11. https://doi.org/10.1016/j.brainresbull.2016.03.003
34. Richards S, Mychasiuk R, Kolb B, Gibb R (2012) Tactile stimulation during development alters behaviour and neuroanatomical

organization of normal rats. Behav Brain Res 231:86–91. https://doi.org/10.1016/j.bbr.2012.02.043

35. Río-Alamos C, Oliveras I, Cañete T et al (2015) Neonatal handling decreases unconditioned anxiety, conditioned fear, and improves two-way avoidance acquisition: a study with the inbred Roman high (RHA-I)- and low-avoidance (RLA-I) rats of both sexes. Front Behav Neurosci 9:174. https://doi.org/10.3389/fnbeh.2015.00174
36. Antoniazzi CTD, Boufleur N, Dolci G, Roversi K, Kuhn F, Pase CS, Dias VT, Roversi K et al (2014a) Influence of neonatal tactile stimulation on amphetamine preference in young rats: parameters of addiction and oxidative stress. Pharmacol Biochem Behav 124:341–349. https://doi.org/10.1016/j.pbb.2014.07.001
37. Antoniazzi CTD, Boufleur N, Pase CS, Kuhn FT, Dias VT, Segat HJ, Roversi K, Roversi K et al (2014b) Tactile stimulation and neonatal isolation affect behavior and oxidative status linked to cocaine administration in young rats. Behav Process 103:297–305. https://doi.org/10.1016/j.beproc.2014.01.0110376-6357
38. Freitas DL, Antoniazzi CT, Segat HJ et al (2015) Neonatal tactile stimulation decreases depression-like and anxiety-like behaviors and potentiates sertraline action in young rats. Int J Dev Neurosci 47:192–197. https://doi.org/10.1016/j.ijdevneu.2015.09.010
39. Gibb RL, Gonzalez CL, Wegenast W, Kolb BE (2010) Tactile stimulation promotes motor recovery following cortical injury in adult rats. Behav Brain Res 214:102–107. https://doi.org/10.1016/j.bbr.2010.04.008
40. Effenberg A, Klein A, Gibb R, Carroll C, Baumgärtner W, Grothe C, Ratzka A (2014) Adult hemiparkinsonian rats do not benefit from tactile stimulation. Behav Brain Res 261:97–105. https://doi.org/10.1016/j.bbr.2013.12.011
41. Bourin M, Poncelet M, Chermat R, Simon P (1983) The value of the reserpine test in psychopharmacology. Arzneimittelforschung 33:1173–1176
42. Nagakura Y, Oe T, Aoki T, Matsuoka N (2009) Biogenic amine depletion causes chronic muscular pain and tactile allodynia accompanied by depression: a putative animal model of fibromyalgia. Pain 146:26–33. https://doi.org/10.1016/j.pain.2009.05.024
43. Porsolt RD, Bertin A, Blavet N, Deniel M, Jalfre M (1979) Immobility induced by forced swimming in rats - effects of agents which modify central catecholamine and serotonin activity. Eur J Pharmacol 57:201–210, 1979. https://doi.org/10.1016/0014-2999(79)90366-2
44. Castagne V, Porsolt RD, Moser P (2009) Use of latency to immobility improves detection of antidepressant-like activity in the behavioral despair test in the mouse. Eur J Pharmacol 616:128–133. https://doi.org/10.1016/j.ejphar.2009.06.018
45. Isingrini E, Camus V, Guisquet AM et al (2010) Association between repeated unpredictable chronic mild stress (UCMS) procedures with a high fat diet: a model of fluoxetine resistance in mice. PLoS One 5(4):e10404. https://doi.org/10.1371/journal.pone.0010404
46. Mutlu O, Gumuslu E, Ulak G, Celikyurt IK, Kokturk S, Kır HM, Akar F, Erden F (2012) Effects of fluoxetine, tianeptine and olanzapine on unpredictable chronic mild stress-induced depression-like behavior in mice. Life Sci 91:1252–1262. https://doi.org/10.1016/j.lfs.2012.09.023
47. Kompagne H, Bárdos G, Szénási G, Gacsályi I, Hársing LG, Lévay G (2008) Chronic mild stress generates clear depressive but ambiguous anxiety-like behaviour in rats. Behav Brain Res 193:311–314. https://doi.org/10.1016/j.bbr.2008.06.008
48. Paxinos G, Watson C (2006) The rat brain in stereotaxic coordinates, 6th ed. Elsevier Academic Press, Cambridge
49. Pase CS, Roversi KR, Roversi K et al (2017) Maternal *trans* fat intake during pregnancy or lactation impairs memory and alters BDNF and TrkB levels in the hippocampus of adult offspring exposed to chronic mild stress. Physiol Behav 169:114–123. https://doi.org/10.1016/j.physbeh.2016.11.009
50. Cryan JF, Valentino RJ, Lucki I (2005) Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. Neurosci Biobehav Rev 29:547–569. https://doi.org/10.1016/j.neubiorev.2005.03.008
51. Antkiewicz-Michaluk L, Wąsik A, Możdżeń E, Romańska I, Michaluk J (2014) Antidepressant-like effect of tetrahydroisoquinoline amines in the animal model of depressive disorder induced by repeated administration of a low dose of reserpine: behavioral and neurochemical studies in the rat. Neurotox Res 26:85–98. https://doi.org/10.1007/s12640-013-9454-8
52. Detke MJ, Rickels M, Lucki I (1995) Active behaviors in the rat forced swimming test differentially produced by serotonergic and noradrenergic antidepressants. Psychopharmacology 121(1):66–72
53. Machado DG, Cunha MP, Neis VB, Balen GO, Colla A, Grando J, Brocardo PS, Bettio LEB et al (2012) Fluoxetine reverses depressive-like behaviors and increases hippocampal acetylcholinesterase activity induced by olfactory bulbectomy. Pharmacol Biochem Behav 103(2):220–229. https://doi.org/10.1016/j.pbb.2012.08.024
54. Boufleur N, Antoniazzi CTD, Pase CS, Benvegnú DM, Dias VT, Segat HJ, Roversi K, Roversi K et al (2013) Neonatal handling prevents anxiety-like symptoms in rats exposed to chronic mild stress: behavioral and oxidative parameters. Stress 16:321–330. https://doi.org/10.3109/10253890.2012.723075
55. Boufleur N, Antoniazzi CTD, Pase CS, Benvegnú DM, Barcelos RCS, Dolci GS, Dias VT, Roversi K et al (2012) Neonatal tactile stimulation changes anxiety-like behavior and improves responsiveness of rats to diazepam. Brain Res 1474:50–59. https://doi.org/10.1016/j.brainres.2012.08.002
56. Estrada-Camarena E, Fernandez-Guasti A, Lopez-Rubalcava C (2004) Interaction between estrogens and antidepressants in the forced swimming test in rats. Psychopharmacology 173(1–2):139–145. https://doi.org/10.1007/s00213-003-1707-4
57. Pawluski JL (2012) Perinatal selective serotonin reuptake inhibitor exposure: impact on brain development and neural plasticity. Neuroendocrinology 95:39–46. https://doi.org/10.1159/000329293
58. Boulle F, Pawluski JL, Homberg JR, Machiels B, Kroeze Y, Kumar N, Steinbusch HWM, Kenis G et al (2016) Developmental fluoxetine exposure increases behavioral despair and alters epigenetic regulation of the hippocampal BDNF gene in adult female offspring. Horm Behav 80:47–57. https://doi.org/10.1016/j.yhbeh.2016.01.017
59. Pisu MG, Garau A, Boero G, Biggio F, Pibiri V, Dore R, Locci V, Paci E et al (2016) Sex differences in the outcome of juvenile social isolation on HPA axis function in rats. Neuroscience 320:172–182. https://doi.org/10.1016/j.neuroscience.2016.02.009
60. Myers B, McKlveen JM, Herman JP (2012) Neural regulation of the stress response: the many faces of feedback. Cell Mol Neurobiol 32:683–694. https://doi.org/10.1007/s10571-012-9801-y
61. Guidotti G, Calabrese F, Anacker C, Racagni G, Pariante CM, Riva MA (2013) Glucocorticoid receptor and FKBP5 expression is altered following exposure to chronic stress: modulation by antidepressant treatment. Neuropsychopharmacol 38:616–627. https://doi.org/10.1038/npp.2012.225
62. Nikkheslat N, Zunszain P A, Carvalho L A, Anacker C, Pariante C M (2017) Antidepressant actions on glucocorticoid receptors A2. In: G Fink (ed), Stress: neuroendocrinology and neurobiology (2nd ed) San Diego, pp. 279-286. https://doi.org/10.1016/B978-0-12-802175-0.00027-9
63. Heydendael W, Jacobson L (2010) Widespread hypothalamic–pituitary–adreno cortical axis-relevant and mood-relevant effects of chronic fluoxetine treatment on glucocorticoid receptor gene expression in mice. Eur J Neurosci 31:892–902. https://doi.org/10.1111/j.1460-9568.2010.07131.x

64. Hussain RJ, Jacobson L (2015) Increased antidepressant sensitivity after prefrontal cortex glucocorticoid receptor gene deletion in mice. Physiol Behav 138:113-177. https://doi.org/10.1016/j.physbeh.2014.10.019
65. Anderson RM, Glanz RM, Johnson SB, Miller MM, Romig-Martin SA, Radley JJ (2016) Prolonged corticosterone exposure induces dendritic spine remodeling and attrition in the rat medial prefrontal cortex. J Comp Neurol 524(18):3729-3746. https://doi.org/10.1002/cne.24027
66. Zhao J, Niu C, Wang J, Yang H, Du Y, Wei L, Li C (2018) The depressive-like behaviors of chronic unpredictable mild stress-treated mice, ameliorated by Tibetan medicine Zuotai: involvement in the hypothalamic-pituitary-adrenal (HPA) axis pathway. Neuropsychiatr Dis Treat 14:129-141. https://doi.org/10.2147/NDT.S151107
67. Wrobel A, Serefko A, Szopa A, Rojek K, Poleszak E, Skalicka-Woźniak K, Dudka J (2017) Inhibition of the CRF1 receptor influences the activity of antidepressant drugs in the forced swim test in rats. Naunyn Schmiedebergs Arch Pharmacol. 390(8):769-774. https://doi.org/10.1007/s00210-017-1377-0
68. Ambrus L, Lindqvist D, Träskman-Bendz L, Westrin Å (2016) Hypothalamic–pituitary–adrenal axis hyperactivity is associated with decreased brain derived neurotrophic factor in female suicide attempters. Nord J Psychiat 70(8):575-581. https://doi.org/10.1080/08039488.2016.1184310
69. Wang Z, Gu J, Wang X et al (2013) Antidepressant-like activity of resveratrol treatment in the forced swim test and tail suspension test in mice: The HPA axis, BDNF expression and phosphorylation of ERK. Pharmacol Biochem Be 112:104-110. https://doi.org/10.1016/j.pbb.2013.10.007
70. Verity AN, Wyatt TL, Lee W et al (1999) Differential regulation of glial cell line-derived neurotrophic factor (GDNF) expression in human neuroblastoma and glioblastoma cell lines. J Neurosci Res 55:187-197. https://doi.org/10.1177/1073858413510208
71. Nakashima S, Matsuyama Y, Yu Y, Kiuchi K, Ishiguro N (2004) Suppression of GDNF production by MPSS treatment following spinal cord injury in the rat. Neuroreport 15:2337-2340. https://doi.org/10.1097/00001756-200410250-00007
72. Antoniazzi CTD, Metz VG, Roversi K et al (2017) Tactile stimulation during different developmental periods modifies hippocampal BDNF and GR, affecting memory and behavior in adult rats. Hippocampus 27:210-220. https://doi.org/10.1002/hipo.22686
73. Yu H, Chen Z Y (2011) The role of BDNF in depression on the basis of its location in the neural circuitry. Acta Pharmacol Sin 32:3-11. https://doi.org/10.1038/aps.2010.184
74. Zhang JC, Wu J, Fujita Y et al (2015) Antidepressant effects of TrkB ligands on depression-like behaviors and dendritic changes in mice after inflammation. Int J Neuropsychopharmacol 18(4): pyu077. https://doi.org/10.1093/ijnp/pyu077
75. Li H, Ishikawa C, Shig T (2018) Effects of postnatal handling on adult behavior and brain mRNA expression of serotonin receptor, brain-derived neurotrophic factor and GABA-A receptor subunit. Int J Dev Neurosci 68:17-25. https://doi.org/10.1016/j.ijdevneu.2018.04.002
76. Yang J, Harte-Hargrove LC, Siao CJ et al (2014) ProBDNF negatively regulates neuronal remodeling, synaptic transmission, and synaptic plasticity in hippocampus. Cell Rep 7:796-806. https://doi.org/10.1016/j.celrep.2014.03.040
77. Shirayama Y, Yang C, Zhang J C et al (2015) Alterations in brain-derived neurotrophic factor (BDNF) and its precursor proBDNF in the brain regions of a learned helplessness rat model and the antidepressant effects of a TrkB agonist and antagonist. Eur Neuropsychopharmacol 25(12): 2449-2458. https://doi.org/10.1016/j.euroneuro.2015.09.002
78. Tripp A, Oh H, Guilloux J P et al (2012) Brain-derived neurotrophic factor signaling and subgenual anterior cingulate cortex dysfunction in major depressive disorder. Am J Psychiatry 169(11):1194-1202. https://doi.org/10.1176/appi.ajp.2012.12020248
79. Lin PY, Tseng PT (2015) Decreased glial cell line-derived neurotrophic factor levels in patients with depression: a meta-analytic study. J Psychiatr Res 63:20-27. https://doi.org/10.1016/j.jpsychires.2015.02.004
80. Ding Q, Li H, Tian X, Shen Z, Wang X, Mo F, Huang J, Shen H (2016) Zinc and imipramine reverse the depression-like behavior in mice induced by chronic restraint stress. J Affect Disord 197:100-106. https://doi.org/10.1016/j.jad.2016.03.017
81. Razavi BM, Sadeghi M, Abnous K, Hasani FV, Hosseinzadeh H (2017) Study of the Role of CREB, BDNF, and VGF Neuropeptide in Long Term Antidepressant Activity of Crocin in the Rat Cerebellum. Iran J Pharm Res 16(4):1452-1462
82. Naumenko VS, Bazovkina DV, Semenova AA et al (2013) Effect of Glial Cell Line-Derived Neurotrophic Factor on Behavior and Key Members of the Brain Serotonin System in Mouse Strains Genetically Predisposed to Behavioral Disorders. J Neurosci Res 91:1628-1638. https://doi.org/10.1002/jnr.23286
83. Tsybko AS, Ilchibaeva TV, Popova NK (2017) Role of glial cell line-derived neurotrophic factor in the pathogenesis and treatment of mood disorders. Rev Neurosci 28(3):219-233. https://doi.org/10.1515/revneuro-2016-0063
84. Maruyama K, Shimoju R, Ohkubo M, Maruyama H, Kurosawa M (2012) Tactile skin stimulation increases dopamine release in the nucleus accumbens in rats. J Physiol Sci 62:259–266. https://doi.org/10.1007/s12576-012-0205-z
85. Fellin T (2009) Communication between neurons and astrocytes: relevance to the modulation of synaptic and network activity. J Neurochem 108:533-544. https://doi.org/10.1111/j.1471-4159.2008.05830.x
86. Cobb J A, O'Neill K, Milner J et al (2016) Density of GFAP-immunoreactive astrocytes is decreased in left hippocampi in major depressive disorder. Neuroscience 316:209-220. https://doi.org/10.1016/j.neuroscience.2015.12.044
87. Mathai S, Fernandez A, Mondkar J, Kanbur W (2001) Effects of tactile-kinesthetic stimulation in preterms: a controlled trial. Indian Pediatr 38:1091-1098

4.2 ARTIGO CIENTÍFICO II

Neonatal Tactile Stimulation Alters Behaviors in Heterozygous Serotonin Transporter Male Rats: Role of the Amygdala

Karine Roversi, Carolina Buizza, Paola Brivio, Francesca Calabrese, Michel M. M. Verheij, Caren T. D. Antoniazzi, Marilise E. Burger, Marco A. Riva, Judith R. Homberg

Frontiers in Behavioral Neuroscience
DOI: 10.3389/fnbeh.2020.00142

frontiers in Behavioral Neuroscience

ORIGINAL RESEARCH
published: 12 August 2020
doi: 10.3389/fnbeh.2020.00142

Neonatal Tactile Stimulation Alters Behaviors in Heterozygous Serotonin Transporter Male Rats: Role of the Amygdala

Karine Roversi[1], Carolina Buizza[2], Paola Brivio[2], Francesca Calabrese[2], Michel M. M. Verheij[3], Caren T. D. Antoniazzi[4], Marilise E. Burger[1], Marco A. Riva[2] and Judith R. Homberg[3]**

[1]Department of Physiology and Pharmacology, Health Sciences Center, Federal University of Santa Maria, Santa Maria, Brazil, [2]Department of Pharmacological and Biomolecular Sciences, Università Degli Studi di Milano, Milan, Italy, [3]Department of Cognitive Neuroscience, Donders Institute for Brain, Cognition, and Behavior, Radboud University Medical Center, Nijmegen, Netherlands, [4]International Centre for Neurotherapeutics, Dublin City University, Dublin, Ireland

OPEN ACCESS

Edited by:
Clement Hamani, University of Toronto, Canada

Reviewed by:
Oliver Ambree, University of Osnabrück, Germany
Carla Dalmaz, Federal University of Rio Grande do Sul, Brazil

***Correspondence:**
Karine Roversi
karineroversi_@hotmail.com
Judith R. Homberg
judith.homberg@radboudumc.nl

Specialty section:
This article was submitted to Behavioral Endocrinology, a section of the journal Frontiers in Behavioral Neuroscience

Received: 09 April 2020
Accepted: 23 July 2020
Published: 12 August 2020

Citation:
Roversi K, Buizza C, Brivio P, Calabrese F, Verheij MMM, Antoniazzi CTD, Burger ME, Riva MA and Homberg JR (2020) Neonatal Tactile Stimulation Alters Behaviors in Heterozygous Serotonin Transporter Male Rats: Role of the Amygdala. Front. Behav. Neurosci. 14:142. doi: 10.3389/fnbeh.2020.00142

The serotonin transporter (SERT) gene, especially the short allele of the human serotonin transporter linked polymorphic region (5-HTTLPR), has been associated with the development of stress-related neuropsychiatric disorders. In line, exposure to early life stress in SERT knockout animals contributes to anxiety- and depression-like behavior. However, there is a lack of investigation of how early-life exposure to beneficial stimuli, such as tactile stimulation (TS), affects later life behavior in these animals. In this study, we investigated the effect of TS on social, anxiety, and anhedonic behavior in heterozygous SERT knockouts rats and wild-type controls and its impact on gene expression in the basolateral amygdala. Heterozygous SERT$^{+/-}$ rats were submitted to TS during postnatal days 8–14, for 10 min per day. In adulthood, rats were assessed for social and affective behavior. Besides, brain-derived neurotrophic factor (Bdnf) gene expression and its isoforms, components of glutamatergic and GABAergic systems as well as glucocorticoid-responsive genes were measured in the basolateral amygdala. We found that exposure to neonatal TS improved social and affective behavior in SERT$^{+/-}$ animals compared to naïve SERT$^{+/-}$ animals and was normalized to the level of naïve SERT$^{+/+}$ animals. At the molecular level, we observed that TS *per se* affected Bdnf, the glucocorticoid-responsive genes Nr4a1, Gadd45β, the co-chaperone Fkbp5 as well as glutamatergic and GABAergic gene expression markers including the enzyme Gad67, the vesicular GABA transporter, and the vesicular glutamate transporter genes. Our results suggest that exposure of SERT$^{+/-}$ rats to neonatal TS can normalize their phenotype in adulthood and that TS *per se* alters the expression of plasticity and stress-related genes in the basolateral amygdala. These findings demonstrate the potential effect of a supportive stimulus in SERT rodents, which are more susceptible to develop psychiatric disorders.

Keywords: neonatal period, tactile stimulation, anxiety, Bdnf, serotonin transporter knockout, amygdala

INTRODUCTION

Vulnerability to psychiatric disorders is thought to be caused by complex interactions between genes and the environment particularly when the interactions take place early in life (Caspi et al., 2010). The serotonin transporter linked polymorphic region (5-HTTLPR) serves as a model for gene-environment interactions, with the short (s) 5-HTTLPR allele playing an important role in the sensitivity to the environment (Homberg and Lesch, 2011; Homberg et al., 2016). In rodents, these behavioral conditions can be evaluated in serotonin transporter (SERT) knockout rats (Homberg et al., 2007). Heterozygous SERT knockout animals ($SERT^{+/-}$) are generally suggested to be most comparable to the human short 5-HTTLPR allele from a gene-dose dependent point of view. Although some studies could not confirm an interaction effect of SERT genotype and early life adversities (see review: Houwing et al., 2017), other studies have shown a significant influence of the $SERT^{+/-}$ genotype on behaviors putatively related to psychiatric disorders (Olivier et al., 2008; Bartolomucci et al., 2010; van der Doelen et al., 2013; Houwing et al., 2019).

SERT knockout animals present enhanced anxiety- and depression-like symptoms (Homberg et al., 2008; Schipper et al., 2015, 2019; Verheij et al., 2018) and most studies examined the increased sensitivity to negative environmental stimuli in SERT knockout rodents. However, a few studies demonstrated that these animals are also very sensitive to positive environmental stimuli such as psychostimulants, conditioned reward, co-housing with a female, and environmental enrichment (Homberg and Lesch, 2011; Nonkes et al., 2012; Kastner et al., 2015; Homberg et al., 2016; Rogers et al., 2017). This is well in line with the differential susceptibility theory from the field of developmental psychology, which postulates that "plastic" individuals, due to (5-HTTLPR s/s) genotype, show increased sensitivity to environmental stimuli, both adverse and supportive ones (Belsky et al., 2009; van Ijzendoorn et al., 2012).

Early life is a critical phase for central nervous system development, during which plasticity levels are high and the brain is very sensitive to environmental influences. Thus far, no studies have addressed the effect of an early life supportive environment on later life behavior in $SERT^{+/-}$ rodents. Neonatal handling is an environmental treatment used to study behavioral mechanisms and neurobiological alterations in rodents (Denenberg, 1964). The handling consists of separating the pups from the mothers for a short period and performing some intervention, such as tactile stimulation (TS; Daskalakis et al., 2009). Neonatal TS is a procedure applied during developmental periods mimicking nonspecific maternal stimulation such as licking and grooming of pups. It has emerged as an efficient tool to improve the behavior by altering brain organization and enhancing hippocampus neurogenesis (Guerrero et al., 2016) and neuroplasticity (Richards et al., 2012). TS decreases anxiety-like behaviors (Río-Alamos et al., 2015), and prevents the negative effects of stress (Boufleur et al., 2013) and the development of depressive-like behaviors (Freitas et al., 2015). These findings raise the possibility that TS has the potential to modify the anxiety- and depression-related phenotypes of $SERT^{+/-}$ animals.

The TS mechanism in the brain is still unclear, but evidence has suggested that TS affects the hypothalamic-pituitary-adrenal (HPA) axis and brain neurotrophic factors such as brain-derived neurotrophic factor (Bdnf; Antoniazzi et al., 2017; Roversi et al., 2019). Further, the GABA and glutamate systems are likely targets. The HPA-axis mediates stress responses through glucocorticoids release from the adrenal cortex. The programming of the HPA-axis is influenced by stress, leading to glucocorticoid receptor (Nr3c1) dysregulation and changes in the expression of glucocorticoid-inducible genes, including the co-chaperone FK506-binding protein 51 (Fkbp5) and the transcription factor Nr4a1 (Kember et al., 2012; van der Doelen et al., 2014). Indeed, the expression of Fkbp5 was found to be altered in peripheral blood cells of patients suffering from psychiatric disorders following early life adversities (Klengel et al., 2013). Furthermore, altered stress-related behavior in adulthood has been associated with changes in Nr4a1 gene expression in the hippocampus of mice (Kember et al., 2012). Also, the growth arrest and DNA-damage-inducible beta (Gadd45β), a glucocorticoid-responsive gene responsible for actively demethylating gene promoter regions, was found to be increased in the hippocampus of rats treated with the multitarget antidepressant vortioxetine and exposed to acute stress (Brivio et al., 2019). Finally, negative experiences in early life seem to affect the levels of Gadd45β, yet also overexpression of this gene has been observed in the amygdala of psychiatric patients (Gavin et al., 2012; Blaze and Roth, 2013). Thus, whether Gadd45β is also responsive to positive stimuli in early life remains to be determined.

Bdnf is a molecule that promotes the development and survival of neurons and is present in high amounts in the basolateral amygdala. Changes in Bdnf levels in this area are associated with stress-dependent learning and important behavioral changes related to fear and depression (Williams et al., 2006; Schulte-Herbruggen et al., 2006). While TS increased Bdnf hippocampus levels and improved memory and anxiety behaviors in normal and depressive-like animals (Antoniazzi et al., 2017; Roversi et al., 2019), transgenic overexpression of Bdnf in the amygdala facilitated the development of anxiety-related behaviors (Govindarajan et al., 2006), thus showing the importance of the modulatory effect of Bdnf in the brain. Neonatal handling also affects the GABAergic system, as demonstrated by increased GABA interneuron density in the lateral amygdala (Giachino et al., 2007). Moreover, there is an important link between the GABAergic and glutamatergic systems in animal models of depression and different stress-related psychiatric disorders (Garcia-Garcia et al., 2009; Luscher et al., 2011), such as depression, which is characterized by an excitation-inhibition imbalance (Sanacora et al., 2004). Furthermore, expression levels of Gad67, the enzyme that converts glutamate in GABA, are decreased in psychiatric disorders (Fatemi et al., 2005). Conversely, positive stimuli like environmental enrichment that can buffer the negative effects of stress also alter GABAergic signaling in the amygdala (Sampedro-Piquero et al., 2016). Finally, there is evidence

that Bdnf affects the excitation-inhibition balance, providing a putative pathway through which positive environmental stimuli may ameliorate stress-induced excitation-inhibition imbalances (Oh et al., 2016).

In this study we sought out to determine the effect of a supportive environment, that is TS, during the postnatal day (PND) 8–14. This period of life was chosen based on a previous study of Antoniazzi et al. (2017), in which the most beneficial effects of TS have been observed during this period. Furthermore, in rats, brain development paces extremely fast during the first weeks of life. Neurogenesis is completed on PND 15 (Rice and Barone, 2000; Babikian et al., 2010), the astrocytes undergo to a rapid period of maturation, with a peak on PND 11–16 (Catalani et al., 2002), and the critical period of synaptogenesis peaks during week 2 after birth (Semple et al., 2013). As readouts we focused on social behavior, anhedonia, and anxiety in SERT$^{+/-}$ and wild-type control rats in adulthood. Additionally, to understand the underlying mechanisms we assessed mRNA expression levels of Bdnf, glucocorticoid (Nr3c1) and mineralocorticoid (Nr3c2) receptors, and glucocorticoid-responsive genes in the basolateral amygdala. We also focused on components of the GABAergic and glutamatergic systems, by analyzing Gad67, vesicular GABA transporter (Vgat), parvalbumin, and vesicular glutamate transporter (Vglut) gene expression.

