Trends
in Psychiatry and Psychotherapy

Review Article

Social defeat protocol and relevant biomarkers, implications for stress response physiology, drug abuse, mood disorders and individual stress vulnerability: a systematic review of the last decade

Protocolo de derrota social e biomarcadores relevantes, implicações para a fisiologia de resposta ao estresse, abuso de drogas, transtornos do humor e vulnerabilidade individual ao estresse: revisão sistemática de estudos na última década

Mailton Vasconcelos,[1] Dirson João Stein,[2] Rosa Maria M. de Almeida[1]

Abstract

Introduction: Social defeat (SD) in rats, which results from male intraspecific confrontations, is ethologically relevant and useful to understand stress effects on physiology and behavior.
Methods: A systematic review of studies about biomarkers induced by the SD protocol and published from 2002 to 2013 was carried out in the electronic databases PubMed, Web of Knowledge and ScienceDirect. The search terms were: social defeat, rat, neurotrophins, neuroinflammatory markers, and transcriptional factors.
Results: Classical and recently discovered biomarkers were found to be relevant in stress-induced states. Findings were summarized in accordance to the length of exposure to stress: single, repeated, intermittent and continuous SD. This review found that the brain-derived neurotrophic factor (BDNF) is a distinct marker of stress adaptation. Along with glucocorticoids and catecholamines, BDNF seems to be important in understanding stress physiology.
Conclusion: The SD model provides a relevant tool to study stress response features, development of addictive behaviors, clinic depression and anxiety, as well as individual differences in vulnerability and resilience to stress.
Keywords: Social stress, affective disorders, drug addiction, glucocorticoids, catecholamines

Resumo

Introdução: A derrota social (*social defeat*, SD) entre ratos, resultado da confrontação intraespecífica entre machos, é etologicamente relevante e útil para o entendimento dos efeitos do estresse na fisiologia e no comportamento.
Métodos: Foi realizada uma revisão sistemática de estudos sobre biomarcadores induzidos pelo protocolo de SD publicados entre 2002 e 2013, usando as bases de dados PubMed, Web of Knowledge e ScienceDirect. Os termos usados na busca foram: derrota social, neurotrofinas, marcadores neuroinflamatórios e fatores de transcrição.
Resultados: Biomarcadores clássicos ou recentemente descobertos mostraram-se relevantes nos estados induzidos pelo estresse. Os achados foram resumidos de acordo com o tempo de exposição ao estresse: SD única, repetida, intermitente ou contínua. O fator neurotrófico derivado do cérebro se mostrou um marcador específico de adaptação ao estresse. Assim como glicocorticóides e catecolaminas, o BDNF parece ser importante para o entendimento da fisiologia do estresse.
Conclusão: O modelo de SD oferece uma ferramenta importante para estudar características da resposta ao estresse, desenvolvimento de comportamentos aditivos, depressão clínica e ansiedade, bem como diferenças individuais de vulnerabilidade e resiliência ao estresse.
Descritores: Estresse social, distúrbios afetivos, adição a drogas, glicocorticóides, catecolaminas.

[1] Instituto de Psicologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil. [2] Hospital de Clínicas de Porto Alegre (HCPA), UFRGS, Porto Alegre, RS, Brazil.

This review was based on an academic dissertation presented at Instituto de Psicologia, Universidade Federal do Rio Grande do Sul (UFRGS), Porto Alegre, RS, Brazil, in 2014, as partial fulfillment of the requirements for the degree of Master in Psychology. The original dissertation was entitled "Studies on social defeat: biomarkers of stress-induced states and influences on individual reactivity to social stress."

Financial support: none.

Submitted Jul 30 2014, accepted for publication Nov 04 2014. No conflicts of interest declared concerning the publication of this article.

Suggested citation: Vasconcelos M, Stein DJ, de Almeida RMM. Social defeat protocol and relevant biomarkers, implications for stress response physiology, drug abuse, mood disorders and individual stress vulnerability: a systematic review of the last decade. Trends Psychiatry Psychother. 2015;37(2):51-66. http://dx.doi.org/10.1590/2237-6089-2014-0034

Introduction

Mental disorders, increasingly characterized by their high worldwide prevalence,[1,2] are neuropsychiatric conditions that lead to significant loss of quality of life for patients and relatives.[3,4] In the World Health Organization (WHO), studies about the global burden of diseases classify mortality and socioeconomic impact according to disease causes.[5] WHO reports aim to provide evidence of the relative impact of health problems worldwide. Calculated projections based on these studies helped to raise awareness about the substantial effect of mental health around the world.[2] According to the 2005 WHO report, 31.7% of all years lived with disability may be attributed to neuropsychiatric conditions. The five major conditions contributing to this are unipolar depression (11.8%), alcohol abuse (3.3%), schizophrenia (2.8%), bipolar depression (2.4%) and dementia (1.6%).[5] The analysis of mortality reveals that 1.2 million deaths every year are attributed to neuropsychiatric conditions, and that 40,000 are associated with mental disorders and 182,000 with drug use and alcohol abuse.[5] The WHO report did not include suicide as a neuropsychiatric cause of death, but almost 800,000 suicides are recorded annually.[2] These numbers seem to confirm that the large prevalence of mental diseases worldwide is an independent contributor, but interactions with other health problems, such as coronary disease, stroke, diabetes, HIV/AIDS and medically unexplained somatic symptoms, should also be taken into consideration.[2] The public health relevance of evidence-based arguments should be evaluated in the study of mental health, and efforts should be directed to social and public policy making throughout the preclinical stages of biomedical investigations. For this purpose, animal models of stress are important tools to construct knowledge about affective and drug-abuse disorders.

