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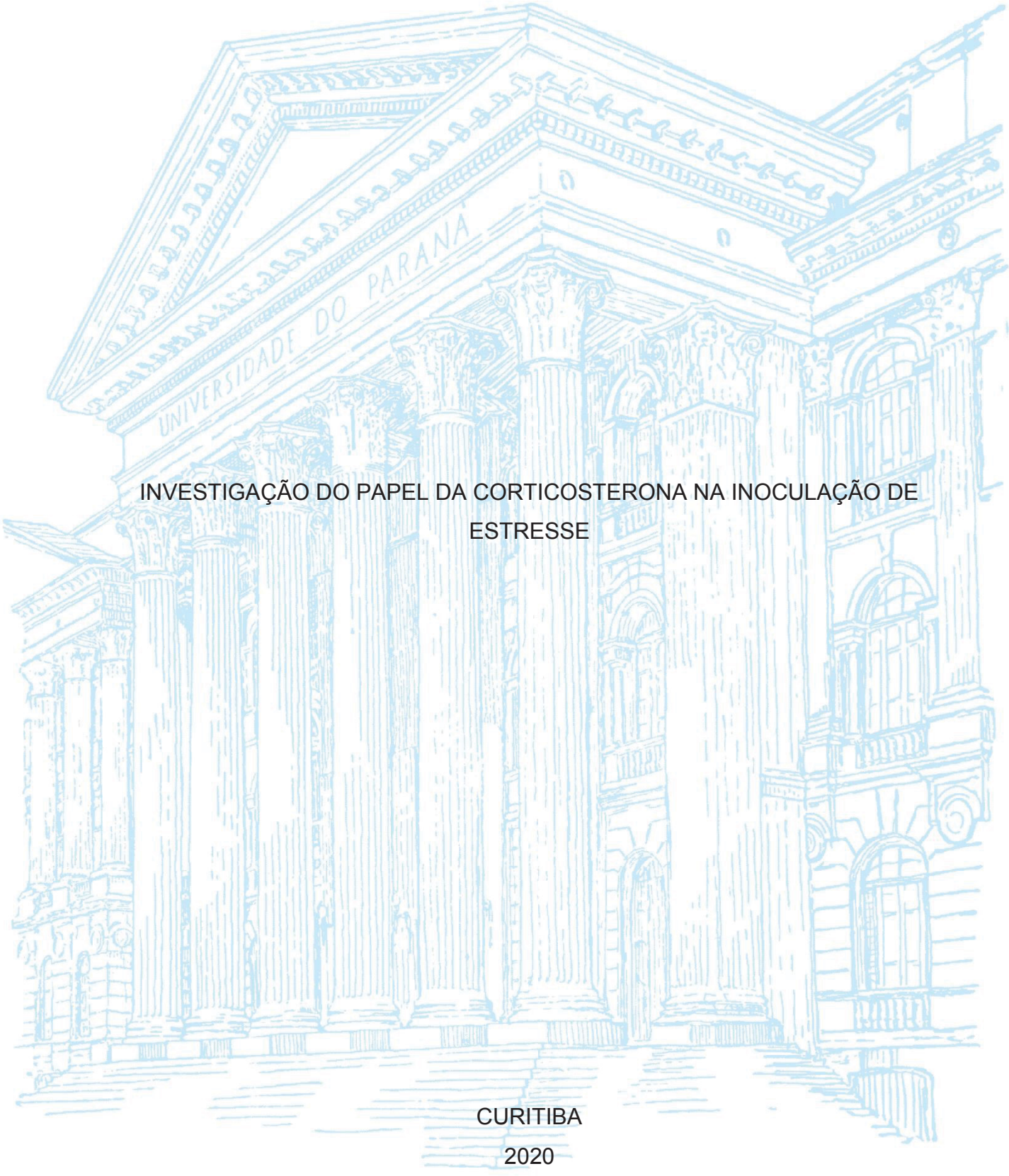

INVESTIGAÇÃO DO PAPEL DA CORTICOSTERONA NA INOCULAÇÃO DE ESTRESSE

CURITIBA

2020

BETINA DITTMAR BLUM

INVESTIGAÇÃO DO PAPEL DA CORTICOSTERONA NA INOCULAÇÃO DE ESTRESSE

Dissertação apresentada como requisito parcial à obtenção do grau de Mestre em Fisiologia, no Programa de Pós- Graduação em Fisiologia, Setor de Ciências Biológicas, Universidade Federal do Paraná.

Orientador: Prof.º Bruno Jacson Martynhak

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Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em FISIOLOGIA da Universidade Federal do Paraná foram convocados para realizar a arguição da dissertação de Mestrado de **BETINA DITTMAR BLUM** intitulada: **INVESTIGAÇÃO DO PAPEL DA CORTICOSTERONA NA INOCULAÇÃO DE ESTRESSE**, sob orientação do Prof. Dr. BRUNO JACSON MARTYNHAK, que após terem inquirido a aluna e realizada a avaliação do trabalho, são de parecer pela sua APROVAÇÃO no rito de defesa.

A outorga do título de mestre está sujeita à homologação pelo colegiado, ao atendimento de todas as indicações e correções solicitadas pela banca e ao pleno atendimento das demandas regimentais do Programa de Pós-Graduação.

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“Sem saber por que, viu nascer dentro de si uma pontinha de esperança”.

(CS Lewis)

RESUMO

O estresse é um fator de risco para o desenvolvimento de diversas psicopatologias. No entanto, há evidências demonstrando que a relação entre estresse e transtornos psiquiátricos pode ocorrer de forma não linear, de forma que tanto o excesso como a insuficiência de situações adversas aumentam o risco de doença, mas quantidades moderadas o diminuem. A exposição a estressores brandos que promovem resiliência é chamada de inoculação de estresse. Até o momento, não se sabe qual é o mecanismo pelo qual a inoculação confere resiliência, mas há um possível envolvimento dos glicocorticoides. Assim, o objetivo deste trabalho foi investigar o papel da corticosterona no efeito pró-resiliência da inoculação de estresse em camundongos. Foram testados três protocolos de inoculação de estresse. Cada um composto de onze sessões de 15 minutos realizadas a cada dois dias. O primeiro protocolo consistiu num estresse social brando em camundongos Swiss jovens. O segundo foi similar ao primeiro, mas realizado em camundongos C57BL/6 adultos. O terceiro consistiu num protocolo de restrição de movimento em C57BL/6. Após as onze sessões de inoculação, os camundongos foram avaliados no campo aberto, no labirinto em cruz levado, no nado forçado, no desamparo aprendido e tiveram a secreção de corticosterona após o nado forçado quantificada. Corticosterona sérica após a primeira e última sessões de inoculação também foi quantificada. Apesar de aumento significativo de corticosterona induzido pela inoculação, nenhum dos protocolos levou ao efeito esperado de resiliência. Não houve redução de comportamento tipo-ansioso no labirinto em cruz, aumento da estratégia ativa de manejo no nado forçado, redução da secreção de corticosterona após um estressor heterotípico, ou redução nas falhas de escape no desamparo aprendido. Apesar disso, foi observado um aumento de locomoção no campo aberto em camundongos C57BL/6 submetidos à inoculação. Para avaliar se essa hiperlocomoção é dependente de corticosterona, metirapona foi administrada antes de cada uma das sessões. No entanto, não foi observada aumento de locomoção em nenhum dos grupos, apesar da elevação de corticosterona no grupo inoculação-veículo. Os resultados sugerem que a corticosterona não é suficiente para levar à hiperlocomoção nem para gerar o efeito de resiliência da inoculação.

Palavras-chave: Transtornos psiquiátricos; Resiliência; Glicocorticoides.

ABSTRACT

Stress is a risk factor for the development of several diseases, including many psychopathologies. However, there is evidence demonstrating that the relation between stress and psychiatric disorders may occur non-linearly, such that both the excess and insufficiency of adverse events increase the risk for disease, but moderate amounts diminish it. Exposure to mild stressors promoting resilience is known as stress inoculation. Up to date, the mechanism through which inoculation confers resilience is unknown, but there is evidence suggesting a possible role for glucocorticoids. Therefore, the objective of this work was to investigate the role of corticosterone on the resilience effect induced by stress inoculation in mice. Three stress inoculation protocols were tested. Each of them comprised eleven 15-minute sessions applied every two days. The first protocol consisted of a mild social stress in juvenile Swiss mice. The second was similar to the first, but performed in adult C57BL/6. The third consisted of a movement restraint protocol in C57BL/6. Following the eleven inoculation sessions, mice were evaluated in the open field, elevated plus maze, forced swim test, learned helplessness and had serum corticosterone dosed following swimming. Corticosterone was also quantified after the first and last inoculation sessions. Despite a significant rise in circulating corticosterone induced by stress inoculation, none of the protocols led to the expected resilience effect. There was not reduction in anxiety-like behavior in the elevated plus maze, shift to an active coping strategy in the forced swim test, reduction of corticosterone secretion following a heterotypic stressor, nor reduction in escape failures in the learned helplessness paradigm. An increase in open field locomotion was observed in C57BL/6 mice subjected to stress inoculation. In order to test if this hyperlocomotion is dependent on corticosterone, metyrapone was administered before each inoculation session. However, locomotion was not increased in any of the groups, despite increased corticosterone in the group inoculation-vehicle group. Results suggest that corticosterone is not sufficient for the hyperlocomotion effect, nor to generate resilience.

Key-words: Psychiatric disorders; Resilience; Glucocorticoids.

SUMÁRIO

1 INTRODUÇÃO

1.1 ESTRESSE

O estresse pode ser definido como um estado de ameaça, real ou percebida, à homeostase e bem-estar do organismo (GOLD, 2015; HERMAN, 2013; SMITH & VALE, 2006; ULRICH-LAI & HERMAN, 2009). Mais recente na literatura é o conceito de alostase e carga alostática. Alostase significa “alcançar estabilidade através da mudança” e se refere ao processo ativo em que o organismo se engaja em restabelecer o equilíbrio homeostático, ao passo que carga e sobrecarga alostática remetem aos efeitos mal adaptativos da resposta muito intensa ou prolongada ao estresse (MCEWEN, 2007). Dentro desse conceito, estressores são os estímulos que ameaçam a homeostase e a resposta ao estresse é a reação desencadeada pelo organismo para restabelecê-la (CHROUSOS, 2009). Os estressores podem ser classificados em duas categorias: estressores físicos, quando produzem perturbações físicas à homeostase, como hemorragia, infecção e alterações significativas de temperatura; ou emocionais, quando geram a percepção de um possível dano real, tais como conflito social, pistas de predador e ambientes novos (DAYAS et al., 2001; ÚBEDA-CONTRERAS, 2018). A resposta ao estresse envolve uma série de mudanças emocionais, cognitivas, comportamentais e fisiológicas que promovem a sobrevivência do organismo (DAYAS et al., 2001; GOLD, 2005; HERMAN, 2013). Ela é coordenada e regulada pelo encéfalo, pois ele é o principal centro de processamento de informações e é responsável por determinar quais estímulos são ameaças reais ou potenciais (MCEWEN & GIANAROS, 2010).

Numa situação de estresse, as informações do ambiente e/ou do interior do organismo chegam por vias sensoriais a diversas estruturas do Sistema Nervoso Central (SNC), como o tálamo, amígdala, hipocampo e regiões do córtex (SAWCHENKO et al., 2000; TAFET & NEMEROFF, 2016; ULRICH-LAI & HERMAN, 2009). Informações sobre estressores físicos ativam primariamente núcleos no tronco encefálico, enquanto estressores emocionais são processados inicialmente pelo córtex pré-frontal e por regiões límbicas superiores (BUIJS & VAN EDEN, 2000; HERMAN et al., 2003; ULRICH-LAI & HERMAN, 2009). Independentemente da natureza do estímulo estressor, a amígdala é ativada e promove um estado aumentado de ansiedade e medo (GOLD, 2015). A ansiedade têm um papel

importante para a sobrevivência do organismo, aumentando a avaliação de risco e evitando que o organismo se exponha a ameaças. Durante o estresse, também ocorrem alterações cognitivas que aumentam o foco no perigo e favorecem a execução de comportamentos simples e automáticos adquiridos durante experiências aversivas prévias em detrimento de ações mais complexas e planejadas (GOLD, 2005). Paralelamente às reações emocionais e cognitivas, os centros encefálicos envolvidos no processamento de estímulos estressores projetam para o hipotálamo, estimulando os dois principais sistemas fisiológicos da resposta ao estresse, a saber, o eixo hipotálamo-pituitária-adrenal (HPA) e o sistema simpático adrenomedular (SAM) (BUIJS & VAN EDEN, 2000).

O sistema simpático adrenomedular é responsável por aumentar os níveis circulantes de adrenalina e noradrenalina. Assim que um estímulo estressor é identificado pelos circuitos neurais e ocorre ativação do sistema simpático, os terminais nervosos liberam noradrenalina na periferia e estimulam a medula da glândula adrenal a secretar catecolaminas, principalmente a adrenalina (ULRICH-LAI & HERMAN, 2009). O aumento da atividade simpática aumenta a frequência e a força dos batimentos cardíacos, além de aumentar a pressão arterial, vasoconstrição periférica, mobilização energética e dilatação brônquica, caracterizando a clássica resposta de luta ou fuga. (GOLD, 2015; ULRICH-LAI & HERMAN, 2009). Também ocorre aumento da sinalização noradrenérgica no SNC pela ativação do *locus ceruleus*, que projeta para diversas áreas do encéfalo e tem papel na ativação da amígdala e inibição do córtex pré-frontal medial. (GOLD, 2015). A sinalização noradrenérgica central funciona como um alarme de emergência e contribui para a ativação do sistema autônomo simpático e para a inibição de funções de repouso como sono, alimentação e atividade sexual, as quais seriam bastante mal adaptativas se realizadas durante uma situação emergencial (GOLD, 2005).

Simultaneamente à atividade autônoma, a estimulação do eixo HPA gera a liberação de fator liberador de corticotrofina (CRF) pelos neurônios parvocelulares do núcleo paraventricular (PVN) do hipotálamo na circulação porta, estimulando a secreção de hormônio adrenocorticotrófico (ACTH) pela pituitária anterior, que por sua vez age sobre o córtex da adrenal estimulando a síntese e liberação de glicocorticoides (GCs) (MARTÍ & ARMARIO, 1998; MIFSUD & REUL, 2018). Os glicocorticoides são sintetizados a partir do colesterol seguindo uma série de etapas enzimáticas cujo último passo envolve a conversão de 11-desoxicortisol em cortisol

pela enzima 11-beta-hidroxilase (NOTI et al., 2009). Em roedores, a mesma enzima converte desoxicorticosterona em corticosterona, o principal glicocorticoide sintetizado por essa ordem de mamíferos. Os GCs realizam feedback negativo sobre o eixo HPA, ligando-se a receptores glicocorticoides localizados no núcleo paraventricular do hipotálamo e na pituitária, reduzindo a secreção de CRH e ACTH, respectivamente. Além disso, os GCs agem sobre o hipocampo, ativando projeções inibitórias para o hipotálamo para reduzir a secreção de CRH (TAFET & NEMEROFF, 2016). Regiões do córtex pré-frontal também inibem o eixo HPA, mas de forma indireta através da ativação de neurônios predominantemente GABAérgicos no núcleo do leito da estria terminal, hipotálamo lateral e outras regiões que projetam para o núcleo paraventricular do hipotálamo (HERMAN et al., 2003). O feedback negativo do eixo HPA tem papel crucial para o controle da resposta ao estresse (KELLER-WOOD, 2015). Os glicocorticoides liberados na circulação sanguínea têm ação sistêmica, atuando sobre diversos órgãos e tecidos como o fígado, músculos e o cérebro. As ações dos GCs são principalmente catabólicas e incluem estimulação de glicogenólise no fígado, proteólise e lipólise, supressão da imunidade, inibição do crescimento ósseo e muscular, potenciação da vaso constrição mediada pelo sistema nervoso autônomo simpático, supressão da função reprodutiva, entre outras (HERMAN et al., 2003; SAPOLSKY et al., 1986). Nas funções mediadas pelo cérebro, as ações dos GCs incluem aumento de ansiedade e medo, aumento de atenção e vigilância, prejuízo da formação de memórias hipocampais em favor do aprendizado de hábito estriatal e potenciação da aquisição e consolidação de memórias emocionais, que facilitam a associação de eventos estressantes ao seu contexto específico para uso em situações semelhantes no futuro (JOËLS, 2018; LEMAIRE et al., 2005; MCEWEN & SAPOLSKY, 1995; SHIRAZI et al., 2015). As mudanças comportamentais induzidas pelos glicocorticoides são importantes para a adaptação e sobrevivência do organismo aos estressores ambientais (SHIRAZI et al., 2015). Ainda é importante mencionar que os GCs também são secretados e exercem uma gama de funções em contextos que não são necessariamente aversivos, como territorialidade, afetividade, ingestão alimentar, predação, comportamentos sociais e exercício físico, representando, de forma geral, uma “prontidão para a ação” (ERICKSON et al., 2003; LEMAIRE et al., 2005).