MATERIALS AND METHODS

Animal and Procedures

Eight wild-type and eight SERT$^{+/-}$ (Slc6a41Hubr) pregnant rats were used. They were derived by crossing heterozygous breeding animals. Dams were checked daily for pups' delivery, and the day of birth was set as postnatal day (PND) 0. On PND8–14, male pups from each litter were assigned to one of the two experimental groups: neonatal TS or not (no TS, naïve), and the pups were marked with a non-toxic colored marker for identification purposes. At PND21, pups were weaned, and ears were punched for identification and genotyping (Homberg et al., 2007). The animals were housed in two animals per cage, in standard polypropylene cages with saw-dust bedding and water and food *ad libitum*, in a temperature (21 ± 1°C) and humidity-controlled room (45%–60% relative humidity), with a 12:12 h light/dark cycle (lights on at 7:00 AM). The experimental procedures were approved by the Committee for Animal Experiments of the Radboud University Nijmegen, The Netherlands, and all efforts were made to minimize animal suffering and to reduce the number of animals used. Only male SERT$^{+/-}$ rats were used in this study. At PND 60, the males from naïve and TS were subdivided between treatment and genotype [wild-type (SERT$^{+/+}$) or heterozygous (SERT$^{+/-}$)] groups, resulting in the following groups: naïve SERT$^{+/+}$ (n = 9); naïve SERT$^{+/-}$ (n = 7); TS SERT$^{+/+}$ (n = 9); TS SERT$^{+/-}$ (n = 9; **Figure 1A**). All tests were performed in the dark phase.

Neonatal Tactile Stimulation

Neonatal TS was applied from PND 8 to PND 14, between 10 AM and 2 PM. Pups were removed from the nest, gently held by the experimenter, and stroked with the index finger on the dorsal surface, in the rostral-caudal direction for 10 min, once a day. At the end of the TS, pups were returned to their litters. The naïve group (no TS) remained in their nest without any touch by human hands (Freitas et al., 2015; Antoniazzi et al., 2017).

Behavioral Procedures

Social Interaction Test

The social interaction test protocol is based on social interest and interaction with an unfamiliar animal. The arenas consisted of a black floor and transparent walls (45 cm × 50 cm). Before the test, animals were put individually in the chamber for a 1-h habituation session and immediately after habituation, two rats from different cages with the same genotype and manipulation were put together in the arena and the behavior was video recorded for 15 min. The recorded behavior was measured as social contact (time animals spent in body-contact, sniffing, grooming), social interest (time animal was following or approaching), social undergoing (passive behavior: when the rat's partner was mounting, sniffing, pinning, attacking the rat), and non-social behavior (time animal was solitary). One observer blind to the test subjects' genotype and manipulation, manually scored video recordings by using Boris v.7. Both animals were equally observed. By using a reliability analysis feature in Boris (Behavioral Observation Research Interactive Software), the percentages of agreement between two observers were calculated and resulted in 78% of agreement (data not shown).

Elevated Plus-Maze

This test is based on the innate fear rodents have for open and elevated spaces and was performed as described by de Jong et al. (2006). The apparatus consisted of a plus-shaped platform elevated 50 cm from the floor. Two opposite arms (50 cm × 10 cm) were enclosed by 40 cm high walls whereas the other two arms had no walls. The four arms had at their intersection a central platform, which gave access to any of the four arms. At the beginning of each test, the rat was placed on the central platform facing an open arm. The movements and position of the animals were recorded and processed afterward using EthoVision XT (Noldus Information Technology, The Netherlands). Entries were counted when all four paws were placed in one of the arms. Data were expressed as the mean percentage of the time spent on open arms [(time on open arms/300 s) × 100%], the mean of the entries number into open arms [(entries on open arms/total entries) × 100%] and the time spent on closed arms (s). The mean of the time spent (s) on and number of entries to open arms were used as the standard anxiety indices. Locomotor activity was expressed as the total distance moved (cm) by the animal in the entire maze.

Sucrose Consumption

Anhedonia is typically measured using the sucrose consumption test in which the animals have in their homecage a free choice between a bottle with water and a bottle with a sucrose solution. A decrease in sucrose intake and/or preference is considered as a measure of anhedonia (Willner et al., 1987). Animals were housed individually and habituated to the two-bottle paradigm by offering them water in two plastic drinking bottles, one

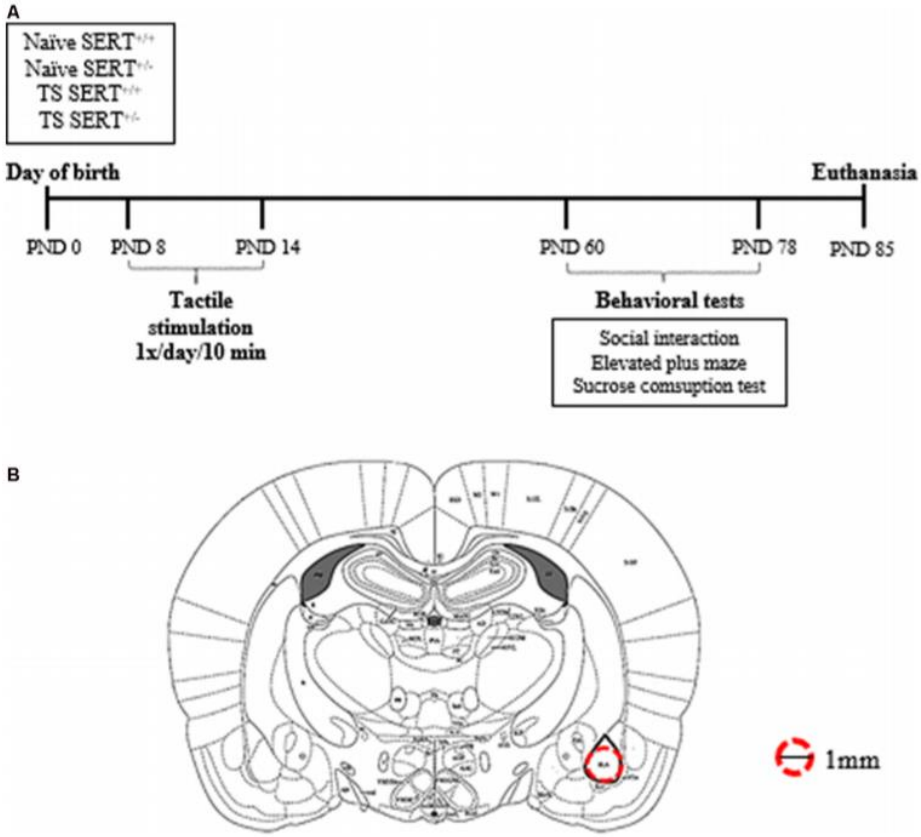


FIGURE 1 | Schematic representation of the experimental paradigm performed in heterozygous serotonin transporter knockout (SERT$^{+/-}$) and wild-type (SERT$^{+/+}$) male rats exposed daily to tactile stimulation (TS) from postnatal day (PND) 8–14 or a control treatment (no TS-naïve; **A**). Diagrams represent the location where basolateral amygdala punches were taken for molecular analyses (adapted from Paxinos and Watson brain Atlas; **B**).

on each side, for a total of 3 days. After the third day of the habituation period, the sucrose test started. Animals were presented with two bottles, one containing water and the other one containing a 3% sucrose solution. Fluid consumption (g) was measured at 2 h, 5 h, and 24 h, and body weight was measured daily (g). Both measures were used to calculate sucrose preference (sucrose intake in ml divided by total intake × 100%) and the intake in grams relative to body weight in Kg (intake in grams divided by body weight in grams/1,000; adapted from Olivier et al., 2008).

Tissue Collection and Preparation

One week after the last day of the sucrose test, the animals were euthanized by decapitation within 10 s. Immediately after the decapitation, the brains were isolated and frozen on dry ice in an aluminum foil and stored in a −80° C. The brains were prepared in 200 μm thick coronal slices in a cryostat (−11°C) to obtain punches of basolateral amygdala bilaterally (Bregma ≈ −2.28: −3.30 mm; Interaural ≈ −6.72: −5.80 mm; anterior-posterior ≈2.8 posterior to bregma; DV ≈6.5 from skull surface) using a 1.00 mm brain puncher (**Figure 1B**). The brain puncher was cleaned with alcohol after each punch. The punches were used for RNA isolation.

RNA Preparation and Gene Expression Analysis by Quantitative Real-Time RT-PCR

Total RNA was isolated from the basolateral amygdala using PureZol RNA isolation reagent (Bio-Rad Laboratories S.r.l.; Segrate, Italy) following the manufacturer's instructions. The RNA concentration was then quantified by spectrophotometric analysis (OD 260/280 1.8 < ratio <2). Afterward, the samples were prepared for real-time polymerase chain reaction (RT-PCR) to measure the mRNA expression of total Bdnf, Bdnf long 3' UTR, Bdnf isoforms IV and VI, Nr3c1, Nr3c2, Nr4a1, Gadd45β, Fkbp5, Gad67, Pvalb, Vgat, Vglut. After RNA isolation, an aliquot of each sample was treated with DNAse to avoid contamination by DNA. The samples were then prepared for the analyses by TaqMan qRT-PCR instrument (CFX384

real-time system, Bio-Rad Laboratories S.r.l.) using the iScript one-step RT-PCR kit for probes (Bio-Rad Laboratories S.r.l.). The primers and probes sequences used were acquired from Eurofins MWG-Operon and Life Technologies and are shown in **Table 1**. The samples (10 ng/ul) were run in a 348-well format in triplicates as multiplexed reactions with the normalizing internal control 36b4. Thermal cycling started with incubation at 50°C for 10 min (RNA retrotranscription), and 95°C for 5 min (TaqMan polymerase activation). After this step, 39 cycles of PCR were performed. Each PCR cycle consisted of heating the samples at 95°C for 10 s to facilitate the melting process and then at 60°C for 30 s for the annealing and extension reactions. A comparative cycle threshold (Ct) method was used to calculate the relative target gene expression by applying the $2^{(-\Delta\Delta CT)}$ method (Livak and Schmittgen, 2001).

Data Analysis

Behavioral data were analyzed by two-way ANOVA followed by *post hoc* of Newman-keuls. Genotype ($SERT^{+/+}$ vs. $SERT^{+/-}$) and manipulation (naïve vs. TS) were assessed as independent variables. The level of significance was set at $P < 0.05$. For the molecular data two-way ANOVA followed by *post hoc* of Fisher's protected least significant difference (PLSD) was used. Benjamini–Hochberg multiple testing corrections for false discovery rate (FDR) was applied and the significance was set to FDR adjusted p-value < 0.05. All statistical analyses were performed using the Statistics Software version 13.3 (Tulsa, OK, USA) and the graphs were made by GraphPad Prism version 7. Data are presented as means ± standard error (SEM). In the graphs of the molecular data, the group of naïve $SERT^{+/+}$ is set at 100%.

RESULTS

Behavioral Tests

Social Interaction Test

Our main readout in the social interaction test was the time spent on social interaction. For this parameter, we found a significant effect of TS ($F_{(1,30)} = 10.16$; $p = 0.003$). After *post hoc* testing, we found that naïve $SERT^{+/-}$ rats spent less time on social contact ($p = 0.014$) compared to naïve $SERT^{+/+}$ rats while in TS $SERT^{+/-}$ rats this parameter was increased ($p = 0.001$) compared to naïve $SERT^{+/-}$ rats (**Figure 2A**).

For non-social behavior a significant interaction between genotype and TS ($F_{(1,30)} = 15.47$; $p = 0.0005$) was found. *Post hoc* testing showed that naïve $SERT^{+/-}$ rats spent more time on non-social contact ($p = 0.0002$) compared to naïve $SERT^{+/+}$ rats, while TS $SERT^{+/-}$ rats spent less time on non-social behavior ($p = 0.0001$) compared to naïve $SERT^{+/-}$ rats (**Figure 2D**).

For social interest and passive undergoing behavior, we did not find any difference between groups (**Figures 2B,C**).

Elevated Plus-Maze Test

The standard readouts for anxiety as measured in the elevated plus-maze test involve the percentage time spent on the open arms and the percentage of entries onto the open arms. As shown in **Figure 3A**, two-way ANOVA showed that there was a significant interaction between genotype and TS ($F_{(1,30)} = 9.29$; $p = 0.004$) for the mean percentage of open arms time. *Post hoc* testing revealed that naïve $SERT^{+/-}$ animals remained less time on the open arms compared to naïve $SERT^{+/+}$ rats ($p = 0.012$) and TS $SERT^{+/-}$ rats ($p = 0.016$; **Figure 3A**). For the relative number of open arm entries, a significant effect of genotype ($F_{(1,30)} = 5.45$; $p = 0.026$) and an interaction between genotype and TS ($F_{(1,30)} = 6.05$; $p = 0.020$) was observed. In particular, naïve $SERT^{+/-}$ animals showed fewer entries on to the open arm compared to naïve $SERT^{+/+}$ ($p = 0.010$) and TS $SERT^{+/-}$ rats ($p = 0.049$; **Figure 3B**). These data show that TS ameliorated the anxiety observed in naïve $SERT^{+/-}$ rats.

Regarding the time spent on closed arms, no statistical differences were observed (**Figure 3C**). For total time traveled, also no significant genotype or TS manipulation effect was found. The latter confirms that the differences seen in anxiety were not due to differences in locomotor behavior (**Figure 3D**).

Sucrose Consumption Test

As shown in **Figure 4A**, there was a significant effect of genotype ($F_{(1,30)} = 8.19$; $p = 0.007$) for sucrose preference over 5 h. Naïve $SERT^{+/-}$ rats showed a reduction in sucrose preference after 5 h of exposure to the water and sucrose solutions (−25%; $p = 0.049$; Newman-Keuls) compared to the naïve $SERT^{+/+}$ group. After 24 h of exposure, TS $SERT^{+/-}$ rats increased their sucrose preference (+5%; $p = 0.034$) compared to naïve $SERT^{+/-}$ rats.

Figure 4B illustrates the sucrose intake data. We found a significant interaction between genotype and TS ($F_{(1,30)} = 6.29$; $p = 0.018$) effect over 2 h. A *post hoc* test revealed a trend towards a reduction (−58%; $p = 0.053$) in sucrose intake in naïve $SERT^{+/-}$ rats after 2 h of exposure compared to naïve $SERT^{+/+}$ rats, while TS $SERT^{+/-}$ rats increased the sucrose intake after 2 h (+177%; $p = 0.016$) compared to naïve $SERT^{+/-}$ rats.

Furthermore, for sucrose intake over 5 h, there was a TS effect ($F_{(1,30)} = 6.24$ $p = 0.018$). *Post hoc* testing showed that the sucrose intake in naïve $SERT^{+/-}$ rats were lower than in naïve $SERT^{+/+}$ rats (−36%; $p = 0.048$) while TS $SERT^{+/-}$ rats showed an increase in sucrose intake after 5 h (+148%; $p = 0.036$) compared to naïve $SERT^{+/-}$ rats. After 24 h of exposure, no significant differences were found in the sucrose intake.

Molecular Results

Bdnf mRNA Expression Levels in the Basolateral Amygdala

We initially investigated total Bdnf mRNA levels in the basolateral amygdala of naïve or TS $SERT^{+/-}$ and $SERT^{+/+}$ animals. We found a significant effect of TS ($F_{(1,31)} = 6.053$, $p = 0.020$) on total Bdnf mRNA levels. *Post hoc* analysis showed that the TS $SERT^{+/+}$ group had a significant decrease of Bdnf mRNA levels compared to the naïve $SERT^{+/+}$ group (−44%, $p = 0.025$; **Figure 5A**).

Based on this result, we decided to evaluate if TS could affect the expression of major Bdnf transcripts. In particular, we quantified the expression levels of the long 3'UTR Bdnf transcripts, associated with dendritic targeting of specific neurotrophin transcripts. Additionally, we measured Bdnf

TABLE 1 | (a) Sequences of primers, reverse primers, and probes used in real-time polymerase chain reaction (RT-PCR) analyses and purchased from Eurofins MWG-Operon.

(a) Gene	Forward primer	Reverse primer	Probe
Total Bdnf	AAGTCTGCATTACATTCCTCGA	GTTTTCTGAAAGAGGGACAGTTTAT	TGTGGTTTGTTGCCGTTGCCAAG
Nr3c1	GAAAAGCCATCGTCAAAAGGG	TGGAAGCAGTAGGTAAGGAGA	AGCTTTGTCAGTTGGTAAAACCGTTGC
Nr3c2	TCGCTTTGAGTTGGAGATCG	ACGAATTGAAGGCTGATCTGG	AGTCTGCCATGTATGAACTGTGCCA
Pvalb	CTGGACAAAGACAAAAGTGGC	GACAAGTCTCTGGCATCTGAG	CCTTCAGAATGGACCCCAGCTCA
Vgat	ACGACAAACCCAAGATCACG	GTAGACCCAGCACGAACATG	TTCCAGCCCGCTTCCCACG
Gad67	ATACTTGGTGTGGCGTAGC	AGGAAAGCAGGTTCTTGGAG	AAAACTGGGCCTGAAGATCTGTGGT
Vglut	ACTGCCTCACCTTGTCATG	GTAGCTTCCATCCCGAAACC	CTTTCGCACATTGGTCGTGGACATT
Fkbp5	GAACCCAATGCTGAGCTTATG	ATGTACTTGCCTCCCTTGAAG	TGTCCATCTCCCAGGATTCTTTGGC
36b4	TTCCCACTGGCTGAAAAGGT AAGGCCTTCCTGGCCGATCCATC	CGCAGCCGCAAATGC	
(b) Gene	**Accession number**	**Assay ID**	
Bdnf long 3' UTR	EF125675	Rn02531967_s1	
Bdnf isoform IV	EF125679	Rn01484927_m1	
Bdnf isoform VI	EF125680	Rn01484928_m1	
Gadd45β	BC085337.1	Rn01452530_g1	
Nr4a1	BC097313.1	Rn01533237_m1	

(b) Probes purchased from Life Technologies which did not disclose the sequence.

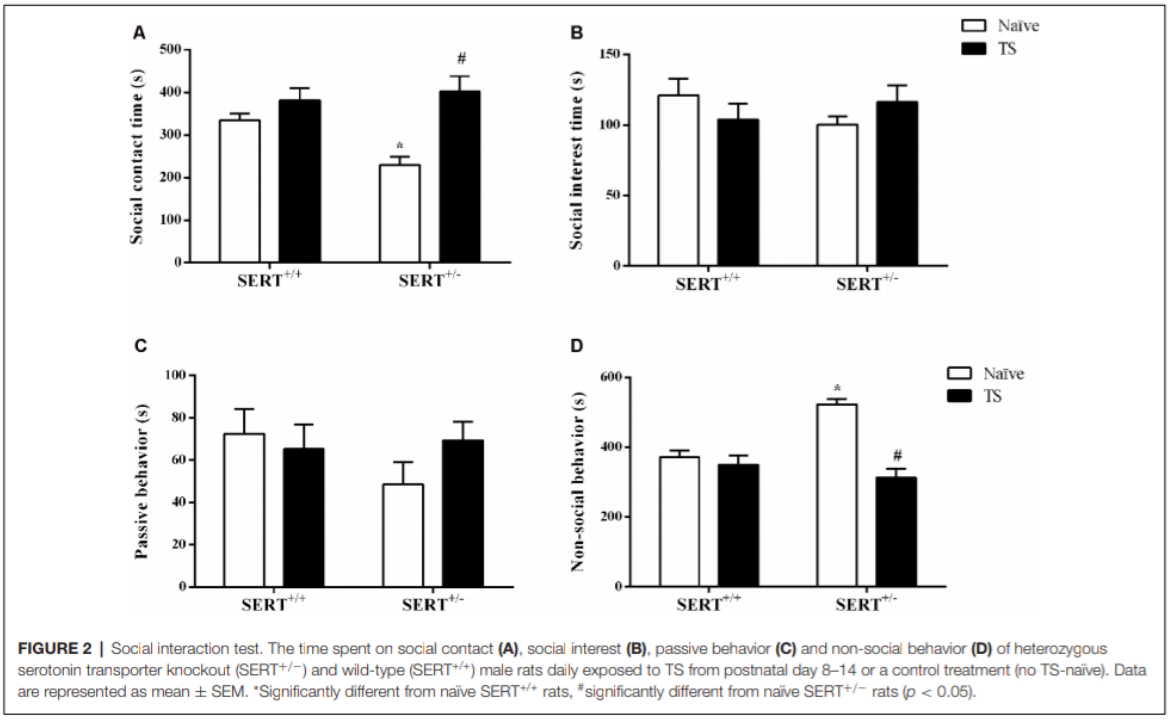


FIGURE 2 | Social interaction test. The time spent on social contact **(A)**, social interest **(B)**, passive behavior **(C)** and non-social behavior **(D)** of heterozygous serotonin transporter knockout ($SERT^{+/-}$) and wild-type ($SERT^{+/+}$) male rats daily exposed to TS from postnatal day 8–14 or a control treatment (no TS-naïve). Data are represented as mean ± SEM. *Significantly different from naïve $SERT^{+/+}$ rats, #significantly different from naïve $SERT^{+/-}$ rats ($p < 0.05$).

isoforms IV and VI. While isoform IV is localized in the soma, is an indication of altered neuronal activity (Pattabiraman et al., 2005), isoform VI is targeted to dendrites (Chiaruttini et al., 2008). Changes in isoform IV and VI have been associated with mood disorders (Molteni et al., 2010).

No significant differences were found for long 3'UTR Bdnf mRNA levels, indicating that there was no modulation of this pool of transcript on total Bdnf mRNA levels (**Figure 5B**). Two-way ANOVA revealed a significant effect of TS ($F_{(1,31)} = 4.484$, $p = 0.043$) for Bdnf isoform IV, with its mRNA levels being decreased in TS $SERT^{+/+}$ animals (−54% vs. naïve $SERT^{+/+}$, $p = 0.024$, Fisher PLSD) in comparison to the naïve counterpart (**Figure 5C**).

Regarding Bdnf exon VI, two-way ANOVA showed a similar result with a trend in the TS group ($F_{(1,31)} = 3.794$, $p = 0.062$). Indeed, we observed a decrease in Bdnf isoform VI levels in the TS $SERT^{+/+}$ group (−44% vs. naïve $SERT^{+/+}$, $p = 0.035$, Fisher PLSD; **Figure 5D**).

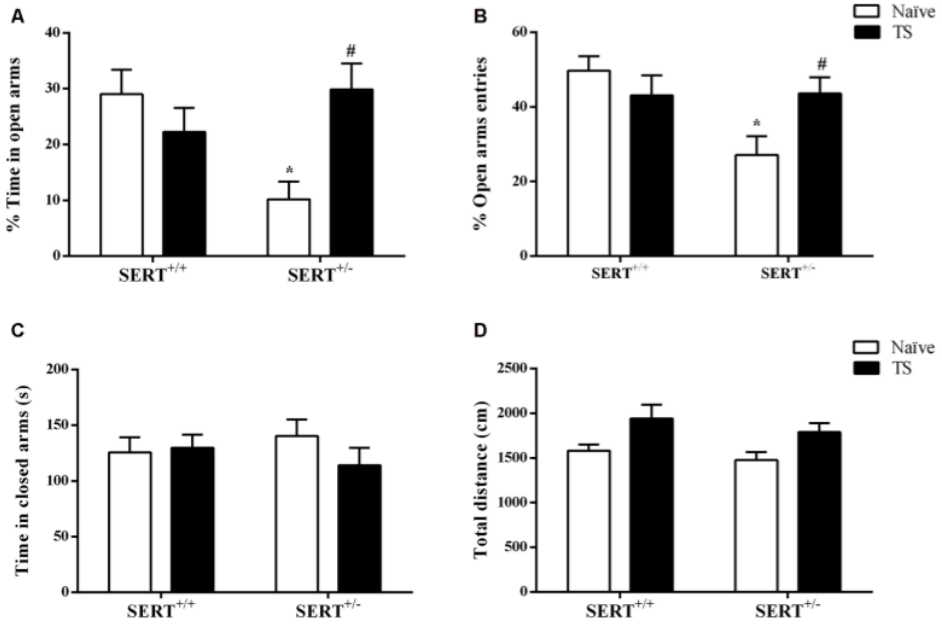


FIGURE 3 | Elevated plus maze test. The mean time of spent in open arms (%; **A**), the mean of entries number in open arms (%; **B**), time spent in closed arms (s; **C**) and total distance traveled (cm; **D**) of heterozygous serotonin transporter knockout ($SERT^{+/-}$) and wild-type ($SERT^{+/+}$) male rats daily exposed to TS from postnatal day 8–14 or a control treatment (no TS-naïve). Data are represented as mean ± SEM. *Significantly different from naïve $SERT^{+/+}$ rats, #significantly different from naïve $SERT^{+/-}$ rats ($p < 0.05$).

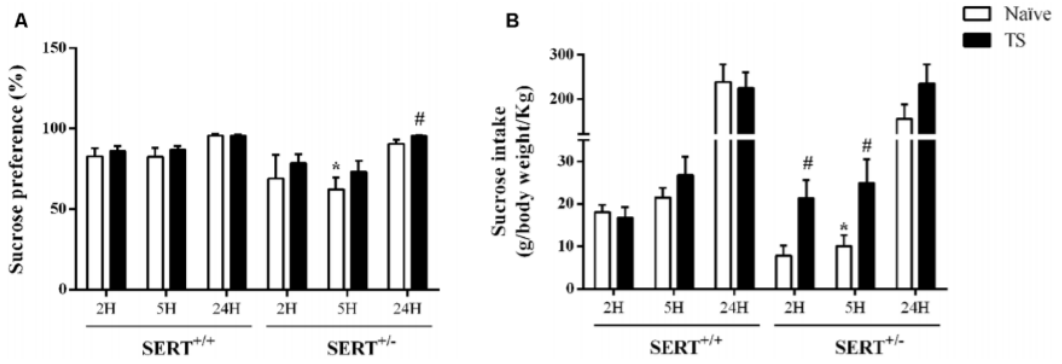


FIGURE 4 | Sucrose consumption test. Sucrose preference **(A)** and sucrose intake **(B)** in heterozygous serotonin transporter knockout ($SERT^{+/-}$) and wild-type ($SERT^{+/+}$) male rats daily exposed to TS from postnatal day 8–14 or a control treatment (no TS-naïve). Data are represented as mean ± SEM. *Significantly different from naïve $SERT^{+/+}$ rats, #significantly different from naïve $SERT^{+/-}$rats ($p < 0.05$).

Mineralocorticoid and Glucocorticoid Receptor mRNA Expression Levels in the Basolateral Amygdala

We next investigated if mRNA expression levels of both corticosterone receptors, Nr3c1, and Nr3c2 could be affected by the SERT genotype or TS. Furthermore, the expression of glucocorticoid-responsive genes, including Nr4a1, Gadd45β, and the co-chaperone Fkbp5 were measured in the basolateral amygdala.