Animal models of stress are particularly useful, because they focus on social life events that generalize across many mammal species, including humans.[6] Social stress, a common stressor readily translated across species, is a recurrent factor in the life of all social species.[7,8] Animal models of social stress have different temporal and intensity characteristics: single, intermittent or continuous exposure of an individual to another or other conspecifics. Interactions are specific to the animal species, sex, age, life history, and distinctive environment.[6] Within these models, the influence of social status may be determined by several factors: the frequency, duration, and intensity of agonistic interactions, their outcome, and the perception of controllability.[9] A common protocol to generate social defeat (SD) among rodents is the resident-intruder paradigm.[10,11] Under precise experimental conditions, it is possible to measure the number of salient acts, postures and displays, and to exert experimental control by determining whether the rat prevails as dominant, or is defeated. The animal can perceive this confrontation as extremely stressful, inducing characteristic neuroendocrine and behavioral responses.[12-14] This pattern confers the model with ethological relevance and translational value for the elucidation of the physiologic and behavioral adaptations to stress.

Animal models of social stress share many response characteristics with models that use other environmental stressors.[15] After decades of studies about social stress, the neuroendocrine responses to social challenges are largely understood as events that begin with the activation of the sympathetic-adrenal-medullary (SAM) axis, followed by stimulation of the hypothalamus-pituitary-adrenal (HPA) axis[16] in response to stress. They activate metabolism of energetic and immune responses that are crucial in coping with stressors. Glucocorticoids and catecholamines are a well-studied set of molecules that act as effectors of those mechanisms.[17] Corticosterone (CORT) secretion in animals under stress is mostly regulated by the activity of the HPA-axis and the negative feedback exerted by the levels of circulating glucocorticoids acting upon glucocorticoid receptors (GR).[18] This mechanism is triggered by a set of stress hormones, such as corticotropin releasing hormone (CRH), adrenocorticotropic hormone (ACTH) and arginine vasopressin,[19,20] and is also determined by stressor modality and its characteristic time course, as in the case of its effect on social status.[21] The involvement of catecholamines is more pronounced and well studied because of their rapid peripheral activation, as a result of sympathetic discharges.[22,23] Norepinephrine and epinephrine are synthesized and released by cells in the adrenal medullary region; also, nerve fibers in contact with target tissues release norepinephrine. As a result of acute activation of this catecholaminergic system, these amines provide the necessary boost to master immediate stress. This response is characterized by rapid increases in heart rate and increased blood flow to skeletal muscles and other regions of the body.[24-27] Individuals need these endocrine responses to survive challenging situations, but inadequate or excessive adrenocortical and autonomic functions are harmful to the body and brain. A characteristic of intermittent exposure to social stress is the continuous activation of these SAM and HPA axes, which indicates lack of habituation to the stressful situation.[28]

In addition to the hypothalamus, particularly the paraventricular nucleus, which is essential for autonomic and neuroendocrine responses to stress,

cortical and brainstem areas are implicated in the neural sites that are critical for adaptations to social stress. Dopaminergic and glutamatergic connections in the ventral tegmental area (VTA), nucleus accumbens (NAc) and prefrontal cortex (PFC) are essential for coping with stressful environmental situations, as well for drug addiction.[29,30] The association between social stress, with its consequent physiological response, and neural substrates involved in neuroadaptations is of particular interest. Behavioral changes induced by social stress seem to be relevant in increased drug taking and affective disturbances in rodents.[28] For instance, socially stressed animals respond more robustly to low doses of psychostimulants and increase self-administration of psychomotor stimulants.[31,32] This behavioral sensitization may contribute to drug-related behaviors, such as craving and relapse,[33] and acquisition of drug self-administration.[34] An augmented behavioral response to a drug challenge produced by an agent other than the challenge drug is termed cross-sensitization. The establishment of this behavior occurs initially due to intermittent exposure to social stress and, in the case of intense and chronic exposure, behavioral sensitization deteriorates and behavioral impairments emerge. The association of stress exposure and addictive behaviors follows an inverted U-shaped curve, in accordance with the Yerkes-Dodson Law,[35] and has a powerful example in alcohol consumption. This biphasic effect of stress, which may also be found in cognitive processes, such as memory and emotion, is relevant in affective psychopathology.[36]

Brain-derived neurotrophic factor (BDNF), an important neurotrophin for synaptic plasticity, is one of the molecular candidates underlying the development of persistent neuroplastic adaptation to social and other types of stress. It is also a candidate molecule that may trigger cross-sensitization induced by SD stress. Mesocorticolimbic elevated BDNF in the VTA is a risk factor for drug sensitivity.[37,38] BDNF mediates synaptic plasticity and cell responses to stress and drugs of abuse. Stress-induced lasting changes of BDNF signaling in mesocorticolimbic regions may regulate the reward circuit.[39] This neurotrophin has some opposite role differences: episodically defeated and continuously subordinated rats may show, respectively, increased and suppressed BDNF responses. These divergent neuroadaptations to social stress may be representative of the substrates for the intensification of cocaine bingeing due to the anhedonia-like deterioration of reward processes during subordination stress.[37]