A maioria dos efeitos do hormônio é mediada pelo receptor glicocorticoide (GR), membro da superfamília de receptores nucleares (ZHOU & CIDLOWSKI, 2005). Os glicocorticoides atravessam livremente as membranas plasmáticas devido à sua lipofilicidade e, uma vez ligados ao GR difundido no citoplasma, promovem a translocação do complexo hormônio-receptor para o núcleo, que atua como um fator de transcrição de diversas proteínas (VITELLIUS et al., 2018; ZHOU & CIDLOWSKI, 2005). Os glicocorticoides também se ligam ao receptor mineralocorticoide (MR), que, assim como o GR, é um receptor nuclear e atua como um fator de transcrição dependente de ligante. Nos rins e outros tecidos, os MRs se ligam primariamente à aldosterona, devido à inativação dos glicocorticoides pela enzima 11-beta-hidroxiesteroide desidrogenase, enquanto no cérebro os MRs ligam-se principalmente aos glicocorticoides (HERMAN et al., 2016). Os MRs têm afinidade dez vezes maior pelos glicocorticoides do que os GRs e, por esse motivo, os MRs encontram-se ocupados mesmo em níveis relativamente baixos de glicocorticoides, observados durante a variação circadiana da secreção do hormônio (MCEWEN, 1988). Os receptores MRs são encontrados principalmente no hipocampo e septo lateral, além do núcleo olfatório, córtex e tronco encefálico (GRAY et al., 2017; PACKARD et al., 2016). Por sua vez, os GRs são ativados principalmente durante uma resposta ao estresse, quando os níveis de glicocorticoides estão altos, sendo expressos de forma ubíqua no cérebro, mas em maior concentração em regiões responsáveis pelo controle do eixo HPA como o hipocampo, hipotálamo e a pituitária (MCEWEN, 1988; PACKARD et al., 2016). Tanto receptores GR como MR medeiam o feedback negativo dos glicocorticoides no eixo HPA, sendo que os MRs regulam o eixo HPA durante as variações circadianas e os GRs, por serem ativados apenas em altas concentrações de glicocorticoides, realizam o feedback durante um episódio de estresse (LEMAIRE et al., 2005; SHIRAZI et al., 2015). Além das funções genômicas, GCs exercem funções não genômicas através de interações inespecíficas com a membrana celular ou interações específicas com receptores citosólicos ou ligados à membrana (PANETTIERI et al., 2019).

As mudanças causadas pela resposta ao estresse são positivas na medida em que favorecem a adaptação do indivíduo ao ambiente e auxiliam no processo de restaurar ou manter a homeostase durante situações de ameaça. No entanto, suas ações infligem um custo para o organismo quando a resposta se prolonga no tempo ou é ativada em excesso (KORTE et al., 2005; MCEWEN & STELLAR, 1993). Há

diversas evidências de que a exposição a estresse está relacionado com maior risco de asma, diabetes, transtornos gastrointestinais, infarto do miocárdio, câncer, infecções virais e autoimunidade, além de transtornos psiquiátricos (MCEWEN & STELLAR, 1993). Os prejuízos da secreção prolongada ou excessiva de glicocorticoides também são evidenciados pela grande prevalência de sintomas psiquiátricos entre pacientes com síndrome de Cushing (LOOSEN, 1994), pela observação de que pacientes em episódio depressivo apresentam maior secreção de cortisol em resposta a um estressor psicossocial (MORRIS & RAO, 2014) e pela correlação entre a redução da resposta glicocorticoide e medidas reduzidas de comportamento tipo-ansioso e tipo-depressivo em estudos animais (BROCKHURST et al., 2015; LEE et al., 2014; LYONS et al., 1999). Korte et al. (2005) utiliza uma metáfora para exemplificar esta discrepância entre efeitos agudos e crônicos da resposta ao estresse, comparando-a com a água utilizada por bombeiros para apagar um incêndio. A água tem um papel crucial no controle do fogo, mas quando usada em excesso pode causar mais dano do que o fogo em si. Semelhantemente, a resposta ao estrese é benéfica e essencial enquanto limitada.

1.2 ESTRESSE E PSICOPATOLOGIAS

Como citado acima, o estresse está envolvido na etiologia de diversas doenças psiquiátricas. Está intimamente relacionado com o diagnóstico dos transtornos relacionados a trauma e a estressores, como o transtorno do estresse pós-traumático (AMERICAN PSYCHATRIC ASSOCIATION, 2013), e é um dos principais fatores de risco para o desenvolvimento de transtornos psicóticos (BEARDS et al., 2013; ZUBIN & SPRING,1977), transtornos de ansiedade (KLAUKE et al., 2010) e transtornos de humor, como o transtorno bipolar (ETAIN et al., 2008) e a depressão maior (NESTLER et al., 2002). Segundo a Organização Mundial da Saúde (2017) a prevalência de transtornos depressivos era de aproximadamente 4,4% da população mundial em 2015 e de transtornos de ansiedade, aproximadamente 3,6%. A depressão é a maior causa de incapacidade no mundo, sendo responsável por 7,5% dos anos vividos com incapacidade (WHO, 2017), e é um dos principais fatores de risco para o suicídio (KESSLER et al., 1999).

Uma das principais teorias que incorporam o papel do estresse na origem de transtornos psiquiátricos é a diátese-estresse, a qual afirma que o risco para o desenvolvimento de psicopatologias envolve uma interação entre uma pré-

disposição inerente ao indivíduo e os desafios aos quais ele é exposto. (BEBBINTON, 1987; COLODRO-CONDE et al., 2018; MONROE & SIMONS, 1991; ROBINS & BLOCK, 1989; ZUBIN & SPRING, 1977). Existem diversos modelos teóricos que utilizam a base da diátese-estresse em suas conceituações. Dentre eles, alguns consideram a diátese como sendo definida principalmente por fatores biológicos (BEBBINGTON, 1987; COLODRO-CONDE et al., 2018) enquanto outros também consideram o papel de propensões adquiridas (MCEWEN & GIANAROS, 2010; ZUBIN & SPRING, 1977). Comumente incorporada às teorias de diátese-estresse está a noção de que estresse e o risco para psicopatologia seguem um padrão linear, sendo que quanto maior a exposição a estressores, maior o risco de doença (COLODRO-CONDE et al.; 2018; MONROE & SIMONS, 1991; ZUBIN & SPRING, 1977). Monroe e Simons (1991) ressaltam que, apesar dos estressores levarem a respostas fisiológicas similares, eles podem ser muito distintos em outros aspectos como duração (estresse agudo vs. crônico), natureza (eventos desejáveis vs. indesejáveis) e intensidade (alta vs. baixa) e destacam que algumas formas de estresse possivelmente têm um papel mais importante para desencadear um episódio depressivo do que outros. Ainda assim, prevalece a ideia de que à medida que aumentam os episódios de estresse, aumenta-se a vulnerabilidade.

No entanto, evidências recentes têm demonstrado que a relação entre estresse e psicopatologia pode ocorrer de forma não linear ao invés de diretamente proporcional (LIU, 2015), de forma que tanto o excesso como a insuficiência de situações adversas aumentam o risco de doença, mas quantidades moderadas o diminuem. Dessa forma, a exposição a quantidades moderadas de estresse pode contribuir para a redução da vulnerabilidade e consequente aumento da resiliência. A resiliência, no contexto de transtornos psiquiátricos, é a habilidade de manter níveis normais de funcionamento físico e psicológico quando o indivíduo é exposto a grandes níveis de estresse e trauma (RUSSO et al., 2012). Russo et al. (2012) também destacam que a resiliência é um processo principalmente ativo de enfrentamento a situações e não apenas a ausência de respostas patológicas.

1.3 INOCULAÇÃO DE ESTRESSE

A exposição a estressores moderados, controláveis e previsíveis que promovem resiliência é chamada de inoculação de estresse, também conhecida

pelos termos *steeling, toughening* ou efeito anti-fragilidade (LIU, 2015). O termo inoculação foi inspirado na imunologia: uma vacina contendo versões enfraquecidas de um vírus ou bactéria é inoculada no organismo e prepara o sistema imune para combater versões mais virulentas do patógeno (MEICHENBAUM, 2007). Por sua vez, a inoculação de estresse prepara o organismo para suportar estressores de maiores intensidades sem desenvolver doença. No entanto, ao contrário das vacinas, nas quais a proteção ocorre para um tipo específico de vírus ou bactéria, a inoculação de estresse tem um efeito generalizado, protegendo contra estressores de diversas naturezas (CROFTON et al., 2015).

O efeito da inoculação pode surgir de um senso de controle e domínio advindos da superação de desafios, o que favoreceria o manejo de situações estressantes subsequentes (MEICHENBAUM, 2007; MINEKA & ZINBARG, 2006; RUSSO et al., 2012). Assim, algumas características dos estressores que favorecem sua natureza de inoculação são a previsibilidade, a possibilidade de controle e o espaçamento entre eles para permitir recuperação e aprendizado (DIENSTBIER, 1989; LEE et al., 2016; LIU et al., 2015). Além disso, grande parte das evidências experimentais indicam que os estressores levam a um efeito positivo mais pronunciado quando vivenciados durante o início da vida, mas alguns estudos demonstram a ocorrência de inoculação mesmo em indivíduos adultos (BROCKHURST et al., 2015; LEE et al., 2014; LYONS et al., 2010, 2018).

Esse efeito vem sendo estudado em humanos e há algumas evidências demonstrando a relação curvilínea entre estresse e resiliência. No estudo de Seery et al. (2013), indivíduos expostos a quantidades moderadas de adversidades ao longo da vida relataram menor afeto negativo após o teste de imersão da mão em água gelada em comparação com os grupos expostos a poucas ou muitas adversidades. O estudo de Edge et al. (2009) mostrou níveis reduzidos de ansiedade implícita em pessoas que passaram por quantidades moderadas de adversidade na infância. Gunnar e colaboradores (2009) estudaram os efeitos de diferentes níveis de estresse em crianças e observaram que indivíduos do grupo estresse moderado apresentaram menores níveis de cortisol em resposta ao Teste do Estresse Social de Trier em comparação com os grupos pouco e muito estresse.

Além de ser observada experimentalmente, a inoculação de estresse é usada na psicologia como uma terapia principalmente preventiva. É uma terapia cognitivo-comportamental desenvolvida por Donald Meichenbaum (1985) que tem o

objetivo de ensinar os pacientes a lidar com o estresse de forma a maximizar performance em situações de estresse intenso e reduzir seus efeitos negativos (SAUNDERS et al., 1996). É utilizada principalmente em pessoas expostas a altos níveis de estresse em seu ambiente de trabalho, como policiais, bombeiros e equipes médicas (MEICHENBAUM & NOVACO, 1985; NOVACO et al., 1989; SAUNDERS et al., 1996). Uma das etapas da terapia consiste em simular situações de exposição a estresse, que preparam os pacientes para lidar com situações reais quando estas surgirem (MEICHENBAUM & NOVACO, 1985).

Em animais, os estudos da inoculação de estresse foram iniciados por Levine na década de 1950. Ele observou que ratos retirados de seus ninhos por 3 minutos diariamente desde o nascimento até o desmame apresentaram menor responsividade a um estresse fisiológico aplicado aos 70 dias de idade (LEVINE, 1957). O estresse fisiológico consistia numa injeção de solução de glicose 20%, após a qual os animais manipulados na infância apresentaram menores adrenais e tomaram mais água em comparação aos não manipulados, indicando uma menor secreção de ACTH e ADH. Ambos os resultados sugerem que a inoculação de estresse vivenciada na infância levou a uma menor reatividade fisiológica em resposta ao estresse na vida adulta. Levine e colaboradores (1967) também demonstraram os efeitos comportamentais da inoculação de estresse: na vida adulta, os animais retirados de seus ninhos por 3 minutos diários até o desmame apresentaram menor taxa de defecação e maior atividade no campo aberto em comparação aos não manipulados, possivelmente indicando uma resposta emocional menos exacerbada. Além disso, os animais submetidos a inoculação de estresse na infância apresentaram menores níveis de corticosterona plasmática após serem submetidos ao campo aberto, também sugerindo uma resposta mais controlada e adaptativa a uma situação de estresse na vida adulta. Os efeitos comportamentais da inoculação também foram demonstrados em macacos, nos quais foram observados menores intensidade e frequência de chamado materno durante períodos de ausência da mãe (LYONS et al., 1999), maior consumo de solução de sacarose durante uma sessão de isolamento social (LEE et al., 2014) e menor quantidade de erros num teste de avaliação de resposta inibitória (PARKER et al., 2005). Além disso, foi demonstrado recentemente que a ansiedade em macacos varia de forma não linear de acordo com o número de estressores no início da vida: animais não submetidos a estresse são mais ansiosos do que animais

submetidos a um ou dois estressores, mas três estressores induzem efeitos ansiogênicos (PARKER et al., 2019). Os estudos em macacos também investigaram os correlatos fisiológicos e neurobiológicos da inoculação, e demonstraram menores níveis de cortisol secretados numa situação de estresse (LYONS et al., 1999), expansão e maior mielinização do córtex pré-frontal ventromedial (KATZ et al., 2009), aumento da expressão do gene para o receptor de glicocorticoide no córtex cingulado anterior (LEE et al., 2014) e maior disponibilidade de receptores dopaminérgicos D2/3 no estriado ventral (LEE et al., 2016).

Até o momento, não se sabe exatamente qual é o mecanismo pelo qual a exposição a estressores moderados confere resiliência. As evidências do estudo da inoculação de estresse em animais indicam que essa manipulação pode regular a reatividade do eixo HPA, já que uma das características dos animais que passaram por inoculação de estresse é uma menor secreção de corticosterona em resposta a estressores (BROCKHURST et al., 2015; LEVINE et al., 1967; PARKER et al., 2004) e um aumento na expressão dos receptores para glicocorticoides no córtex cingulado anterior (LEE et al., 2014). Além disso, durante as sessões de inoculação ocorre um aumento na secreção de corticosterona em comparação com os níveis basais (BROCKHURST et al., 2015). Dessa forma, é possível que a inoculação de estresse atue modulando o eixo HPA, de forma que sua resposta seja controlada e adaptativa em resposta a outros estressores.