Two-way ANOVA showed that TS significantly affected mRNA levels of Nr3c2 ($F_{(1,32)} = 11.243$, $p = 0.002$; **Figure 6B**). We observed that TS induced an increase of its mRNA levels specifically in $SERT^{+/-}$rats (+42% vs. naïve $SERT^{+/-}$, $p = 0.003$). We did not observe any change in Nr3c1 gene expression

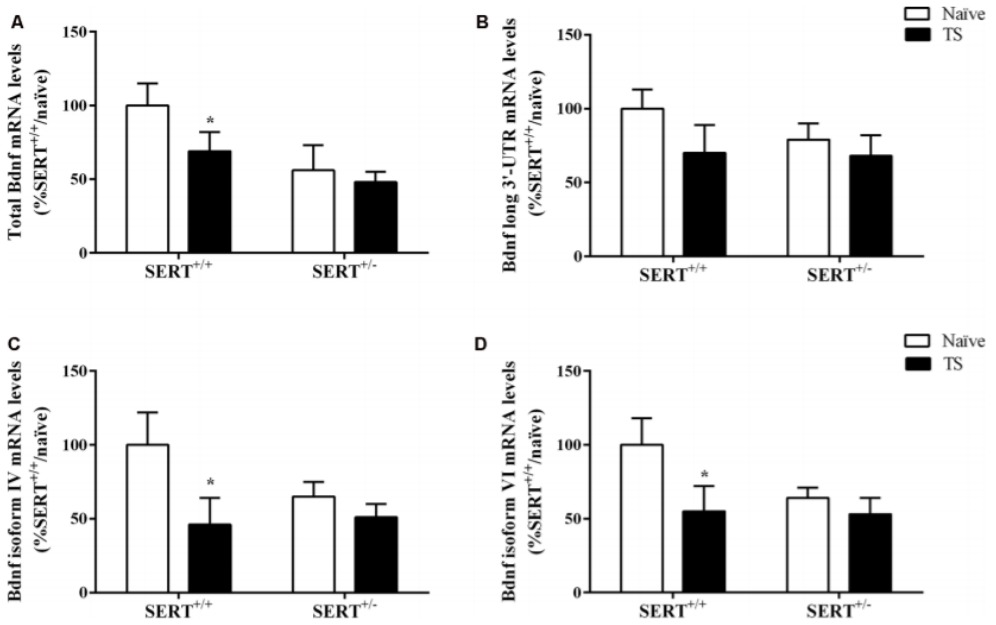


FIGURE 5 | Total Bdnf **(A)**, Bdnf long 3'-UTR **(B)**, Bdnf exon IV **(C)** and exon VI **(D)** mRNA levels in the basolateral amygdala of heterozygous serotonin transporter knockout ($SERT^{+/-}$), and wild-type ($SERT^{+/+}$) male rats daily exposed to TS from postnatal day 8–14 or a control treatment (no TS-naïve). Data are expressed as a percentage of naïve $SERT^{+/+}$ rats (set at 100%) and are presented as mean ± SEM. *Significantly different from naïve $SERT^{+/+}$ rats ($p < 0.05$).

(**Figure 6A**). As a consequence, the Nr3c1/Nr3c2 ratio was significantly affected by TS ($F_{(1,32)} = 11.541$, $p = 0.014$). Fisher PLSD showed a decrease in this ratio in TS $SERT^{+/+}$ rats compared to their naïve counterparts (−32% vs. naïve $SERT^{+/+}$, $p = 0.002$; **Figure 6C**).

Two-way ANOVA of Nr4a1 mRNA levels revealed a significant effect of TS ($F_{(1,33)} = 13.460$, $p = 0.0009$; **Figure 7A**). *Post hoc* testing revealed a decrease in Nr4a1 mRNA levels both in TS $SERT^{+/+}$ (−34%, $p = 0.005$) and TS $SERT^{+/-}$ rats (−32%, $p = 0.0008$) compared to their naïve counterparts.

Gadd45β gene expression was significantly affected by TS ($F_{(1,33)} = 4.887$, $p = 0.034$, two-way ANOVA) and we found that TS induced a significant reduction of its mRNA levels in TS $SERT^{+/+}$rats compared to naïve $SERT^{+/-}$ rats (−28% vs. naïve $SERT^{+/+}$, $p = 0.009$, Fisher PLSD; **Figure 7B**).

As shown in **Figure 7C**, we found a significant effect of TS on Fkbp5 gene expression ($F_{(1,33)} = 10.377$, $p = 0.003$, two-way ANOVA). Accordingly, we observed a significant increase in Fkbp5 mRNA levels due to TS in $SERT^{+/+}$ rats compared to naïve $SERT^{+/+}$ rats (+39% vs. naïve $SERT^{+/+}$, $p = 0.013$, Fisher PLSD).

mRNA Expression Levels of Key Elements of the GABAergic and Glutamatergic Systems in the Basolateral Amygdala

Finally, we investigated the expression levels of genes encoding key elements of the GABAergic synapses, which are the GABA-producing enzyme (Gad67), vesicular GABA transporter (Vgat), and parvalbumin (Pvalb). Additionally, we measured one glutamatergic marker, vesicular glutamate transporter (Vglut), in the basolateral amygdala.

Two-way ANOVA showed a significant effect of genotype ($F_{(1,33)} = 17.158$, $p = 0.000$) and an interaction between genotype × TS ($F_{(1,33)} = 4.971$, $p = 0.033$) for Gad67 gene expression (**Figure 8A**). We found a significant increase in its mRNA levels specifically in TS $SERT^{+/+}$ animals in comparison to naïve $SERT^{+/+}$ rats (+30%, $p = 0.007$, Fisher PLSD). We did not observe any alteration in Pvalb mRNA levels and Vgat mRNA levels (**Figures 8B,C**).

As shown in **Figure 9A**, we observed a significant effect of TS on Vglut gene expression ($F_{(1,33)} = 7.071$, $p = 0.015$, two-way ANOVA). TS $SERT^{+/+}$ animals showed a decrease in its mRNA levels (−47% vs. naïve $SERT^{+/+}$, $p = 0.009$, Fisher PLSD).

Finally, we calculated the Vglut/Vgat ratio. Two-way ANOVA revealed that there was a trend effect for TS on this ratio ($F_{(1,32)} = 3.812$, $p = 0.061$). Further, *post hoc* testing revealed that the ratio was significantly reduced in TS $SERT^{+/+}$ rats compared to naïve $SERT^{+/+}$ rats (−55%, $p = 0.047$, Fisher PLSD; **Figure 9B**).

DISCUSSION

In this study, we investigated the effect of neonatal TS in male $SERT^{+/-}$ rats on social and affective behavior, as well

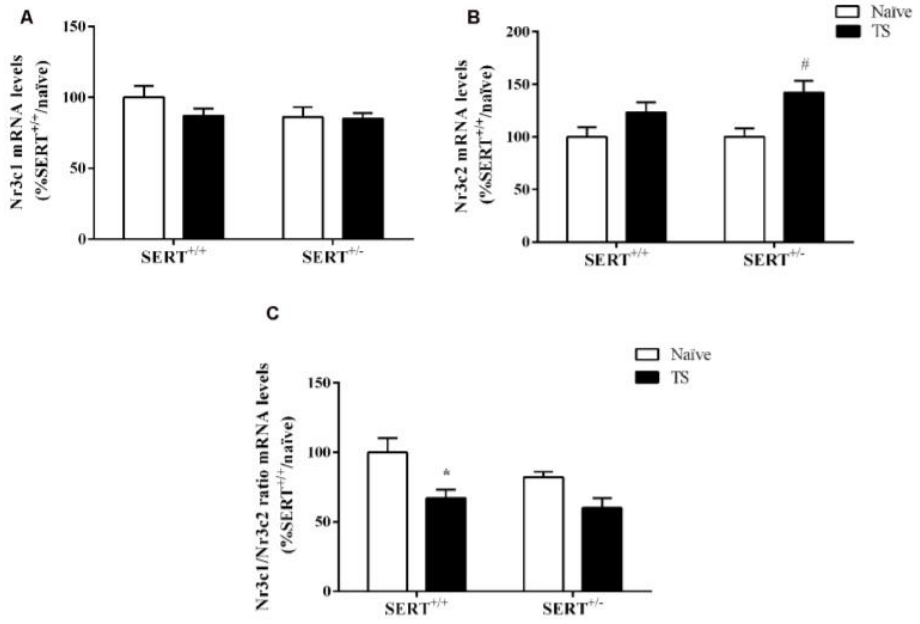


FIGURE 6 | Glucocorticoid receptor (Nr3c1) **(A)**, mineralocorticoid receptor (Nr3c2) **(B)**, and the ratio between Nr3c1 and Nr3c2 **(C)** mRNA levels in the basolateral amygdala of heterozygous serotonin transporter knockout ($SERT^{+/-}$), and wild-type ($SERT^{+/+}$) male rats daily exposed to TS from postnatal day 8–14 or a control treatment (no TS-naïve). Data are expressed as a percentage of naïve SERT naïve rats (set at 100%) and are presented as mean ± SEM. *Significantly different from naïve $SERT^{+/+}$ rats, #significantly different from naïve $SERT^{+/-}$ rats ($p < 0.05$).

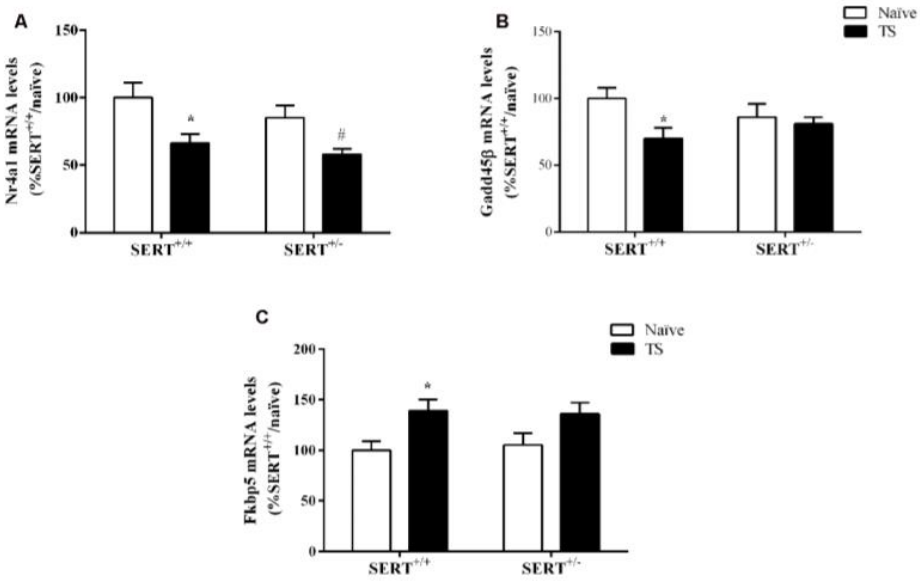


FIGURE 7 | Nr4a1**(A)**, Gadd45β **(B)** and Fkbp5 **(C)** mRNA levels in the basolateral amygdala of heterozygous serotonin transporter knockout ($SERT^{+/-}$), and wild-type ($SERT^{+/+}$) male rats daily exposed to TS from postnatal day 8–14 or a control treatment (no TS-naïve). Data are expressed as a percentage of naïve $SERT^{+/+}$ rats (set at 100%) and are presented as mean ± SEM. *Significantly different from naïve $SERT^{+/+}$ rats, #significantly different from naïve $SERT^{+/-}$ rats ($p < 0.05$).

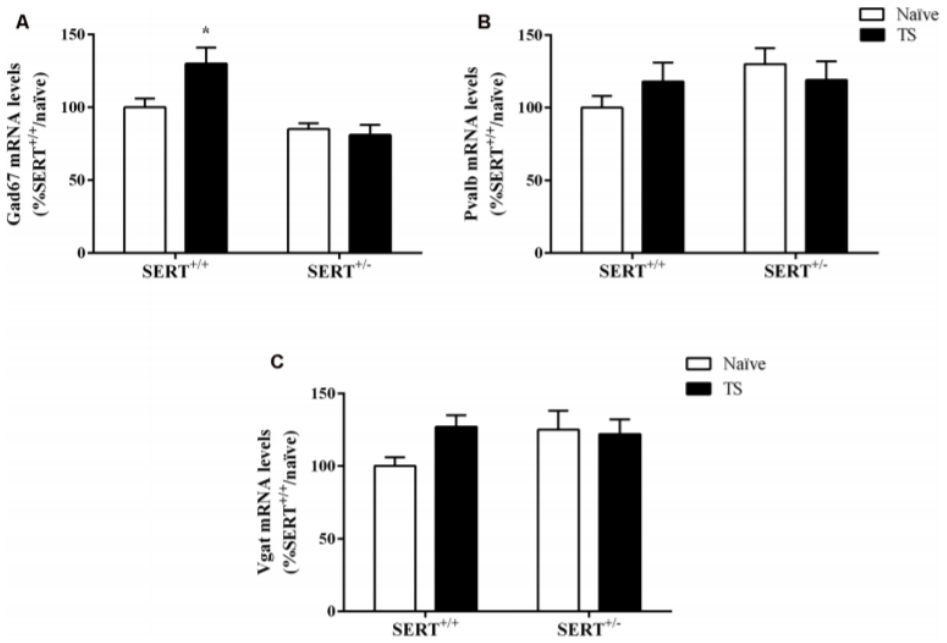


FIGURE 8 | Gad67 **(A)**, Parvalbumin (Pvalb; **B**) and vesicular GABA transporter (Vgat; **C**) mRNA levels in the basolateral amygdala of heterozygous serotonin transporter knockout (SERT$^{+/-}$) and wild-type (SERT$^{+/+}$) male rats exposed to TS from postnatal day 8–14 or a control treatment (no TS-naïve). Data are expressed as a percentage of naïve SERT$^{+/+}$ (set at 100%) and are represented as mean ± SEM. *Significantly different from naïve SERT$^{+/+}$ rats ($p < 0.05$).

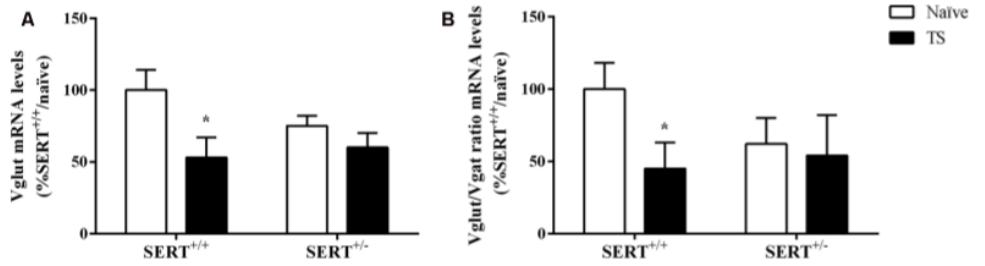


FIGURE 9 | Vesicular glutamate transporter (Vglut; **A**) and the ratio between Vglut and vesicular GABA transporter (Vgat; **B**) mRNA levels in the basolateral amygdala of heterozygous serotonin transporter knockout (SERT$^{+/-}$) and wild-type (SERT$^{+/+}$) male rats exposed to TS from postnatal day 8–14 or a control treatment (no TS-naïve). Data are expressed as a percentage of naïve SERT$^{+/+}$ (set at 100%) and are represented as mean ± SEM. *Significantly different from naïve SERT$^{+/+}$ rats ($p < 0.05$).

as gene expression in the basolateral amygdala as readouts. By applying TS 1 × day/10 min from PND 8–14 we observed that diminished social contact and increased non-social behavior in naïve SERT$^{+/-}$ rats were normalized in TS SERT$^{+/-}$ animals. Also, we observed that increased anxiety as measured in the elevated plus-maze test and reduced anhedonia as measured in the 3% sucrose consumption test was normalized in TS SERT$^{+/-}$ rats, suggesting that neonatal TS had a beneficial influence on the development of the effective behavior in SERT$^{+/-}$animals. Interestingly, at the molecular level, we observed a strong effect of TS on mRNA expression levels of genes encoding key elements of the Bdnf, GABA and glutamate systems, and on mRNA expression levels of genes encoding glucocorticoid-responsive genes in the basolateral amygdala of SERT$^{+/+}$ but not SERT$^{+/-}$ animals.

Previous work has demonstrated that inherited down-regulation of SERT is associated with increased sensitivity to the adverse effects of early life stress, resulting in increased anxiety- and depression-like behavior (Houwing et al., 2019). However, the SERT does not only increase sensitivity to negative environmental stimuli, since this would preclude the existence of the high frequency of the 5-HTTLPR in the human

population. Given that, it is unlikely that a so common gene variance is maintained throughout evolution only exerting negative effects. Indeed, there is evidence, particularly from human studies, that the 5-HTTLPR also increases sensitivity to positive environmental stimuli, in line with the differential susceptibility theory (Belsky et al., 2009). TS is a positive manipulation that when applied during neonatal periods can prevent the development of anxiety and depression (Boufleur et al., 2012; Freitas et al., 2015). Accordingly, we observed that naïve SERT$^{+/-}$ rats displayed increased anxiety in the elevated plus-maze test and that this behavior was normalized in TS SERT$^{+/-}$ rats. Also, we observed a reduction in sucrose preference and intake in naïve SERT$^{+/-}$ animals in the sucrose 3% preference test, indicative of an anhedonic state in the animals, while TS SERT$^{+/-}$ animals showed a normalization of the sucrose preference up to the level of SERT$^{+/+}$ rats. Based on these findings we can hypothesize that animals submitted to TS did not develop the anxiety- and anhedonia-like behaviors as observed in naïve SERT$^{+/-}$ animals. Since we did not test the influence of negative stimuli on SERT$^{+/-}$ rats, our findings would reflect vantage sensitivity, which reflects a disproportionally increased sensitivity to positive stimuli (Pluess and Belsky, 2013).

According to literature, SERT$^{+/-}$ animals present an increase in social avoidance after stress exposure (Bartolomucci et al., 2010). Here, naïve SERT$^{+/-}$ animals showed a reduction in social contact time and an increase in non-social behavior time, while TS normalized these observations in SERT$^{+/-}$ animals. Sociability has been strongly associated with amygdala function (Hitti and Siegelbaum, 2014) since the neuroplastic changes accompanying the social decisions in rodents involves modulation of the amygdala. For instance, amygdala lesions have been related to alterations in social behavior in juvenile and adult rats (Daenen et al., 2002) and changes in amygdala circuits also have been associated with sociability deficits and anxiety symptoms in mice (Li et al., 2019).

At the molecular level, in general, we did not find significant alterations in naïve SERT$^{+/-}$ rats compared to naïve SERT$^{+/+}$ rats, while in TS SERT$^{+/+}$ animals there were basolateral amygdala modifications when compared to naïve SERT$^{+/+}$ animals. Possibly, SERT$^{+/+}$ animals exhibit a large dynamic space for adjustments to buffer environment influences while SERT$^{+/-}$ animals may lack such a dynamic space because of a tonic elevation in neuronal activity. This may render them susceptible to environmental influences, including supportive stimuli (Homberg et al., 2016). A previous study reported that in SERT$^{-/-}$ mice, compared to wild-type mice, spine density in the amygdala was significantly increased. Interestingly, after stress exposure, behavioral changes were observed selectively in the SERT$^{-/-}$ mice, while spine density remained unaltered in these mice. In wild-type controls, spine density increased up to the level of that of SERT$^{-/-}$ mice. These findings suggest that a tonic increase in excitability reduces plasticity when a further increase in excitability is required to process the stress, failing to buffer the effects of stress and exaggerated behavioral stress response (Nietzer et al., 2011). It is plausible that a comparable mechanism is at play in the present study, with SERT$^{+/-}$ rats responding behaviorally to TS due to a lack of a dynamic range to molecularly "neutralize" the effects of TS.

Previous works demonstrated that Bdnf levels were reduced in the prefrontal cortex and hippocampus of SERT$^{-/-}$ animals throughout life (Molteni et al., 2010; Calabrese et al., 2013). Bdnf is a key player in neurodevelopment and neuronal plasticity. Here, we observed a decrease in the expression of total Bdnf and its isoform IV and VI in TS SERT$^{+/+}$ animals compared to naïve SERT$^{+/+}$ rats. Although an increase in Bdnf expression in the brain is generally related to an antidepressant effect, Bdnf has been reported to have an opposite role in the amygdala. Indeed, overexpression of Bdnf in the amygdala has been related to an anxiogenic response (Govindarajan et al., 2006). Also, a Bdnf up-regulation in the central amygdala of SERT$^{-/-}$ rats is related to enhance negative emotional state, contributing to a compulsive drug self-administration behavior (Caffino et al., 2019). Not only the total Bdnf but also alterations in Bdnf isoform VI and IV were related to increased anxiety in male rats after acute stress exposure (Luoni et al., 2016; Pandey et al., 2017). Although a previous study demonstrated that TS in Wistar rats led to an increase in Bdnf levels in the hippocampus, along with a beneficial effect on anxiety and an improvement in working memory as evaluated using the Y-maze test (Antoniazzi et al., 2017), in our study the decrease of Bdnf expression in the basolateral amygdala in TS animals could be indicative of a protective mechanism against anxiety and anhedonic behaviors.

To assess if the HPA axis is modulated by TS in SERT animals, we investigated the expression of both glucocorticoid (Nr3c1) and mineralocorticoid (Nr3c2) receptor encoding genes. There was an increase in Nr3c2 gene expression only in TS SERT$^{+/-}$ animals compared to TS SERT$^{+/+}$ animals and there were no changes in Nr3c1 gene expression. Nonetheless, the ratio of Nr3c1/Nr3c2 was found to be increased in the basolateral amygdala of both SERT$^{+/+}$ and SERT$^{+/-}$ TS exposed animals. In previous work, this ratio was found to be reduced in the paraventricular nucleus and hippocampus of stressed animals (Brydges et al., 2014; Murgatroyd et al., 2015). The ratio between glucocorticoid and mineralocorticoid expression plays a key role in the promotion of health, homeostasis, and adaptation (de Kloet et al., 1993). An increase in this ratio as observed in TS SERT$^{+/-}$ animals could indicate a change in homeostasis, leading to neuroadaptation and improving the ability to cope with different environmental situations and explaining the normalization of anxiety behavior observed in this group. Furthermore, we measured expression levels of glucocorticoid-regulator FK506-binding protein 51 (Fkbp5), Nr4a1, and growth arrest and DNA damage-inducible factor 45 (Gadd45β). We observed that FKBP5 was increased in TS SERT$^{+/+}$ animals compared to naïve SERT$^{+/+}$. Literature is controversial regarding Fkbp5 expression in the brain. Some studies showed increased expression levels of Fkbp5 in the basolateral amygdala of stressed rats (Xu et al., 2017) as well as increased FKBP5 mRNA in the ventral prefrontal cortex of homozygous SERT rats after early life stress exposure (van der Doelen et al., 2014). In contrast, maternal separation reduced Fkbp5 expression in the hippocampus and did not alter in the amygdala of adult male mice (Candemir et al., 2019).

Furthermore, we observed that Nr4a1 expression was reduced in both TS manipulated SERT$^{+/+}$ and SERT$^{+/-}$ animals. Nr4a1 is an activity-dependent immediate early-gene responding to a variety of sensory stimuli, adapting the synaptic activity in response to stimuli. Prolonged expression of Nr4a1 can lead to mitochondrial malfunction and altered synaptic plasticity (Chen et al., 2014; Jeanneteau et al., 2018). Accordingly, the reduction of Nr4a1 expression in TS groups could explain the mechanism of protection against brain disturbances. Moreover, Gadd45β has been associated with amygdala-related learning tasks and the epigenetic programming of social behavior (Kigar et al., 2015). Despite Gadd45β's effects on social behavioral function, we did not observe a change in Gadd45β expression in both naïve and TS SERT$^{+/-}$ animals. Only in TS SERT$^{+/+}$ animals, we found a change, possibly contributing to the maintenance of brain homeostasis. This could explain why there was no change in behavior, as a response to TS in SERT$^{+/+}$ rats.

The basolateral amygdala is a brain region involved in the processing of emotional signals and contains GABAergic interneurons. The amygdala undergoes developmental changes in early life and is fully mature around adolescence. Environmental events during early life or the developmental stage of the amygdala can have long-lasting persisting effects (Bessières et al., 2019). The balance between inhibitory and excitatory neurotransmission is necessary for brain development (Chamberland and Topolnik, 2012). Here, we investigated the expression levels of Gad67, Pvalb, vesicular GABA transporter (Vgat), and vesicular glutamate transporter (Vglut) to reflect the excitatory/inhibitory balance in the basolateral amygdala. We observed an increase in the GABAergic marker Gad67 in TS SERT$^{+/+}$ rats compared to naïve SERT$^{+/+}$ rats. Gad67 serves as an inhibitory marker that helps in the GABA synthesis under basal conditions. Stress is related to decreases in Gad67 expression in the medial prefrontal cortex, impairing social behavior in rats, and reducing inhibitory synapses in the brain (Ohta et al., 2019). Evidence has shown that treatment with the antidepressant duloxetine can restore the reduction of Gad67 in stress-depressive state animals (Guidotti et al., 2012). Moreover, inhibitory and excitatory amygdaloid circuits were found to be affected in patients with depression, bipolar disorder, or schizophrenia, which was paralleled by a decrease in GAD67 and an increase in VGLUT levels in various nuclei of the amygdala in all patients (Varea et al., 2012). The increase in Gad67 observed in TS SERT$^{+/+}$ rats may suggest that the manipulation increased the number of GABAergic terminals in the basolateral amygdala, preventing anxious-depressive behaviors. Regarding excitatory neurotransmission, TS SERT$^{+/+}$ animals showed a reduction in both Vglut expression levels and Vglut/Vgat ratio. Stress has been associated with enhancement of glutamatergic neurotransmission in prefrontal cortex and amygdala, and peripubertal stress has been related to increasing the Vglut/Vgat ratio, which in turn was associated with the development of psychiatric disorders (Yuen et al., 2011; Tzanoulinou et al., 2014). Although we did not find alterations in SERT$^{+/-}$ groups, the maintenance of normal anxiety behavior observed in TS SERT$^{+/+}$ animals could be explained by the reduction in this glutamatergic expression, in which these reduced levels could prevent sensitivity to adversity in these animals.

Summarized, our findings indicate that exposure to neonatal TS in SERT$^{+/-}$ and SERT$^{+/+}$ animals result in lasting changes in emotional and social parameters and molecular changes in the basolateral amygdala. Generally, the data suggest that TS in SERT$^{+/-}$ animals had pronounced effects on anxiety and social behaviors, but not on gene expression in the basolateral amygdala, possibly because of SERT$^{+/-}$ animals present at baseline a tonic elevation in neuronal activity, hindering further changes in gene expression upon environmental challenges. On the other hand, TS in SERT$^{+/+}$ animals altered molecular parameters in the basolateral amygdala but these effects were not accompanied by behavioral changes, suggesting that SERT$^{+/+}$ animals might have a greater dynamic range for adjustments, allowing them to remain unaffected by TS. One limitation of the present study is that we did not include female rats. Since women show more susceptibility to depression (Kuehner, 2017), studies comparing male and female are still needed to better understand the relation between TS and SERT$^{+/-}$ animals in both sexes. To conclude, this is the first study to investigate the beneficial effects of an early-life supportive environmental stimulus on later life behavior of stress-sensitive SERT$^{+/-}$ animals, with results that will further our understanding of how a supportive environment particularly benefits vulnerable individuals.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The animal study was reviewed and approved by Committee for Animal Experiments of the Radboud University Nijmegen, The Netherlands.