Psychiatric disorders are directly linked to social stress etiologically. For example, defeated animals have signs of anhedonia and neuroendocrine stress-responses that correspond to those seen in patients with depression.[6,40] Several of these behavioral and neuroendocrine effects caused by psychosocial stress may be reversed with antidepressants and anxiolytics.[41,42] Not all individuals experiencing social stress develop cardinal symptoms of depression or anxiety, which suggests that the response to stress is, to a significant extent, determined by individual vulnerability or resilience, in both humans[43] and several animal species.[44,45] In animal models, individual differences in the endophenotypes have been studied to act as substrates for stress vulnerability.[46-48] One such example is attributing differences in hedonic temperament between individuals as a candidate mechanism.[49] Rats exposed to the mild stress of a novel environment that respond with increased exploratory behaviors are termed high responders (HR), and those responding with decreased exploration are termed low responders (LR).[48,49] HR animals are more vulnerable to depressive-like symptoms, such as decreased behavioral responses to SD stress, sweet solution preference, forced swim test and social avoidance.[50,51]

The molecular changes in the central nervous system (CNS) that trigger and sustain behavioral and physiologic changes in socially defeated animals are not completely known. This systematic literature review will discuss information about the extensive brain and CNS molecular changes induced by the rat SD protocol. Its main focus is to provide an overview of this protocol by using systematic and explicit methods to search, critically review and synthesize selected information. The current review focuses on CNS biomarkers induced by the use of this SD protocol in the last 10 years.

Methods

A systematic literature review was conducted in the second half of 2013 by means of an electronic search of articles indexed in the databases Web of Knowledge, PubMed, and ScienceDirect. Articles published in the last ten years (2002-2013) were selected using the following terms: social defeat, rat, neurotrophins, neuroinflammatory markers, and transcriptional factors. Only empirical studies written in English were included. The preselected studies were assessed independently by two authors according to inclusion criteria: experimental studies; abstract available; rats as animal model; SD protocol used in the methods; and analysis of CNS biomarkers. After excluding duplicates, concordance between authors was analyzed, as recommended by Lopes, Viacava & Bizarro (personal communication), to define the final selection of studies. Figure 1 shows the flowchart of steps of this systematic search.