Assim, a hipótese deste trabalho é de que a secreção de corticosterona é essencial para o efeito pró-resiliência da inoculação de estresse. Além disso, hipotetizamos que a corticosterona não seja suficiente para gerar este efeito, uma vez que a inoculação fornece treinamento em diversos aspectos da resposta ao estresse.

2 OBJETIVOS

2.1 OBJETIVO GERAL

Avaliar o envolvimento da corticosterona no efeito pró-resiliência gerado por inoculação de estresse em camundongos.

2.2 OBJETIVOS ESPECÍFICOS

- Buscar padronizar a inoculação de estresse pela investigação dos efeitos de um protocolo de inoculação de estresse social em camundongos Swiss machos jovens, testando seus efeitos no campo aberto, labirinto em cruz elevado, nado forçado e secreção de corticosterona após o nado forçado;
- Buscar padronizar a inoculação de estresse pela avaliação dos efeitos de um protocolo de inoculação de estresse social em camundongos C57BL/6 machos adultos, testando seus efeitos no campo aberto, labirinto em cruz elevado, nado forçado e secreção de corticosterona após o nado forçado;
- Verificar a ocorrência de ativação do eixo HPA pelo protocolo de inoculação de estresse social em camundongos C57BL/6 machos adultos através da quantificação de corticosterona sérica após a primeira e última sessões;
- Buscar padronizar a inoculação de estresse pela observação dos efeitos de um protocolo de inoculação de estresse de restrição de movimento em camundongos C57BL/6 machos adultos, testando seus efeitos no campo aberto, labirinto em cruz elevado, nado forçado e secreção de corticosterona após o nado forçado;
- Verificar a ocorrência de ativação do eixo HPA pelo protocolo de inoculação de estresse por restrição de movimento em camundongos C57BL/6 machos adultos através da quantificação de corticosterona sérica após a primeira e última sessões;
- Avaliar os efeitos do protocolo de inoculação de estresse de restrição de movimento em camundongos C57BL/6 adultos fêmeas, testando

seus efeitos no campo aberto após uma sessão aguda de restrição de movimento, labirinto em cruz elevado e nado forçado;

- Determinar o envolvimento da secreção de corticosterona durante as sessões de inoculação de estresse por restrição de movimento no efeito hiperlocomotor no campo aberto através da administração de metirapona, um inibidor da síntese de corticosterona, durante as sessões de inoculação de estresse;
- Comprovar o bloqueio da secreção de corticosterona através da dosagem de corticosterona sérica após a administração de metirapona seguida de uma sessão de inoculação de estresse por restrição de movimento;
- Testar o efeito da inoculação de estresse por restrição de movimento no paradigma do desamparo aprendido.

3 ARTIGO CIENTÍFICO

Corticosterone secreted during stress inoculation is not sufficient to generate resilience

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ABSTRACT

The stress inoculation hypothesis of resilience states that exposure to mild stressful situations can strengthen an individual's ability to cope and increase adaptability to future stressors. This hypothesis receives supporting data from several studies in humans, monkeys, rats and mice. Up to date, no study explored the role played by the glucocorticoids in the effect. Therefore, the objective of this work was to investigate the involvement of corticosterone in stress inoculation (SI) in mice. We tested three different stress inoculation protocols, each one comprising eleven 15-minute sessions applied every two days. The first protocol consisted of a mild social stress (Social SI) in juvenile Swiss mice. The second was similar to the first (Social SI), but performed in adult C57BL/6. The third was a movement restraint protocol (Restraint SI) in C57BL/6. Following the eleven inoculation sessions, we evaluated the effects in the open field (OF), elevated plus maze (EPM), forced swim test (FST), corticosterone secretion following the FST and learned helplessness. We also quantified corticosterone secretion induced by SI. We observed robust secretion of corticosterone following the inoculation sessions, but none of the protocols tested led to a resilience effect. Social and restraint SI in C57BL/6 mice led to an increase in locomotion in the open field. We then administered the inhibitor of corticosterone synthesis metyrapone during restraint SI to test if hyperlocomotion was dependent on corticosterone secretion. Metyrapone decreased the secretion of corticosterone induced by restraint but we did not observe increased locomotion in any of the groups, despite increased corticosterone in the vehicle treated group. This result suggests that corticosterone secreted during SI sessions is not sufficient to induce hyperlocomotion. The results also indicate that corticosterone is not sufficient for the resilience effect.

Keywords: Social stress; Restraint stress; Swiss mice; C57BL/6 mice; HPA axis; Resilience.

1 INTRODUCTION

Stress is an important risk factor for the development of several psychiatric disorders, especially depression [1]. However, exposure to mild stressful situations can strengthen an individual's ability to cope and increase adaptability to future stressors [2,3]. This is known as the stress inoculation hypothesis of resilience, and it receives supporting data from several studies in humans, monkeys, rats and mice [4–9]. Brockhurst et al. [8] showed that male C57BL/6 mice intermittently exposed to a dominant same-sex stranger through a wire mesh secrete less corticosterone after acute restraint stress, present a shift towards active coping in the tail suspension test, exhibit less freezing in a novel environment and present decreased novel-object exploration latencies. Lyons et al. [9] observed similar results in females

So far, some structures and mechanisms have been implicated in the stress inoculation effect. Katz et al. [10] showed that juvenile squirrel monkeys that underwent intermittent social separations presented, in puberty, increased ventromedial prefrontal cortical volumes. Neuroimaging demonstrated that this increase in volume reflects expansion of surface area and increased prefrontal myelination. Lee et al. [11] observed that stress inoculated juvenile female monkeys had increased DRD2/3 availability in ventral striatum. Lee et al. [12] also published a study with adult female squirrel monkeys and their results showed that inoculated animals had increased glucocorticoid receptor (NR3C1) gene expression in the anterior cingulate cortex. Nevertheless, to our knowledge no study has attempted to explore the involvement of glucocorticoids release in stress inoculation. In support of this approach, Brockhurst et al. [8] and Lyons et al. [9] showed that social stress inoculation increases circulating corticosterone both in male and female mice and suggested that the hormone probably has a major role in the effect.

Therefore, the objective of this study was to investigate the role of corticosterone in the stress inoculation effect. We first attempted to model a stress inoculation protocol in our laboratory, testing the effects of three different procedures in locomotion, anxiety and depression-like tests and in corticosterone response to a heterotypic stressor. We also aimed to block corticosterone secretion with the inhibitor of corticosterone synthesis metyrapone during stress inoculation sessions. We hypothesized that corticosterone secreted during mild stressors is necessary for the inoculation effect.

2 METHODS

2.1 Animals

This study used 21 days old male Swiss mice and 60-80 days old male and female C57BL/6 mice. Adult Swiss mice (80-275 days old) were also used in the social stress inoculation experiments. Apart from experiment 1, adult Swiss mice had reproductive experience. Animals used were from our own breeding stock or purchased from Carlos Chagas Institute – ICC/Fiocruz Paraná. Animals purchased from ICC were allowed to acclimate for at least 1 week in our facilities before the start of experiments. All mice were maintained in a temperature-controlled room (22 ºC ± 2 ºC) under a light/dark cycle of 12 h (lights on at 7h00) with food and water available *ad libitum*. Except for the adult Swiss mice which were individually housed to increase aggressiveness, animals were group-housed.

All experiments were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals and approved by the Ethics Committee on the Use of Animals of the Biological Division of the Federal University of Parana (CEUA nº 1207).

2.2 Behavioral tests

2.2.1 Open field

In the experiment with Swiss mice, the open field used was a 50 cm diameter round black arena. In the experiments in which C57BL/6 mice were used, it consisted of a 45 cm diameter round white arena. The animals were allowed to explore for 5 minutes and data were analysed with the software Optimouse version 3.0 (available at: https://github.com/yorambenshaul/optimouse).

2.2.2 Elevated plus maze

The elevated plus-maze was comprised of two opposite closed arms, 30 x 5 x 15 cm, and two opposite open arms, 30 x 5 x 0.5 cm, elevated 50 cm above the floor. Animals were placed in the center of the maze facing one of the closed arms and allowed to explore. The test was recorded and analyzed for 5 minutes. Percentage of time in the open arms was calculated dividing time spent in the open arms by time in the open and closed arms.

2.2.3 Forced Swim Test

For the forced swim test, animals were placed in a transparent cylinder of 20 cm diameter containing water at 24 ºC ± 1 ºC and at a depth of 13 cm. The test was recorded for 6 minutes and immobility in the last 4 minutes was analysed.

2.2.4 Learned helplessness

The learned helplessness procedure consisted in exposing mice for 2 consecutive days to 360 scrambled inescapable foot shocks (0.1 mA) in a shuttle box (307 x 333 x 540 mm) divided in 4 compartments. The foot shocks varied randomly in duration (1 – 3 s) and interval time (1 - 15 s). Total session duration was approximately 52 min. Shocks during the 2 training days were applied to 4 animals simultaneously, each in one of the compartments. In the next day after the second inescapable shock procedure, animals were tested one at a time in the shuttle box. Testing was comprised of 30 trials, each trial consisting of a light stimulus of 5 seconds and a subsequent shock of 10 seconds (0.2 mA). The light signaled the upcoming shock and the stimuli were interrupted when the animal shuttled to the other compartment. Intertrial intervals lasted 30 seconds. An avoidance was recorded when the animal shuttled to the other compartment during light presentation to avoid the shock; escape was counted when shuttling was performed during the shock and a failure occurred when shuttling did not occur during light nor shock presentation. Shuttles were also recorded during the 2 minutes preceding the first trial, as a measure of general activity. Testing lasted 20-24 minutes and was performed one animal at a time.

The learned helplessness experiment took place from 12 PM to 6 PM, under dim light.

2.3 Blood collection and corticosterone quantification

For blood collection, animals were decapitated and approximately 0.75 ml trunk blood was collected. Blood was allowed to clot and one hour after collection it was centrifuged at 1.4 g for 10 min in a microcentrifuge at room temperature. The serum was recovered and stored at -20ºC until analysis. Blood was collected between 08:30 AM and 11:00 AM.

Serum corticosterone was quantified by competitive ELISA. The antibody for corticosterone (Polyclonal CJM006; 1:15000 dilution) and the competitive ligand

(HRP conjugated corticosterone; 1:80000) were obtained from the University of California, Davis, CA, USA. Dilution of serum samples varied according to mouse strain and experimental group and is reported in each experiment. Intra and inter-assay coefficient variation were <10%.

2.4 Experiments

2.4.1 Experiment 1: Effects of social stress inoculation (SI) in juvenile male swiss mice

21 days old male Swiss mice were allocated in three groups: control, intraperitoneal saline injection and social stress inoculation. Social SI consisted in placing a juvenile mouse inside a small perforated plastic box in the home cage of an adult mouse (Fig. 1). This barrier allowed for interaction between the animals, such as nose pokes, but prevented wounding. SI sessions lasted 15 minutes and took place once every two days for 21 days, totalizing 11 exposures. Encounters always occurred between the same pair of mice. Animals from the injection group were injected intraperitoneally with saline once every two days for 21 days. The injection method used did not require immobilizing the animals, which has been shown to attenuate corticosterone secretion and still provide the same peritoneal distribution of the solution [13]. Injections were administered in order to identify the effect of the injection stress on behavior and corticosterone secretion, which would be important for future experiments. Controls were left undisturbed except for routine animal care.

Figure 1. Social stress inoculation in 21-day old Swiss mice. Experimental subjects were placed inside a perforated plastic box in the home-cage of an adult Swiss.

One day after the last SI session and IP injection, animals were tested in the EPM and OF. On the next day, half of the animals in each group were subjected to the forced swim test (FST) and had trunk blood collected 30 minutes later for corticosterone dosage (Fig. 2). Parallelly, mice not subjected to FST also had trunk

blood collected to provide a basal measure for each group. For dosage, serum samples were diluted 1:6 (controls) and 1:33 (FST). Experiments were performed from 8 AM to 2 PM under 300 lux light intensity.

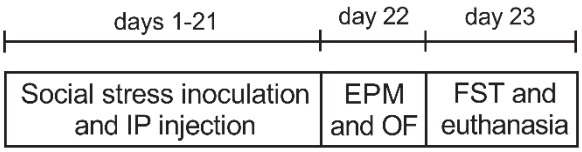

Figure 2. Experiment 1 timeline. Swiss male mice were tested one and two days after the last social stress inoculation session and intraperitoneal injection.

2.4.2 Experiment 2: Effects of social stress inoculation in adult male C57BL/6 mice

As we did not observe the expected resilience effect in experiment 1, we decided to use C57BL/6 mice as experimental subjects in order to make our protocol more analogous to the work of Brockhurst et al. (2015). Adult male C57BL/6 mice were divided into 4 groups: control, transport, injection, social SI. The social SI protocol was conducted essentially equally to experiment one, altering only the strain of the experimental subjects and the fact that the adult Swiss mouse was the one kept inside the perforated plastic box instead of the experimental subject (Fig. 3). The injection procedure was performed as described above. Animals in the group transport were taken in their home cages to the experimental room once every two days for 21 days and remained there for the duration of the inoculation session (approx. 1h) and then returned to the animal facility. This group was included in this experiment in an attempt to isolate effects of different stressors, such as transportation, on the inoculation effect.

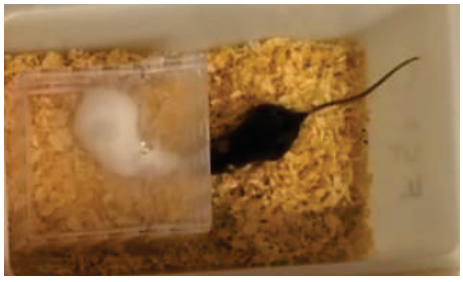

Figure 3. Social stress inoculation in adult C57BL/6 mice. Experimental subjects were placed inside the home-cage of an adult Swiss. The resident was kept inside the perforated plastic box.

Two days after the last SI session, IP injection and transport animals were tested in the EPM and OF. In the next day, half of them were subjected to the FST and had trunk blood collected 30 minutes later for corticosterone dosage (Fig. 4). Mice not subjected to FST also had trunk blood collected. Dilution of serum samples were 1:12 for controls and 1:65 for FST. Experiments were performed from 8 AM to 1 PM under 50 lux light intensity.

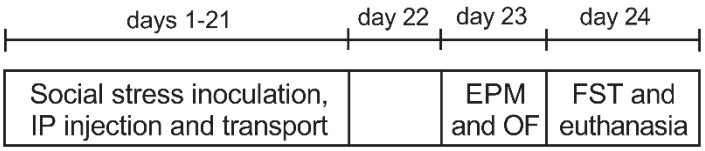

Figure 4. Experiment 2 timeline. C57BL/6 male mice were tested two and three days after the last social stress inoculation session, intraperitoneal injection and transport.

Two extra cohorts of mice were used to assess corticosterone secretion after the first and last social SI session, in order to evaluate if our social SI protocol was eliciting a corticosterone response. The protocols were performed as described above and 20 minutes after the end of the inoculation, injection and transportation to the experimental room, animals were euthanized for blood collection. Samples collected after the first session were diluted 1:8 (control and transport), 1:73 (injection) and 1:101 (SI) and the ones collected after the last session were diluted 1:8 (control and transport), 1:65 (injection) and 1:101 (SI).