AUTHOR CONTRIBUTIONS

KR and CB experimented and collected the behavioral data. CB, FC, and PB performed and analyzed the molecular data. KR analyzed the data and wrote the manuscript. JH, MV, CA, MB, and KR designed the study and interpreted the data. JH, MV, and MB reviewed and edited the manuscript. JH and MR were responsible for the funding acquisition. All authors contributed to the article and approved the submitted version.

FUNDING

This work was supported in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance Code 001, as well the Eranid consortium project "STANDUP" awarded to JH.

REFERENCES

Antoniazzi, C. T. D., Metz, V. G., Roversi, K., Freitas, D. L., Vey, L. T., Dias, V. T., et al. (2017). Tactile stimulation during different developmental periods modifies hippocampal BDNF and GR, affecting memory and behavior in adult rats. *Hippocampus* 27, 210–220. doi: 10.1002/hipo.22686

Babikian, T., Prins, M. L., Cai, Y., Barkhoudarian, G., Hartonian, I., Hovda, D. A., et al. (2010). Molecular and physiological responses to juvenile traumatic brain injury: focus on growth and metabolism. *Dev. Neurosci.* 32, 431–441. doi: 10.1159/000320667

Bartolomucci, A., Carola, V., Pascucci, T., Puglisi-Allegra, S., Cabib, S., Lesch, K.-P., et al. (2010). Increased vulnerability to psychosocial stress in heterozygous serotonin transporter knockout mice. *Dis. Model. Mech.* 3, 459–470. doi: 10.1242/dmm.004614

Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., and Williams, R. (2009). Vulnerability genes or plasticity genes? *Mol. Psychiatry* 14, 746–754. doi: 10.1038/mp.2009.44

Bessières, B., Jia, M., Travaglia, A., and Alberini, C. M. (2019). Developmental changes in plasticity, synaptic, glia and connectivity protein levels in rat basolateral amygdala. *Learn. Mem.* 26, 436–448. doi: 10.1101/lm.049866.119

Blaze, J., and Roth, T. L. (2013). Exposure to caregiver maltreatment alters expression levels of epigenetic regulators in the medial prefrontal cortex. *Int. J. Dev. Neurosci.* 31, 804–810. doi: 10.1016/j.ijdevneu.2013.10.001

Boufleur, N., Antoniazzi, C. T., Pase, C. S., Benvegnú, D. M., Barcelos, R. C., Dolci, G. S., et al. (2012). Neonatal tactile stimulation changes anxiety-like behavior and improves responsiveness of rats to diazepam. *Brain Res.* 1474, 50–59. doi: 10.1016/j.brainres.2012.08.002

Boufleur, N., Antoniazzi, C. T. D., Pase, C. S., Benvegnu, D. M., Dias, V. T., Segat, H. J., et al. (2013). Neonatal handling prevents anxiety-like symptoms in rats exposed to chronic mild stress: behavioral and oxidative parameters. *Stress* 16, 321–330. doi: 10.3109/10253890.2012.723075

Brivio, P., Corsini, G., Riva, M. A., and Calabrese, F. (2019). Chronic vortioxetine treatment improves the responsiveness to an acute stress acting through the ventral hippocampus in a glucocorticoid-dependent way. *Pharmacol. Res.* 142, 14–21. doi: 10.1016/j.phrs.2019.02.006

Brydges, N. M., Jin, R., Seckl, J., Holmes, M. C., Drake, A. J., and Hall, J. (2014). Juvenile stress enhances anxiety and alters corticosteroid receptor expression in adulthood. *Brain Behav.* 4, 4–13. doi: 10.1002/brb3.182

Caffino, L., Mottarlini, F., Diniz, D. M., Verheij, M. M., Fumagalli, F., and Homberg, J. R. (2019). Deletion of the serotonin transporter perturbs BDNF signaling in the central amygdala following long-access cocaine self-administration. *Drug Alcohol Depend.* 205:107610. doi: 10.1016/j.drugalcdep.2019.107610

Calabrese, F., Guidotti, G., Middelman, A., Racagni, G., Homberg, J. R., and Riva, M. A. (2013). Lack of serotonin transporter alters BDNF expression in the rat brain during early postnatal development. *Mol. Neurobiol.* 48, 244–256. doi: 10.1007/s12035-013-8449-z

Candemir, E., Post, A., Dischinger, U. S., Palme, R., Slattery, D. A., O'Learya, A., et al. (2019). Limited effects of early life manipulations on sex-specific gene expression and behavior in adulthood. *Behav. Brain Res.* 369:111927. doi: 10.1016/j.bbr.2019.111927

Caspi, A., Hariri, A. R., Holmes, A., Uher, R., and Moffitt, T. E. (2010). Genetic sensitivity to the environment: the case of the serotonin transporter gene and its implications for studying complex diseases and traits. *Am. J. Psychiatry* 167, 509–527. doi: 10.1176/appi.ajp.2010.09101452

Catalani, A., Sabbatini, M., Consoli, C., Cinque, C., Tomassoni, D., Azmitia, E., et al. (2002). Glial fibrillary acidic protein immunoreactive astrocytes in developing rat hippocampus. *Mech. Ageing Dev.* 123, 481–490. doi: 10.1016/s0047-6374(01)00356-6

Chamberland, S., and Topolnik, L. (2012). Inhibitory control of hippocampal inhibitory neurons. *Front. Neurosci.* 6:165. doi: 10.3389/fnins.2012.00165

Chen, Y., Wang, Y., Erturk, A., Kallop, D., Jiang, Z., Weimer, R. M., et al. (2014). Activity-induced Nr4a1 regulates spine density and distribution pattern of excitatory synapses in pyramidal neurons. *Neuron* 83, 431–443. doi: 10.1016/j.neuron.2014.05.027

Chiaruttini, C., Sonego, M., Baj, G., Simonato, M., and Tongiorgi, E. (2008). BDNF mRNA splice variants display activity-dependent targeting to distinct hippocampal laminae. *Mol. Cel. Neurosci.* 37, 11–19. doi: 10.1016/j.mcn.2007.08.011

Daenen, E. W. P., Wolterink, G., Gerrits, M. A. F., and van Ree, J. M. (2002). The effects of neonatal lesions in the amygdala or ventral hippocampus on social behavior later in life. *Behav. Brain Res.* 136, 571–582. doi: 10.1016/s0166-4328(02)00223-1

Daskalakis, N. P., Kaperoni, M., Koros, C., de Kloet, E. R., and Kitraki, E. (2009). Environmental and tactile stimulation modulates the neonatal handling effect on adult rat spatial memory. *Int. J. Dev. Neurosc.* 27, 747–755. doi: 10.1016/j.ijdevneu.2009.08.013

de Jong, T. R., Snaphaan, L. J., Pattij, T., Veening, J. G., Waldinger, M. D., Cools, A. R., et al. (2006). Effects of chronic treatment with fluvoxamine and paroxetine during adolescence on serotonin-related behavior in adult male rats. *Eur. Neuropsychopharmacol.* 16, 39–48. doi: 10.1016/j.euroneuro.2005.06.004

de Kloet, E. R., Sutanto, W., van den Berg, D. T., Carey, M. P., van Haarst, A. D., Hornsby, C. D., et al. (1993). Brain mineralocorticoid receptor diversity: functional implications. *J. Steroid Biochem. Mol. Biol.* 47, 183–190. doi: 10.1016/0960-0760(93)90073-6

Denenberg, V. H. (1964). Critical periods, stimulus input and emotional reactivity: a theory of infantile stimulation. *Psychol. Rev.* 71, 335–351. doi: 10.1037/h0042567

Fatemi, S. H., Stary, J. M., Earle, J. A., Araghi-Niknam, M., and Eagan, E. (2005). GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65 and 67 kDa and reelin proteins in cerebellum. *Schizophr. Res.* 72, 109–122. doi: 10.1016/j.schres.2004.02.017

Freitas, D., Antoniazzi, C. T. D., Segat, H. J., Metz, V. G., Vey, L. T., Barcelos, R. C. S., et al. (2015). Neonatal tactile stimulation decreases depression-like and anxiety-like behaviors and potentiates sertraline action in young rats. *Int. J. Dev. Neurosci.* 47, 192–197. doi: 10.1016/j.ijdevneu.2015.09.010

Garcia-Garcia, A. L., Elizalde, N., Matrov, D., Harro, J., Wojcik, S. M., Venzala, E., et al. (2009). Increased vulnerability to depressive-like behavior of mice with decreased expression of VGLUT1. *Biol. Psychiat.* 3, 275–282. doi: 10.1016/j.biopsych.2009.02.027

Gavin, D. P., Sharma, R. P., Chase, K. A., Matrisciano, F., Dong, E., and Guidotti, A. (2012). Growth arrest and DNA-damage-inducible, beta (GADD45β)-mediated DNA demethylation in major psychosis. *Neuropsychopharmacol.* 37, 531–542. doi: 10.1038/npp.2011.221

Giachino, C., Canalia, N., Capone, F., Fasolo, A., Alleva, E., Riva, M. A., et al. (2007). Maternal deprivation and early handling affect density of calcium binding protein-containing neurons in selected brain regions and emotional behavior in periadolescent rats. *Neuroscience* 145, 568–578. doi: 10.1016/j.neuroscience.2006.12.042

Govindarajan, A., Rao, B. S., Nair, D., Trinh, M., Mawjee, N., Tonegawa, S., et al. (2006). Transgenic brain-derived neurotrophic factor expression causes both anxiogenic and antidepressant effects. *Proc. Natl. Acad. Sci. U S A.* 103, 13208–13213. doi: 10.1073/pnas.0605180103

Guerrero, M. A. A., Del Carmen, R. O., Wendy, P. M., and Socorro, R. M. (2016). Tactile stimulation effects on hippocampal neurogenesis and spatial learning and memory in prenatally stressed rats. *Brain Res. Bull.* 124, 1–11. doi: 10.1016/j.brainresbull.2016.03.003

Guidotti, G., Calabrese, F., Auletta, F., Olivier, J., Racagni, G., Homberg, J. R., et al. (2012). Developmental influence of the serotonin transporter on the expression of Npas4 and GABAergic markers: modulation by antidepressant treatment. *Neuropsychopharmacology.* 37, 746–758. doi: 10.1038/npp.2011.252

Hitti, F. L., and Siegelbaum, S. (2014). The hippocampal CA2 region is essential for social memory. *Nature* 508, 88–92. doi: 10.1038/nature13028

Homberg, J. R., and Lesch, K. P. (2011). Looking on the bright side of serotonin transporter gene variation. *Biol. Psychiatry* 69, 513–519. doi: 10.1016/j.biopsych.2010.09.024

Homberg, J. R., Olivier, J. D. A., Smits, B. M. G., Mul, J. D., Mudde, J., Verheul, M., et al. (2007). Characterization of the serotonin transporter knockout rat: a selective change in the functioning of the serotonergic system. *Neuroscience* 146, 1662–1676. doi: 10.3410/f.1087654.541731

Homberg, J. R., Schubert, D., Asan, E., and Aron, E. (2016). Sensory processing sensitivity and serotonin gene variance: insights into mechanisms shaping

environmental sensitivity. *Neurosci. Biobehav. Rev.* 71, 472–483. doi: 10.1016/j.neubiorev.2016.09.029

Homberg, J. R., de Boer, S. F., Halfdan, S. R., Olivier, J. D. A., Verheul, M., Ronken, E., et al. (2008). Adaptations in pre- and postsynaptic 5-HT1A receptor function and cocaine supersensitivity in serotonin transporter knockout rats. *Psychopharmacology* 200, 367–380. doi: 10.1007/s00213-008-1212-x

Houwing, D. J., Buwalda, B., van der Zee, E. A., de Boer, S. F., and Olivier, J. D. A. (2017). The serotonin transporter and early life stress: translational perspectives. *Front. Cell. Neurosci.* 11:117. doi: 10.3389/fncel.2017.00117

Houwing, D. J., Ramsteijn, A. S., Riemersma, I. W., and Olivier, J. D. A. (2019). Maternal separation induces anhedonia in female heterozygous serotonin transporter knockout rats. *Behav. Brain Res.* 356, 204–207. doi: 10.1016/j.bbr.2018.08.031

Jeanneteau, F., Barrère, C., Vos, M., De Vries, C. J. M., Rouillard, C., Levesqu, D., et al. (2018). The stress-induced transcription factor NR4A1 adjusts mitochondrial function and synapse number in prefrontal cortex. *J. Neurosci.* 38, 1335–1350. doi: 10.1523/JNEUROSCI.2793-17.2017

Kastner, N., Richter, S. H., Lesch, K.-P., Schreiber, R. S., Kaiser, S., and Sachser, N. (2015). Benefits of a "vulnerability gene"? A study in serotonin transporter knockout mice. *Behav. Brain Res.* 283, 116–120. doi: 10.1016/j.bbr.2015.01.031

Kember, R. L., Dempster, E. L., Lee, T. H. A., Schalkwyk, L. C., Mill, J., and Fernandes, C. (2012). Maternal separation is associated with strain-specific responses to stress and epigenetic alterations to Nr3c1, Avp and Nr4a1 in mouse. *Brain Behav.* 2, 455–467. doi: 10.1002/brb3.69

Kigar, S. L., Chang, L., and Auger, A. P. (2015). Gadd45β is an epigenetic regulator of juvenile social behavior and alters local pro-inflammatory cytokine production in the rodent amygdala. *Brain Behav. Immun.* 46, 60–69. doi: 10.1016/j.bbi.2015.02.018

Klengel, T., Mehta, D., Anacker, C., Rex-Haffner, M., Pruessner, J. C., Pariante, C. M., et al. (2013). Allele-specific FKBP5 DNA demethylation mediates gene-childhood trauma interactions. *Nat. Neurosci.* 16, 33–41. doi: 10.1038/nn.3275

Kuehner, C. (2017). Why is depression more common among women than among men? *Lancet Psychiatry* 4, 146–158. doi: 10.1016/s2215-0366(16)30263-2

Li, A., Jing, D., Dellarco, D. V., Hall, B. S., Yang, R., Heilberg, R. T., et al. (2019). Role of BDNF in the development of an OFC-amygdala circuit regulating sociability in mouse and human. *Mol. Psychiatry* doi: 10.1038/s41380-019-0422-4 [Epub ahead of print]

Livak, K. J., and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the $2(-\Delta\Delta C(T))$ method. *Methods* 25, 402–408. doi: 10.1006/meth.2001.1262

Luoni, A., Berry, A., Raggi, C., Bellisario, V., Cirulli, F., and Riva, M. A. (2016). Sex-specific effects of prenatal stress on bdnf expression in response to an acute challenge in rats: a role for Gadd45β. *Mol. Neurobiol.* 53, 7037–7047. doi: 10.1007/s12035-015-9569-4

Luscher, B., Shen, Q., and Sahir, N. (2011). The GABAergic deficit hypothesis of major depressive disorder. *Mol. Psychiatry* 16, 383–406. doi: 10.1038/mp.2010.120

Molteni, R., Cattaneo, A., Calabrese, F., Macchi, F., Olivier, J. D., Racagni, G., et al. (2010). Reduced function of the serotonin transporter is associated with decreased expression of BDNF in rodents as well as in humans. *Neurobiol. Dis.* 37, 747–755. doi: 10.1016/j.nbd.2009.12.014

Murgatroyd, C. A., Peña, C. J., Podda, G., Nestler, E. J., and Nephew, B. C. (2015). Early life social stress induced changes in depression and anxiety associated neural pathways which are correlated with impaired maternal care. *Neuropeptides* 52, 103–111. doi: 10.1016/j.npep.2015.05.002

Nietzer, S. L., Bonn, M., Jansen, F., Heiming, R. S., Lewejohann, L., Sachser, N., et al. (2011). Serotonin transporter knockout and repeated social defeat stress: impact on neuronal morphology and plasticity in limbic brain areas. *Behav. Brain Res.* 220, 42–54. doi: 10.1016/j.bbr.2011.01.011

Nonkes, L. J., van de Vondervoort, I. I., de Leeuw, M. J. C., Wijlaars, L. P., Maes, J. H. R., and Homberg, J. R. (2012). Serotonin transporter knockout rats show improved strategy set-shifting andreduced latent inhibition. *Learn. Mem.* 19, 190–193. doi: 10.1101/lm.025908.112

Oh, H., Lewis, D. A., and Sibille, E. (2016). The role of BDNF in age-dependent changes of excitatory and inhibitory synaptic markers in the human prefrontal cortex. *Neuropsychopharmacology* 41, 3080–3091. doi: 10.1038/npp.2016.126

Ohta, K., Suzuki, S., Warita, K., Sumitani, K., Tenkumo, C., Ozawa, T., et al. (2019). The effects of early life stress on the excitatory/inhibitory balance of the medial prefrontal cortex. *Behav. Brain Res.* 379:112306. doi: 10.1016/j.bbr.2019.112306

Olivier, J. D. A., van der Hart, M. G. C., Van Swelm, R. P. L., Dederen, P. J., Homberg, J. R., Cremers, T., et al. (2008). A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders. *Neuroscience* 152, 573–584. doi: 10.1016/j.neuroscience.2007.12.032

Pandey, S. C., Kyzar, E. J., and Zhang, H. (2017). Epigenetic basis of the dark side of alcohol addiction. *Neuropharmacology* 122, 74–84. doi: 10.1016/j.neuropharm.2017.02.002

Pattabiraman, P. P., Tropea, D., Chiaruttini, C., Tongiorgi, E., Cattaneo, A., and Domenici, L. (2005). Neuronal activity regulates the developmental expression and subcellular localization of cortical BDNF mRNA isoforms *in vivo*. *Mol. Cel. Neurosci.* 28, 556–570. doi: 10.1016/j.mcn.2004.11.010

Pluess, M., and Belsky, J. (2013). Vantage sensitivity: individual differences in response to positive experiences. *Psychol. Bull.* 139, 901–916. doi: 10.1037/a0030196

Rice, D., and Barone, S. Jr. (2000). Critical periods of vulnerability for the developing nervous system: evidence from humans and animal models. *Environ. Health Perspect.* 108, 511–533. doi: 10.1289/ehp.00108s3511

Richards, S., Mychasiuk, R., Kolb, B., and Gibb, R. (2012). Tactile stimulation during development alters behavior and neuroanatomical organization of normal rats. *Behav. Brain Res.* 231, 86–91. doi: 10.1016/j.bbr.2012.02.043

Río-Alamos, C., Oliveras, I., Cañete, T., Blázquez, G., Martínez-Membrives, E., Tobeña, A., et al. (2015). Neonatal handling decreases unconditioned anxiety, conditioned fear and improves two-way avoidance acquisition: a study with the inbred Roman high (RHA-I)- and low-avoidance (RLA-I) rats of both sexes. *Front. Behav. Neurosci.* 9:174. doi: 10.3389/fnbeh.2015.00174

Rogers, J., Li, S., Lanfumey, L., Hannan, A. J., and Renoir, T. (2017). Environmental enrichment reduces innate anxiety with no effect on depression-like behavior in mice lacking the serotonin transporter. *Behav. Brain Res.* 332, 355–361. doi: 10.1016/j.bbr.2017.06.009

Roversi, K., de Antoniazzi, C. T. D., Milanesi, L. H., Rosa, H. Z., Kronbauer, M., and Rossato, D. R., et al (2019). Tactile stimulation on adulthood modifies the HPA axis, neurotrophic factors and GFAP signaling reverting depression-like behavior in female rats. *Mol. Neurobiol.* 56, 6239–6250. doi: 10.1007/s12035-019-1522-5

Sampedro-Piquero, P., Castilla-Ortega, E., Zancada-Menendez, C., Santín, L. J., and Begega, A. (2016). Environmental enrichment as a therapeutic avenue for anxiety in aged wistar rats: effect on cat odor exposition and GABAergic interneurons. *Neuroscience* 330, 17–25. doi: 10.1016/j.neuroscience.2016.05.032

Sanacora, G., Gueorguieva, R., Epperson, C. N., Wu, Y. T., Appel, M., and Rothman, D. L., et al (2004). Subtype-specific alterations of gamma-aminobutyric acid and glutamate in patients with major depression. *Arch. Gen. Psychiatry* 61, 705–713. doi: 10.1001/archpsyc.61.7.705

Schipper, P., Brivio, P., de Leest, D., Madder, L., Asrar, B., Rebuglio, F., et al. (2019). Impaired fear extinction recall in serotonin transporter knockout rats is transiently alleviated during adolescence. *Brain Sci.* 9:118. doi: 10.3390/brainsci9050118

Schipper, P., Lopresto, D., Reintjes, R. J., Joosten, J., Henckens, M. J. A. G., Kozicz, T., et al. (2015). Improved stress control in serotonin transporter knockout rats: involvement of the prefrontal cortex and dorsal raphe nucleus. *ACS Chem. Neurosci.* 6, 1143–1150. doi: 10.1021/acschemneuro.5b00126

Schulte-Herbruggen, O., Chourbaji, S., Ridder, S., Brandwein, C., Gass, P., Hortnagl, H., et al. (2006). Stress-resistant mice overexpressing glucocorticoid receptors display enhanced BDNF in the amygdala and hippocampus with unchanged NGF and serotonergic function. *Psychoneuroendocrinology* 31, 1266–1277. doi: 10.1016/j.psyneuen.2006.09.008

Semple, B. D., Blomgren, K., Gimlin, K., Ferriero, D. M., and Noble-Haeusslein, L. J. (2013). Brain development in rodents and humans: Identifying benchmarks of maturation and vulnerability to injury across species. *Prog. Neurobiol.* 106-107, 1–16. doi: 10.1016/j.pneurobio.2013.04.001

Tzanoulinou, S., Riccio, O., and de Boer, M. (2014). Peripubertal stress-induced behavioral changes are associated with altered expression of genes involved

in excitation and inhibition in the amygdala. *Transl. Psychiatry* 4:e410. doi: 10.1038/tp.2014.54

van der Doelen, R. H., Calabrese, F., Guidotti, G., Geenen, B., Riva, M. A., Kozicz, T., et al. (2014). Early life stress and serotonin transporter gene variation interact to affect the transcription of the glucocorticoid and mineralocorticoid receptors and the co-chaperone FKBP5, in the adult rat brain. *Front. Behav. Neurosci*, 8:355. doi: 10.3389/fnbeh.2014.00355

van der Doelen, R. H., Kozicz, T., and Homberg, J. R. (2013). Adaptive fitness: early life adversity improves adult stress coping in heterozygous serotonin transporter knockout rats. *Mol. Psychiatry* 18, 1244–1245. doi: 10.1038/mp.2012.186

van Ijzendoorn, M. H., Belsky, J., and Bakermans-Kranenburg, M. J. (2012). Serotonin transporter genotype 5HTTLPR as a marker of differential susceptibility? A meta-analysis of child and adolescent gene-by-environment studies. *Transl. Psychiatry* 2:e147. doi: 10.1038/tp.2012.73

Varea, E., Guirado, R., Gilabert-Juan, J., Martí, U., Castillo-Gomez, E., and Blasco-Ibáñez, J. M., et al (2012). Expression of PSA-NCAM and synaptic proteins in the amygdala of psychiatric disorder patients. *J. Psychiatr. Res.* 46, 189–197. doi: 10.1016/j.jpsychires.2011.10.011

Verheij, M. M. M., Contet, C., Karel, P., Latour, J., van der Doelen, R. H. A., Geenen, B., et al. (2018). Median and dorsal raphe serotonergic neurons control moderate versus compulsive cocaine intake. *Biol. Psychiatry* 83, 1024–1035. doi: 10.1016/j.biopsych.2017.10.031

Williams, L. M., Kemp, A. H., Felmingham, K., Barton, M., Olivieri, G., Peduto, A., et al. (2006). Trauma modulates amygdala and medial prefrontal responses to consciously attended fear. *Neuroimage* 29, 347–357. doi: 10.1016/j.neuroimage.2005.03.047

Willner, P., Towell, A., Sampson, D., Sophokleous, S., and Muscat, R. (1987). Reduction of sucrose preference by chronic mild stress and its restoration by a tricyclic antidepressant. *Psychopharmacology* 93, 358–364. doi: 10.1007/bf00187257

Xu, J., Wang, R., Liu, W., Liu, D., Jiang, H., and Pan, F. (2017). FKBP5 and specific microRNAs *via* glucocorticoid receptor in the basolateral amygdala involved in the susceptibility to depressive disorder in early adolescent stressed rats. *J. Psychiatr. Res.* 95, 102–113. doi: 10.1016/j.jpsychires.2017.08.010

Yuen, E. Y., Liu, W., Karatsoreos, I. N., Ren, Y., Feng, J., McEwen, B. S., et al. (2011). Mechanisms for acute stress-induced enhancement of glutamatergic transmission and working memory. *Mol. Psychiatry* 16, 156–170. doi: 10.1038/mp.2010.50

Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

4.3 MANUSCRITO I

Este manuscrito apresenta-se em forma de revisão sistemática da literatura, e encontra-se em fase de redação.

The impact of tactile stimulation in early and adult life: a systematic review of behavioral and neurobiological changes in rats

Karine Roversi, Caren T. D. Antoniazzi, Marilise E. Burger

Abstract

Tactile stimulation (TS) is a supportive strategy that exerts impact on different aspects of behavioral, physiological, and neurobiological functioning in early and later life stages. Here, we conduct a systematic review to identify and provide information regarding the TS methodological procedures, mechanisms involved in its beneficial action, besides general influences evidenced in experimental studies. Our search was conducted using the PUBMED database, and the findings were categorized into experimental protocols, behavioral outcomes, and peripheral and central nervous system (CNS) outcomes. We identified 42 studies on the searched database and a further 13 studies were added based on authors' knowledge, totalizing 55 studies included in this systematic review. The acquired data demonstrated that TS application in rats showed important beneficial influences on behaviors, and most of these positive outcomes were observed on emotional behaviors. Also, the effects on the stress modulatory system, as well as in brain plasticity were the most common findings among all the reviewed studies. These results suggest that independently of the wide variations found in TS protocol, this procedure is a promising strategy for improving behaviors and neurobiological functioning in rodents. The current review provides important insights regarding the influence of TS on rats and this could help to devise strategies in the future to buffer different problems in humans.

Keywords: handling; neonatal; adulthood; anxiety; memory; brain, stress

1 Introduction

Gentle touch is known to induce pleasant sensation in the body (Loken et al., 2009). In humans, studies have shown that close physical contact or social support intervention (touch) is a key regulator of physiological, emotional, and behavioral as well as touch can “buffer” disadvantageous circumstances, such as stressful situations (Hofer, 1994; Morrison, 2016; Moszkowski & Stack, 2007). In rodents, different types of contact support, used as environmental interventions, have been used to study the relation of environmental effect on brain and behavior. Noxious stimuli applied in early life, such as sensory deprivation, social and maternal isolation, are widely used as a translational model of psychopathologies and have been associated to impair the development of the pups and alter brain anatomy and behavior, which can persist throughout life (Newson et al., 2005; Lai et al., 2008; Iguchi et al., 2020). Although negative experiences are the majority studied, positive environmental stimuli such as visual stimulation, enriched environment, and tactile stimulation (TS), have shown beneficial effects on neural circuits, psychiatric disorders, improving cerebral development, learning process, and skill abilities (Prusky et al., 2008; Sampedro-Piquero and Begega, 2016; Antoniazzi et al., 2017).