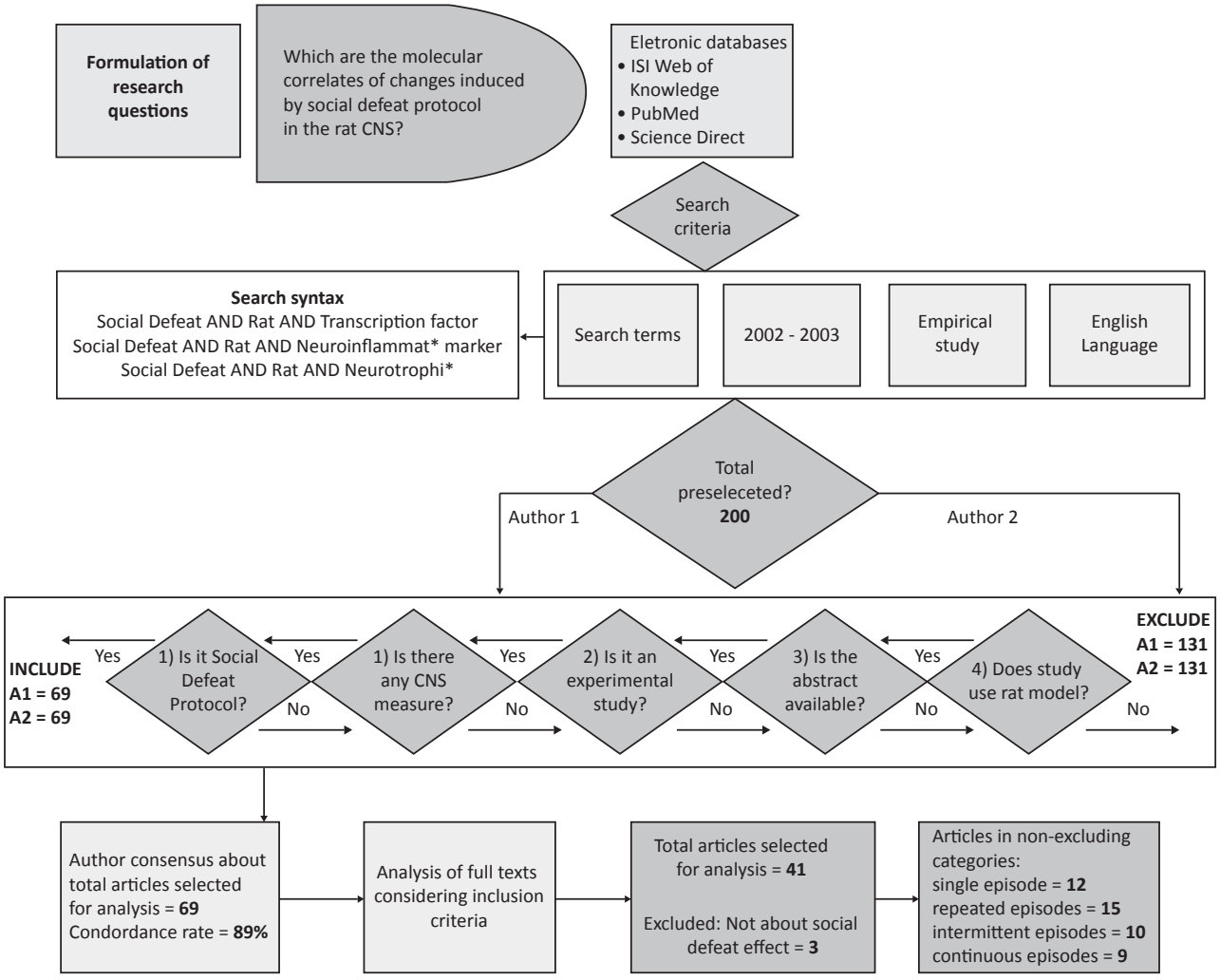

Figure 1 - Flow diagram of articles selected for inclusion

Results

The searches in the three databases using the search terms resulted in 200 articles. After the inclusion of studies that met the defined criteria and the exclusion of duplicates, 69 were selected by author 1 (MV) and 69 by author 2 (DJS). The authors discussed and reached the consensus that 41 studies met the previously established criteria. This consensus resulted in a concordance rate of 89%, calculated using the following equation: total compatible studies / total compatible studies + total incompatible studies = compatibility index. However, during analysis, 3 studies were excluded because they did not discuss the SD effect. Therefore, the final sample had 38 studies, which were analyzed and classified according to the length of SD episodes during the protocol. Some studies had more than one type of occurrence: single episode (12), repeated episodes (15), intermittent episodes (10) and continuous exposure (9). The analysis of the methods, summarized in Table 1, revealed studies that used the rat SD protocol to obtain behavioral and molecular changes associated with specific brain areas or with the CNS. Excluded studies adopted a combination of distinct stress protocols together with SD, and did not, therefore, clearly identify a SD effect on the results.[52-54] The analysis of results was conducted separately for each length of SD protocol.

Table 1 - Behavioral and molecular impact of distinct lengths of SD exposure and related brain areas

Behavioral impact and molecular effects	Brain areas	References
Adult single SD		
22 kHz USV		
IGF-I protein levels	Frontal and parietal cortices	Burgdorf et al.[55]
BDNF, TrkB.T1 mRNA levels, BDNF epigenetic factors	Hippocampus	Duclot & Kabbaj[46]
Fos protein and CRH_2	Arcuate nucleus, ventromedial hypothalamus, posterior medial amygdala	Fekete et al.[56]
c-Fos mRNA, CRH mRNA	mPFC, LC and VTA/vBNST	Funk et al.[57]
Increased passive coping behavior in maternally separated animals		
c-Fos, TrpOH	Dorsal raphe nucleus	Gardner et al.[58]
*Tph*2 mRNA	Dorsal raphe nucleus	Gardner et al.[59]
slc6a4 mRNA	Dorsal raphe nucleus	Gardner et al.[60]
NOP receptor mRNA	Amygdala, PVN	Green & Devine[61]
Higher immobility in FST and lower latency to immobility, decreased total line crossing in OF		
CAM-L1 protein, increased CORT concentrations, ratio pCREB/CREB, GAP-43 expression	Amygdala and hippocampus	Kavushansky et al.[62]
Increased 22 kHz USV, reduced number of line crossing in OF		
Increased CORT serum levels, increased CRH and GR mRNA levels	Hippocampus	Marini et al.[15]
Increased ACTH, CORT and leptin plasma levels	HPA-axis	Razzoli et al.[51]
Short-term locomotor sensitization under amphetamine challenge		
BDNF and ΔFosB	VTA, NAc and PFC	Wang et al.[38]
Adult repeated SD		
Passive coping behaviors		
c-Fos/TrpOH	Dorsal raphe nucleus	Paul et al.[63]
Increased anxiety-like behaviors		
Proteins, including protein folding, signal transduction, synaptic plasticity, cytoskeleton regulation and energy metabolism	Hippocampus	Carboni et al.[64]
TrkB.FC-induced social avoidance in LR rats and 7,8-DHF-induced social approach in HR rats		
TrkB.FC and TrkB.T1 mRNA levels, PKB and pCREB, BNDF promoter 6	Hippocampus	Duclot & Kabbaj[46]
Grooming frequency and time in OF		
Decreased T3 and T4 serum levels		Olivares et al.[65]
Reduced saccharin intake		
$5\text{-}HT_{1b}$ mRNA expression	NAcSh, dorsolateral striatum	Furay et al.[66]
NOP receptor mRNA	PVN	Green & Devine[61]
Increased self-grooming, shorter rearing duration, presence of risk assessment, lower sucrose preference, lower climbing in FST, lower general activity and sociability in social avoidance test	HPA-axis	Razzoli et al.[51]
Increased rearing, decreased partition exploration, higher risk assessment		
Increased ACTH and CORT plasma levels in long-term condition	HPA-axis	Razzoli et al.[23]
Reduced sucrose consumption of HR rats		
Histone 3, 4 and 2B	Hippocampus	Hollis et al.[48]
Lower FGF2 and FGFR1 mRNA levels	Hippocampus	Turner et al.[67]
IL-1β	PVN	Hueston et al.[68]
CAM-KIIb gene	Hippocampus	Kabbaj et al.[69]
Freezing during exposure, 22 kHz USV		
Increased expression of CHRNB2 and ACHE genes	PAG	Kroes et al.[70]
Reduced mounting in copulatory behavior		
Reduced testosterone plasma levels, c-Fos mRNA	Medial preoptic area	Niikura et al.[71]

(cont.)