2.4.3 Experiment 3: Effects of restraint stress inoculation in adult male C57BL/6 mice

In experiment 2 we also failed to observe the expected resilience effect of stress inoculation, therefore, in experiment 3 we substituted social SI for a protocol of movement restraint in an attempt to minimize the possible sources of unpredictability of social stress. Adult C57BL/6 male mice were restrained in perforated 50 ml Falcon tubes for 15 minutes, once every two days for 21 days. Because the effects of transport and IP injection had been evaluated in C57BL/6 mice in the previous experiment, we did not repeat these groups. Control animals were left undisturbed except for routine animal care.

Two days after the last SI session, animals were tested in the OF and EPM. On the next day, all of them were subjected to the FST and had trunk blood collected 30 minutes later for corticosterone dosage (Fig. 5). A naïve group, which did not

undergo any procedure or behavior test, was euthanized in parallel to obtain basal values of serum corticosterone. Serum samples from animals subjected to FST and naïves were diluted 1:101 and 1:8, respectively. Experiments were performed from 8 AM to 1 PM. OF and EPM were conducted under 50 lux and the FST was conducted under 300 lux light intensity.

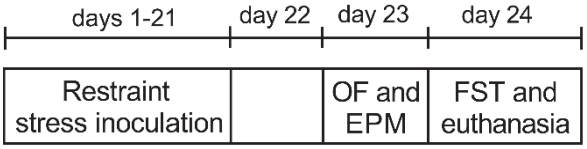

Figure 5. Experiment 3 timeline. C57BL/6 male mice were tested two and three days after the last restraint stress inoculation session.

Two extra cohorts were used to evaluate corticosterone secretion following the first and last restraint SI sessions. Blood collection and corticosterone dosage were performed as described above. Dilution of control serum samples was 1:8 and SI samples from the first and last session were diluted 1:101 and 1:51, respectively.

2.4.4 Experiment 4: Effects of restraint stress inoculation in adult female C57BL/6 mice

Experiment 4 was designed to observe stress reactivity of inoculated female C57BL/6 in the open field. Zimprich et al. [14] reported a noninvasive method to observe stress reactivity in mice. Basically, animals are restrained for 2 hours in Falcon tubes and 20 minutes later present a significant increase in locomotor activity in the open field. In our study, the restraint SI procedure was performed as described for males: animals were restrained in Falcon tubes for 15 minutes once every two days for 21 days. Controls were left undisturbed except for routine animal care. Two days after completion of the protocol, half of the animals in each group was submitted to a 2h restraint in Falcon tubes and 20 minutes later animals were tested in the open field. Unrestrained mice were also tested in the OF. In the following day, all mice were tested in the elevated plus maze and forced swim test (Fig. 6). Testing occurred from 2 PM to 6 PM under 300 lux light intensity.

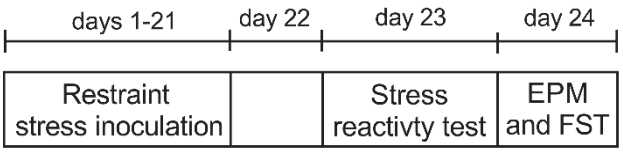

Figure 6. Experiment 4 timeline. C57BL/6 female mice were tested two and three days after the last restraint stress inoculation session.

2.4.5 Experiment 5: Effects of blocking corticosterone secretion during restraint stress inoculation in adult male and female C57BL/6 mice

This experiment was aimed at investigating the role of corticosterone secreted during the inoculation sessions on the hyperlocomotion effect observed in the open field. For this aim, corticosterone secretion during the inoculation sessions was blocked administering 100 mg/kg metyrapone (Sigma-Aldrich), an inhibitor of the 11-beta-hydroxylase. C57BL/6 mice from both sexes were randomized into four groups: control-vehicle (con-veh), control-metyrapone (con-met), inoculation-vehicle (SI-veh) and inoculation-metyrapone (SI-met).

Animals in the inoculation group were injected with either saline or metyrapone 30 minutes before being placed in the Falcon tubes. The restraint protocol was performed as described earlier. Parallelly, controls were transported to the experimental room and received either saline or metyrapone. For technical reasons, we used the traditional immobilization method to administer the solutions.

Two days after the last inoculation session, animals were tested in the OF under 50 lux light intensity. In the following day, we began the learned helplessness procedure in an attempt to identify a resilience effect in an animal model of depression. Animals were submitted to the learned helplessness procedure in three separate cohorts, matched by age, sex and experimental group, the first from days 24-26, the second from days 27-29 and the third from days 30-32 (Fig. 7).

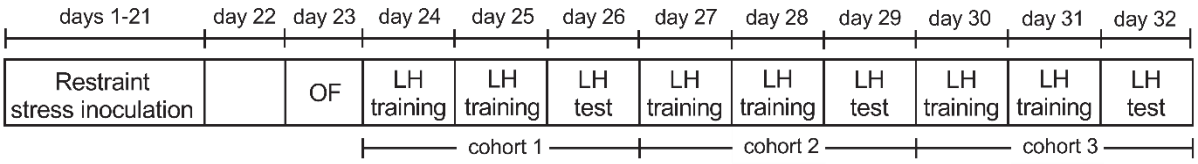

Figure 7. Experiment 5 timeline. C57BL/6 male and female mice were tested in the OF two days after the last restraint stress inoculation session. Three cohorts were then submitted to the learned helplessness procedure.

Another cohort of animals was used to determine corticosterone secretion after the first inoculation session and its blockade by metyrapone. Animals were divided into the four groups and trunk blood was collected 20 minutes after removal from the Falcon tube or 50 minutes after IP injection for the control groups. Dilution of serum samples was 1:8 for the groups con-veh, con-met and SI-met. Samples from the group SI-veh were diluted 1:101.

2.5 Statistical Analysis

All data were tested for outliers using the ROUT test with a Q coefficient of 1%, available on the GraphPad Prism 7.0 software. Data were analysed by one- or two-way ANOVA followed by the Newman-Keuls post hoc and the Student's t test for parametric data. Kruskal-Wallis and Mann-Whitney tests were used for nonparametric distributions. Data are presented as mean ± SEM when parametric and as median ± interquartile range when non-parametric.

3 RESULTS

3.1 Experiment 1: Effects of social stress inoculation in juvenile male Swiss mice

In the first attempt to validate an stress inoculation protocol, there was no effect of group in distance traveled in the open field ($F_{(2,54)}$=0.12, p=0.89) (Fig. 1A). In the elevated plus maze there was an effect of group in percentage of time in the open arms ($H_{(2,56)}$=8.11, p=0.017). The group SI had decreased percentage of time in open arms in comparison to the injection group (p=0.017) but not controls (p=0.14) (Fig. 1B). There was no effect of group in immobility time in the last 4 minutes of the forced swim test ($H_{(2,29)}$= 2.85, p =0.24) (Fig. 1C).

There was an effect of group ($F_{(2,51)}$=6.09, p=0.0042) and FST ($F_{(1,51)}$=200.88, p=0.000) in corticosterone collected 30 min after the FST. There was also an interaction between the factors ($F_{(2,51)}$=6.35, p=0.0035). Newman-Keuls post hoc showed that all groups subjected to FST had significantly higher corticosterone in comparison to its control (con-FST vs. con-con p=0.00012; inj-FST vs. inj-con p=0.00013; SI-FST vs. SI-con p=0.00018). In addition, the group injection subjected to FST presented higher levels of serum corticosterone in comparison both to con-FST (p=0.00028) and SI-FST (p=0.00026) (Fig. 1D).

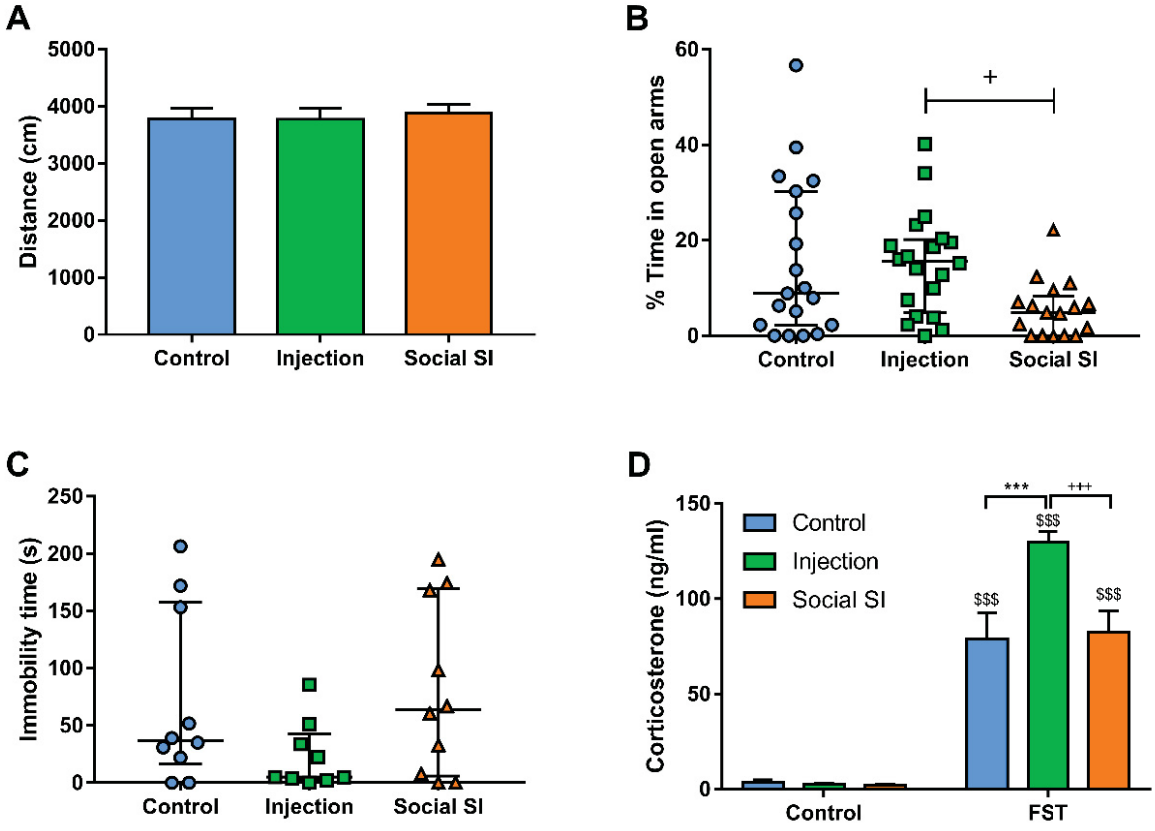

Figure 1. Effects of social stress inoculation in juvenile male Swiss mice. (A) Total distance travelled in the open field; n=18-20/group. (B) Percentage of time spent in the open arms of the elevated plus maze; n=17-20/group. (C) Immobility time during the last 4 minutes of the forced swim test; n=9-10/group. (D) Serum corticosterone secreted after forced swim test; n=8-10/group. In graph A data are presented as mean ± SEM and analysed by one-way ANOVA; in graphs B and C data are presented as median and interquartile range and analysed by the Kruskal-Wallis test; in graph D data are presented as mean ± SEM and analysed by two-way ANOVA. +p<0.05 in comparison to injection; $$$p<0.001 in comparison to each respective control; ***p<0.001 in comparison to con-FST; +++p<0.001 in comparison to SI-FST.

3.2 Experiment 2: Effects of social stress inoculation in adult male C57BL/6 mice

In the second stress inoculation protocol, there was a significant effect of group for distance traveled in the open field ($F_{(3,69)}$=5.66, p=0.0012). Newman-Keuls post hoc indicated that the group SI ambulated significantly more than all other groups (p=0.002 vs. con; p=0.009 vs. trans; p=0.011 vs. inj) (Fig. 1A). In the elevated plus maze, there was no significant difference in percentage of time spent in the open arms ($H_{(3,70)}$=6.83, p=0.078) (Fig. 1B). There was also no difference in

immobility during the last 4 minutes of the forced swim test ($F_{(3,34)}$=0.92, p=0.44) (Fig. 1C).

For serum corticosterone secreted in response to the forced swim test, there was an effect of FST ($F_{(1,62)}$=229.16, p=0.0000), indicating again a robust increase in corticosterone secretion in animals submitted to FST. There was also an effect of group ($F_{(3,62)}$=3.88, p=0.013), but no interaction between the factors ($F_{(3,62)}$=0.81, p=0.49). Post hoc only for the factor group indicated that SI had significantly lower corticosterone levels in comparison to control (p=0.007) and transport (p=0.045) but not injection (p=0.21) (Fig. 1D).

In the first transport, injection and inoculation session there was an effect of group ($F_{(3,31)}$=29.27, p=0.000) for serum corticosterone. Newman-Keuls post-hoc indicated that SI was significantly greater than control (p=0.0002), transport (p=0.0001) and injection (p=0.0005). The injection group was also different from both control (p=0.003) and transport (p=0.003). Transport was not different from control (p=0.73) (Fig. 1E). In the last session, there was also an effect of group ($F_{(3,12)}$=8.17, p=0.0031). Newman-Keuls post-hoc indicated that SI was different from control (p=0.011) and transport (p=0.007) and that the group injection was also different from control (p=0.030) and transport (p=0.013) (Fig. 1F). In a two-way ANOVA performed with data from the first and last sessions, there was an effect of session ($F_{(1,41)}$17.64, p=0.0001), group ($F_{(3,41)}$=14.39, p=0.000) and interaction between the factors ($F_{(3,41)}$=5.17, p=0.004). Newman-Keuls post-hoc indicated that social SI in the last session was significantly lower than SI in the first session (p=0.00013).