TS consists of a variety of external sensorial stimuli that when applied in early life mimics the mother’s caring and grooming behavior towards the pups, and in adulthood, TS is categorized as social contact enrichment. TS in neonatal periods was found to prevent damage from ischemia (Rodrigues et al., 2004) and cortical injury (Gibb et al., 2010), prevent psychostimulants addiction (Antoniazzi et al., 2014a,b) reverse the stress effects (Boufleur et al., 2013) and acid valproic exposure (Raza et al., 2015). Also, neonatal TS prevented alterations in brain organization, enhancing hippocampus neurogenesis (de los Angeles et al., 2016) and neuroplasticity (Richards et al., 2012). Regarding the effects of TS in adulthood, studies have shown that TS reverts depression-like behaviors (Roversi et al., 2019; Costa et al., 2020), prevents cortical lesion (Gibb et al., 2010), increasing neurotrophins levels in the prefrontal cortex (Roversi et al., 2019) and dendritic length in the striatum (Effenberg et al., 2014; Roversi et al., 2019). The TS mechanism in the brain remains unclear, however, there is evidence suggesting that TS affects the hypothalamic-pituitary-adrenal (HPA) axis and brain neurotrophic factors, but still is a lack of full understanding of the mechanism.

From a translational perspective, in humans, TS is used in the form of massage therapy in preterm infants and children and it has been an important tool used for the newborns' development (Pepino; Mezzacappa, 2015; Fields et al., 1996) while in adults, massage therapy, involving gentle touching of the skin, influences the autonomic nervous system and alters the stress response, increasing the wellbeing of patients with emotional disturbance, such as anxiety and depression (Lindgren, 2012; Weze et al., 2007).

To date, no reviews are detailing the mechanisms, methodological procedures, and effects of TS on rats. Given the wide variety of alterations in behavior and brain circuits found in animals submitted to TS, this systematic review aims to analyze the current literature and address the potential impacts of TS in early life and adulthood in rats. Here we provide an overview of different methodological TS applications, and the observed effects on physiological, behavioral, and neurobiological outcomes in different periods of rats' life.

2 Methods

A systematic literature review on PubMed was conducted on July, 18th, 2020. No limits were included for publication year or the language of publication. The search consisted of the following entry: Tactile stimulation AND (handling OR neonatal OR adult) AND rat. Additional scientific papers of interest were obtained via assessments through the relevant literature review and based on the authors' knowledge. A publication was excluded if it was a review and if it did not specifically focus on the skin/body tactile stimulation, such as stimulation on paws or whiskers, stimulation with anesthetized animals, tactile discrimination, electrode stimulation, stimulation only on anogenital area, and stimulation on artificially reared pups.

3 Results

3.1 Study selection

The literature search resulted in 241 papers and after applying the exclusion criteria, 42 articles were considered relevant and 13 other articles were added to the current review based on authors' knowledge and assessment of the relevant citations reference sections, totalizing 55 articles.

3.2 Experimental protocols for tactile stimulation

The neonatal TS procedure generally varies among research groups, but it mainly consists of removing the dam from the nest, followed by the removal or not of the pups from the home cage, to finally have the TS applied.

The first evidence of tactile manipulation in rats was described in a review by Schanberg and Field (1987) in which the pups were kept in the nest and submitted to TS applied with a small brush to mimics the mother's tongue-licking behavior. A total of 22 of the reviewed studies used the brush methodology, including, baby brush, Swiffer duster, bristled brush, camel hairbrush, toothbrush, and paintbrush. In 3 studies, the TS was applied with the felt part of a marking pen. Additionally, studies applying TS stroke directly with the experimenter's hands or fingers are also usually observed in the literature. In this procedure, the animal is removed from the nest and placed in a novel cage, such as a plastic cage with paper towel bedding or an individual round container without bedding (n=4 experiments); or the most commonly observed, on the experimenter lap/hand (n=21) and the animals/offspring were stroked with gloved hands or fingers. 3 articles applied TS with the hand/fingers but did not report in which bed the animals were placed and 2 articles did not report how the TS was applied.

Another important observation of different strategies used for TS is related to the frequency and total duration of the stroke. The daily amount of stimulation ranged from 0.5 min to 45 min while the total days of TS ranged between 1 day to 3 months of stroke. TS was applied in a variety of body locations, but the majority of experiments (n=42) reported the TS being applied on the dorsal surface, which includes the head, neck, and back. TS stroke was less commonly observed when applied on limbs, abdomen, and tail (n=7) and 6 experiments did not report which specific body part was submitted to the stroke.

3.3 Tactile stimulation and period of life

The early life stage is an important period for brain development when different stimuli can influence neurobiological and neuroendocrine systems, and these changes are known to remain for the rest of the rat's life. Due to these important changes occurring on the neural systems in the early life period (Levine, 1962), this stage of life is the most common choice among the studies for handling manipulation to be applied. The majority studies with TS are applied in early life (n=45 studies). The TS on early life is used to mimic the maternal behavior of licking and grooming. It

is well established that an increase of these specific maternal behaviors towards the offspring can improve the pups' emotionality-related behaviors in a long-term response (Liu et al., 1997; Macri, Mason, and Wurbel, 2004). Moreover, evidence has shown that after a brief mother-pup separation, an increase in mother licking behavior was observed what could be related to the improved emotionality-behavior response of the pups in adulthood (de Azevedo et al., 2010).

As previously described, TS is also applied on adulthood, but only in a fewer number of studies (n=9 studies) when compared to neonatal periods. This approach is related to the fact that not only the brain in development but also the adult nervous system has great neural plasticity, including the generation of new neurons and new connections among neurons (Gage, 2004). The TS application on adulthood confirms the hypothesis that the brain is always changing, since, changes in behavior and neuroanatomical differences were observed after TS on adult animals (see item 3.5.2). Among all reviewed studies, only 1 paper had TS applied from birth to adult period (from birth to the age of three months), to investigate the long-term effects of TS in rats.

3.4 Behavioral outcomes

3.4.1 Anxiety and depression behaviors

Several environmental factors are known to influence the risk for psychiatric disorders development, such as anxiety and depression. In both early periods and adulthood, adverse life events such as maternal separation, stress, or social isolation can predispose to anxiety and depression symptoms (Boufleur et al., 2013b; Grippo et al., 2014; Alves et al., 2020). Meanwhile, tactile stimulation is used as a supportive environment for a better adaptation to cope with adverse situations throughout life.

Many of the reviewed studies report beneficial TS effects on anxiety- and depression-like behaviors. 17 experiments assessed the TS effects on anxiety behaviors and among these, 11 found anxiety-like symptoms reduced/reverted by TS procedure (Antoniazzi, Boufleur, Dolci, et al., 2014; Antoniazzi, Boufleur, Pase, et al., 2014; Boufleur et al., 2012, 2013; Antoniazzi et al., 2017; Costa et al., 2012; Imanaka et al., 2008; Riul et al., 1999; Roversi et al., 2020; Soares et al., 2013). 5 studies established that TS did not affect anxiety-like behaviors (Silveira et al., 2005; Muhammad and Kolb, 2011; Muhammad et al., 2011; Richards et al., 2012; Raza et

al., 2015) and 1 experiment reported higher levels of anxiety in animals submitted to TS (Soares et al., 2014). Regarding depression-like behaviors, 5 studies observed that TS has beneficial effects on these symptoms, being able to reduce the depression-like behaviors (Boufleur et al., 2013; Costa et al., 2012; Freitas et al., 2015; Roversi et al., 2019; 2020).

3.4.2 Cognitive function: memory and learning

It is well established that environmental interventions may have prolonged effects on the brain. Given that, positive interventions have shown beneficial outcomes on memory and learning (Harati et al., 2013; Kambali et al., 2019). Different types of memory - function, content, duration, nature, or motivation memory - can be assessed to evaluate learning and memory performances of rodents (Antunes and Biala, 2012; Vorhees and Williams, 2014). In the reviewed studies, 14 experiments investigated the TS role in memory and learning behaviors. 10 experiments reported positive outcomes on learning and memory (Antoniazzi et al., 2017; Costa et al., 2012; Daskalakis et al., 2009; de los Angeles et al., 2016; Gschanes et al., 1995, 1998; Kolb & Gibb, 2010; Richards et al., 2012; Stara et al., 2018; Zhang & Cai, 2008) and 5 studies found no effect of TS on these behaviors (Wong, 1966; Muhammad and Kolb, 2011; Muhammad et al., 2011; Raza et al., 2015; Antoniazzi et al., 2017). Among these 14 studies, only 1 experiment showed TS impairments on learning and memory, depending on the duration of TS application (Daskalakis et al., 2009), which confirms the hypothesis that supportive environments have been positively correlated with cognition function.

3.4.3 Locomotor and drug addiction effects

Due to the important evidence provided by the outcomes of TS on psychiatric disorders, this positive approach was also tested on drug addiction models and their psychomotor effects. Among the reviewed studies, 4 studies investigated the influence of TS on drug effects, and 2 experiments found a reduced preference for psychostimulants (cocaine and amphetamine) (Antoniazzi et al., 2014a,b), and the other 2 observed an attenuated amphetamine behavioral sensitization, assessed in a locomotor behavioral test (Muhammad et al., 2011; Muhammad and Kolb, 2011). Furthermore, 9 studies investigated the locomotor and exploratory effects of TS. Among these, 6 experiments found locomotion increase (Silveira et al., 2005;

Imanaka et al., 2008; Muhammad and Kolb, 2011; Boufleur et al., 2012; Raza et al., 2015) and 3 observed no effect of TS on locomotor behavior (de Azevedo et al., 2010; Muhammad et al., 2011; Kentner et al., 2018).

Also, among the reviewed studies, 4 experiments assessed motor tasks after brain lesions, and 2 experiments evaluated motor skills in rats without brain lesions. Related to this, 3 experiments found improved skilled locomotor behaviors after cortical lesion (Gibb et al., 2010; Kolb and Gibb, 2010; Zucchi et al., 2014), 1 experiment found improved skilled locomotor behaviors in normal rats (Richards et al., 2012) while 2 experiments observed no effect of TS on motor behaviors after valproic acid administration (Raza et al., 2015) and after striatal lesion (Effenberg et al., 2014;). Moreover, 1 study assessed the developmental motor recovery after exposure to a stressful model mimicking the neonatal intensive care unit and TS was able to improve surface righting and rooting reflexes (Kentner et al., 2018).

3.4.4 Other behaviors outcomes

Regarding emotional behaviors, 9 studies investigated the TS influence on social behavior, and among them, 3 experiments observed an increase in social play behavior (Aguilar, 2010; Aguilar et al., 2009; Roversi et al., 2020) and 2 found a reduction on play attacks behavior (Muhammad et al., 2011; Muhammad and Kolb, 2011). TS also was found to reduce social behavior in 1 study (Kentner et al., 2018) and in 1 experiment TS showed no effect on play attacks in social behavioral tests (Riul et al., 1999). 1 experiment observed an increase in the social 50 kHz ultrasonic vocalizations, an index of positive emotion (Okabe et al., 2015) while 1 experiment did not observe any effect in the 192 kHz vocalizations, in which represents a social behavior (Kentner et al. 2018).

2 studies assessed thermal pain sensitivity, and both studies observed increased latency for paw withdrawal from the heating source (Stephan et al., 2002a; Imanaka et al., 2008). 2 studies evaluated the food ingestion and the findings demonstrated an increase in palatable foods intake (Silveira et al., 2004, 2005). 1 study investigated visual acuity development and the researchers found increased visual acuity in TS-treated rats (Guzzetta et al., 2009). On the other hand, 1 experiment showed an aggravation in the clinical scores measured in the experimental autoimmune encephalomyelitis model, used to mimic the symptoms observed in multiple sclerosis in humans (Stephan et al., 2002b) and 1 experiment

observed heightened clinic signs in the experimental allergic encephalomyelitis model, an autoimmune disease of the central nervous system (Laban et al., 1995). 1 study assessed the odor preference, and the TS procedure showed improvements in the learning odor preference (Sullivan et al., 1991), and 1 study evaluated the TS activity after deprivation states and TS did not alter the active behavior observed in the wheel running test (Wong et al., 1967).

3.5 Peripheral and central nervous system outcomes

3.5.1 Stress systems regulation

The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system have been investigated in handling procedures due to the important neuroendocrine and catecholaminergic alterations that external stimuli can cause. The HPA axis is the first hormonal response to homeostatic challenges (Herman et al., 2016). In rodents, the HPA axis regulates the release of corticosterone, a glucocorticoid hormone related to stressful situations. Once the HPA axis is activated, the corticotrophin-release hormone (CRH) is released, and CRH signals the pituitary gland to secretes the adrenocorticotrophic hormone (ACTH) in the bloodstream. This sequence of events results in glucocorticoid secretion, the corticosterone, by the adrenal glands. In the brain, the glucocorticoid and mineralocorticoid receptors (GR and MR, respectively) regulate the HPA axis through a negative feedback response. 14 studies assessed corticosterone serum levels and among them, 11 experiments found decreased serum levels of corticosterone after TS (Stephan et al., 2002b; Jutapakdeegul et al., 2003; Daskalakis et al., 2009; Haley et al., 2013; Soares et al., 2013; Zucchi et al., 2014; Freitas et al., 2015; Antoniazzi et al., 2017b; Kentner et al., 2018; Roversi et al., 2019; Costa et al., 2020). 2 experiments found increased levels (Daskalalaskis et al., 2009; Soares et al., 2014) and 2 observed no alterations in corticosterone serum levels (Guzzeta et al., 2009; Costa et al., 2012). 1 study investigated the serum ACTH levels, and the researchers observed a reduction of its levels (Roversi et al., 2019) and 1 study observed a reduction in cortisol plasma levels, a hormone also considered as a general stress measurement for adrenocortical function (Boufleur et al., 2012). 3 studies evaluated the adrenal weight/body weight ratio, and TS treatment reduced/restored this relation in all 3 studies (Boufleur et al., 2013; Zucchi et al., 2014; Roversi et al., 2019). In the brain, 5 studies investigated the GR levels in different brain areas related to stress,

anxiety, and depression. 4 studies observed increased levels of GR on the hippocampus, prefrontal cortex, and midbrain after TS treatment (Jutapakdeegul et al., 2003; Antoniazzi et al., 2017; Kentner et al., 2018; Roversi et al., 2019) while 1 study did not find alterations on this receptor (Roversi et al., 2020). Regarding MR levels on the brain, only 1 study evaluated its levels on the amygdala, and it was reported an increase in the receptor levels (Roversi et al., 2020). Furthermore, the same study also investigated the glucocorticoid inducible genes FK506-binding protein 51 (Fkbp5), Nr4a1, and the growth arrest and DNA-damage-inducible beta (Gadd45b), finding reduced levels of Nr4a1 and Gadd45b on the amygdala besides an increased Fkbp5 mRNA levels (Roversi et al., 2020).

The sympathetic nervous system, a key role for the release of the catecholamines, is also related to the homeostatic regulation for stressful situations. Thus, in response to adverse external stimuli, the sympathetic nervous system will signal the adrenal glands for releasing epinephrine, norepinephrine, and cortisol, which can act directly on autonomic nerves, and trigger the physical stress reaction (Renard et al., 2005; Olausson et al., 2008; Costa et al., 2020). Plasma norepinephrine and epinephrine levels can represent an index of sympathoneural activity. In the reviewed studies, 2 experiments analyzed adrenaline and norepinephrine serum levels and both studies found a reduction in norepinephrine levels (Costa et al., 2012; 2020). Regarding epinephrine levels, 1 study found reduction (Costa et al., 2020), and 1 found no alterations on these levels (Costa et al., 2012). 1 study investigated the angiotensin II levels on the hypothalamus, which is a component of the renin-angiotensin system that participates in the regulation of the stress response, and the researchers found a reduction of this peptide (Costa et al., 2020).

3.5.2 Neuronal morphology and plasticity

The brain is known to have a high ability to change in response to different stimuli, also known as neuroplasticity, which can potentially affect the brain's morphology by the reorganization of its structure, connections, and functions (Shors et al., 2012; Mateos-Aparicio and Rodríguez-Moreno, 2019). In the reviewed studies, 5 studies investigated anatomical changes in the brain, mainly evaluated by the dendritic length, arborization, and spinal density. 1 study found that TS increased dendritic length on layer III and V of the frontal cortex, and no alterations on dendritic

branching and spine density (Gibb et al., 2010). 1 study revealed that TS reversed the reduction of dendritic lengths on the parietal but not on the frontal cortex after a brain lesion. Also, TS increased the spinal density in the frontal cortex of animals submitted to a lesion in this brain area and did not alter on the parietal cortex, but decreased the spinal density on control animals (Kolb and Gibb, 2010). 1 study observed an increase in spine density in the amygdala after TS, and besides that, spine density, dendritic length, and branching were also increased in the prefrontal cortex (Richards et al., 2012). 1 study found increased dendritic length in the striatum, while in the apical or basilar prefrontal cortex it was not altered by TS. The dendritic bifurcation and number of spines were not altered as well (Effenberg et al., 2014). 1 study found that TS increased/reverted the reduction caused by exposure to valproic acid, observed on dendritic length, branching, and spinal density on apical and basilar cg3 of the frontal cortex (Raza et al., 2015).

Furthermore, 3 studies investigated brain volume and thickness. 1 study reported that TS prevented the reduction of the whole hippocampus and the dentate gyrus after exposure to neonatal hypoxia-ischemia event while no differences in the thickness of ipsilateral and contralateral cortex were observed (Rodrigues et al., 2004). 2 studies showed that TS after amphetamine administration promoted an increase in the cortical thickness of female but not male rats, and increased anterior striatum in male but not in female rats. Also, with the TS application it was observed a reduction in total brain weight after amphetamine administration was observed in males but not in female rats (Muhammad et al., 2011; Muhammad and Kolb, 2011).

Our observations showed that 3 studies investigated neuroanatomical changes in the optical nerve. 1 study found an increase of oligodendrocyte in the optical nerve and blood vessel density, lower density of damaged fibers, and no alterations on astrocytes density and diameter of the optical nerve (Horiquini-Barbosa and Lachat, 2016). 1 study showed that TS reversed the alterations of oligodendrocytes, damaged fiber, and myelinated fiber density but did not alter the astrocyte density on the optical nerve (Horiquini-Barbosa et al., 2017). 1 study reported that TS reversed axon, fiber, and myelin size of small fibers while on large fibers TS did not affect (Horiquini-Barbosa et al., 2020).

It is known that brain plasticity is influenced by different exposures to a stimulating environment (Sale et al., 2014). Neurotrophic factors such as brain-derived neurotrophic factor (BDNF), and glial cell line-derived neurotrophic factor (GDNF),

and growth factors such as glial fibrillary acidic protein (GFAP), fibroblast growth factor- 2 (FGF-2), and insulin-like growth factor-1 (IGF-1) are strongly involved on synaptic plasticity. In the reviewed database, 4 studies investigated the TS influence on BDNF and GDNF, and these 4 studies found increased levels of BDNF on the hippocampus, amygdala, and prefrontal cortex (Effenberg et al., 2014; Antoniazzi et al., 2017; Roversi et al., 2019; 2020). 1 study showed increased levels of GDNF on the prefrontal cortex (Roversi et al., 2019) and 1 study found no alterations on GDNF striatum levels after the TS procedure (Effenberg et al., 2014). 3 studies assessed the TS role upon the growth factors FGF-2, GFAP, and IGF-1, in which 1 experiment observed increased levels of FGF-2 on the hippocampus and striatum but no alterations on the ventral midbrain (Effenberg et al., 2014), 1 paper reported increased levels of GFAP on the prefrontal cortex (Roversi et al., 2019) and 1 experiment observed increased IGF-1 levels in visual cortex (Guzzeta et al., 2009). Moreover, 1 study evaluated different gene growth factors in the motor cortex, and the researchers found increased gene growth factors, which were related to increased brain plasticity (Zucchi et al., 2014).

3.5.3 Neurochemical outcomes

The metabolism of the nervous system can be affected by different environmental factors and the brain tissue is highly sensitive to the oxidative imbalance. Alterations on the maintenance of the redox state can compromise the brain physiology, damaging its function (Mármol et al., 2017; Marcon et al., 2018). 5 of the reviewed studies investigated oxidative and antioxidant biochemical markers in different brain areas. 1 study found that TS favors the protein carbonyl reduction in the cortex and subthalamic regions, but did not cause alterations in the hippocampus (Boufleur et al., 2012). 1 study found a reduction in protein carbonyl, catalase activity in the hippocampus and cortex and reduction in SOD activity in the hippocampus of rats submitted to TS (Boufleur et al., 2013). 2 experiments found reduced protein carbonyl and catalase activity in the cortex, striatum, and hippocampus after TS (Antoniazzi et al., 2014a, b). 1 study observed that TS promoted a reduction in thiobarbituric acid reactive substances (TBARS) and increased catalase activity in the hippocampus (Antoniazzi et al., 2017).

3.5.4 Other systems outcomes

The short time of removing the pup from the mother, also known as handling, can cause a variety of physiological changes (Raineki et al., 2014). 1 study evaluated glucose and lactate serum levels and the researchers found that lactate serum was increased after the TS procedure, which is related to increased brain development in early periods of life, while glucose levels were not affected (Alasmi et al., 1997). 1 study investigated the levels of interferon-γ, a cytokine that plays an important role in inducing and modulating an array of immune responses, and interleukin-4 and -10, involved in the anti-inflammatory response, and TS treatment increased interferon-γ and interleukin-4 levels while did not alter interleukin-10 serum levels (Stephan et al., 2002b). 2 studies investigated the impact of TS on bone mineralization, and 1 found an increase in bone mineralization content and higher levels of growth factors in serum and bone (Haley et al., 2011) while the other study observed the maintenance of the bone mineralization content and growth factors on serum (Haley et al., 2013). 2 studies evaluated the abdominal fat deposition and both studies showed that TS induced a normalization on the visceral fat deposition, minimizing the metabolic consequences (Moyer-Mileur et al., 2011; Haley et al., 2013b). 1 study investigated fos protein expression in oxytocin-immunoreactive neurons and it was observed an increase of the expression on the supraoptic nucleus and the paraventricular nucleus, while no alterations were observed on the bed nucleus of the stria terminalis (Okabe et al., 2015). 1 study investigated the pCREB activation and it was found an increase in the pCREB on the olfactory bulbs when the animals were exposed to a specific odor and submitted to the TS treatment, but no alterations were found in animals receiving only the TS treatment (McLean et al., 1999). 1 study evaluated the dopaminergic receptor 1 activation and the researchers observed an optimal activation of prefrontal D1 receptors in TS treated rats (Zhang and Cai, 2008). 1 study evaluated the influence of TS on the polyamines levels in the hippocampus and the results showed no alterations on spermine and spermidine revealing no alterations in the anabolic and catabolic process (Soares et al., 2013).

4 Discussion

The purpose of this systematic review was to access the TS impact on behavioral, neuroendocrine, and physiologic parameters in experimental studies performed in rats. Our findings suggest that although the heterogeneity observed among the protocols for TS, TS promotes beneficial influences on different

behaviors, including emotional, cognition, drug addiction as well as on brain and other systems activity. Also, it was well described that TS exerts a positive effect when applied in early periods, being also positive in both male and female adulthood animals. These positive outcomes go in line with previous studies looking at the effects of supportive environments (Raineki et al., 2014; Sampedro-Piquero and Begega, 2016).

A wide variety of protocols regarding the duration – the amount of TS, time in one session, or the total time in days - of the TS application was compared in the reviewed studies. A study compared if two different amounts (10 back-strokes or 20 back-strokes per session) of neonatal TS could affect behavior or corticosterone levels in adulthood. In both amounts of strokes, the researchers observed positive and negative outcomes, leading to the conclusion that the intensity of TS exerts a high impact on modifying behavioral aspects and stress hormonal production (Daskalalaskis et al., 2009). Also, another study investigated the number of days necessary for TS treatment to modify expression levels of some genes in different brain areas of animals in adulthood. The findings showed that independent of how many days of TS application, it changed brain gene expressions, suggesting that even a minimal amount of TS (5 days) can cause significant modifications in the CNS (Effenberg et al., 2014). Besides, the postnatal day in which TS was started was also a parameter considered in the current review, considering the period more effective for external stimulus during the stress-hyporesponsive period. 2 studies compared the TS application in the preweaning and postweaning periods, and although TS presented a positive approach in both phases of development, the learning behavior was better in the groups receiving TS during the first postnatal week than in the groups stimulated after the weaning period (Gschanes et al., 1995; 1998). Furthermore, Antoniazzi et al (2017) compared the stimulation on 3 different stages of preweaning and TS applied in the postnatal days (PND) 8-14 improved the rats' performance in behavioral tests and molecular brain analysis when compared to the periods of PND 1-7 or PND 15-21. The study showed that the intermediate phase of the preweaning period was the best period for the TS intervention because this is the phase in which the adrenal response to the stress is minimal and it is considered a stress hyporesponsive period (Antoniazzi et al., 2017). However, a study comparing the TS stroking on PND 2-9 and PND 10-17 did not observe significant differences between the two assessed periods (Zhang et al., 2008). Overall, we could

hypothesize that although the differences detected in the duration or the neonatal period of TS application, the TS procedure reflected positive effects in a range of behaviors, neurological, and physiological factors.

Behavioral studies were investigated in 44 of the 55 reviewed studies and the majority of the behavior quantifications were related to affective behaviors, cognition, locomotion activity, and these behavioral outcomes were generally related to biochemical or neuroendocrinal adaptations. Indeed, glucocorticoid corticosterone was the stress hormonal more assessed in these reviewed studies. Among all of them, the relationship between corticosterone and behavioral outcomes were investigated. The decreased serum corticosterone/cortisol was highly associated with the improved performance of learning and memory (Antoniazzi et al., 2017, Daskalalaskis et al., 2009), minor levels of anxiety, besides depressive-like behaviors (Antoniazzi et al., 2017; Freitas et al., 2016; Roversi et al., 2019). Similarly, glucocorticoid- (GR) and mineralocorticoid- (MR) receptors were associated with emotional behavioral adaptations (Antoniazzi et al., 2017; Roversi et al., 2019; 2020). In fact, glucocorticoids exert a strong influence on memory and, in adaptative conditions, the release of corticosterone or the activation of GR and MR can mediate and modulate the learning and memory behavior (Kelemen et al., 2014). Furthermore, the imbalance in the regulation of corticosterone release is often associated with psychopathologies development, such as anxiety and depression disorders, and GR and MR support the HPA axis to maintain a proper regulation (Finsterwald and Alberini, 2014). On TS animals, it was observed an increase of GR and MR levels on different brain areas, and this increase was closely related to enhanced negative feedback of the HPA axis which provides a better response against adverse situations (Pham et al., 1997; Antoniazzi et al., 2017). Then, as suggested that the positive effects of TS are associated with its ability to alter the HPA axis sensitivity, as well as the glucocorticoid receptors concentration, and as consequence, reduce emotionality and improve cognition.