(cont.)

Adolescent repeated SD		
Adult increased locomotion behavior, CPP for amphetamine		
D2 receptor	NAc	Burke et al.[72]
Adult intermittent SD		
Sensitized locomotor response to amphetamine		
BDNF and ΔFosB	Mesocorticolimbic structures	Nikulina et al.[39]
Increased play initiation, increased submission in adolescence and less and later submission in adulthood		Buwalda et al.[73]
Sensitized locomotor response to amphetamine		
Long-term Fos-like immunoreactivity	VTA and AMY	Nikulina et al.[32]
Anhedonic sexual disinterest		
Reduced orexin and dynorphin	VTA, mPFC and hypothalamus	Nocjar et al.[74]
Decreased horizontal and vertical locomotion, increased inactivity in OF, decreased sucrose preference		
Decreased T3, T4 and CORT serum levels	HPA-axis	Olivares et al.[65]
Increased cocaine intake in 24 h binge		
*zif*268 mRNA expression	Amygdala and frontal cortex	Covington III et al.[75]
BDNF and/or mRNA expression	mPFC, AMY, substantia nigra, VTA	Fanous et al.[76]
Reduced social behavior		
BDNF	VTA	Fanous et al.[77]
Reduced saccharin intake		Furay et al.[66]
Increased cocaine self-administration		
Increased dopamine levels and BDNF	NAc and VTA	Miczek et al.[37]
Adult continuous SD		
Anhedonia		
Increased CORT, mRNA and protein NET, decreased PKA, PKC and pCREB	Locus coeruleus, and its terminal regions	Chen et al.[18]
Increased defeat-related behaviors		
Increased BrdU-positive labeling	Hippocampus	Buwalda et al.[73]
Increased CORT, decreased serotonin, increased MAO A mRNA expression, increased KLF11 mRNA and protein expression	Cortex and thalamus	Grunewald et al.[78]
TNF-α	BNST	Hueston et al.[68]
Increased immobility in FS test		
Phosphorylated MEK1/2, phosphorylated ERK1/2 and MKP-1	Hippocampus	Iio et al.[79]
Reduced sucrose consumption and increased struggling of HR rats		
Decreased expression of *Gsk3b* and *Taf2*, increased expression of *HMox3* and *Eno3*	Frontal cortex and hippocampus	Kanarik et al.[80]
Reduced sucrose consumption		
Lower BMP7 gene expression	LC	Ordway et al.[81]
Increased CORT level, 5-HT_{1a} receptor mRNA	PFC	Kieran et al.[82]
Suppressed cocaine intake, decreased preference and intake of sugar and decreased exploratory behavior		
Suppression of DA and BDNF responses	NAc and VTA	Miczek et al.[37]

5-HT_{1a} = 5-hydroxytryptamine receptor 1a or serotonin receptor 1a; 5-HT_{1b} = 5-hydroxytryptamine receptor 1b or serotonin receptor 1b; ACHE = acetylcholinesterase subunit T gene; ACTH = adrenocorticotropic factor; AMY = amygdala; BMP7 = bone morphogenetic protein 7; BNDF = brain-derived neurotrophic factor; BNST = bed nucleus of stria terminalis; BrdU = bromodeoxyuridine; CAMKIIb = calcium/calmodulin-dependent protein kinase type II β; CAM-L1 = L1 cell adhesion molecule; CORT = corticosterone; CPP = conditioned place preference; CREB = cAMP response element-binding protein; CRH = corticotropin-releasing hormone; CRHNB2 = nicotinic acetylcholine receptor subunit β2 gene; DA = dopamine; DHF = dihydroxyflavone; *Eno3* = β-enolase gene; ERK= extracellular-signal-regulated kinase; FGF = fibroblastic growth factor; FGFR1 = fibroblastic growth factor receptor 1; FST = forced swim test; GAP = growth-associated protein 43; GR = glucocorticoid receptors; *Gsk3b* = glycogen synthase kinase 3b gene; *HMox3* = heme oxygenase gene; HPA = hypothalamus-pituitary-adrenal; HR = high responders; IGF-I = insulin-like growth factor I; IL-1β = interleukin 1β; LC = locus coeruleus; LR = low responders; MAO A = monoamine oxidase A; MEK = mitogen/extracellular signal-regulated kinase; MKP-1 = mitogen-activated protein kinase phosphatase-1; mPFC = medial prefrontal cortex; mRNA = messenger ribonucleic acid; NAc = nucleus accumbens; NAcSh = nucleus accumbens shell; NET = norepinephrine transporter; NOP = nociceptin/orphanin receptor; OF = open field; PAG = periaqueductal gray area; pCREB = phosphorylated cAMP response element-binding protein; PFC = prefrontal cortex; PKA = protein kinase A; PKB = protein kinase B; PKC = protein kinase C; PVN = paraventricular nucleus; SD = social defeat; slc6a4 = serotonin transporter gene; *Taf2* = transcription initiation factor subunit 2 gene; TNF-α = tumor necrosis factor α; TrkB.FC = recombinant tropomyosin-related kinase B receptor; TrkB.T1 = truncated tropomyosin-related kinase B receptor; *Tph2* = tryptophan hydroxylase 2 gene; TrpOH = tryptophan hydroxylase; USV = ultrasound vocalizations; VTA = ventral tegmental area.