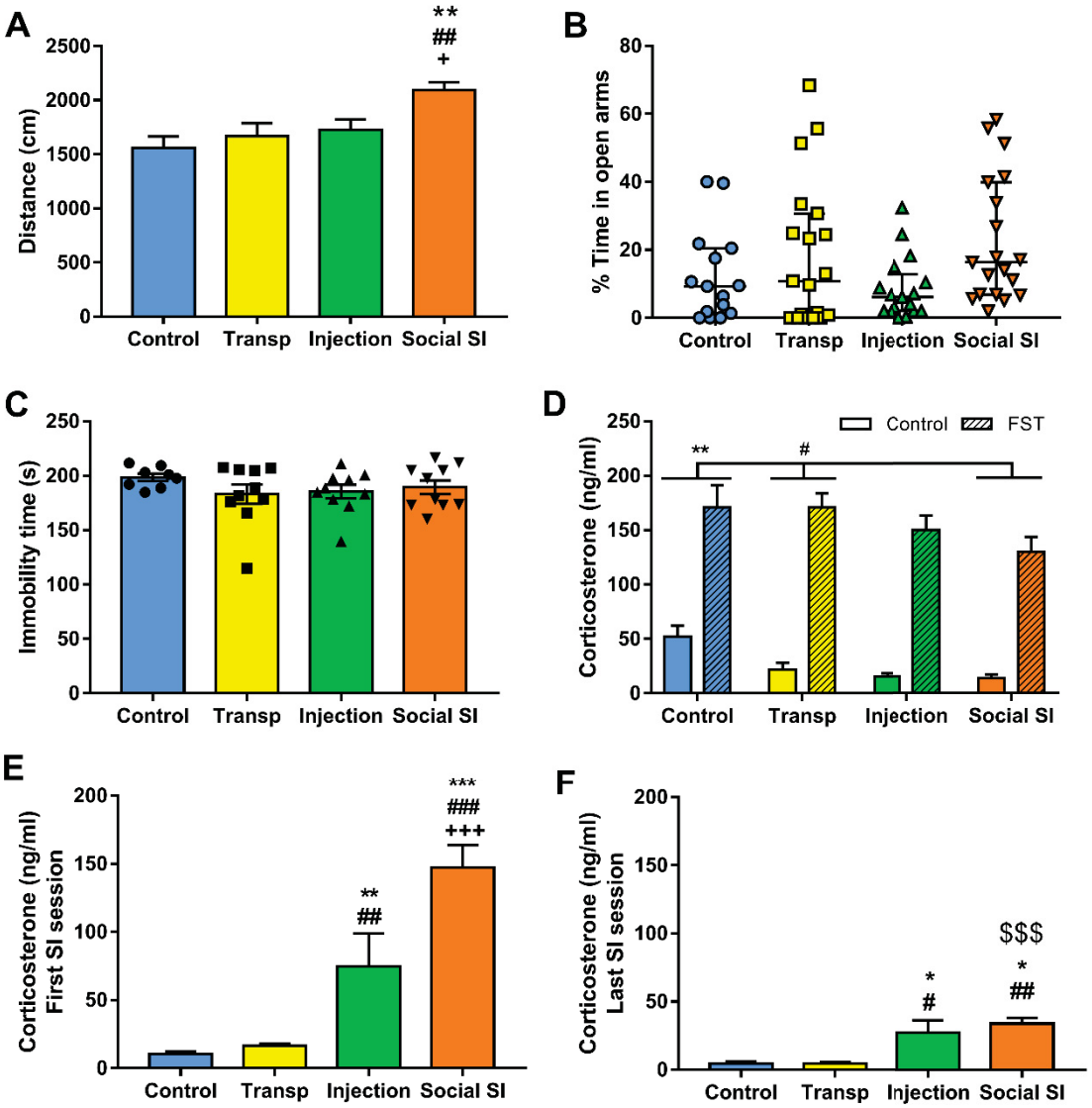

Figure 2. Effects of social stress inoculation in adult male C57BL/6 mice. (A) Total distance travelled in the OF; n=15-20/group. (B) Percentage of time spent in the open arms of the EPM; n=15-20/group. (C) Immobility time during the last 4 minutes of the FST; n=8-10/group. (D) Serum corticosterone secreted after FST; n=7-10/group. (E) Serum corticosterone secreted after the first inoculation session, IP injection and transport; n=6-10/group. (F) Serum corticosterone secreted after the last inoculation session, IP injection and transport; n=3-5/group. In graphs A, C, E and F data are presented as mean ± SEM and analysed by one-way ANOVA; in graph D data are presented as mean ± SEM and analysed by two-way ANOVA; in graph B data are presented as median and interquartile range and analysed by the Kruskal-Wallis test. *p<0.05 in comparison to control; **p<0.01 in comparison to control; ***p<0.001 in comparison to control; #p<0.05 in comparison to transport; ##p<0.01 in comparison to transport; ###p<0.001 in comparison to transport; +p<0.05 in comparison to injection; +++p<0.001 in comparison to injection; $$$p<0.001 in comparison to Social SI in the first session (two-way ANOVA).

3.3 Experiment 3: Effects of restraint stress inoculation in adult male C57BL/6 mice

In the third stress inoculation protocol, there was a significant difference between groups in distance traveled in the open field ($t_{(40)}$=4.53, p=0.000052) (Fig. 3A), but no difference in percentage of time spent in the open arms of the EPM (U=137.0, p=0.09) (Fig. 3B) nor immobility time in the last 4 minutes of the FST ($t_{(37)}$=1.32, p=0.19) (Fig. 3C). For corticosterone secreted during the forced swim test, there was not a significant difference ($t_{(38)}$=0.70, p=0.49) (the group naïve was not included in the analysis) (Fig. 3D).

In the first inoculation session, restraint significantly increased serum corticosterone ($t_{(13)}$=9.49, p=0.000) (Fig. 3E), as well as in the last ($t_{(11)}$=5.92, p=0.000) (Fig. 3F). In a two-way ANOVA performed with data from the first and last sessions, there was an effect of session ($F_{(1,24)}$=8.10, p=0.000), group ($F_{(1,24)}$=117.88, p=0.0089) and interaction between the factors ($F_{(1,24)}$=8.86, p=0.0065). Newman-Keuls post-hoc indicated that restraint SI in the last session was significantly lower than SI in the first session (p=0.00053).

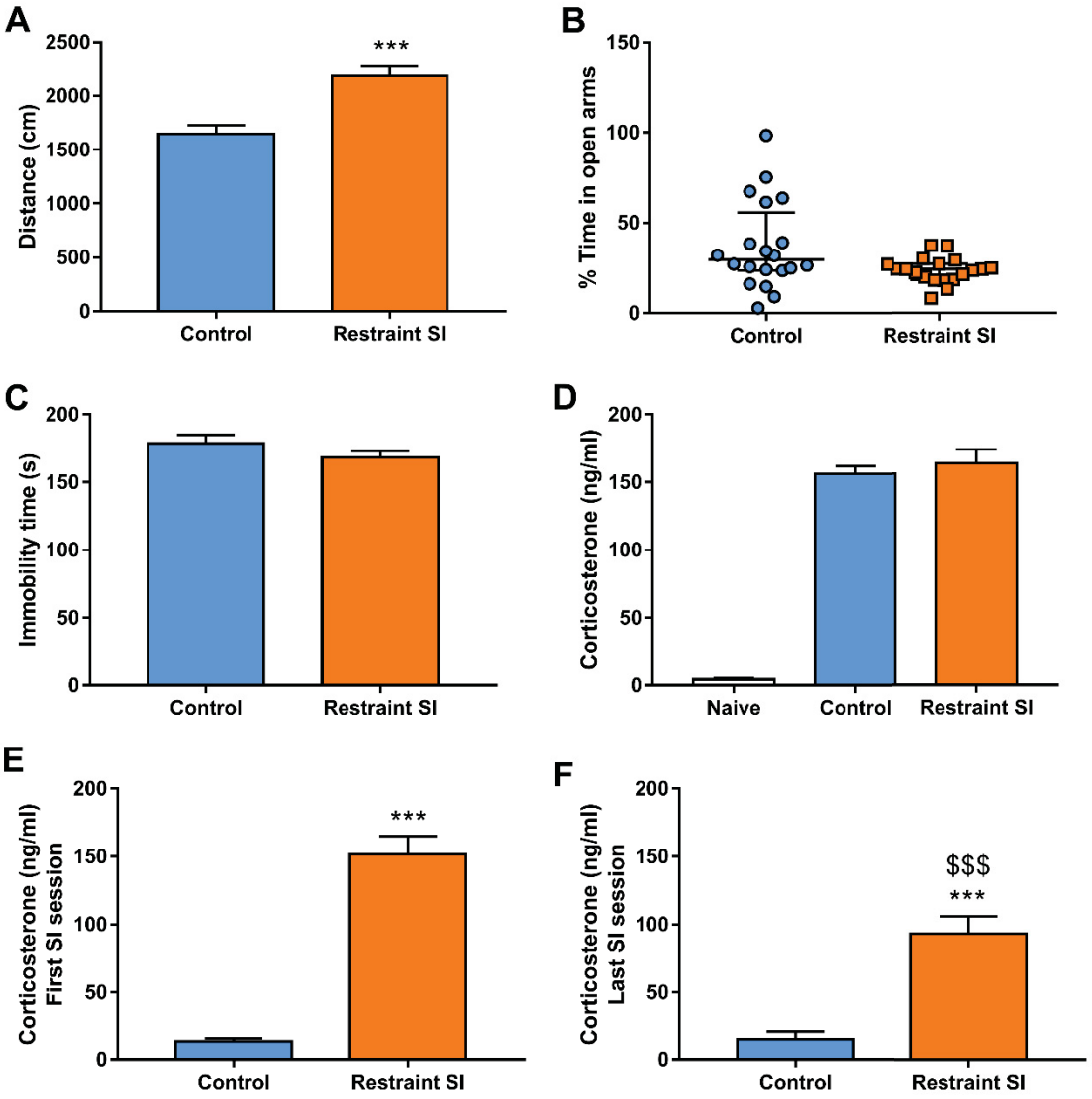

Figure 3. Effects of restraint stress inoculation in adult male C57BL/6 mice. (A) Total distance travelled in the OF; n=20-21/group. (B) Percentage of time spent in the open arms of the EPM; n=19-20/group. (C) Immobility time during the last 4 minutes of the FST; n=19-20/group. (D) Serum corticosterone secreted after FST; n=20/group. (E) Serum corticosterone secreted after the first inoculation session; n=7-8/group. (F) Serum corticosterone secreted after the last inoculation session; n=6-7/group. In graphs A, C, D, E and F data are presented as mean ± SEM and analysed by Student's t-test; in graph B data are presented as median and interquartile range and analysed by the Mann-Whitney test. ***p<0.001 in comparison to control; $$$p<0.001 in comparison to restraint SI in the first session (two-way ANOVA).

3.4 Experiment 4: Effects of restraint stress inoculation in adult female C57BL/6 mice

When evaluating the effects of restraint SI in females, there was an effect of both stress inoculation ($F_{(1,23)}$=9.56, p=0.0052) and acute stress ($F_{(1,23)}$=13.86,

p=0.0011) on distance traveled in the open field, but there was no interaction between the factors ($F_{(1,23)}$=0.76, p=0.39) (Fig. 4A). For both the EPM and FST, there was no effect of the factor acute stress in a two-way ANOVA (EPM: $F_{(1,22)}$=1.50, p=0.2; FST: $F_{(1,23)}$=0.038, p=0.85), so data was combined and analyzed considering only the factor stress inoculation. There was no difference in percentage of time in the open arms ($t_{(23)}$=0.49, p=0.63) (Fig. 4B) nor in immobility in the last 4 minutes of the test (U=88, p=0.90) (Fig. 4C).

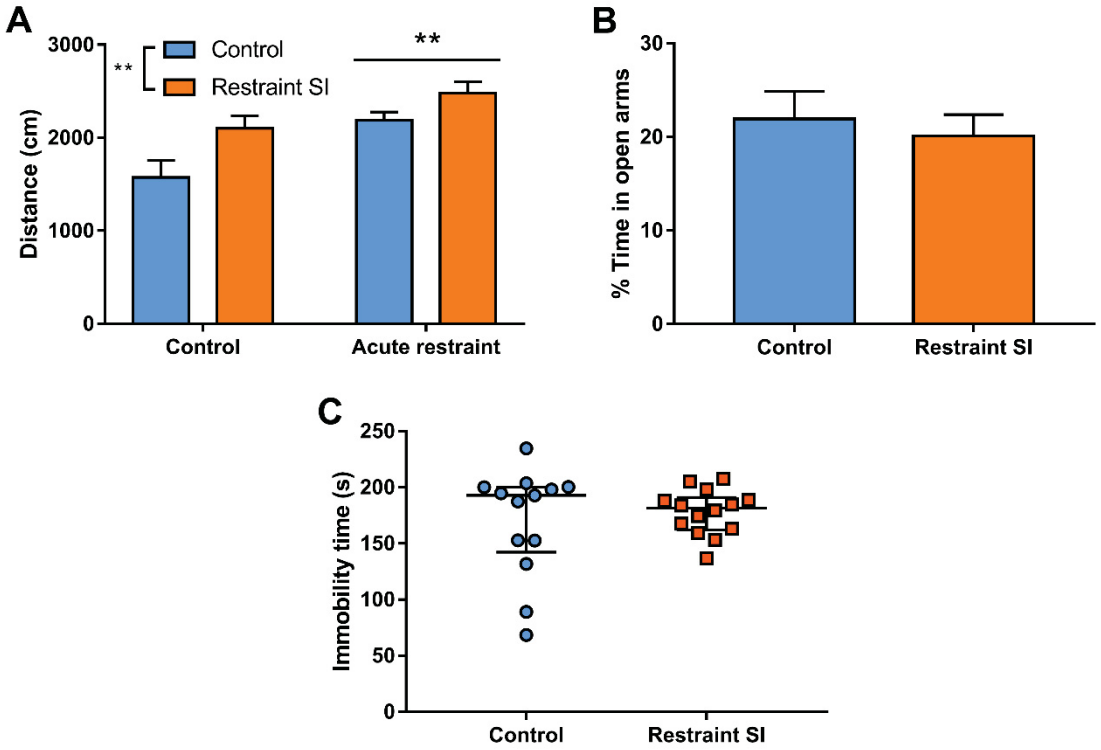

Figure 4. Effects of restraint stress inoculation in adult female C57BL/6 mice. (A) Total distance travelled in the OF; data are presented as mean ± SEM and analysed by two-way ANOVA; n=6-7/group. (B) Percentage of time spent in the open arms of the EPM; data presented as mean ± SEM and analysed by Student's t-test; n=11-14/group. (C) Immobility time during the last 4 minutes of the FST; data presented as median and interquartile range and analysed by the Mann-Whitney test; n=13-14/group. **p<0.01.

3.5 Experiment 5: Effects of blocking corticosterone secretion during restraint stress inoculation in adult male and female C57BL/6 mice

When corticosterone secretion during restraint SI was blocked by metyrapone, there was no effect of group ($F_{(1,46)}$=0.10, p=0.75), treatment ($F_{(1,46)}$=0.11, p =0.74)

nor interaction between factors ($F_{(1,46)}$=0.024, p=0.88) in distance travelled in the open field (Fig. 5A). In the learned helplessness paradigm, there was no effect of SI ($F_{(1,28)}$=0.082, p=0.776974), treatment ($F_{(1,28)}$=1.604, p=0.215725) nor interaction between factors ($F_{(1,28)}$=2.285, p=0.141795) for failures to escape (Fig. 5B). There was also no effect of group, treatment or interaction for escapes and shuttles before the first shock (data not shown).

When analyzing corticosterone secreted during restraint, there was a significant effect of group ($F_{(1,24)}$=47.71, p=0.000), treatment ($F_{(1,24)}$=51.93, p=0.000) and interaction between the factors ($F_{(1,24)}$=44.56, p=0.000). Restraint significantly increased corticosterone (SI-veh vs con-veh: p=0.00015). This rise was abolished by metyrapone (SI-met vs. SI-veh: p=0.00013)

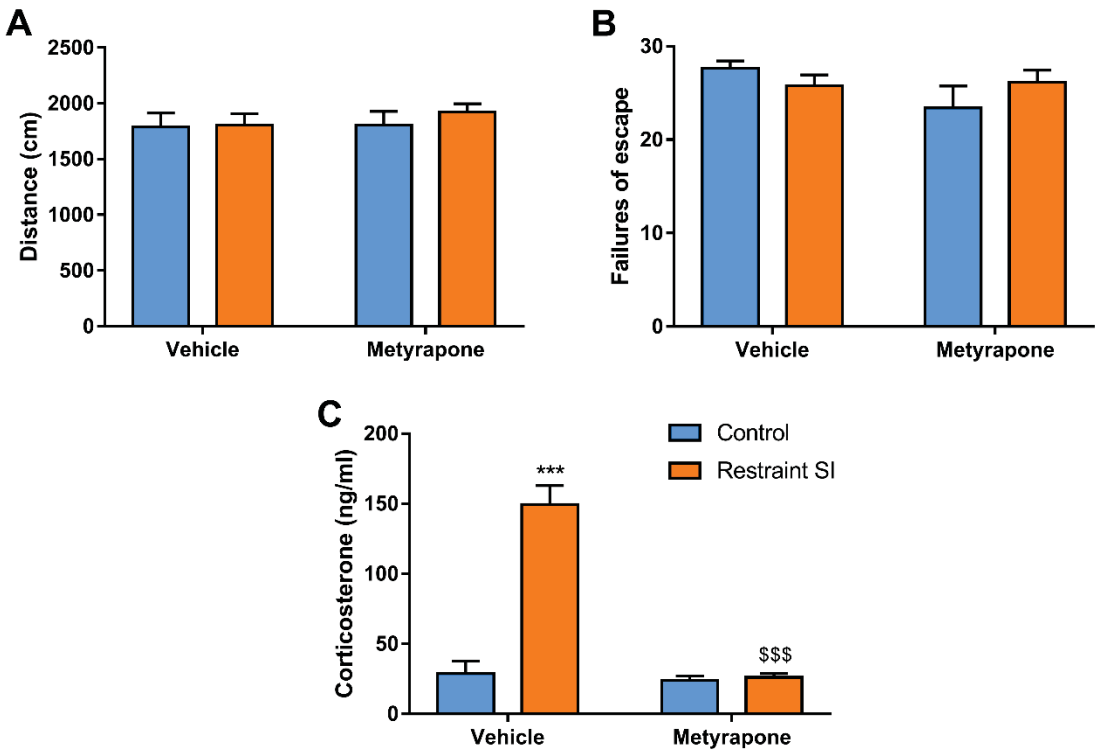

Figure 5. Effects of blocking corticosterone secretion during restraint stress inoculation in adult male and female C57BL/6 mice. (A) Total distance travelled in the OF; n=12-13/group. (B) Failures to escape in the learned helplessness paradigm; n=8/group. (C) Corticosterone secreted during the first stress inoculation session and metyrapone treatment; n=6-8/group. Data are presented as mean ± SEM and analysed by two-way ANOVA. ***p<0.001 in comparison to con-veh; $$$p<0.001 in comparison to SI-veh.