Different handling procedures can influence both brain -function and -morphology through neuroplasticity. Also, literature reports that separate pups from the mother can hinder the pups' development, but it seems clear that removing pups from their dams and adding the TS procedure favors a better development to the subjects involved. Among the studies evaluating the brain's anatomical organization or function, it was possible to observe that TS positively affects these parameters

and, in some cases, TS application even reverted the behavioral and anatomical deficits observed in rats following brain injuries. The brain is known to have the ability to change after lesions. In the papers that TS improved brain morphology after an injury, the hypothesis is that ocurr a sum effect of both the brain's ability to reorganize itself and TS influence on interact with these changes to produce further enhancement (Gibb et al., 2010). Also, it seems that the positive influence of TS on neurogenesis is associated with the hypothesis that TS is acting as a stressor and is changing the brain's capacity to adapt to new situations, according to other studies with different environment stimuli (Lehmann and Herkenham, 2011; González-Pardo et al., 2019). Another interesting topic relies on the fact that the majority of the reviewed studies associated the neurogenesis with TS in early life because it is a critical and sensitive period that the brain is especially affected by external experiences (Vazquez et al., 2006). However, a few studies also showed this effect of TS on adulthood, suggesting that TS exerts potential benefits in all phases of life (Effenberg et al., 2015; Roversi et al., 2019).

As with all systematic reviews, this study has its limitations. It is possible that some relevant studies were not identified as our search strategy was limited to only one electronic database (PUBMED) as well as only studies published in the English language were included, subjecting this review to a language bias. Besides, a considerable amount of heterogeneity related to the standardization of the TS application protocol was found, making it difficult to compare the results between the studies.

In conclusion, under our point of view, this is the first systematic review pointing to the effects of the TS on behavioral, physiological, and brain alterations in rats. Affective state, cognition, locomotion, drug addiction, and other behaviors in male and female rats and early life and adulthood were favorably modified by TS application. Also, a range of neuroplastic and neuroendocrine pathways markers were affected by TS as well. These findings demonstrate the many positive effects of TS suggest that this procedure can be an efficient tool applied in both early life and adulthood able to modify brain functions that regulate different behaviors in rats.

References

Aguilar, R. (2010). Infantile experience and play motivation. *Soc. Neurosci.* 5.

doi:10.1080/17470919.2010.503618.

Aguilar, R., Caramés, J. M., and Espinet, A. (2009). Effects of Neonatal Handling on Playfulness by Means of Reversal of the Desire to Play in Rats (Rattus norvegicus). *J. Comp. Psychol.* 123. doi:10.1037/a0016437.

Alasmi, M. M., Pickens, W. L., and Hoath, S. B. (1997). Effect of tactile stimulation on serum lactate in the newborn rat. *Pediatr. Res.* 41. doi:10.1203/00006450-199706000-00010.

Alves, R. L., Oliveira, P., Lopes, I. M., Portugal, C. C., Alves, C. J., Barbosa, F., et al. (2020). Early-life stress affects drug abuse susceptibility in adolescent rat model independently of depression vulnerability. *Sci. Rep.* 10. doi:10.1038/s41598-020-70242-4.

Antoniazzi, C. T. D., Boufleur, N., Dolci, G., Roversi, K., Kuhn, F., Pase, C. S., et al. (2014a). Influence of neonatal tactile stimulation on amphetamine preference in young rats: Parameters of addiction and oxidative stress. *Pharmacol. Biochem. Behav.* 124. doi:10.1016/j.pbb.2014.07.001.

Antoniazzi, C. T. D., Boufleur, N., Pase, C. S., Kuhn, F. T., Dias, V. T., Segat, H. J., et al. (2014b). Tactile stimulation and neonatal isolation affect behavior and oxidative status linked to cocaine administration in young rats. *Behav. Processes* 103. doi:10.1016/j.beproc.2014.01.011.

Antoniazzi, C. T. D., Metz, V. G., Roversi, K., Freitas, D. L., Vey, L. T., Dias, V. T., et al. (2017). Tactile stimulation during different developmental periods modifies hippocampal BDNF and GR, affecting memory and behavior in adult rats. *Hippocampus* 27. doi:10.1002/hipo.22686.

Antunes, M., and Biala, G. (2012). The novel object recognition memory: Neurobiology, test procedure, and its modifications. *Cogn. Process.* 13. doi:10.1007/s10339-011-0430-z.

Boufleur, N., Antoniazzi, C. T. D., Pase, C. S., Benvegnú, D. M., Barcelos, R. C. S., Dolci, G. S., et al. (2012). Neonatal tactile stimulation changes anxiety-like behavior and improves responsiveness of rats to diazepam. *Brain Res.* 1474. doi:10.1016/j.brainres.2012.08.002.

Boufleur, N., Antoniazzi, C. T. D., Pase, C. S., Benvegnú, D. M., Dias, V. T., Segat, H. J., et al. (2013). Neonatal handling prevents anxiety-like symptoms in rats exposed to chronic mild stress: Behavioral and oxidative parameters. *Stress* 16. doi:10.3109/10253890.2012.723075.

Costa, R., Tamascia, M. L., Nogueira, M. D., Casarini, D. E., and Marcondes, F. K. (2012). Handling of adolescent rats improves learning and memory and decreases anxiety. *J. Am. Assoc. Lab. Anim. Sci.* 51, 548–553.

Costa, R., Tamascia, M. L., Sanches, A., Moreira, R. P., Cunha, T. S., Nogueira, M. D., et al. (2020). Tactile stimulation of adult rats modulates hormonal responses,

depression-like behaviors, and memory impairment induced by chronic mild stress: Role of angiotensin II. *Behav. Brain Res.* 379. doi:10.1016/j.bbr.2019.112250.

Daskalakis, N. P., Kaperoni, M., Koros, C., de Kloet, E. R., and Kitraki, E. (2009). Environmental and tactile stimulation modulates the neonatal handling effect on adult rat spatial memory. *Int. J. Dev. Neurosci.* 27. doi:10.1016/j.ijdevneu.2009.08.013.

de Azevedo, M. S., de Souza, F. L., Donadio, M. V. F., Lucion, A. B., and Giovenardi, M. (2010). Interventions in the neonatal environment in rats and their relationship to behavior in adulthood and maternal behavior. *Psychol. Neurosci.* 3. doi:10.3922/j.psns.2010.1.009.

de los Angeles, G. A. M., del Carmen, R. O. M., Wendy, P. M., and Socorro, R. M. (2016). Tactile stimulation effects on hippocampal neurogenesis and spatial learning and memory in prenatally stressed rats. *Brain Res. Bull.* 124. doi:10.1016/j.brainresbull.2016.03.003.

Effenberg, A., Klein, A., Gibb, R., Carroll, C., Baumgärtner, W., Grothe, C., et al. (2014). Adult hemiparkinsonian rats do not benefit from tactile stimulation. *Behav. Brain Res.* 261. doi:10.1016/j.bbr.2013.12.011.

Field, T., Grizzle, N., Scafidi, F., Abrams, S. (1996). Massage therapy for infants of depressed mothers. Inf. Behav. Develp., 19, 107–112.doi: 10.1016/S0163-6383(96)90048-X.

Finsterwald, C., Alberini, C.M. (2014). Stress and glucocorticoid receptor-dependent mechanisms in long-term memory: from adaptive responses to psychopathologies. *Neurobiol. Learn. Mem.* 112, 17-29. doi: 10.1016/j.nlm.2013.09.017.

Freitas, D., Antoniazzi, C. T. D., Segat, H. J., Metz, V. G., Vey, L. T., Barcelos, R. C. S., et al. (2015). Neonatal tactile stimulation decreases depression-like and anxiety-like behaviors and potentiates sertraline action in young rats. *Int. J. Dev. Neurosci.* 47. doi:10.1016/j.ijdevneu.2015.09.010.

Gage, F. H. (2004). Structural plasticity of the adult brain. *Dialogues Clin. Neurosci.* 6.

Gibb, R. L., Gonzalez, C. L. R., Wegenast, W., and Kolb, B. E. (2010). Tactile stimulation promotes motor recovery following cortical injury in adult rats. *Behav. Brain Res.* 214. doi:10.1016/j.bbr.2010.04.008.

González-Pardo, H., Arias, J. L., Vallejo, G., and Conejo, N. M. (2019). Environmental enrichment effects after early stress on behavior and functional brain networks in adult rats. *PLoS One* 14. doi:10.1371/journal.pone.0226377.

Grippo, A. J., Ihm, E., Wardwell, J., Mcneal, N., Scotti, M. A. L., Moenk, D. A., et al. (2014). The effects of environmental enrichment on depressive and anxiety-relevant behaviors in socially isolated prairie voles. *Psychosom. Med.* 76. doi:10.1097/PSY.0000000000000052.

Gschanes, A., Eggenreich, U., Windisch, M., and Crailsheim, K. (1995). Effects of postnatal stimulation on the passive avoidance behaviour of young rats. *Behav. Brain Res.* 70. doi:10.1016/0166-4328(95)80008-5.

Gschanes, A., Eggenreich, U., Windisch, M., and Crailsheim, K. (1998). Early postnatal stimulation influences passive avoidance behaviour of adult rats. *Behav. Brain Res.* 93. doi:10.1016/S0166-4328(97)00143-5.

Guzzetta, A., Baldini, S., Bancale, A., Baroncelli, L., Ciucci, F., Ghirri, P., et al. (2009). Massage accelerates brain development and the maturation of visual function. *J. Neurosci.* 29. doi:10.1523/JNEUROSCI.5548-08.2009.

Haley, S., Gulliver, K., Baldassarre, R., Miller, S., Lane, R. H., and Moyer-Mileur, L. J. (2013). Tactile and kinesthetic stimulation (TKS) intervention improves outcomes in weanling rat bone in a neonatal stress model. *J. Musculoskelet. Neuronal Interact.* 13.

Haley, S., et al. (2013b). Mechanical-tactile stimulation (MTS) intervention in a neonatal stress model alters adult adipose tissue deposition and prevents hyperinsulinemia in male rats. *Early Hum. Dev.* 89, 387-392. doi: 10.1016/j.earlhumdev.2012.12.005

Haley, S., O'Grady, S., Gulliver, K., Bowman, B., Baldassarre, R., Miller, S., et al. (2011). Mechanical-tactile stimulation (MTS) intervention in a neonatal stress model improves long-term outcomes on bone. *J. Musculoskelet. Neuronal Interact.* 11.

Harati, H., Barbelivien, A., Herbeaux, K., Muller, M. A., Engeln, M., Kelche, C., et al. (2013). Lifelong environmental enrichment in rats: Impact on emotional behavior, spatial memory vividness, and cholinergic neurons over the lifespan. *Age (Omaha).* 35. doi:10.1007/s11357-012-9424-8.

Herman, J. P., McKlveen, J. M., Ghosal, S., Kopp, B., Wulsin, A., Makinson, R., et al. (2016). Regulation of the hypothalamic-pituitary- adrenocortical stress response. *Compr. Physiol.* 6. doi:10.1002/cphy.c150015.

Horiquini-Barbosa, E., Gibb, R., Kolb, B., Bray, D., and Lachat, J. J. (2020). Neonatal tactile stimulation reverses alterations in fine structure of small, but not large myelinated fibers, from the optic nerve of iron-deficient rats: A size-based selectivity. *Behav. Brain Res.* 379. doi:10.1016/j.bbr.2019.112357.

Horiquini-Barbosa, E., and Lachat, J. J. (2016). Tactile stimulation during development alters the neuroanatomical organization of the optic nerve in normal rats. *Exp. Brain Res.* 234. doi:10.1007/s00221-016-4586-8.

Horiquini-Barbosa, E., Gibb, R., Kolb, B., Bray, D., Lachat, J.J. (2017). Tactile stimulation partially prevents neurodevelopmental changes in visual tract caused by early iron deficiency. *Brain Res.* 1657, 130-139. doi: 10.1016/j.brainres.2016.12.003.

Iguchi, R., Tanaka, S., and Okabe, S. (2020). Neonatal social isolation increases the proportion of the immature spines in the layer 2/3 pyramidal neurons of the

somatosensory cortex. *Neurosci. Res.* 154. doi:10.1016/j.neures.2019.05.004.

Imanaka, A., Morinobu, S., Toki, S., Yamamoto, S., Matsuki, A., Kozuru, T., et al. (2008). Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male and female adult Sprague-Dawley rats. *Behav. Brain Res.* 186. doi:10.1016/j.bbr.2007.07.039.

Jutapakdeegul, N., Casalotti, S. O., Govitrapong, P., and Kotchabhakdi, N. (2003). Postnatal touch stimulation acutely alters corticosterone levels and glucocorticoid receptor gene expression in the neonatal rat. *Dev. Neurosci.* 25. doi:10.1159/000071465.

Kambali, M. Y., Anshu, K., Kutty, B. M., Muddashetty, R. S., and Laxmi, T. R. (2019). Effect of early maternal separation stress on attention, spatial learning and social interaction behaviour. *Exp. Brain Res.* 237. doi:10.1007/s00221-019-05567-2.

Kelemen, E., Bahrendt, M., Born, J., and Inostroza, M. (2014). Hippocampal corticosterone impairs memory consolidation during sleep but improves consolidation in the wake state. *Hippocampus* 24. doi:10.1002/hipo.22266.

Kentner, A. C., Scalia, S., Shin, J., Migliore, M. M., and Rondón-Ortiz, A. N. (2018). Targeted sensory enrichment interventions protect against behavioral and neuroendocrine consequences of early life stress. *Psychoneuroendocrinology* 98. doi:10.1016/j.psyneuen.2018.07.029.

Kolb, B., and Gibb, R. (2010). Tactile stimulation after frontal or parietal cortical injury in infant rats facilitates functional recovery and produces synaptic changes in adjacent cortex. *Behav. Brain Res.* 214. doi:10.1016/j.bbr.2010.04.024.

Laban, O., Dimitrijević, M., von Hoersten, S., Marković, B. M., and Janković, B. D. (1995). Experimental allergic encephalomyelitis in adult DA rats subjected to neonatal handling or gentling. *Brain Res.* 676. doi:10.1016/0006-8993(95)00106-Z.

Lai, M. C., Yang, S. N., and Huang, L. T. (2008). Neonatal Isolation Enhances Anxiety-like Behavior Following Early-life Seizure in Rats. *Pediatr. Neonatol.* 49. doi:10.1016/S1875-9572(08)60006-6.

Lehmann, M. L., and Herkenham, M. (2011). Environmental enrichment confers stress resiliency to social defeat through an infralimbic cortex-dependent neuroanatomical pathway. *J. Neurosci.* 31. doi:10.1523/JNEUROSCI.0577-11.2011.

Levine, S (1962). The effects of infantile experience on adult behavior. In: Bachrach, A.J. Experimental foundations of clinical psychology. New York: Basic Books, 139–169.

Lindgren, L. (2012). Pleasant human touch is represented in pregenual anterior cingulate cortex. Neuroimage, 59, 3427-3432. doi: 10.1016/j.neuroimage.2011.11.013.

Liu, D., Diorio, J., Tannenbaum, B., Caldji, C., Francis, D., et al. (1997). Maternal

care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. *Science*. 277(5332), 1659-1662. doi: 10.1126/science.277.5332.1659.

Löken, L.S. et al (2009). Coding of pleasant touch by unmyelinated afferents in humans", Nat. Neurosci. 12(5):547-8. doi: 10.1038/nn.2312.

Macrí, S., Mason, G.J., Würbel, H. (2004). Dissociation in the effects of neonatal maternal separations on maternal care and the offspring's HPA and fear responses in rats. Eur. J. Neurosci. 20, 4, 1017-1024. doi: 10.1111/j.1460-9568.2004.03541.x

Marcon, M., Mocelin, R., Sachett, A., Siebel, A. M., Herrmann, A. P., and Piato, A. (2018). Enriched environment prevents oxidative stress in zebrafish submitted to unpredictable chronic stress. *PeerJ* 2018. doi:10.7717/peerj.5136.

Mármol, F., Sánchez, J., Torres, M. N., and Chamizo, V. D. (2017). Environmental enrichment in the absence of wheel running produces beneficial behavioural and anti-oxidative effects in rats. *Behav. Processes* 144. doi:10.1016/j.beproc.2017.09.009.

Mateos-Aparicio, P., and Rodríguez-Moreno, A. (2019). The impact of studying brain plasticity. *Front. Cell. Neurosci.* 13. doi:10.3389/fncel.2019.00066.

McLean, J. H., Harley, C. W., Darby-King, A., and Yuan, Q. (1999). pCREB in the neonate rat olfactory bulb is selectively and transiently increased by odor preference-conditioned training. *Learn. Mem.* 6. doi:10.1101/lm.6.6.608.

Morrison, I. (2016). Keep Calm and Cuddle on: Social Touch as a Stress Buffer. *Adapt. Hum. Behav. Physiol.* 2. doi:10.1007/s40750-016-0052-x.

Moszkowski, R. J., and Stack, D. M. (2007). Infant touching behaviour during mother-infant face-to-face interactions. *Infant Child Dev.* 16. doi:10.1002/icd.510.

Moyer-Mileur, L. J., Haley, S., Gulliver, K., Thomson, A., Slater, H., Barrett, B., et al. (2011). Mechanical-tactile stimulation (MTS) during neonatal stress prevents hyperinsulinemia despite stress-induced adiposity in weanling rat pups. *Early Hum. Dev.* 87. doi:10.1016/j.earlhumdev.2010.12.001.

Muhammad, A., Hossain, S., Pellis, S. M., and Kolb, B. (2011). Tactile Stimulation During Development Attenuates Amphetamine Sensitization and Structurally Reorganizes Prefrontal Cortex and Striatum in a Sex-Dependent Manner. *Behav. Neurosci.* 125. doi:10.1037/a0022628.

Muhammad, A., and Kolb, B. (2011). Prenatal tactile stimulation attenuates drug-induced behavioral sensitization, modifies behavior, and alters brain architecture. *Brain Res.* 1400. doi:10.1016/j.brainres.2011.05.038.

Newson, P., Lynch-Frame, A., Roach, R., Bennett, S., Carr, V., and Chahl, L. A. (2005). Intrinsic sensory deprivation induced by neonatal capsaicin treatment induces changes in rat brain and behaviour of possible relevance to schizophrenia. *Br. J.*

Pharmacol. 146. doi:10.1038/sj.bjp.0706349.

Okabe, S., Yoshida, M., Takayanagi, Y., and Onaka, T. (2015). Activation of hypothalamic oxytocin neurons following tactile stimuli in rats. *Neurosci. Lett.* 600. doi:10.1016/j.neulet.2015.05.055.

Olausson, H., Cole, J., Rylander, K., McGlone, F., Lamarre, Y., Wallin, B. G., et al. (2008). Functional role of unmyelinated tactile afferents in human hairy skin: Sympathetic response and perceptual localization. *Exp. Brain Res.* 184. doi:10.1007/s00221-007-1175-x.

Pepino, V. C., and Mezzacappa, M. A. (2015). Application of tactile/kinesthetic stimulation in preterm infants: A systematic review. *J. Pediatr. (Rio. J).* 91. doi:10.1016/j.jped.2014.10.005.

Pham, T.M., Soderstrom, S., Henriksson, B.G., Mohammed, A.H. (1997). Effects of neonatal stimulation on later cognitive function and hippocampal nerve growth factor. *Behav. Brain Res.* 86, 113–120. doi: 10.1016/s0166-4328(96)02252-8.

Prusky, G. T., Silver, B. D., Tschetter, W. W., Alam, N. M., and Douglas, R. M. (2008). Experience-dependent plasticity from eye opening enables lasting, visual cortex-dependent enhancement of motion vision. *J. Neurosci.* 28. doi:10.1523/JNEUROSCI.1940-08.2008.

Raineki, C., Lucion, A. B., and Weinberg, J. (2014). Neonatal handling: An overview of the positive and negative effects. *Dev. Psychobiol.* 56. doi:10.1002/dev.21241.

Raza, S., Harker, A., Richards, S., Kolb, B., and Gibb, R. (2015). Tactile stimulation improves neuroanatomical pathology but not behavior in rats prenatally exposed to valproic acid. *Behav. Brain Res.* 282. doi:10.1016/j.bbr.2014.12.055.

Renard, G. M., Suárez, M. M., Levin, G. M., and Rivarola, M. A. (2005). Sex differences in rats: Effects of chronic stress on sympathetic system and anxiety. *Physiol. Behav.* 85. doi:10.1016/j.physbeh.2005.05.003.

Richards, S., Mychasiuk, R., Kolb, B., and Gibb, R. (2012). Tactile stimulation during development alters behaviour and neuroanatomical organization of normal rats. *Behav. Brain Res.* 231. doi:10.1016/j.bbr.2012.02.043.

Riul, T. R., Carvalho, A. F., Almeida, P. S., De-Oliveira, L. M., and Almeida, S. S. (1999). Ethological analysis of mother-pup interactions and other behavioral reactions in rats: Effects of malnutrition and tactile stimulation of the pups. *Brazilian J. Med. Biol. Res.* 32. doi:10.1590/S0100-879X1999000800007.

Rodrigues, A. L., Arteni, N. S., Abel, C., Zylbersztejn, D., Chazan, R., Viola, G., et al. (2004). Tactile stimulation and maternal separation prevent hippocampal damage in rats submitted to neonatal hypoxia-ischemia. *Brain Res.* 1002. doi:10.1016/j.brainres.2003.12.020.

Roversi, K., de David Antoniazzi, C. T., Milanesi, L. H., Rosa, H. Z., Kronbauer, M., Rossato, D. R., et al. (2019). Tactile Stimulation on Adulthood Modifies the HPA Axis, Neurotrophic Factors, and GFAP Signaling Reverting Depression-Like Behavior in Female Rats. *Mol. Neurobiol.* 56. doi:10.1007/s12035-019-1522-5.

Roversi, K., Buizza, C., Brivio, P., Calabrese, F., Verheij, M.M.M., et al. (2020). Neonatal Tactile Stimulation Alters Behaviors in Heterozygous Serotonin Transporter Male Rats: Role of the Amygdala. *Front. Behav. Neurosci.* 14, 142. doi:10.3389/fnbeh.2020.00142

Sale, A., Berardi, N., and Maffei, L. (2014). Environment and brain plasticity: Towards an endogenous pharmacotherapy. *Physiol. Rev.* 94. doi:10.1152/physrev.00036.2012.

Sampedro-Piquero, P., and Begega, A. (2016). Environmental Enrichment as a Positive Behavioral Intervention Across the Lifespan. *Curr. Neuropharmacol.* 15. doi:10.2174/1570159x14666160325115909.

Schanberg, S.M., Field, T.M. (1987). Sensory deprivation stress and supplemental stimulation in the rat pup and preterm human neonate. *Child Dev* 58,1431–1447. doi: 10.2307/1130683.

Shors, T. J., Anderson, M. L., Curlik, D. M., and Nokia, M. S. (2012). Use it or lose it: How neurogenesis keeps the brain fit for learning. *Behav. Brain Res.* 227. doi:10.1016/j.bbr.2011.04.023.

Silveira, P. P., Portella, A. K., Clemente, Z., Bassani, E., Tabajara, A. S., Gamaro, G. D., et al. (2004). Neonatal handling alters feeding behavior of adult rats. *Physiol. Behav.* 80. doi:10.1016/j.physbeh.2003.12.009.

Silveira, P. P., Portella, A. K., Clemente, Z., Gamaro, G. D., and Dalmaz, C. (2005). The effect of neonatal handling on adult feeding behavior is not an anxiety-like behavior. *Int. J. Dev. Neurosci.* 23. doi:10.1016/j.ijdevneu.2004.07.018.

Soares, R. O., Oliveira, L. M., Marchini, J. S., Rodrigues, J. A., Elias, L. L. K., and Almeida, S. S. (2013). Effects of early protein malnutrition and environmental stimulation on behavioral and biochemical parameters in rats submitted to the elevated plus-maze test. *Nutr. Neurosci.* 16. doi:10.1179/1476830512Y.0000000036.

Soares, R.O., de Oliveira, L.M., and Almeida, S.S. (2014). Effects of environmental stimulation during different periods of central nervous system development in malnourished rats subjected to the elevated plus maze. *Psychol. Neurosci.* 7. doi:10.3922/j.psns.2014.4.11.

Stara, V., Mach, M., Ujhazy, E., Liptak, B., Gasparova, Z. (2018). Beneficial effect of 6 weeks lasting handling of adult rats on spatial memory in experimental model of neurodegeneration. *Interdiscip. Toxicol.* 11(3), 217–220. doi: 10.2478/intox-2018-0020.

Stephan, M., Helfritz, F., Pabst, R., and Von Hörsten, S. (2002a). Postnatally induced

differences in adult pain sensitivity depend on genetics, gender and specific experiences: Reversal of maternal deprivation effects by additional postnatal tactile stimulation or chronic imipramine treatment. *Behav. Brain Res.* 133. doi:10.1016/S0166-4328(01)00468-5.

Stephan, M., Straub, R. H., Breivik, T., Pabst, R., and Von Hörsten, S. (2002b). Postnatal maternal deprivation aggravates experimental autoimmune encephalomyelitis in adult Lewis rats: Reversal by chronic imipramine treatment. *Int. J. Dev. Neurosci.* 20. doi:10.1016/S0736-5748(02)00007-2.

Sullivan, R. M., McGaugh, J. L., and Leon, M. (1991). Norepinephrine-induced plasticity and one-trial olfactory learning in neonatal rats. *Dev. Brain Res.* 60. doi:10.1016/0165-3806(91)90050-S.

Vazquez, D. M., Bailey, C., Dent, G. W., Okimoto, D. K., Steffek, A., López, J. F., et al. (2006). Brain corticotropin-releasing hormone (CRH) circuits in the developing rat: Effect of maternal deprivation. *Brain Res.* 1121. doi:10.1016/j.brainres.2006.08.104.

Vorhees, C. V., and Williams, M. T. (2014). Assessing spatial learning and memory in rodents. *ILAR J.* 55. doi:10.1093/ilar/ilu013.
Wong, R. (1966). Infantile handling and performance in the T-maze. *Psychon. Sci.* 5. doi:10.3758/BF03328353.

Weze, C., Leathard, H.L., Grange, J., Tiplady, P., Stevens, G. (2007) Healing by Gentle Touch Ameliorates Stress and Other Symptoms in People Suffering with Mental Health Disorders or Psychological Stress. *Evid. Based Complement. Altern*. *Med.* 4, 115–123. doi: 10.1093/ecam/nel052.

Wong, R., Bradley, H. W., and Jamieson, J. L. (1967). Infantile handling, activity deprivation, and subsequent wheel running behavior. *Psychon. Sci.* 7. doi:10.3758/BF03328567.

Zhang, M., and Cai, J. X. (2008). Neonatal tactile stimulation enhances spatial working memory, prefrontal long-term potentiation, and D1 receptor activation in adult rats. *Neurobiol. Learn. Mem.* 89. doi:10.1016/j.nlm.2007.10.010.