The rat SD stress protocol

The rat SD protocol consists of the exposure of an experimental animal to a dominant aggressive male. Most of the selected studies followed the procedures established by Miczek et al.,[11,83,84] who developed and characterized this resident-intruder model of social stress in rats and mice. Before beginning the experiments, adult male rats were selected as aggressive residents and placed in large individual cages where they lived in pairs with a sterile female. After an adaptation period and establishment of territorial status of the residents, smaller experimental animals, termed intruders, are placed into the resident's home cage. Before the beginning of interactions between resident and intruder, females are removed from the home cage. The experimental sequences mostly consist of presence or not of a pre-defeat period, generally 10 minutes, a physical or defeat period, lasting up to 10 minutes and, also facultative, a post-defeat period of a variable length of time. Resident animals show a pattern of attack and threat behaviors, while intruders engage in defensive, submissive and flight reactions. It is important to distinguish between brief episodes of SD and continuous subordination stress. There are three classes of exposure. The first, single SD stress differs from repeated SD stress in the number of exposure episodes. Repeated defeat differs from continuous subordination stress, which requires cohabitation, albeit protected, with a dominant opponent and consists of an inescapable and uncontrollable nature of stress. Here we considered a fourth class of stress exposure, the intermittent protocol, which produces clear and distinct effects from the other patterns and should be classified as a separate class of SD stress.

Single SD episode

This category included studies in which the rats were exposed to a single episode of SD. Rats that underwent one single episode of SD had more 22 kHz ultrasound vocalizations (USV),[55,85] more depressive-like symptoms, such as higher immobility and lower latency to engage in immobility in the forced swim test (FST),[62] and reduced line crossings in open field (OF).[62,85] When challenged three days after the SD session, rats had a short-lived locomotor sensitization to amphetamine exposure.[38] Also, maternally separated animals during early infancy had more passive coping behaviors when adults.[58]

As molecular markers of stress, those animals had increased hormonal levels of CORT, CRH, ACTH, and leptin in areas such the HPA-axis, PFC, locus coeruleus (LC), VTA, bed nucleus of stria terminalis (BNST), hippocampus and amygdala (AMY).[51,57,62,85] They also had upregulation of CRH and GR in the arcuate nucleus, AMY, hippocampus and HPA-axis[56,85]; changes in neurotransmitter receptors and metabolites for serotonin in the dorsal raphe nucleus (DR)[58-60]; changes in nociceptin/orphanin receptor (NOP) in the AMY and hypothalamus.[61] Moreover, analyses revealed changes in neurotrophic factor molecules, such as insulin-like growth factor I (IGF-I) in the frontal and parietal cortices[55]; changes in BDNF levels and its receptors and metabolites in the hippocampus, VTA, NAc and PFC[38,46]; changes in growth associated protein 43 in the AMY and also in the hippocampus. A wide variety of proteins were expressed as markers of single exposure to SD: L1 cell adhesion molecule (CAM-L1) and phosphorylated cAMP response element-binding protein (CREB) in the AMY and hippocampus[62]; and, finally Fos family proteins, such as c-Fos and delayed ΔFosB in the PFC, LC, VTA, BNST, DR, and NAc.[38,57,58]

Repeated SD episodes

This category included studies in which rats were exposed to two or more repeated days of SD episodes. Rats that underwent repeated SD on consecutive days had more 22 kHz USV,[70] more passive coping behaviors,[23,63,70] increased anxiety-like behaviors, such as increased self-grooming, increased locomotion in novel environments and risk assessment behavior,[23,51,64,65,72] and increased depressive-like symptoms, such as reduced sweet solution preference and intake, reduced climbing in the FST, lower general activity and sociability in the social avoidance test and reduced mounting in copulatory behavior.[48,51,66,71] Repeatedly stressed LR rats displayed social avoidance under infusion of BDNF antagonist in hippocampus, whereas repeatedly stressed HR rats displayed social approach under infusion of BDNF agonist in hippocampus.[46] Furthermore, rats exposed to the repeated stress protocol during adolescence had more conditioned place preference (CPP) behaviors in adulthood when under amphetamine exposure.[72]

As molecular markers of stress, repeatedly stressed animals had increased hormonal levels of CORT and ACTH,[3] decreased thyroid hormones and decreased testosterone plasma levels,[65,71] increased neurotransmitter receptors and metabolites for serotonin, NOP and dopamine (DA) in the NAc, striatum, hypothalamus and DR[61,63,66,72] and increased cholinergic receptors subunits and enzymes in the periaqueductal gray area (PAG).[70] They also had changes in neurotrophic factor molecules, such as BDNF metabolites, and downregulation of fibroblastic growth factor (FGF) in the hippocampus.[46,67] Changes in neuroinflammatory markers, such as Interleukin-1β in the hypothalamus,[68] were evident after repeated psychosocial stress. Finally, these animals had increased

levels of c-Fos in the PFC and DR brain areas[63,71] and changes in histones and calcium/calmodulin-dependent kinase in the hippocampus.[48,69] In a more comprehensive description, Carboni et al.[64] reported changes in folding-related proteins, signal transduction, synaptic plasticity, cytoskeleton regulation and energy metabolism in the hippocampus after repeated psychosocial stress exposure.