4 DISCUSSION

This study attempted to replicate a stress inoculation model in mice to investigate the involvement of corticosterone in the resilience effect. However, the three attempts to reproduce the model were unsuccessful. None of the protocols shifted coping behavior in the forced swim test to an active strategy, nor reduced anxiety in the elevated plus maze, nor reduced corticosterone secretion in response to a heterotypic stressor. There was also no difference when the effects of restraint SI were tested in the learned helplessness paradigm. In the first experiment social SI actually increased anxiety-like behavior in the EPM compared to the group saline injection. And in the second experiment, social SI groups had lower corticosterone levels, but it is not possible to affirm that they were less responsive to the FST stress in the absence of significant interaction. Our results did not replicate the findings of Brockhurst et al. [8] and Lyons et al. [9] who performed a protocol of social SI in males and females C57BL/6 mice, respectively, and found measures of increased active coping in the open field, object exploration and tail suspension test and decreased corticosterone secretion following acute 15-min restraint. Although the tests we used to evaluate resilience were not identical to theirs, similar results would be expected, especially in experiment 2 in which the stress inoculation procedure was essentially the same. Different results might have arisen due to strain, observer and laboratory variability in procedures and/or different sensitivity of the tests used to detect alterations in behavior.

It has been suggested that resilience or adaptation to a novel stressor following exposure to a homotypic stressor can happen at some degree and for some behavioral and physiological parameters, but usually not all aspects of the stress response [15,16]. For example, Pol et al. [17] showed that rats previously exposed chronically to immobilization and acutely to tail shock struggled more in the forced swim test than controls, but there was no difference in behavior in the hole board test. Pastor-Ciurana et al. [18] observed that previous chronic stress protected rats from the hypo-activity induced by acute immobilization and attenuated the reduction of weight gain and saccharin consumption induced by this acute stressor, but it did not affect active coping during the acute immobilization. Armario et al. [15] showed that previous chronic immobilization did not alter ACTH and lactate response to acute shock but attenuated inhibition of body weight gain caused by chronic shock. Therefore, cross-adaptation seems to depend on the variables chosen to study the

phenomenon. Another factor of importance for the occurrence of cross-adaptation is which stressors are selected for the protocol [19]. Rats handled in infancy with brief maternal separation have shown lowered corticosterone secretion when exposed to the open field [7] but increased when exposed to foot shock [20]. Also, chronic adrenocorticotropic hormone (ACTH) administration blunts corticosterone secretion in response to 1 h, but not to 18 h restraint stress [21]. These observations show that occurrence of cross adaptation is not a straight-forward, easily detected phenomenon. It can be argued that it is not always observed because most studies use middle-intensity to severe stressors, in opposition to stress inoculation whose principle is to expose individuals to low-intensity, intermittent stress. However, our work provides evidence for the non-occurrence of cross adaptation even when mild stressors are used.

In spite of the lack of cross-adaptation, we did observe adaptation to the homotypic stress during both social and restraint SI. Our data from the first and last sessions were obtained from different cohorts of mice, hindering the conduction of a repeated-measures analysis, but we performed a two-way ANOVA and observed significant reduction of corticosterone secretion in the last session. The reduction is clear for both SI paradigms, being approximately 4 times lower for social SI (mean ± SEM: 147.2 ± 16.4 vs. 34.1 ± 4.2; first vs. last session, respectively) and 1.5 times lower in restraint SI (mean ± SEM: 151.6 ± 13.3 vs. 93.7 ± 12.6; first vs. last session, respectively). This result is in accordance with most reports in literature, which describe a decrease in glucocorticoid response following repeated exposure to a homotypic stressor [22–25] but opposes the reports of Brockhurst et al. [8] and Lyons et al. [9], who found that corticosterone response does not diminish across social SI sessions in males [8] and even increases in females [9]. Reduction of corticosterone secretion following repeated exposure to the same stressor seems to depend on the intensity of the stressor and its ethological relevance [26], usually not being observed with more intense stressors [23,27–29]. For example, repeated exposure to restraint stress usually leads to adaptation [30–32] but social defeat has been shown to lead to sensitization [33] and animals witnessing chronic social defeat present increases in serum corticosterone similarly to those acutely exposed to the stress [34]. Although social stress may be more intense than restraint, our results show that adaptation of corticosterone response can occur in social stress as well.

In general, the literature reports that repeated exposure to a homotypic stressor leads to adaptation of the corticosterone response whereas exposure to a novel stressor elicits similar corticosterone secretion despite previous stress experience [30,35], observation with which our results agree. Yet, Brockhurst et al. [8] observed the opposite: non-adaptation to the homotypic stressor but adaptation to a heterotypic one. This raises the question of whether non-adaptation of the corticosterone response is necessary for cross-adaptation. However, Armario et al. [15] demonstrated that rats exposed chronically to immobilization did not show reduced corticosterone to an acute immobilization nor to acute shock, in other words, even in the absence of adaptation to the homotypic stress, cross-adaptation did not occur.

Although we were not able to observe the resilience effect in our experiments, our study does allow for some conclusions regarding the role of corticosterone in stress inoculation. As discussed above, both our SI protocols performed in C57BL/6 elicited a clear corticosterone response, which was still significant in the last session, despite less intense. Therefore, we can conclude that corticosterone is not sufficient for the effect, because the secretion of corticosterone during the protocol did not lead to changes in behavior and HPA axis responsiveness to a novel stressor. However, our study cannot respond our initial question of whether corticosterone is necessary for stress inoculation, because corticosterone would have to be blocked in an SI model that consistently alters behavior and physiological parameters.

Our stress inoculation protocols did not affect most of the parameters we analyzed, but we observed an unexpected increase in locomotion in the open field following both social SI as restraint SI in the C57BL/6 mice. We had previously observed increased locomotion in Swiss mice in the open field following a stress inoculation protocol involving predator threat stress (unpublished data). In the present study, the result was observed both in males and females. Therefore, increased locomotion in the open field following mild stress appears to be rather consistent in our laboratory. However, Lyons et al. [9] reported that the females exposed to social SI did not present alterations of locomotion in the open field and Brockhurst et al. [8] did not mention specific effects on locomotion. On the other hand, both studies reported less freezing in the open field in mice exposed to SI, and, although our mice did not freeze in the open field, the results might share some common behavioral ground in that both outcomes point to SI increasing activity in a

novel environment. Although the literature does not present consistent data on the effects of stress on locomotion, with some authors reporting it decreased or unaltered [18,19,36,37], several studies observe increased open field locomotion as an effect of stress [38–42]. However, the meaning of increased locomotion is still uncertain. Some associate it with a decrease in anxiety and fear [7,40], others with increase in depressive-like behavior [39] and others with an unspecific effect of stress [42]. Strekalova et al. [42] showed that the hyperlocomotion observed in the open field after 4 weeks of chronic stress is abolished when the animals are tested under dim illumination (5 lux) or are administered a low dose of diazepam before testing, suggesting that hyperlocomotion might be triggered under mildly stressful conditions. Our data adds evidence to the interpretation that increased locomotion in the open field is an unspecific effect of stress, as we did not observe alterations in anxiety- and depression-like behavior in the elevated plus maze, forced swim test and learned helplessness. In addition, in experiment 4 we used a non-invasive test for stress reactivity proposed by Zimprich et al. [14] to evaluate the interaction between restraint SI and an acute restraint session in female C57BL/6. The results showed that the effect of restraint SI are very similar to the effects of the acute restraint, in other words, the restraint SI did not reduce stress reactivity as would be predicted by stress inoculation theory.

Nevertheless, neurobiological bases of stress-induced hyperlocomotion are still uncertain, therefore, we decided to block corticosterone secretion during SI sessions using metyrapone to test if this effect is dependent on corticosterone. Curiously, in this experiment stress inoculation did not increase locomotion, limiting our conclusions on the role of corticosterone. It is unclear why this happened, but it is possible that combining the restraint stress with the stress from receiving intraperitoneal injections produces different effects than each stressor individually. To confirm this, further experiments need to be performed. Yet, we can say that corticosterone is not sufficient to generate hyperlocomotion in the open field because we observed increases in corticosterone secretion but not increased locomotion.

Another hypothesis for the increased locomotion observed in stress inoculated mice concerns the expression of dopamine receptors (DR) in the ventral striatum. It is known that the activation of DRs in the nucleus accumbens leads to increased locomotor activity [43-45]. And stress inoculation has been shown to increase the expression of DRD2/3 in the ventral striatum of squirrel monkeys [11]. Therefore, it is

possible that stress inoculation increases locomotion in the open field through greater dopaminergic signaling in the ventral striatum. However, our experiments were not designed to test this hypothesis, not allowing further discussion

Finally, our experiments performed with animals receiving saline injections and being transported to the experimental room demonstrated that these manipulations do not alter locomotion, anxiety-like behavior nor coping strategy. In C57BL/6, transportation did not elicit a corticosterone response detectable 20 minutes following the procedure, but injection did, even after the last injection. This also corroborates our conclusion that corticosterone secretion is not sufficient for the inoculation effect. Swiss mice who received the injections appeared to be sensitized to the FST, secreting more corticosterone, but there were no effects on behavior.

In conclusion, our study shows that corticosterone secretion during mild and intermittent stress is not sufficient to generate resilience. We cannot conclude whether it is necessary because we failed to replicate the behavioral and physiological effects of stress inoculation. We demonstrated a hyperlocomotion induced by stress inoculation, also showing that corticosterone is not sufficient for this effect. We were unable to conclude if it is necessary. Most likely corticosterone plays a role both in creating resilience after mild stress and inducing hyperlocomotion, but other factors might be involved as well. Brockhurst et al. suggest that corticosterone secretion during stress inoculation places glucocorticoid signaling as a potential target in the development of new preventive or therapeutic interventions in psychiatry. Understanding the mechanisms underlying stress inoculation is important in translational research, however, the involvement of glucocorticoids in stress inoculation seems to be complex and more studies are necessary before translating its applicability to clinical research.

REFERENCES

[1] E.J. Nestler, M. Barrot, R.J. DiLeone, A.J. Eisch, S.J. Gold, L.M. Monteggia, Neurobiology of depression., Neuron. 34 (2002) 13–25. http://www.ncbi.nlm.nih.gov/pubmed/11931738.

[2] M.R. Gunnar, K. Frenn, S.S. Wewerka, M.J. Van Ryzin, Moderate versus severe early life stress : Associations with stress reactivity and regulation in 10 — 12-year-old children, (2009) 62–75. https://doi.org/10.1016/j.psyneuen.2008.08.013.

[3] D.M. Lyons, K.J. Parker, Stress inoculation-induced indications of resilience in monkeys, J. Trauma. Stress. 20 (2007) 423–433. https://doi.org/10.1002/jts.20265.

[4] M.D. Seery, R.J. Leo, S.P. Lupien, C.L. Kondrak, J.L. Almonte, An Upside to Adversity?: Moderate Cumulative Lifetime Adversity Is Associated With Resilient Responses in the Face of Controlled Stressors, Psychol. Sci. 24 (2013) 1181–1189. https://doi.org/10.1177/0956797612469210.

[5] K.J. Parker, C.L. Buckmaster, A.F. Schatzberg, D.M. Lyons, Prospective investigation of stress inoculation in young monkeys, Arch Gen Psychiatry. 61 (2004) 933–941. https://doi.org/10.1001/archpsyc.61.9.933.

[6] K.J. Parker, C.L. Buckmaster, S.A. Hyde, A.F. Schatzberg, D.M. Lyons, Nonlinear relationship between early life stress exposure and subsequent resilience in monkeys, Sci. Rep. 9 (2019) 16232. https://doi.org/10.1038/s41598-019-52810-5.

[7] S. Levine, G.C. Haltmeyer, G.G. Karas, V.H. Denenberg, Physiological and behavioral effects of infantile stimulation, Physiol. Behav. 2 (1967) 55–59. https://doi.org/10.1016/0031-9384(67)90011-X.

[8] J. Brockhurst, C. Cheleuitte-Nieves, C.L. Buckmaster, A.F. Schatzberg, D.M. Lyons, Stress inoculation modeled in mice, Transl. Psychiatry. 5 (2015) e537. https://doi.org/10.1038/tp.2015.34.

[9] D.M. Lyons, C.L. Buckmaster, A.F. Schatzberg, Learning to actively cope with stress in female mice, Psychoneuroendocrinology. 96 (2018) 78–83. https://doi.org/10.1016/j.psyneuen.2018.06.010.

[10] M. Katz, C. Liu, M. Schaer, K.J. Parker, M.C. Ottet, A. Epps, C.L. Buckmaster, R. Bammer, M.E. Moseley, A.F. Schatzberg, S. Eliez, D.M. Lyons, Prefrontal plasticity and stress inoculation-induced resilience, Dev. Neurosci. 31 (2009) 293–299. https://doi.org/10.1159/000216540.

[11] A.G. Lee, J.M. Nechvatal, B. Shen, C.L. Buckmaster, M.J. Levy, F.T. Chin, A.F. Schatzberg, D.M. Lyons, Striatal dopamine D2/3 receptor regulation by stress inoculation in squirrel monkeys, Neurobiol. Stress. 3 (2016) 68–73. https://doi.org/10.1016/j.ynstr.2016.02.001.

[12] A.G. Lee, C.L. Buckmaster, E. Yi, A.F. Schatzberg, D.M. Lyons, Coping and glucocorticoid receptor regulation by stress inoculation, Psychoneuroendocrinology. 49 (2014) 272–279. https://doi.org/10.1016/j.psyneuen.2014.07.020.

[13] J.M. Baek, S.C. Kwak, J.Y. Kim, S.J. Ahn, H.Y. Jun, K.H. Yoon, M.S. Lee, J. Oh, Evaluation of a novel technique for intraperitoneal injections in mice, Lab Anim. (NY). 44 (2015) 440–444. https://doi.org/10.1038/laban.880.

[14] A. Zimprich, L. Garrett, J.M. Deussing, C.T. Wotjak, H. Fuchs, C.P. Gilman, A robust and reliable non-invasive test for stress responsivity in mice, 8 (2014) 1–12. https://doi.org/10.3389/fnbeh.2014.00125.