Zucchi, F. C. R., Yao, Y., Ilnytskyy, Y., Robbins, J. C., Soltanpour, N., Kovalchuk, I., et al. (2014). Lifetime stress cumulatively programs brain transcriptome and impedes stroke recovery: Benefit of sensory stimulation. *PLoS One* 9. doi:10.1371/journal.pone.0092130.

5 DISCUSSÃO

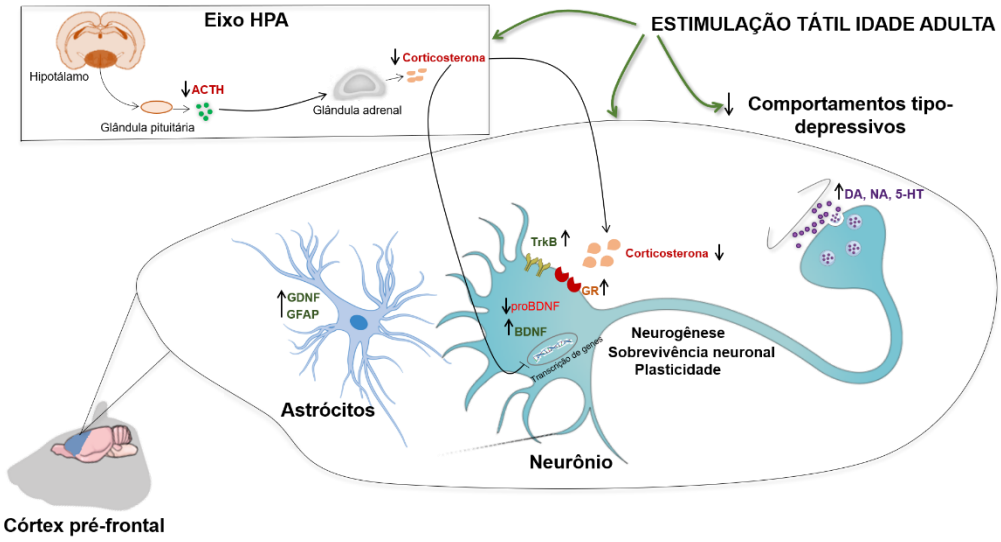
A exposição dos indivíduos a estímulos externos negativos está diretamente relacionada a uma maior vulnerabilidade para desenvolver doenças neuropsiquiátricas (NESTLER; HYMAN, 2010), enquanto os estímulos positivos podem ser associados a um efeito benéfico tanto na prevenção destas doenças neuropsiquiátricas bem como no tratamento (BOUFLEUR et al., 2012; SAMPEDRO-PIQUERO; BEGEGA, 2017). Os dados experimentais apresentados nesta tese confirmam os benefícios de que a exposição à ET, um estímulo externo positivo, tanto no período inicial da vida quanto na idade adulta, exerce influências benéficas na prevenção e reversão de comportamentos tipo depressivos e de ansiedade em ratos.

No presente estudo, avaliou-se inicialmente, a influência da ET sobre parâmetros de comportamento e de neuroadaptações moleculares no córtex pré-frontal, quando aplicada na idade adulta, após a indução de modelo de depressão por reserpina em ratas. Além de reverter os comportamentos tipo depressivos observados nos testes de nado forçado, de preferência pela sacarose e no teste de “splash”, a ET também reduziu os níveis plasmáticos dos hormônios corticosterona e adrenocorticotrófico, também aumentando a expressão do BDNF e do seu receptor Trkb, GFAP e GR. Essas respostas positivas observadas nos testes comportamentais podem ser explicadas pelas adaptações na funcionalidade do eixo HPA, consequentes à ET, observadas pela menor relação entre o peso das adrenais e o peso corporal dos animais, cuja alterações, estão associada com uma maior habilidade de lidar com situações estressantes que possam induzir sintomas de depressão ou ansiedade (BOUFLEUR et al., 2013; FREITAS et al., 2012). Além disto, a diminuição dos níveis plasmáticos de corticosterona e ACTH, e o aumento do GR no córtex pré-frontal estão de acordo com estudos da literatura, os quais apontam um aumento do GR para restabelecer o feedback negativo alterado em situações de hiperatividade do eixo HPA, inibindo então a liberação de glicocorticoides como a corticosterona (GUIDOTTI et al., 2013;) e favorecendo a minimização da resposta comportamental do tipo-depressiva.

A maturação e sobrevivência neuronal exerce grande impacto sobre o desenvolvimento de transtornos neuropsiquiátricos. incluindo a ansiedade e a depressão (DUMAN, 2009). Assim, o BDNF, representa um dos principais fatores

neurotróficos relacionados a depressão, visto que fármacos antidepressivos utilizados na clínica parecem modificar a sinalização do receptor de BDNF, o TrkB, e cujo nível é dependente da concentração de BDNF, o que seria um dos mecanismos responsáveis pelos efeitos terapêuticos dos antidepressivos (COYLE; DUMAN, 2003; SAARELAINEN et al., 2003). Neste sentido, os antidepressivos são reconhecidos por modular a expressão do GDNF, possivelmente pelo aumento dos níveis de serotonina observado (NAUMENKO et al., 2013). Em nosso estudo, a ET aplicada na idade adulta aumentou os níveis corticais de BDNF e do seu receptor TrkB, ao mesmo tempo que diminuiu a expressão dos níveis de GDNF e do receptor $p75^{NTR}$, o qual é associado a apoptose (YANG et al., 2014), sugerindo que essa modificação na sinalização BDNF-TrkB e GDNF pode ser responsável pela resposta antidepressiva observada na ET (Figura 6).

Figura 6- Esquema geral da influência da estimulação tátil aplicada na idade adulta em ratas.



Fonte: Autor.

5-HT: serotonina; BDNF: fator neurotrófico derivado do encéfalo DA: dopamina; HPA: hipotálamo-pituitária-adrenal; NA: noradrenalina, GDNF: fator neurotrófico derivado da glia; GFAP: proteína glial fibrilar ácida; GR: receptor glicocorticoide; TrkB: Receptor tropomiosina quinase B.

Adicionalmente, um restabelecimento dos níveis de GFAP foram observados no córtex pré-frontal dos animais expostos à ET, sugerindo que este manuseio pode afetar tanto a morfologia quanto a proliferação dos astrócitos, melhorando a plasticidade sináptica e em conjunto, melhorando comportamentos do tipo-depressivos. Até o limite do nosso conhecimento, este foi o primeiro estudo a demonstrar que a ET alterou comportamentos de depressão mesmo quando aplicada nos animais adultos, indicando que a plasticidade cerebral, mesmo após formada, pode responder positivamente frente às mudanças ambientais a ponto de modificar um comportamento afetivo.

Estudos anteriores desenvolvidos pelo nosso grupo de pesquisa, mostraram que a ET neonatal potencializou os efeitos do fármaco benzodiazepínico Diazepam, melhorando os comportamentos de ansiedade que estavam prejudicados após a exposição ao estresse (BOUFLEUR et al., 2013), também potencializando a resposta do antidepressivo sertralina, observados em comportamentos de depressão e ansiedade (FREITAS et al., 2016). A partir disto, decidiu-se investigar o papel da ET neonatal sobre comportamentos do tipo depressivo e de ansiedade e sobre alterações moleculares na região cerebral da amígdala basolateral em ratos machos heterozigotos para o transportador de serotonina ($SERT^{+/-}$). A escolha destes animais foi decorrente da sua maior vulnerabilidade para desenvolver sintomas de ansiedade e depressão (OLIVIER et al., 2008). Deste modo, através deste estudo observamos que em semelhança aos estudos do grupo desenvolvidos anteriormente, a ET neonatal foi capaz de prevenir comportamentos de anedonia e de ansiedade na vida adulta, melhorando o comportamento social dos animais nocaute ($SERT^{+/-}$). Em nível cerebral, observamos novamente uma resposta da ET sobre os níveis de BDNF e sobre marcadores da atividade do eixo HPA, como o receptor mineralocorticoide e genes responsivos aos glicocorticoides.

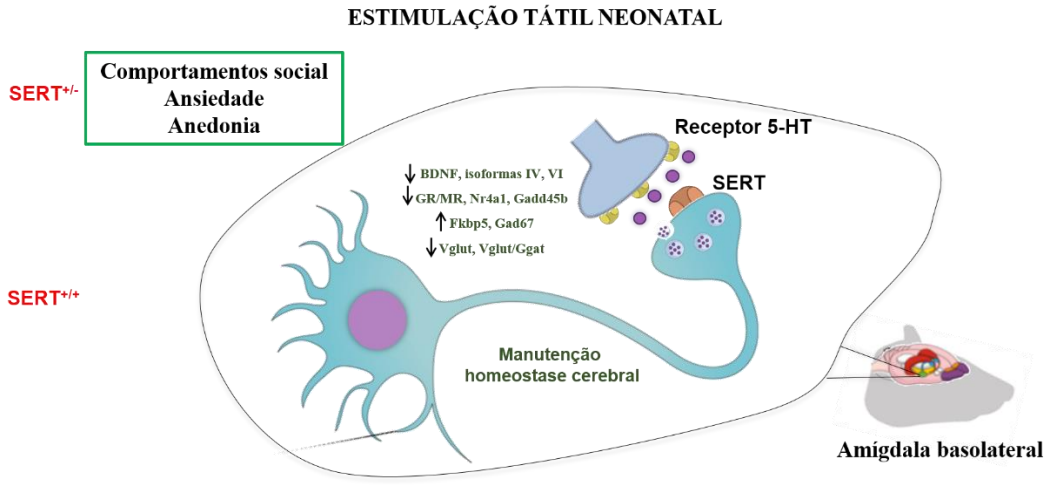
Sabe-se que o polimorfismo 5HTTLPR em humanos não só aumenta a sensibilidade a estímulos negativos, mas também a estímulos positivos (BELSKY et al., 2009). Nossos resultados experimentais confirmam esta hipótese, visto que a ET neonatal preveniu o desenvolvimento de comportamentos de ansiedade e anedonia nos animais. Também, estes animais $SERT^{+/-}$ apresentam características fenotípicas de prejuízo no comportamento social quando expostos a situações estressantes (BARTOLOMUCCI et al., 2010), e aqui, a ET neonatal melhorou comportamentos associados a sociabilidade. Interessantemente, na região cerebral da amígdala

basolateral, as respostas da ET neonatal só foram observadas nos animais selvagens, porém não nos animais SERT$^{+/-}$. Acreditamos que essa ausência de respostas cerebrais nos animais SERT$^{+/-}$ possa ser decorrente a uma diminuição da capacidade de autoajuste frente a alterações causadas por influências externas. Esta diminuição está relacionada ao aumento da elevação tônica na atividade neuronal a nível basal de animais SERT$^{+/-}$, portanto quando expostos a diferentes estímulos externos, um novo aumento da atividade neuronal somente pode ser vista nos animais controles, porém não nos animais SERT$^{+/-}$ (NIETZER et al., 2011; HOMBERG et al., 2016). Por outro lado, a ET mostrou diversas alterações moleculares *per se* na amígdala basolateral. Mesmo que estudos anteriores do nosso grupo tenham mostrado que a ET foi capaz de aumentar a expressão dos níveis de BDNF no hipocampo e no córtex pré-frontal (ANTONIAZZI et al., 2017), no presente estudo observamos uma diminuição tanto nos níveis de BDNF quanto de suas isoformas IV e VI. Esta diminuição pode estar relacionada a região cerebral investigada, visto que além de a amígdala basolateral ser reconhecida por suas funções ansiogênicas, níveis elevados de BDNF já foram associados com um aumento do estado negativo emocional em ratos SERT$^{+/-}$. Portanto, esta diminuição na amígdala basolateral causada pela ET poderia configurar uma resposta ansiolítica de proteção nestes animais.

Da mesma forma, os resultados encontrados dos marcadores do eixo HPA, foram diferentes encontrados em estudos anteriores do grupo, quando um aumento nos níveis de GR foi observado tanto no hipocampo quanto no córtex pré-frontal (ANTONIAZZI et al., 2017). Embora diferenças não significativas da expressão de GR e MR foram observadas na amígdala basolateral dos animais expostos à ET *per se*, uma diminuição na razão entre esses dois receptores foi observada, além de uma diminuição em genes responsivos aos glicocorticoides e um aumento na proteína ligante FK506. Apesar de diferentes resultados em relação aos estudos prévios, estes resultados corroboram com hipóteses anteriores sobre a ET, a qual desempenha um importante papel no controle da funcionalidade do eixo HPA. Também, pela primeira vez mostramos uma possível influência da ET no balanço entre os sistemas glutamatérgico e GABAérgico. Aqui observamos que a ET aumentou *per se* os níveis da enzima GAD67, a qual converte glutamato em GABA, também diminuindo a expressão do transportador vesicular de glutamato (VGLUT) e a razão entre o VGLUT e o transportador vesicular de GABA (VGAT). A partir desses

resultados podemos inferir que as respostas normais observadas tanto no comportamento social quanto nos comportamentos de ansiedade e anedonia dos animais expostos à ET, estariam mais uma vez relacionados a estas alterações glutamatérgicas e gabaérgicas, já que em outros estudos, fatores externos estressantes foram relacionados a um aumento na razão do VGLUT/VGAT (TZANOULINOU et al., 2014), bem como a diminuição da GAD67, observada em diferentes transtornos psiquiátricos (VAREA et al., 2012). De modo geral, podemos propor que a ET melhorou comportamentos associados a emoção, mas esta resposta não estaria relacionada às alterações na amígdala basolateral, levando-nos a considerar sobre a necessidade da continuidade dos estudos a fim de esclarecer o mecanismo molecular de ação benéfica da ET nesses animais $SERT^{+/-}$ (Figura 7).

Figura 7- Esquema geral da influência da estimulação tátil neonatal em ratos $SERT^{+/+}$ e $SERT^{+/-}$.



Fonte: Autor.

5-HT: serotonina; BDNF: Fator neurotrófico derivado do encéfalo; FKBP5: proteína ligante de FK506 de 51 kDa; GADD45β: Growth arrest and DNA damage inducible beta; GR: receptor glicocorticoide; MR: receptor mineralocorticoide; SERT: Transportador de Serotonina; VGAT: Transportador vesicular de GABA; VGLUT: Transportador vesicular de monoaminas.

Considerando a ampla variedade de estudos com a ET e a ausência de uma revisão que abordasse detalhadamente as metodologias utilizadas, as respostas

comportamentais, fisiológicas e neurobiológicas da ET, nós decidimos realizar uma revisão sistemática da literatura que mostrasse o impacto da ET neonatal e na idade adulta em ratos. A partir desta revisão sistemática, observamos que independente do tempo, da forma ou da idade da aplicação, a ET exerceu influências benéficas sobre diferentes tipos de comportamento aqui avaliados, como por exemplo, cognição, emoção, locomoção, drogadição, dor, entre outros. Também, uma significativa influência da ET foi observada na regulação do estresse, principalmente pela via do eixo HPA, desde que na maioria dos estudos ocorreu uma redução da hiperatividade do eixo HPA, melhorando a resposta a estímulos externos negativos (BOUFLEUR et al., 2012; FREITAS et al., 2016; ROVERSI et al., 2019) e via sistema nervoso simpático, no qual a ET diminuiu a liberação de catecolaminas em nível periférico, favorecendo a resposta ao estresse (COSTA et al., 2012; 2020). Já em nível central, a ET se mostrou importante sobre a morfologia e a plasticidade neuronal, cuja melhoria anatômica dos neurônios pode ser observada (GIBB et al., 2010; KOLB; GIBB, 2010; RICHARDS et al., 2012), juntamente com a maior expressão de fatores neurotróficos em diferentes áreas cerebrais (EFFENBERG et al., 2014; ANTONIAZZI et al., 2017; ROVERSI et al., 2019; 2020). Assim sendo, podemos afirmar que a ET pode ser uma interessante ferramenta que pode ser utilizada para modificar ou melhorar a função neuronal que regula diferentes respostas comportamentais.

Tomados em conjunto, os dados apresentados nesta tese sugerem que a exposição à ET no início da vida ou na vida adulta pode beneficiar respostas comportamentais, principalmente àquelas relacionadas à emocionalidade, mesmo que tais respostas benéficas da ET se mostrem presentes tanto agudamente quanto ao longo da vida. A ET também mostrou influências positivas em nível cerebral, atuando sobre fatores neurotróficos como BDNF e seu receptor TrkB, GDNF, e sobre fatores relacionados a funcionalidade do eixo HPA, também em ambos os períodos de sua aplicação. Assim, levando em consideração os resultados dos dois protocolos experimentais e dos estudos apresentados na revisão sistemática, é possível sugerir que a ET é capaz de atuar como um tratamento e também prevenir situações neuropsiquiátricas ao longo da vida.

6 CONCLUSÕES

Através dos resultados apresentados nesta tese, podemos propor que:

- A ET, quando aplicada na idade adulta, é capaz de reverter comportamentos tipo depressivos induzidos pela reserpina, indicando que essa terapia manual exerce influências benéficas mesmo quando o SNC já se encontra desenvolvido.
- A ET é capaz de reduzir o nível plasmático de glicocorticoides e aumentar a imunoreatividade dos receptores glicocorticoides (GR), em resposta à reduzida hiperatividade do eixo HPA. A ET também é capaz de aumentar a imunoreatividade de fatores associados à neurogênese e sobrevivência neuronal, como BDNF, GDNF, TrkB, GFAP, reduzindo os níveis de proBDNF, associado à morte neuronal, com respostas superiores ao fármaco antidepressivo imipramina,
- A ET neonatal é capaz de prevenir o desenvolvimento de comportamentos de ansiedade e anedonia, melhorando o comportamento social de ratos heterozigotos para o transportador de serotonina ($SERT^{+/-}$).
- A ET neonatal pode, *per se*, modificar marcadores moleculares na região da amígdala basolateral, tais como: BDNF e suas isoformas IV e VI , a razão entre os receptores GR e mineralocorticoides (MR), genes responsivos ao GR, além da expressão do transportador de glutamato e a enzima GAD67, a qual converte glutamato em GABA.
- Experimentalmente, a ET mostra importante influência em diferentes comportamentos, desde cognitivos, emocionais, entre outros, mostrando também ser capaz de exercer alterações periféricas e neuronais envolvidas em diferentes patologias.
- Por fim, podemos propor que a ET apresenta eficácia ao modificar a neurogênese e outros fatores que encontram-se prejudicados em situações de depressão; a ET pode ser considerada uma interessante ferramenta para aplicação terapêutica, não invasiva, de baixo custo e desprovida efeitos tóxicos, tão presentes nos atuais tratamentos utilizados na depressão e outras condições neuropsiquiátricas.

REFERÊNCIAS

AID, T., et al. Mouse and Rat BDNF Gene Structure and Expression Revisited. **J. Neurosci. Res.**, v. 3253, p. 3244–3253, 2007.

AKATSU, S., et al. Effects of prenatal stress and neonatal handling on anxiety, spatial learning and serotonergic system of male offspring mice. **Neurosc. Res.**, v. 101, p.15-23, Dez. 2015.

AL-HARBI, K.S. Treatment-resistant depression: therapeutic trends, challenges, and future directions. **Patient Prefer Adher.** v. 6, p. 369–388, Mai 2016.

ALBERT, P. Why is depression more prevalent in women? **J. Psychiatry Neurosci**. v. 40, n. 4, p. 219–221, Jul. 2015.

AMERICAN PSYCHIATRIC ASSOCIATION (APA). **What is depression?** Revisão, 2017. Disponível em: https://www.psychiatry.org/patients-families/depression/what-is-depression . Acesso em 05 de setembro de 2020.

ANTONIAZZI, C.T.D. et al. Influence of neonatal tactile stimulation on amphetamine preference in young rats: parameters of addiction and oxidative stress. **Pharmacol. Biochem. Behav.**, v. 124, p. 341–349, 2014a.

ANTONIAZZI, C.T.D. et al. Tactile stimulation and neonatal isolation affect behavior and oxidative status linked to cocaine administration in young rats. **Behav. Process.**, v.103, p. 297–305, 2014b.

ANTONIAZZI, C.T.D. et al. Tactile stimulation during different developmental periods modifies hippocampal BDNF and GR, affecting memory and behavior in adult rats. **Hippocampus**, v. 27, n. 2, p. 210-220, 2017.

ARORA, V. et al. Curcumin ameliorates reserpine-induced pain-depression dyad: behavioural, biochemical, neurochemical and molecular evidences. **Psychoneuroendocrinology**, v. 36, n. 10, p. 1570-1581, 2011.

BARANOVA, K.A., RYBNIKOVA, E.A. & SAMOILOV, M.O. The neurotrophin BDNF is involved in the development and prevention of stress-induced psychopathologies. **Neurochem. J.**, v. 9, p. 108-115, Abr. 2015.

BARTOLOMUCCI, A., et al. Increased vulnerability to psychosocial stress in heterozygous serotonin transporter knockout mice. **Dis. Model. Mech.**, v. 3, p. 459–470, 2010.

BELMAKER RH, AGAM G. Major depressive disorder. **N. Engl. J. Med.**, v. 358, p. 55-68, 2008.

BELSKY, J., et al. Vulnerability genes or plasticity genes? **Mol. Psychiatry**, v. 14, p. 746–754, 2009.

BINDER, E. B. The role of FKBP5, a co-chaperone of the glucocorticoid receptor in the pathogenesis and therapy of affective and anxiety disorders. **Psychoneuroendocrinology**, v. 34, n. 1, p. 186-195, 2009.

BIRCH, A.M.; MCGARRY, N.B.; KELLY, A.M. Short-term environmental enrichment, in the absence of exercise, improves memory, and increases NGF concentration, early neuronal survival, and synaptogenesis in the dentate gyrus in a time-dependent manner. **Hippocampus**, v. 23, p. 437-450, 2013.

BJÖRKHOLM, C.; MONTEGGIA, L.M. BDNF - a key transducer of antidepressant effects. **Neuropharmacology**, v. 102, p. 72-79, Mar 2016.

BLACKBURN-MUNRO, G.; BLACKBURN-MUNRO, R.E. Chronic pain, chronic stress and depression: coincidence or consequence? **J. Neuroendocrinol.**, v. 13, p. 1009-1023, 2001.

BOSSONG, F. In memory of Roland Kunn (1912- 2005) and 50 years of imipramine. **Nervenarzt**, v. 79, n. 9, p. 1080-1086, Set. 2008.

BOUFLEUR, N. et al. Neonatal tactile stimulation changes anxiety-like behavior and improves responsiveness of rats to diazepam. **Brain Res.**, v. 1474, p. 50–59, 2012.

BOUFLEUR, N. et al. Neonatal handling prevents anxiety-like symptoms in rats exposed to chronic mild stress: behavioral and oxidative parameters. **Stress**, v. 16, p. 321–330, 2013.

BOULLE, F. et al. Developmental fluoxetine exposure increases behavioral despair and alters epigenetic regulation of the hippocampal BDNF gene in adult female offspring. **Hormon. Behav.**, v. 80, p. 47–5, 2016.

BLUM, R.; KONNERTH, A. Neurotrophin-mediated rapid signaling in the central nervous system: mechanisms and functions. **Physiology**, v. 20, p. 70-77, 2005.

BRUNTON, L. et al. **Goodman e Gilman: As bases farmacológicas da terapêutica.** 11. ed. Porto Alegre (RS): Ed. Mc Graw Hill/Artmed, 2010. p. 238.

CALABRESE, E.J.; BALDWIN, L.A. U-shaped dose-responses in biology, toxicology, and public health. **Annu. Rev. Public Health**, v. 22, p. 15-33, 2001.

CAMERON, H.A., MCKAY, R.D. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. **J. Comp. Neurol.**, v. 435, p. 406-417, 2001.

CAMPBELL, L.C.; CLAUW, D.J.; KEEFE, F.J. Persistent pain and depression: a biopsychosocial perspective. **Biol. Psychiatry,** v. 54, p. 399-409, 2003.

CHAO, M.V. Neurotrophins and their receptors: a convergence point for many signalling pathways. **Nat. Rev. Neurosci.**, v. 4, p. 299-309, 2003.

CHIARUTTINI, C. et al. BDNF mRNA splice variants display activity-dependent targeting to distinct hippocampal laminae. **Mol. Cel. Neurosci.**, v. 37, p. 11-19, 2008

CHO, L., TSUNODA, M., SHARMA, R.P. Effects of endotoxin and tumor necrosis factor α on regional brain neurotransmitters in mice. **Nat. Toxin**, v. 7, n. 5, p. 187-195, Out. 1999.

COPPEN, A., et al. Letter: Total and free plasma-tryptophan concentration and oral contraception. **Lancet** v. 2, p. 1498, 1973.

COSTA, R., et al. Handling of Adolescent Rats Improves Learning and Memory and Decreases Anxiety. **J. Am. Assoc. Lab. Anim. Sci.**, v. 51, n. 5, p. 548-553, 2012.

COSTA, R., et al. Tactile stimulation of adult rats modulates hormonal responses, depression-like behaviors, and memory impairment induced by chronic mild stress: role of angiotensin II. **Behav. Brain Res.**, v. 379, p. 112250, 2020.

COWEN, P.J., PARRY-BILLINGS, M., NEWSHOLME, E.A. Decreased plasma tryptophan levels in major depression. **J. Affect. Disord.**, v, 16, p. 27–31, 1989.

COYLE, J.T., DUMAN, R.S. Finding the intracellular signaling pathways affected by mood disorders treatment. **Neuron**, v. 38, p. 157-160.

CRYAN, J.F., VALENTINO, R.J., LUCKI, I. Assessing substrates underlying the behavioral effects of antidepressants using the modified rat forced swimming test. **Neurosci. Biobehav. Rev.,** v. 29, p. 547–69, 2005.

CULLEN, L.A., et al. Positive touch, the implications for parents and their children with autism: an exploratory study. **Complement. Ther. Clin. Pract.**, v. 11, p. 182-189, 2005.

DANTZER, R., et al. From inflammation to sickness and depression: when the immune system subjugates the brain. **Nat. Rev. Neurosci.**, v. 9, p. 46–56, 2008.

DASKALAKIS N.P. et al. Environmental and tactile stimulation modulates the neonatal handling effect on adult rat spatial memory. **Int. J. Devl. Neurosc.**, v. 27, p. 747–755, 2009.

DUCRAY, A., et al. (2006). GDNF family ligands display distinct action profiles on cultured GABAergic and serotonergic neurons of rat ventral mesencephalon. **Brain Res.**, v. 1069, p. 104–112.

DUMAN, R.S., HENINGER, G.R., NESTLER, E.J., 1997. A molecular and cellular theory of depression. **Arch. Gen. Psychiatry,** v. 54, p. 597–606, 2006.

DUMAN, R.S. Neuronal damage and protection in the pathophysiology and treatment of psychiatric illness: stress and depression. **Dialogues Clin. Neurosci.**, v.11, n. 3, p. 239–255, 2009.

DUMAN, R.S., AGHAJANIAN, G.K. Synaptic Dysfunction in Depression: Potential Therapeutic Targets. **Science**, v. 338, p. 68–72, 2012.

EFFENBERG, A., et al. Adult hemiparkinsonian rats do not benefit from tactile stimulation. **Behav. Brain Res.**, v. 261, p. 97-105, 2014.