Intermittent SD

This category included rats that were exposed to two or more episodes of SD on non-consecutive days, often termed acute defeat. In adolescence, these rats had more submissive behaviors and increased play initiation behaviors.[73] As adults, they displayed less frequent and longer latency to submit and reduced social behavior.[73,77] For anxiety-like and depressive symptoms, rats acutely defeated displayed anhedonic sexual disinterest[74] and decreased locomotion and line crossings, as well as increased immobility in the OF[65] and decreased sucrose preference.[65,66] They also had drug-related behaviors, such as sensitized locomotion under amphetamine challenge[32,39] and increased cocaine self-administration.[37,75]

The analysis of molecular markers of stress revealed that repeatedly stressed animals had decreased serum levels of thyroid hormones.[65] Decreased orexin neurotransmitter and dynorphin protein in the VTA, PFC and hypothalamus[74] were also found, as well as increased DA levels in the VTA and NAc.[37] They might also have alterations in neurotrophic factors molecules, such as BDNF metabolites in brain areas, including VTA, NAc, PFC, AMY and substantia nigra.[37,39,76,77] Finally, these animals had increased levels of Fos family proteins such as ΔFosB and c-Fos, as well as *zif*268 in the VTA, NAc, PFC, and AMY brain areas.[32,39,75]

Continuous SD

This category included rats that were exposed to continuous SD episodes, often termed as chronic exposure. Rats that were exposed to continuous SD stress had increased defeat-related behaviors,[73] increased depressive-like symptoms such as anhedonia, immobility in the FST, reduced sweet solution preference and decreased exploratory behavior.[18,37,79-81] Chronic SD stress experiences resulted in suppressed cocaine intake.[37] HR rats displayed reduced struggling in the FST after continuous SD stress.[80]

Molecular markers of stress indicated that continuously socially defeated rats had markedly increased hormonal levels of CORT,[18,78,82] and decreased serotonin neurotransmitter metabolites and receptors in the cortex and thalamus.[78,82] Chronic exposure upregulates norepinephrine transporter (NET) expression in the LC and its terminal regions[18] and suppresses DA in the NAc and VTA.[37] As for neurotrophic factors, chronically stressed rats have lower bone morphogenetic protein 7 (BMP7) gene expression and suppression of BDNF in the LC, NAc and VTA.[37,81] There were changes in the inflammatory marker TNF-α, specifically in the Long Evans strain of defeated rats.[68]

A wide variety of molecules may function as markers of continuous stress, including BrdU-positive labeling nucleosides in the hippocampus[73] and neurotransmitter degradation molecules in the cortex and thalamus.[78] Cell signaling proteins, glucose metabolism, transcription factors and cell functioning enzymes in the frontal cortex and the hippocampus may also be found in chronically stressed animals.[79,80]

Discussion

Molecular basis of stress-response

Few studies selected in this systematic review focused only on the understanding of the molecular basis of stress-response. These studies contributed to the clarification of several and perhaps interconnected mechanisms of response and adaptation to stress. Therefore, several studies selected for this review measured basic stress hormone levels in animals exposed to single social stress. The other stress exposure protocols relied not only on hormone levels to examine the stressful conditions of SD, but also on behavioral measures.

Fekete et al.[56] discussed the role of CRH in responses to stress. They found that this peptide is present in the paraventricular nucleus (PVN) of the hypothalamus, induces ACTH release from pituitary[86] and is also present in extra hypothalamic regions, where it acts as a non-neuroendocrine stress-related modulator.[87] For instance, its presence is confirmed after a single SD episode in the hippocampus[85] and in vBNST and ceAMY.[57] CRH binds to two known receptors in brain tissue, CRH_1 and CRH_2. CRH_1 is expressed in more specific brain areas and the cerebellum and is involved in the activational and anxiety-like components of stress-related behaviors.[87] The CRH_2 receptor is discretely distributed, and its role in endogenous stress responses was unclear, as suggested by Fekete et al.,[56] before this study. After a single exposure to SD, rats had an increased Fos expression in CRH_2 positive neurons in the mAMY.[56] The role of the mAMY in stress response may be associated with defeat behaviors, such as avoidance of aggressive

mates[88] and defensive responses to predator noxious stimuli.[89] The mAMY seems to be another regulator of HPA-axis activity.[90] Furthermore, functional activation of the mAMY will be discussed together with the analysis of intermittent SD protocols involved in drug-related behaviors (see following topic).

Marini et al.[64] investigated several hippocampal molecular changes in protein levels induced by single psychosocial stress exposure. The pattern of changes after repeated social stress events were quantitatively and qualitatively different when compared to single exposures.[64] Green & Devine[61] found a possible plasticity effect on the expression of NOP FQ receptor mRNA: a single exposure to social stress elicited increasing levels of NOP mRNA in the central and mAMY and PVN, but this effect was no longer evident after repeated stress exposure. The authors suggested a habituation process of these neurotransmitter peptides with continuation of stress exposure.[61] Two hours after the application of the intermittent SD protocol, BDNF and BDNF mRNA levels were differentially upregulated in the mPFC, substantia nigra and AMY regions.[76] The prominent finding in this study was the persistence of BDNF changes up to 28 days after stress discontinuation; this effect was evident in the mAMY and VTA regions. These BDNF changes are indices of long-lasting neuronal adaptation to social stress. The authors discussed implications of their study in the understanding of the dopaminergic system functioning.[76] A lack of discrepancies between protocols was reported by Hueston et al.[68] Different cohorts of Sprague-Dawley rats exposed to repeated or continuous SD protocols did not have changes in inflammatory responses. Despite the well-established role of sympathetic and noradrenergic functioning as mediators of inflammatory processes in the brain and blood,[40,91-93] acute measures of SD did not impact gene expression of inflammatory markers at any timepoint.[68] A careful analysis of the protocol revealed that a very mild type of SD was implemented in these studies: the stimulus animals were not highly aggressive. The residents were not allowed to wound or bite to prevent inflammatory tissue processes.