[15] A. Armario, J. Hidalgo, M. Giralt, Evidence that the pituitary-adrenal axis does not cross-adapt to stressors: comparison to other physiological variables., Neuroendocrinology. 47 (1988) 263–7. https://doi.org/10.1159/000124921.

[16] E. Chauhan, A. Bali, N. Singh, A.S. Jaggi, Cross stress adaptation: Phenomenon of interactions between homotypic and heterotypic stressors, Life Sci. 137 (2015) 98–104. https://doi.org/10.1016/j.lfs.2015.07.018.

[17] O. Pol, L. Campmany, M. Gil, A. Armario, Behavioral and neurochemical changes in response to acute stressors: Influence of previous chronic exposure to immobilization, Pharmacol. Biochem. Behav. 42 (1992) 407–412. https://doi.org/10.1016/0091-3057(92)90133-Z.

[18] J. Pastor-Ciurana, C. Rabasa, J.A. Ortega-Sánchez, M. Sanchís-Ollè, M. Gabriel-Salazar, M. Ginesta, X. Belda, N. Daviu, R. Nadal, A. Armario, Prior exposure to repeated immobilization or chronic unpredictable stress protects from some negative sequels of an acute immobilization, Behav. Brain Res. 265 (2014) 155–162. https://doi.org/10.1016/j.bbr.2014.02.028.

[19] E. Chauhan, A. Bali, N. Singh, A.S. Jaggi, Pharmacological investigations on cross adaptation in mice subjected to stress immobilization, Life Sci. 127 (2015) 98–105. https://doi.org/10.1016/j.lfs.2015.02.015.

[20] S. Levine, Plasma-Free Corticosteroid Response to Electric Shock in Rats Stimulated in Infancy, Science (80-.). 135 (1962) 795–796. https://doi.org/10.1126/science.135.3506.795-a.

[21] M. Giralt, C. Garcia-Marquez, A. Armario, Previous chronic ACTH administration does not protect against the effects of acute or chronic stress in male rats, Physiol. Behav. 40 (1987) 165–170. https://doi.org/10.1016/0031-9384(87)90202-2.

[22] C. Rabasa, H. Gagliano, J. Pastor-Ciurana, S. Fuentes, X. Belda, R. Nadal, A. Armario, Adaptation of the hypothalamus-pituitary-adrenal axis to daily repeated stress does not follow the rules of habituation: A new perspective, Neurosci. Biobehav. Rev. 56 (2015) 35–49.

https://doi.org/10.1016/j.neubiorev.2015.06.013.

[23] O. Martí, A. Armario, Anterior pituitary response to stress: Time-related changes and adaptation, Int. J. Dev. Neurosci. 16 (1998) 241–260. https://doi.org/10.1016/S0736-5748(98)00030-6.

[24] G.J. Kant, J.R. Leu, S.M. Anderson, E.H. Mougey, Effects of chronic stress on plasma corticosterone, ACTH and prolactin, Physiol. Behav. 40 (1987) 775–779. https://doi.org/10.1016/0031-9384(87)90282-4.

[25] J.P. Herman, Neural control of chronic stress adaptation., Front. Behav. Neurosci. 7 (2013) 61. https://doi.org/10.3389/fnbeh.2013.00061.

[26] C.J. Barnum, P. Blandino, T. Deak, Adaptation in the corticosterone and hyperthermic responses to stress following repeated stressor exposure, J. Neuroendocrinol. 19 (2007) 632–642. https://doi.org/10.1111/j.1365-2826.2007.01571.x.

[27] B.H. Natelson, J.E. Ottenweller, J.A. Cook, D. Pitman, R. McCarty, W.N. Tapp, Effect of stressor intensity on habituation of the adrenocortical stress response, Physiol. Behav. 43 (1988) 41–46. https://doi.org/10.1016/0031-9384(88)90096-0.

[28] C. Rabasa, R. Delgado-Morales, A. Gomez-Roman, R. Nadal, A. Armario, Adaptation of the pituitary-adrenal axis to daily repeated forced swim exposure in rats is dependent on the temperature of water, Stress. 16 (2013) 698–705. https://doi.org/10.3109/10253890.2013.824964.

[29] D.L. Pitman, J.E. Ottenweller, B.H. Natelson, Effect of Stressor Intensity on Habituation and Sensitization of Glucocorticoid Responses in Rats, Behav. Neurosci. 104 (1990) 28–36. https://doi.org/10.1037/0735-7044.104.1.28.

[30] J.A. Babb, C. V. Masini, H.E.W. Day, S. Campeau, Habituation of hypothalamic–pituitary–adrenocortical axis hormones to repeated homotypic stress and subsequent heterotypic stressor exposure in male and female rats, Stress. 17 (2014) 224–234. https://doi.org/10.3109/10253890.2014.905534.

[31] M. Girotti, T.W.W. Pace, R.I. Gaylord, B.A. Rubin, J.P. Herman, R.L. Spencer, Habituation to repeated restraint stress is associated with lack of stress-induced c-fos expression in primary sensory processing areas of the rat brain, Neuroscience. 138 (2006) 1067–1081. https://doi.org/10.1016/j.neuroscience.2005.12.002.

[32] S. Gong, Y.L. Miao, G.Z. Jiao, M.J. Sun, H. Li, J. Lin, M.J. Luo, J.H. Tan, Dynamics and correlation of serum cortisol and corticosterone under different physiological or stressful conditions in mice, PLoS One. 10 (2015) 1–14. https://doi.org/10.1371/journal.pone.0117503.

[33] H. Engler, A. Engler, M.T. Bailey, J.F. Sheridan, Tissue-specific alterations in the glucocorticoid sensitivity of immune cells following repeated social defeat in

mice, J. Neuroimmunol. 163 (2005) 110–119. https://doi.org/10.1016/j.jneuroim.2005.03.002.

[34] B.L. Warren, V.F. Vialou, S.D. Iñiguez, L.F. Alcantara, K.N. Wright, J. Feng, P.J. Kennedy, Q. Laplant, L. Shen, E.J. Nestler, C.A. Bolaños-Guzmán, Neurobiological sequelae of witnessing stressful events in adult mice, Biol. Psychiatry. 73 (2013) 7–14. https://doi.org/10.1016/j.biopsych.2012.06.006.

[35] A. Armario, A. Lopez-Calderon, T. Jolin, J. Balasch, Response of anterior pituitary hormones to chronic stress. The specificity of adaptation, Neurosci. Biobehav. Rev. 10 (1986) 245–250. https://doi.org/10.1016/0149-7634(86)90011-4.

[36] C. Hu, Y. Luo, H. Wang, S. Kuang, G. Liang, Y. Yang, S. Mai, J. Yang, Re-evaluation of the interrelationships among the behavioral tests in rats exposed to chronic unpredictable mild stress, PLoS One. 12 (2017) e0185129. https://doi.org/10.1371/journal.pone.0185129.

[37] A. Armario, C. Restrepo, J.M. Castellanos, J. Balasch, Dissociation between adrenocorticotropin and corticosterone responses to restraint after previous chronic exposure to stress., Life Sci. 36 (1985) 2085–92. https://doi.org/10.1016/0024-3205(85)90304-2.

[38] M.C. Pardon, F. Pérez-Diaz, C. Joubert, C. Cohen-Salmon, Age-dependent effects of a chronic ultramild stress procedure on open- field behaviour in B6D2F1 female mice, Physiol. Behav. 70 (2000) 7–13. https://doi.org/10.1016/S0031-9384(00)00216-X.

[39] S.J. Alonso, C. Damas, E. Navarro, Behavioral despair in mice after prenatal stress, J. Physiol. Biochem. 56 (2000) 77–82. https://doi.org/10.1007/BF03179902.

[40] E. Lee, M. Tsai, C. Chai, Region Activity of Mice in an Open Field, Physiol. Behav. 37 (1986) 659–662.

[41] S. Zhu, R. Shi, J. Wang, J.F. Wang, X.M. Li, Unpredictable chronic mild stress not chronic restraint stress induces depressive behaviours in mice, Neuroreport. 25 (2014) 1151–1155. https://doi.org/10.1097/WNR.0000000000000243.

[42] T. Strekalova, R. Spanagel, O. Dolgov, D. Bartsch, Stress-induced hyperlocomotion as a confounding factor in anxiety and depression models in mice, (2001) 171–180.

[43] J.K. Dreher, D.M. Jackson, Role of D1 and D2 dopamine receptors in mediating locomotor activity elicited from the nucleus accumbens of rats, Brain Res. 487 (1989) 267–277. https://doi.org/10.1016/0006-8993(89)90831-7.

[44] S. Barik, R. De Beaurepaire, Dopamine D3 modulation of locomotor activity and sleep in the nucleus accumbens and in lobules 9 and 10 of the cerebellum

in the rat, Prog. Neuro-Psychopharmacology Biol. Psychiatry. 29 (2005) 718–726. https://doi.org/10.1016/j.pnpbp.2005.04.020.

[45] J. Anyan, S. Amir, Too Depressed to Swim or Too Afraid to Stop? A Reinterpretation of the Forced Swim Test as a Measure of Anxiety-Like Behavior, Neuropsychopharmacology. 43 (2018) 931–933. https://doi.org/10.1038/npp.2017.260.

4 CONCLUSÕES

O presente estudo procurou investigar o envolvimento da corticosterona no efeito de resiliência da inoculação de estresse. Para este fim, três modelos de inoculação foram testados para validação. O primeiro consistiu num protocolo de estresse social em camundongos Swiss jovens. O segundo foi similar ao primeiro com a exceção dos sujeitos experimentais, os quais foram substituídos por camundongos C57BL/6. O terceiro, também realizado em camundongos C57BL/6, consistiu num protocolo de restrição de movimento.

Os resultados demonstraram que a secreção de corticosterona durante sessões de inoculação de estresse não é suficiente para induzir resiliência. Apesar do aumento de secreção de corticosterona, significativo mesmo após a última sessão, não foram observadas mudanças comportamentais ou na responsividade do eixo HPA a um estressor heterotípico. No entanto, nosso estudo não foi capaz de responder se a corticosterona é necessária para o efeito, pois para este fim o hormônio deveria ser bloqueado num modelo em que se verificassem os efeitos esperados.

De fato, nenhum dos três protocolos realizados foi capaz de gerar resiliência. Não foi constatado aumento da estratégia de manejo ativa no nado forçado, nem redução da ansiedade no labirinto em cruz ou redução da secreção de corticosterona após exposição a um estressor heterotípico. Os efeitos da inoculação de estresse por restrição de movimento foram testados no paradigma do desamparo aprendido, mas também não se observaram alterações.

Apesar da ausência de efeitos nos testes para avaliação de comportamento tipo-depressivo e tipo-ansioso, foi detectado um aumento da locomoção no campo aberto induzida pelos dois protocolos de inoculação de estresse realizados em camundongos C57BL/6 . Além disso, o efeito também foi identificado em fêmeas. Na tentativa de investigar se a hiperlocomoção é dependente de corticosterona, administramos metirapona, um inibidor de síntese de glicocorticoides, antes de cada uma das sessões de inoculação de estresse por restrição de movimento. No entanto, nesse experimento a inoculação não levou a um aumento de locomoção. Apesar desta limitação, observou-se o aumento de corticosterona sérica durante as sessões, e seu bloqueio pela metirapona, indicando que a liberação de

corticosterona induzida pelas sessões de inoculação de estresse não é suficiente para o efeito hiperlocomotor.

Em consonância com a literatura, os dois protocolos em que a secreção de corticosterona induzida pela inoculação foi avaliada demonstraram habituação da resposta de corticosterona, com clara redução após a última sessão em comparação com a primeira.

Por último, os experimentos realizados com animais recebendo injeções intraperitoneais de salina e sendo transportados para a sala de experimentos demonstraram que essas manipulações não afetam locomoção, comportamento tipo-ansioso ou estratégia de manejo a um estressor agudo.

Em conclusão, os resultados deste trabalho indicam que a secreção de corticosterona durante estressores brandos e intermitentes não é suficiente para levar ao efeito de resiliência.

REFERÊNCIAS

AMERICAN PSYCHIATRIC ASSOCIATION. **Diagnostic and Statistical Manual of Mental Disorders**, Fifth Edition (DSM-V). Arlington, VA: American Psychiatric Association, 2013.

BEARDS, S.; GAYER-ANDERSON, C.; BORGES, S.; et al. Life events and psychosis: A review and meta-analysis. **Schizophrenia Bulletin**, v. 39, n. 4, p. 740–747, 2013.

BEBBINGTON, P. Misery and the of Disease Theories of Depression. **International Journal of Social Psychiatry**, v. 33, n. 1, p. 13–20, 1987. Disponível em: <http://journals.sagepub.com/doi/10.1177/002076408703300102>. .

BROCKHURST, J.; CHELEUITTE-NIEVES, C.; BUCKMASTER, C. L.; SCHATZBERG, A. F.; LYONS, D. M. Stress inoculation modeled in mice. **Translational Psychiatry**, v. 5, n. 3, p. e537, 2015. Nature Publishing Group. Disponível em: <http://www.nature.com/doifinder/10.1038/tp.2015.34>. .

BUIJS, R. M.; VAN EDEN, C. G. The integration of stress by the hypothalamus, amygdala and prefrontal cortex: Balance between the autonomic nervous system and the neuroendocrine system. **Progress in Brain Research**, v. 126, n. June 2013, p. 117–132, 2000.

CHROUSOS, G. P. Stress and disorders of the stress system. **Nature Reviews Endocrinology**, v. 5, n. 7, p. 374–381, 2009. Nature Publishing Group. Disponível em: <http://dx.doi.org/10.1038/nrendo.2009.106>. .

COLODRO-CONDE, L.; COUVY-DUCHESNE, B.; ZHU, G.; et al. A direct test of the diathesis–stress model for depression. **Molecular Psychiatry**, v. 23, n. 7, p. 1590–1596, 2018.

CROFTON, E. J.; ZHANG, Y.; GREEN, T. A. Inoculation stress hypothesis of environmental enrichment. **Neuroscience and Biobehavioral Reviews**, v. 49, p. 19–31, 2015. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.neubiorev.2014.11.017>. .

DAYAS, C. V; BULLER, K. M.; CRANE, J. W.; XU, Y.; DAY, T. A. Stressor categorization: acute physical and psychological stressors elicit distinctive recruitment. **Eur J Neurosci**, v. 14, n. 7, p. 1143–1152, 2001.

DIENSTBIER, R. A. Arousal and physiological toughness: Implications for mental and physical health. **Psychological Review**, v. 96, n. 1, p. 84–100, 1989. Disponível em: <http://doi.apa.org/getdoi.cfm?doi=10.1037/0033-295X.96.1.84>. .

EDGE, M. D.; RAMEL, W.; DRABANT, E. M.; et al. For better or worse? Stress inoculation effects for implicit but not explicit anxiety. **Depression and Anxiety**, v. 26, n. 9, p. 831–837, 2009.

ERICKSON, K.; DREVETS, W.; SCHULKIN, J. Glucocorticoid regulation of diverse

cognitive functions in normal and pathological emotional states. **Neuroscience and Biobehavioral Reviews**, v. 27, n. 3, p. 233–246, 2003.

ETAIN, B.; HENRY, C.; BELLIVIER, F.; MATHIEU, F.; LEBOYER, M. Beyond genetics: Childhood affective trauma in bipolar disorder. **Bipolar Disorders**, v. 10, n. 8, p. 867–876, 2008.