EGELAND, M., ZUNSZAIN, P., PARIANTE, C. Molecular mechanisms in the regulation of adult neurogenesis during stress. **Nat. Rev. Neurosci.** v.16, p.189-200 2015.

ESCALONA, A., et al. Brief report: improvements in the behavior of children with autism following massage therapy. **J. Autism Dev. Disord.**, v. 31, p. 513-516, 2001.

FATEMI, S.H. et al. GABAergic dysfunction in schizophrenia and mood disorders as reflected by decreased levels of glutamic acid decarboxylase 65 and 67 kDa and reelin proteins in cerebellum. **Schizophr. Res.**, v. 72, p. 109-122, 2005.

FERRARI, A.J. et al. Burden of depressive disorders by country, sex, age and year: findings from the global burden of disease study. **Plos Med.**, v. 10, n. 11, p. 1001547, Nov. 2013.

FOLTRAN, R.B., DIAZ, S.L. BDNF isoforms: a round trip ticket between neurogenesis and serotonin? **J. Neurochemistry**, v. 138, n.2, p. 204-221, 2016.

FREITAS D.L. et al. Neonatal tactile stimulation decreases depression-like andanxiety-like behaviors and potentiates sertraline action in young rats. **Int. J. Devl. Neuroscience**, v. 47, p. 192–197, 2015.

GARDNER, A., BOLES, R.G. Beyond the serotonin hypothesis: Mitochondria, inflammation and neurodegeneration in major depression and affective spectrum disorders. **Prog. Neuropsychopharmacol. Biol. Psychiatry**, v. 35, p. 730– 743, 2011.

GARTLEHNER, G. et al. Comparative benefits and harms of second-generation antidepressants for treating major depressive disorder: an updated meta-analysis. **Ann. Intern. Med.**, v. 6, p. 772-785, Dez. 2011.

GASS, P., HELLWEG, R. Peripheral brain-derived neurotrophic factor (BDNF) as a biomarker for affective disorders? **Int. J. Neuropsychopharmacol.**, v. 13, n. 1, p. 1-4, Fev. 2010.

GIBB, R.L., et al. Tactile stimulation promotes motor recovery following cortical injury in adult rats. **Behav. Brain Res.**, v. 214, p. 102-107, 2010.

GOES, T.C., ANTUNES, F.D., TEIXEIRA-SILVA, F. Environmental enrichment for adult rats: Effects on trait and state anxiety. **Neurosc. Lett.**, v. 584, p. 93-96, Jan. 2015.

GOLAN, D.E. **Princípios de farmacologia: a base fisiopatológica da farmacoterapia**. 2. ed. Rio de Janeiro: Guanabara Koogan, 2009, p. 194.

GOS, T. et al. 2013. S100B-immunopositive astrocytes and oligodendrocytes in the hippocampus are differentially afflicted in unipolar and bipolar depression: a postmorten study. **J. Psychiatr. Res.**, v. 47, p. 1694–1699, 2013.

GRAD. I., PICARD D. The glucocorticoid responses are shaped by molecular chap erones. **Mol. Cell. Endocrinol.**, v. 275, p. 2-12, 2007.

GRAEFF, F.G., et al. Role of 5-HT in stress, anxiety, and depression. **Pharmacol. Biochem. Behav.**, v. 54, p. 129–141, 1996.

GUIDOTTI, G., et al. Glucocorticoid receptor and FKBP5 expression is altered following exposure to chronic stress: modulation by antidepressant treatment. **Neuropsychopharmacol.**, v. 38, p. 616–627, 2013.

GUR, T.L. et al. cAMP response element-binding protein deficiency allows for increased neurogenesis and a rapid onset of antidepressant response. **J. Neurosci.**, v. 27, p. 7860-7868, 2007.

HASLER, G. Pathophysiology of depression: do we have any solid evidence of interest to clinicians? **World Psychiatry**, v. 9, p. 155-161, 2010.

HENDERSON, T.A. Practical application of the neuroregenerative properties of ketamine: real world treatment experience, **Neural Regen. Res.**, v. 11, p. 195-200, 2016.

HERTING, G., AXELROD, J., WHITBY, L.G. Effect of drugs on the uptake and metabolism of H3-norepinephrine. **J. Pharmacol. Exp. Ther.**, v. 134, p. 146–153, 1961.

HOMBERG, J. R. et al. Characterization of the serotonin transporter knockout rat: A selective change in the functioning of the serotonergic system. **Neuroscience**, v.146, n. 4, p. 1662-1676, 2007.

HOMBERG, J.R., LESCH, K.P. Looking on the bright side of serotonin transporter gene variation. **Biol. Psychiatry**, v. 69, n. 6, p. 513–519, 2011.

HOMBERG, J.R. et al. Sensory processing sensitivity and serotonin gene variance: insights into mechanisms shaping environmental sensitivity. **Neurosc. Biobehav. Rev.**, v. 71, p. 472-483, 2016.

HORIQUINI-BARBOSA, E., LACHAT, J.J. Tactile stimulation during development alters the neuroanatomical organization of the optic nerve in normal rats. **Exp Brain Res**, v. 234, n. 6, p. 1737-1746, Jun. 2016

HUANG, E.G., REICHARDT, L.F. Neurotrophins: roles in neuronal development and function. **Annu. Rev. Neurosci**., v. 24, p. 677-736, 2001.

HUANG, L. et al. Glial cell line-derived neurotrophic factor (GDNF) is required for differentiation of pontine noradrenergic neurons and patterning of central respiratory output. **Neuroscience**, v. 130, p. 95–105, 2005.

IRITANI, S. et al. Immunohistochemical study of the serotonergic neuronal system in an animal model of the mood disorder. **Exp. Neurol.**, v. 201, n. 1, p. 60–65, 2006.

IMANAKA, A. et al. Neonatal tactile stimulation reverses the effect of neonatal isolation on open-field and anxiety-like behavior, and pain sensitivity in male and female adult Sprague–Dawley rats. **Behav. Brain Res.**, v. 186, p. 91–97, 2008.

JANGRA A., et al. Sodium phenylbutyrate and edaravone abrogate chronic restraint stress-induced behavioral deficits: implication of oxido-nitrosative, endoplasmic reticulum stress cascade, and neuroinflammation. **Cell. Mol. Neurobiol.**, v. 37, n. 1, p. 65–81, 2017.

JANKE K.L., et al. Investigating the role of hippocampal BDNF in anxiety vulnerability using classical eyeblink conditioning. **Front. Psychiatry.**, v. 6, p. 106, 2015.

JANSSEN, C.I. et al. Impact of dietary n−3 polyunsaturated fatty acids on cognition, motor skills and hippocampal neurogenesis in developing C57BL/6J mice. **J. Nutr. Biochem.**, v. 26, n. 1, p. 24-35, Jan. 2015.

JUTAPAKDEEGUL, N. et al. Postnatal touch stimulation acutely alters corticosterone levels and receptor gene expression in the neonatal rat. **Dev. I. Neurosc.**, v. 25, p. 26-33, 2003.

KAREGE, F. et al. Neurotrophin levels in postmortem brains of suicide victims and the effects of antemortem diagnosis and psychotropic drugs. **Brain Res. Mol. Brain Res.**, v.136, p. 29–37, Mai. 2004.

KEERS, R., UHER, R. Gene-environment interaction in major depression and antidepressant treatment response. **Curr. Psychiatry Rep.**, v. 14, p. 129–137, 2012.

KINDERMAN, P., et al. Psychological Processes Mediate the Impact of Familial Risk, Social Circumstances and Life Events on Mental Health. **PLoS ONE**, v. 8, n. 10, p. e76564, 2013.

KOLB, B., GIBB, R. Tactile stimulation after frontal or parietal cortical injury in infant rats facilitates functional recovery and produces synaptic changes in adjacent cortex. **Behav. Brain Res.**, v. 214, p. 115–120, 2010.

KRISHNAN, V. et al. Linking Molecules to Mood: New Insight into the Biology of Depression. **Am. J. Psychiat.**, v. 167, n. 11, p. 1305–1320, Nov. 2010

KROEZE, Y. et al. Perinatal reduction of functional serotonin transporters results in developmental delay. **Neuropharmacology**, v. 109, p. 96-111, 2016.

KRUGEL, U. et al. Antidepressant effects of TNF-alpha blockade in an animal model of depression. **J. Psychiatr. Res.**, v. 47, p. 611-616, 2013.

LAPIN, I.P., OXENKRUG, G.F. Intensification of the central serotoninergic processes as a possible determinant of the thymoleptic effect. **Lancet**, v. 1, p. 132–136, 1969.

LARSEN, M.H. et al. Regulation of brainderived neurotrophic factor (BDNF) in the chronic unpredictable stress rat model and the effects of chronic antidepressant treatment. **J. Psychiatr. Res.**, v. 44, n. 13, p. 808–816, 2010.

LEITH, N.J., BARRETT, R.J. Effects of chronic amphetamine or reserpine on self-stimulation responding: animal model of depression? **Psychopharmacology**, v. 72, n. 1, p. 9-15, 1980.

LENER, M.S. et al. Glutamate and Gamma-Aminobutyric Acid Systems in the Pathophysiology of Major Depression and Antidepressant Response to Ketamine. **Biol Psychiatry.**, v. 81, n. 10, p. 886-897, 2017.

LESCH, K.P. Association of Anxiety-Related Traits. **Science**, v. 42, n.11, p. 2482–2488, 1996.

LESCH, K.P., WAIDER, J. Serotonin in the modulation of neural plasticity and networks: implications for neurodevelopmental disorders. **Neuron**, v. 76, p. 175-191, 2012.

LEVINE, S. Infantile experience and resistance to physiological stress. **Science,** v. 126, p. 405, 1957.

LICHT, C., MORTENSEN, E.L., KNUDSEN, G.M. Association between sensory processing sensitivity and the serotonin transporter polymorphism 5-HTTLPR short/short genotype. **Biol. Psychiatry**, v. 69, p. 152S–153S, 2011.

LIN, L.F., et al. GDNF: a glial cell line-derived neurotrophic factor for midbrain dopaminergic neurons. **Science**, v. 260, p. 1130–1132, 1993

LIU, D. et al. Maternal care, hippocampal glucocorticoid receptors, and hypothalamic-pituitary-adrenal responses to stress. **Science**, v. 277, p. 1659-1662, 1997.

LIU, B. et al. From Serotonin to Neuroplasticity: Evolvement of Theories for Major Depressive Disorder. **Front. Cell. Neurosci.**, v. 11, p. 305, Set. 2017.

LÓPEZ-MUÑOZ, F., ALAMO, C. Monoaminergic neurotransmission: the history of the discovery of antidepressants from 1950s until today. **Curr. Pharm. Des.**, v. 15, n. 14, p. 1563-1586, 2009.

LUKKES, J.L. et al. Consequences of post-weaning social isolation on anxiety behavior and related neural circuits in rodents. **Front. Behav. Neurosci.**, v. 3, p. 2009.

MATHAI, S., et al. Effects of tactile- kinesthetic stimulation in preterms: a controlled trial. **Indian Pediatr.**, v.38, p.1091-1098, 2001.

MCEWEN, B.S. Physiology and neurobiology of stress and adaptation: central role of the brain. **Physiol. Rev.**, v. 87, p. 873–904, 2007.

MCEWEN, B.S., NASCA, C., GRAY, J.D. Stress Effects on Neuronal Structure: Hippocampus, Amygdala, and Prefrontal Cortex. **Neuropsychopharmacology**, v. 41, n. 1, p. 3-23, 2016.

MEANEY, M.J. et al. The effects of neonatal handling on the development of the adrenocortical response to stress: implications for neuropathology and cognitive deficits in later life. **Psychoneuroendocrinology**, v.16, p. 85–103, 1991.

MEANEY, M.J. et al. Basal ACTH, corticosterone and corticosterone-binding globulin levels over the diurnal cycle, and age-related changes in hippocampal type I and type II corticosteroid receptor binding capacity in young and aged, handled and non-handled rats. **Neuroendocrinology**, v. 55, n. 2, p. 204-211, Fev. 1992.

MÉNARD, C., HODES, G.E., RUSSO, S.J. Pathogenesis of depression: Insights from human and rodent studies. **Neuroscience**, v. 321, p. 138–162, 2016.

MENDES, E.W., PROCIANOY, R.S. Massage therapy reduces hospital stay and occurrence of late-onset sepsis in very preterm neonates. **J. Perinatol.**, v. 28, p. 815-820, 2008.

MESSLINGER, K. What is a nociceptor? **Anaesthesist,** v. 46, n. 2, p. 142-53, 1997.

MEYER, J.H. et al. Elevated monoamine oxidase A levels in the brain: an explanation for the monoamine imbalance of major depression. **Arch. Gen. Psychiat.**, v. 63, p. 1209-1216, 2006.

MIDDELDORP, J., HOL, E.M. GFAP in health and disease. **Prog. Neurobiol.**, v. 93, n. 3, p. 421-443, Mar. 2011.

MIGUEL-HIDALGO, J.J. et al. Glial fibrillary acidic protein immunoreactivity in the prefrontal cortex distinguishes younger from older adults in major depressive disorder. **Biol. Psychiatry**, v. 48, p. 861–873, 2000.

MILLER, H.L. et al. Clinical and biochemical effects of catecholamine depletion on antidepressant-induced remission of depression. **Arch. Gen. Psychiat.**, v. 53, p. 117–128, 1996.

MINELLI, A. et al. Role of Allelic Variants of Fk506-Binding Protein 51 (Fkbp5) Gene in the Development of Anxiety Disorders. **Depression Anxiety**, v. 30, n. 12, p. 1170-1176, 2013.

MITCHELL, C. et al. Role of mother's genes and environment in postpartum depression. **Proc. Natl. Acad. Sci. USA.**, v. 108, n. 20, p. 8189–8193, 2011.

MOLDOFSKY, H. Rheumatic pain modulation syndrome: the interrelationships between sleep, central nervous system serotonin, and pain. **Adv. Neurol**., v.33, p. 51-57, 1982.

MOORE, S.R., DEPUE, R.A. Neurobehavioral foundation of environmental reactivity. **Psychol. Bull.**, v. 142, n. 2, p. 107–164, 2015.

MU, Y., GAGE, F.H. Adult hippocampal neurogenesis and its role in Alzheimer's disease. **Mol. Neurodegener.**, v. 6, p. 85, 2011.

MUMTAZ, F. et al. Neurobiology and consequences of social isolation stress in animal model- A comprehensive review. **Biomed. Pharmacother.**, v. 105, p.1205–1222, 2018.

NAGAKURA, Y. et al. Biogenic amine depletion causes chronic muscular pain and tactile allodynia accompanied by depression: a putative animal model of fibromyalgia. **Pain**, v. 146, p. 26–33, 2009.

NAUMENKO, V.S., et al. Effect of Glial Cell Line-Derived Neurotrophic Factor on Behavior and Key Members of the Brain Serotonin System in Mouse Strains Genetically Predisposed to Behavioral Disorders. **J. Neurosci. Res.**, v. 91, p. 1628-1638, 2013.

NEMEROFF, C.B. The corticotropin-releasing factor (CRF) hypothesis of depression: new findings and new directions**. Mol. Psychiatry**, v. 1, p. 336-342, 1996.

NEMEROFF, C.B., VALE, W.W. The neurobiology of depression: inroads to treatment and new drug discovery. **J. Clin. Psychiatry**, v. 66, n. 7, 5-13, 2005.

NESTLER, E.J., HYMAN, S.E. Animal models of neuropsychiatric disorders. **Nat. Neurosci.**, v.13, n. 10, p. 1161-1169, 2010.

NIBUYA, M., NESTLER, E.J., DUMAN, R.S. Chronic antidepressant administration increases the expression of cAMP response element binding protein (CREB) in rat hippocampus. **J. Neurosci.**, v. 16, n. 7, p. 2365-2372, 1996.

NICOLAIDES, N.C. et al. Stress, the stress system and the role of glucocorticoids', **NeuroImmunoModulation**, v. 22, p. 6-19, 2014.

NIETZER, S. L., et al. Serotonin transporter knockout and repeated social defeat stress: impact on neuronal morphology and plasticity in limbic brain areas. **Behav. Brain Res.**, v. 220, p. 42–54, 2011.

NONKES, L.J., de POOTER, M., HOMBERG, J.R. Behavioural therapy based on distraction alleviates impaired fear extinction in male serotonin transporter knockout rats. **J. Psychiatry Neurosci.**, v.37, p. 224-230, 2012.

NONKES, L.J., HOMBERG, J.R. Perseverative instrumental and Pavlovian responding to conditioned stimuli in serotonin transporter knockout rats. **Neurobiol. Learn. Mem.**, v. 100, p. 48-55, 2013.

OLIVIER, J.D.A. et al. A study in male and female 5-HT transporter knockout rats: an animal model for anxiety and depression disorders. **Neuroscience**, v. 152, p. 573-584, 2008.

PANAGIOTARAPOULOS, T. Effects of neonatal handling and sex on basal and chronic stress-induced corticosterone and leptin secretion. **Neuroendocronology**, v. 79, n. 2, p. 109-118, 2004.

PARIANTE, C.M., LIGHTMAN, S.L. The HPA axis in major depression: classical theories and new developments. **Trends Neurosci.**, v. 31, n. 9, p. 464–468, 2008.

PATTABIRAMAN, P.P. et al. Neuronal activity regulates the developmental expression and subcellular localization of cortical BDNF mRNA isoforms in vivo. **Mol. Cel. Neurosci.**, v. 28, p. 556-570, 2005

PHILLIPS, C. Brain-Derived Neurotrophic Factor, Depression, and Physical Activity: Making the Neuroplastic Connection. **Neural plast.**, p. 7260130, 2017.

PLUESS, M. et al. 5-HTTLPR moderates effects of current life events on neuroticism: differential susceptibility to environmental influences. **Prog. Neuropsychopharmacol. Biol. Psychiatry**, v. 34, n. 6, p. 1070–1074, 2010.

PORSOLT, R.D. et al. Immobility induced by forced swimming in rats - effects of agents which modify central catecholamine and serotonin activity. **Eur. J. Pharmacol.**, v. 57. P. 201–210, 1979.

QIAO, H., et al. Role of proBDNF and BDNF in dendritic spine plasticity and depressive-like behaviors induced by an animal model of depression. **Brain Res.**, v. 1663, p. 29–37, 2017

QUEVEDO, J., SILVA, A.G. **Depressão: Teoria e Clínica**. 1. Ed., Porto Alegre, Artmed ABP, 2013.

RAABE, F.J.; SPENGLER, D. Epigenetic risk factors in PTSD and depression. **Front. Psyachiatric**, v. 4, p. 80, 2013.

RAJKOWSKA, G., STOCKMEIER, C.A. Astrocyte pathology in major depressive disorder: insights from human postmortem brain tissue. **Curr. Drug Targets**, v. 14, p. 1225–1236, 2013.

RAZA, S. et al. Tactile stimulation improves neuroanatomical pathology but not behavior in rats prenatally exposed to valproic acid. **Behav. Brain Res.**, v. 282, p. 25-36, 2015.

RICHARDS, S. et al. Tactile stimulation during development alters behaviour and neuroanatomical organization of normal rats. **Behav. Brain Res**., v. 231, p. 86-91, 2012.

RÍO-ALAMOS, C. et al. Neonatal handling decreases unconditioned anxiety, conditioned fear, and improves two-way avoidance acquisition: a study with the inbred Roman high (RHA-I)- and low-avoidance (RLA-I) rats of both sexes. **Front. Behav. Neurosci.**, v. 9, p. 174, 2015.

ROVERSI, K., et al. Tactile Stimulation on Adulthood Modifies the HPA Axis, Neurotrophic Factors, and GFAP Signaling Reverting Depression-Like Behavior in Female Rats. **Mol. Neurobiol.**, v.56, p. 6239-6250, 2019.

ROVERSI, K., et al. Neonatal Tactile Stimulation Alters Behaviors in Heterozygous Serotonin Transporter Male Rats: Role of the Amygdala. **Front. Behav. Neurosci.**, v. 14, p.142, 2020.

RUHÉ, H.G., MASON, N.S., SCHENE, A.H. Mood is indirectly related to serotonin, norepinephrine and dopamine levels in humans: a meta-analysis of monoamine depletion studies. **Mol. Psychiatry**, v. 12, p. 331–359, 2007.

SAARELAINEN, T., et al. Activation of the Trkb neurotrophin receptor is induced by antidepressant drugs and is required for antidepressant-induced behavioral effects. **J. Neurosci.**, v. 23, p. 349-357.

SAKATA, K., et al. Critical role of promoter IV-driven BDNF transcription in GABAergic transmission and synaptic plasticity in the prefrontal cortex. **Proc. Natl. Acad. Sci. U.S.A.**, v. 106, p. 5942–5947, 2009.

SALOMONS, A.R. et al. Susceptibility of a potential animal model for pathological anxiety to chronic mild stress. **Behav. Brain Res.**, v. 209, p. 241–248, 2010.

SAMPEDRO-PIQUERO, P., BEGEGA, A. Environmental Enrichment as a Positive Behavioral Intervention Across the Lifespan. **Curr. Neuropharmacol.**, v. 15, n. 4, p. 459–470, 2017.

SCHANBERG, S., FIELD, T. Sensory deprivation stress and supplemental stimulation in the rat pup and preterm neonate. **Child. Devl.**, v. 58, p. 1431-1447, 1987.

SCHILDKRAUT, J.J. The catecholamine hypothesis of affective disorders: a review of supporting evidence. **Am. J. Psychiatry**, v.122, p. 509–522, 1965.

SCHAPIRO, S., VUKOVICH, K.R. Early experience effects upon cortical dendrites: a proposed model for development. **Science**, v. 167, p. 292–294, 1970.

SCHIPPER, P. et al. Acute inescapable stress alleviates fear extinction recall deficits caused by serotonin transporter abolishment. **Behav. Brain Res.**, v. 346, p. 16-20, Jul. 2017.

SCHULDINER, S., SHIRVAN, A., LINIAL, M. Vesicular neurotransmitter transporters: from bacteria to humans. **Physiol. Rev.**, v. 75, p. 369-92, 1995.

SIMONS, S.S.J. Glucocorticoid receptor cofactors as therapeutic targets. **Curr. Opin. Pharmacol.**, **v.**10, p. 613–619, 2010.

SKILLETER, A.J. et al. Peripheral BDNF: a candidate biomarker of healthy neural activity during learning is disrupted in schizophrenia. **Psychol. Med.**, v. 45, n. 4, p. 841-854, Mar. 2015.

SNYDER J.S. et al. Adult hippocampal neurogenesis buffers stress responses and depressive behaviour. **Nature**, v. 476, p. 458–461, 2011.

SOARES, R.O., OLIVEIRA, L.M., ALMEIDA, S.S. Effects of environmental stimulation during different periods of central nervous system development in malnourished rats subjected to the elevated plus maze. **Psychol. Neurosci.**, v. 7, n. 4, p. 521-529, 2004.

SOLKOFF, N., et al. Effects of handling on the subsequent development of premature infants. **Dev. Psychol.**, v. 1, p. 765-769, 1969.

STAHL, S.M. **Psicofarmacologia- base neurocientífica e aplicações práticas**. Rio de Janeiro, Ed. Guanabara Koogan, 2014.

STAMATAKIS, A. et al. Effect of neonatal handling on adult rat spatial learning and memory following acute stress. **Stress**, v. 11, p. 148-159, 2008.

STEPHAN, M. et al. Postnatally induced differences in adult pain sensitivity depend on genetics, gender and specific experiences: reversal of maternal deprivation effects by additional postnatal tactile stimulation or chronic imipramine treatment. **Behav. Brain Res.**, v.133, p. 149-158, 2002.

SUN, G.G. et al. Chinese herbal medicines promote hippocampal neuroproliferation, reduce stress hormone levels, inhibit apoptosis, and improve behavior in chronically stressed mice. **J. Ethnopharmacol.**, v.193, p. 159–168, 2016.

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

TAKAHASHI, T. **Neonatal Social Isolation: Effects on Neural Function**. International Encyclopedia of the Social and Behavioral Sciences. 2. Ed. Elsevier, 2015, Pages 489-492

TZANOULINOU, S., et al. Peripubertal stress-induced behavioral changes are associated with altered expression of genes involved in excitation and inhibition in the amygdala. **Transl. Psychiatry**, v. 4, n. e410, 2014.

VAREA, E., et al. Expression of PSA-NCAM and synaptic proteins in the amygdala of psychiatric disorder patients. **J. Psychiatr. Res.**, v. 46, p. 189–197, 2012.

VAZQUEZ, D.M. et al. Brain corticotropin-releasing hormone (CRH) circuits in the developing rat: Effect of maternal deprivation. **Brain Res.**, v. 1121, p. 83–94, 2006.

WATANABE, K., et al. Effect of antidepressants on brain-derived neurotrophic factor (BDNF) release from platelets in the rats. **Prog. Neuro-Psychopharmacol.**, v. 34, n. 8, p. 1450-1454, Dez. 2010.

WILLNER, P. Chronic mild stress (CMS) revisited: consistency and behavioural-neurobiological concordance in the effects of CMS. **Neuropsychobiology**, v. 52, n. 2, p. 90–110, 2005.

WILLNER, P., SCHEEL-KRÜGER, J., BELZUNG, C.The neurobiology of depression and antidepressant action. **Neurosci. Biobehav. Rev.**, v. 37, p. 2331–2371, 2013.

WITTCHEN, H.U. et al. The size and burden of mental disorders and other disorders of the brain in Europe 2010. **Eur. Neuropsychopharmacol.**, v. 21, p. 655–679, Set. 2011.

WHOOLEY, M. A., WONG, J. M. Depression and cardiovascular disorders. **Annu. Rev. Clin. Psychol.**, v. 9, p. 327–354, 2013.

WORLD HEALTH ORGANIZATION. **Depression and Other Common Mental Disorders: Global Health Estimates**. Geneva: World Health Organization, 2017. License: CC BY-NC-SA 3.0 IGO

WORLD HEALTH ORGANIZATION. **Depression**, 2020. Acesso em: https://www.who.int/health-topics/depression#tab=tab_1. Acesso em 05 setembro de 2020.

YANG, J., et al. ProBDNF negatively regulates neuronal remodeling, synaptic transmission, and synaptic plasticity in hippocampus. **Cell. Rep.**, v. 7, p. 796-806, 2014.

ZAMAN, V. et al. The noradrenergic system of aged GDNF heterozygous mice. **Cell Transplant.**, v. 12, p. 291–303, 2003.

ZARATE, C.A. et al. A randomized trial of an N-methyl-D-aspartate antagonist in treatment-resistant major depression. **Arch. Gen. Psychiatry**, v. 63, p. 856–864, 2006.

ZHANG, Y., PARDRIDGE, W.M. Blood-brain barrier targeting of BDNF improves motor function in rats with middle cerebral artery occlusion. **Brain Res.**, v. 1111, p. 227-229, 2006.

ZHOU, Z. et al. Decreased serum brain-derived neurotrophic factor (BDNF) is associated with post-stroke depression but not with BDNF gene Val66Met polymorphism. **Clin. Chem. Lab. Med.**, v. 49, n. 2, p. 185-189, Fev. 2011.