GOLD, P. W. The neurobiology of stress and its relevance to psychotherapy. **Clinical Neuroscience Research**, v. 4, n. 5-6 SPEC. ISS., p. 315–324, 2005.

GOLD, P. W. The organization of the stress system and its dysregulation in depressive illness. **Molecular Psychiatry**, v. 20, n. 1, p. 32–47, 2015. Nature Publishing Group. Disponível em: <http://dx.doi.org/10.1038/mp.2014.163>. .

GRAY, J. D.; KOGAN, J. F.; MARROCCO, J.; MCEWEN, B. S. Genomic and epigenomic mechanisms of glucocorticoids in the brain. **Nature Reviews Endocrinology**, v. 13, n. 11, p. 661–673, 2017. Nature Publishing Group. Disponível em: <http://dx.doi.org/10.1038/nrendo.2017.97>. .

GUNNAR, M. R.; FRENN, K.; WEWERKA, S. S.; VAN RYZIN, M. J. Moderate versus severe early life stress: Associations with stress reactivity and regulation in 10–12-year-old children. **Psychoneuroendocrinology**, v. 34, n. 1, p. 62–75, 2009. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0306453008002175>. .

HERMAN, J. P. Neural control of chronic stress adaptation. **Frontiers in behavioral neuroscience**, v. 7, n. August, p. 61, 2013. Disponível em: <http://journal.frontiersin.org/article/10.3389/fnbeh.2013.00061/abstract>. .

HERMAN, J. P.; FIGUEIREDO, H.; MUELLER, N. K.; et al. Central mechanisms of stress integration: Hierarchical circuitry controlling hypothalamo-pituitary-adrenocortical responsiveness. **Frontiers in Neuroendocrinology**, v. 24, n. 3, p. 151–180, 2003.

HERMAN, J. P.; MCKLVEEN, J. M.; GHOSAL, S.; et al. Regulation of the hypothalamic-pituitary- adrenocortical stress response. **Comprehensive Physiology**, v. 6, n. 2, p. 603–621, 2016.

JOËLS, M. Corticosteroids and the brain. **Journal of Endocrinology**, v. 238, n. 3, p. R121–R130, 2018.

KATZ, M.; LIU, C.; SCHAER, M.; et al. Prefrontal plasticity and stress inoculation-induced resilience. **Developmental Neuroscience**, v. 31, n. 4, p. 293–299, 2009.

KELLER-WOOD, M. Hypothalamic-pituitary-adrenal axis-feedback control. **Comprehensive Physiology**, v. 5, n. 3, p. 1161–1182, 2015.

KESSLER, R. C.; BORGES, G.; WALTERS, E. E. Prevalence of and risk factors for lifetime suicide attempts in the National Comorbidity Survey. **rchives of General Psychiatry**, v. 56, n. 7, p. 617–626, 1999.

KLAUKE, B.; DECKERT, J.; REIF, A.; PAULI, P.; DOMSCHKE, K. Life Events in panic disorder-An update on “candidate stressors”. **Depression and Anxiety**, v. 27, n. 8, p. 716–730, 2010.

KORTE, S. M.; KOOLHAAS, J. M.; WINGFIELD, J. C.; MCEWEN, B. S. The Darwinian concept of stress: Benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. **Neuroscience and Biobehavioral Reviews**, v. 29, n. 1 SPEC. ISS., p. 3–38, 2005.

LEE, A. G.; BUCKMASTER, C. L.; YI, E.; SCHATZBERG, A. F.; LYONS, D. M. Coping and glucocorticoid receptor regulation by stress inoculation. **Psychoneuroendocrinology**, v. 49, n. 1, p. 272–279, 2014. Elsevier Ltd. Disponível em: <http://dx.doi.org/10.1016/j.psyneuen.2014.07.020>. .

LEE, A. G.; NECHVATAL, J. M.; SHEN, B.; et al. Striatal dopamine D2/3 receptor regulation by stress inoculation in squirrel monkeys. **Neurobiology of Stress**, v. 3, p. 68–73, 2016. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S2352289515300357>. .

LEMAIRE, V.; PIAZZA, P. V.; LE MOAL, M. Glucocorticoids and motivated behaviour. **Techniques in the Behavioral and Neural Sciences**. v. 15, p.341–358, 2005. Elsevier Masson SAS. Disponível em: <https://linkinghub.elsevier.com/retrieve/pii/S0921070905800203>. .

LEVINE, S. Infantile experience and resistance to physiological stress. **Science (New York, N.Y.)**, v. 126, n. 3270, p. 405, 1957. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/13467220>. .

LEVINE, S.; HALTMEYER, G. C.; KARAS, G. G.; DENENBERG, V. H. Physiological and behavioral effects of infantile stimulation. **Physiology & Behavior**, v. 2, n. 1, p. 55–59, 1967. Disponível em: <http://www.sciencemag.org/cgi/doi/10.1126/science.126.3270.405>. .

LIU, R. T. A developmentally informed perspective on the relation between stress and psychopathology: when the problem with stress is that there is not enough. **Journal of abnormal psychology**, v. 124, n. 1, p. 80–92, 2015. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/25688435>. .

LOOSEN, P. T. Cushing’s Syndrome and Depression. **The Endocrinologist**, v. 4, n. 5, p. 373–382, 1994. Disponível em: <https://journals.lww.com/theendocrinologist/abstract/1994/09000/cushing_s_syndrome_and_depression.8.aspx>. .

LYONS, D. M.; BUCKMASTER, C. L.; SCHATZBERG, A. F. Learning to actively cope with stress in female mice. **Psychoneuroendocrinology**, v. 96, n. June, p. 78–83, 2018. Elsevier. Disponível em: <https://doi.org/10.1016/j.psyneuen.2018.06.010>. .

LYONS, D. M.; BUCKMASTER, P. S.; LEE, A. G.; et al. Stress coping stimulates hippocampal neurogenesis in adult monkeys. **Proceedings of the National**

Academy of Sciences of the United States of America, v. 107, n. 33, p. 14823–14827, 2010.

LYONS, D. M.; MARTEL, F. L.; LEVINE, S.; RISCH, N. J.; SCHATZBERG, A. F. Postnatal Experiences and Genetic Effects on Squirrel Monkey Social Affinities and Emotional Distress. **Hormones and Behavior**, v. 36, n. 3, p. 266–275, 1999. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0018506X99915477>. .

MARTÍ, O.; ARMARIO, A. Anterior pituitary response to stress: Time-related changes and adaptation. **International Journal of Developmental Neuroscience**, v. 16, n. 3–4, p. 241–260, 1998.

MCEWEN, B. S. Glucocorticoid receptors in the brain . **Hosp.Pract.(Off Ed)** , v. 23, n. 8750–2836 (Print), p. 107–121, 1988.

MCEWEN, B. S. Physiology and neurobiology of stress and adaptation: Central role of the brain. **Physiological Reviews**, v. 87, n. 3, p. 873–904, 2007.

MCEWEN, B. S.; GIANAROS, P. J. Central role of the brain in stress and adaptation: Links to socioeconomic status, health, and disease. **Annals of the New York Academy of Sciences**, v. 1186, n. 1, p. 190–222, 2010. Disponível em: <http://doi.wiley.com/10.1111/j.1749-6632.2009.05331.x>. .

MCEWEN, B. S.; SAPOLSKY, R. M. Stress and cognitive function. **Current Opinion in Neurobiology**, v. 5, n. 2, p. 205–216, 1995.

MCEWEN, B. S.; STELLAR, E. Stress and the individual. Mechanisms leading to disease. **Archives of internal medicine**, v. 153, n. 18, p. 2093–101, 1993. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/8379800>. .

MEICHENBAUM, D. Stress Inoculation Training: A Preventative and Treatment Approach. **Principles and Practice of Stress Management**, 2007. Disponível em: <http://psycnet.apa.org/record/2007-14796-019>. .

MEICHENBAUM, D.; NOVACO, R. Stress Inoculation: A Preventative Approach. **Issues in Mental Health Nursing**, v. 7, n. 1–4, p. 419–435, 1985.

MIFSUD, K. R.; REUL, J. M. H. M. Mineralocorticoid and glucocorticoid receptor-mediated control of genomic responses to stress in the brain. **Stress**, v. 21, n. 5, p. 389–402, 2018. Informa UK Ltd. Disponível em: <https://doi.org/10.1080/10253890.2018.1456526>. .

MINEKA, S.; ZINBARG, R. A contemporary learning theory perspective on the etiology of anxiety disorders: It's not what you thought it was. **American Psychologist**, v. 61, n. 1, p. 10–26, 2006.

MONROE, S. M.; SIMONS, A. D. Diathesis stress theories in the context of life stress research - implications for the depressive-disorders. **Psychological Bulletin**, v. 110, n. 3, p. 406–425, 1991.

MORRIS, M. C.; RAO, U. Cortisol response to psychosocial stress during a depressive episode and remission. **Stress**, v. 17, n. 1, p. 51–58, 2014. Disponível em: <http://www.tandfonline.com/doi/full/10.3109/10253890.2013.857398>. .

NESTLER, E. J.; BARROT, M.; DILEONE, R. J.; et al. Neurobiology of depression. **Neuron**, v. 34, n. 1, p. 13–25, 2002. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/11931738>. .

NOTI, M.; SIDLER, D.; BRUNNER, T. Extra-adrenal glucocorticoid synthesis in the intestinal epithelium: More than a drop in the ocean? **Seminars in Immunopathology**, v. 31, n. 2, p. 237–248, 2009.

NOVACO, R. W.; COOK, T. M.; SARASON, I. G. Military Recruit Training. **Stress Reduction and Prevention**, p. 377–418, 1989.

PACKARD, A. E. B.; EGAN, A. E.; ULRICH-LAI, Y. M. HPA axis interactions with behavioral systems. **Comprehensive Physiology**, v. 6, n. 4, p. 1897–1934, 2016.

PANETTIERI, R. A.; SCHAAFSMA, D.; AMRANI, Y.; et al. Non-genomic Effects of Glucocorticoids: An Updated View. **Trends in Pharmacological Sciences**, v. 40, n. 1, p. 38–49, 2019. Elsevier Ltd. Disponível em: <https://doi.org/10.1016/j.tips.2018.11.002>. .

PARKER, K. J.; BUCKMASTER, C. L.; HYDE, S. A.; SCHATZBERG, A. F.; LYONS, D. M. Nonlinear relationship between early life stress exposure and subsequent resilience in monkeys. **Scientific Reports**, v. 9, n. 1, p. 16232, 2019. Nature Publishing Group. Disponível em: <http://www.nature.com/articles/s41598-019-52810-5>. .

PARKER, K. J.; BUCKMASTER, C. L.; JUSTUS, K. R.; SCHATZBERG, A. F.; LYONS, D. M. Mild early life stress enhances prefrontal-dependent response inhibition in monkeys. **Biological Psychiatry**, v. 57, n. 8, p. 848–855, 2005. Disponível em: <http://linkinghub.elsevier.com/retrieve/pii/S0006322304013642>. .

PARKER, K. J.; BUCKMASTER, C. L.; SCHATZBERG, A. F.; LYONS, D. M. Prospective investigation of stress inoculation in young monkeys. **Arch Gen Psychiatry**, v. 61, n. 9, p. 933–941, 2004. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/15351772>. .

ROBINS, C. J.; BLOCK, P. Cognitive theories of depression viewed from a diathesis-stress perspective: Evaluations of the models of Beck and of Abramson, Seligman, and Teasdale. **Cognitive Therapy and Research**, v. 13, n. 4, p. 297–313, 1989.

RUSSO, S. J.; MURROUGH, J. W.; HAN, M.-H.; CHARNEY, D. S.; NESTLER, E. J. Neurobiology of resilience. **Nature Neuroscience**, v. 15, n. 11, p. 1475–1484, 2012. Nature Publishing Group. Disponível em: <http://www.nature.com/doifinder/10.1038/nn.3234>. .

SAPOLSKY, R. M.; KREY, L. C.; MCEWEN, B. S. The Neuroendocrinology of Stress and Aging: The Glucocorticoid Cascade Hypothesis*. **Endocrine Reviews**, v. 7, n. 3,

p. 284–301, 1986. Disponível em: <http://press.endocrine.org.proxy.kib.ki.se/doi/pdf/10.1210/edrv-7-3-284>. .

SAUNDERS, T.; DRISKELL, J. E.; JOHNSTON, J. H.; SALAS, E. The Effect of Stress Inoculation Training on Anxiety and Performance. , v. 1, n. 2, p. 170–186, 1996.

SAWCHENKO, P. E.; LI, H. Y.; ERICSSON, A. Circuits and mechanisms governing hypothalamic responses to stress: A tale of two paradigms. **Progress in Brain Research**, v. 122, p. 61–78, 2000.

SEERY, M. D.; LEO, R. J.; LUPIEN, S. P.; KONDRAK, C. L.; ALMONTE, J. L. An Upside to Adversity?: Moderate Cumulative Lifetime Adversity Is Associated With Resilient Responses in the Face of Controlled Stressors. **Psychological Science**, v. 24, n. 7, p. 1181–1189, 2013.

SHIRAZI, S. N.; FRIEDMAN, A. R.; KAUFER, D.; SAKHAI, S. A. Glucocorticoids and the Brain: Neural Mechanisms Regulating the Stress Response. In: J.-C. Wang; C. Harris (Orgs.); **Advances in experimental medicine and biology**, Advances in Experimental Medicine and Biology. v. 872, p.235–252, 2015. New York, NY: Springer New York. Disponível em: <http://www.ncbi.nlm.nih.gov/pubmed/26215998>. .

SMITH, S. M.; VALE, W. W. The role of the hypothalamic-pituitary-adrenal axis in neuroendocrine responses to stress. **Dialogues in Clinical Neuroscience**, v. 8, n. 4, p. 383–395, 2006.

TAFET, G. E.; NEMEROFF, C. B. The links between stress and depression: Psychoneuroendocrinological, genetic, and environmental interactions. **Journal of Neuropsychiatry and Clinical Neurosciences**, v. 28, n. 2, p. 77–88, 2016.

ÚBEDA-CONTRERAS, J.; MARÍN-BLASCO, I.; NADAL, R.; ARMARIO, A. Brain c-fos expression patterns induced by emotional stressors differing in nature and intensity. **Brain Structure and Function**, v. 223, n. 5, p. 2213–2227, 2018. Springer Berlin Heidelberg. Disponível em: <http://dx.doi.org/10.1007/s00429-018-1624-2>. .

ULRICH-LAI, Y. M.; HERMAN, J. P. Neural regulation of endocrine and autonomic stress responses. **Nature Reviews Neuroscience**, v. 10, n. 6, p. 397–409, 2009.

VITELLIUS, G.; TRABADO, S.; BOULIGAND, J.; DELEMER, B.; LOMBÈS, M. Pathophysiology of Glucocorticoid Signaling. **Annales d'Endocrinologie**, v. 79, n. 3, p. 98–106, 2018. Elsevier Masson SAS. Disponível em: <https://doi.org/10.1016/j.ando.2018.03.001>. .

WORLD HEALTH ORGANIZATION. Depression and other common mental disorders: global health estimates. **World Health Organization**, p. 1–24, 2017.

ZHOU, J.; CIDLOWSKI, J. A. The human glucocorticoid receptor: One gene, multiple proteins and diverse responses. **Steroids**, v. 70, n. 5-7 SPEC. ISS., p. 407–417, 2005.

ZUBIN, J.; SPRING, B. Vulnerability : A New View of Schizophrenia Vulnerability — A New View of Schizophrenia. **Journal of Abnormal Pschology**, v. 86, n. 2, p. 103–126, 1977.