These studies reinforced the hypothesis that different modalities and courses of exposure to stress lead to different outcomes, and therefore the type of adaptive response specific to a certain stressor should be previously clarified in the study objective. Findings reinforced the idea of important brain reactions to stress in the form of neural plasticity, implicating BDNF as an important biomarker. Also, distinct neurobiological pathways other than HPA-axis stress-response mechanisms may play an important role; pathways involving AMY, physiologic responses to immunologic markers, and different stress-related neurotransmitters were here mentioned as examples.

Drug-related studies

In our review, studies about drug addictions focused on neurobiological and behavioral stress-induced adaptations. By taking the mesocorticolimbic DA system as the prominent SD neurobiological substrate,[34] Nikulina et al.[32] found that behavioral sensitization to d-amphetamine challenge was a stress induced-effect of intermittent exposure to SD. This effect is accompanied by activation of mesocorticolimbic structures one week and two months after stress discontinuation, and is specifically evident in the VTA and AMY. These effects were evaluated using an immunohistochemical technique to measure Fos-like immunoreactive (Fos-LI) proteins. The amygdaloid region, as well as other mesocortical regions, is an important site for behavioral sensitization. Additionally, Fos-LI activation pattern may be associated with the expression of chronic Fos-related antigens. Covington III et al.[94] investigated a different immediate early gene expression after exposure to intermittent social stress, *zif*268. Amphetamine challenge decreased *zif*268 mRNA expression in the AMY, and this effect was also found 60 days after SD stress. Further studies confirmed the involvement of the AMY in the sensitization processes,[39] and the medial region of the AMY seemed to be responsible for dealing with emotional stressors.[90]

In subsequent studies, Nikulina et al.[39] confirmed the presence of a more stable Fos family protein member, ΔFosB,[95] involved in behavioral sensitization and cross-sensitization. They analyzed the role of neurotrophic factor, BDNF, and ΔFosB signaling in mesocorticolimbic structures 10 days after intermittent SD exposure. A sustained activation pattern of infralimbic innervations projecting to the VTA was found after SD stress, consistent with the role of the VTA and its reciprocal connection to PFC in the development of behavioral sensitization.[96,97] This mesocorticolimbic and corticotegmental functional activation were interpreted as a possible neural circuit underlying stress-induced cross-sensitization to psychostimulants.[39] The role of NAc in drug-related behaviors was further discussed in Furay et al.[66]: after repeated, but not intermittent, exposure to SD exposure, mRNA levels of 5-HT_{1b} receptors were increased in the rostral NAc shell. The NAc, an important region mediating stress and fear responses, assess emotional valence of incoming stimuli. The shell of the NAc is part of the postulated extended AMY, according to Fanselow & Dong.[98] Opposing expressions of BDNF and DA in the NAc and VTA were implicated in distinct outcomes of different lengths of rat SD protocols. Miczek et al.[37] demonstrated that intermittently or continuously defeated rats tested for psychomotor stimulation and binge-like cocaine self-administration had behaviors

either intensified or deteriorated. According to these authors, the patterns of results among intermittently stressed animals suggested that BDNF in the VTA and NAc DA cells are part of a stress-induced cascade promoting behavioral and neural sensitization associated with drug abuse. Also, the decreases in VTA BDNF in continuously stressed animals were associated with the persistent suppression of cocaine and sweet solution reward intake, which suggests that these animals had an anhedonia-like profile.[37] More recent studies conducted by Wang et al.[38] implicated the increased BDNF expression in VTA phenotype as a risk factor for drug sensitization at both the neural and the behavioral levels.[38]

Further studies discussed whether the effects on amphetamine- and cocaine-elicited sensitization were also found in adolescent rats and, therefore, persistent after weeks of discontinuation of the stress exposure in adulthood. Rats repeatedly stressed during adolescence developed CPP for amphetamine intake cues when adults more easily than foot-shock stressed animals.[72] The authors suggested that stress exposure had effects associated with drug abuse behaviors extending through the period of transition from adolescence to adulthood. The adolescent effects of social stress may be reversed by housing with an age-matched mate during stress exposure. Moreover, rats experiencing continuous SD during adolescence had minor changes in BDNF, and no behavioral or physiological effect persisting into adulthood.[73]

The studies discussed above agreed that intermittent and continuous SD protocols were powerful tools in the understanding of the association between stress and drug abuse. Moreover, the continuous SD protocol provided some clues about stress and depressive-like symptoms associated with behaviors and molecular markers. Finally, the evidence contributed to the well-established knowledge of the participation of the mesocorticolimbic structures in drug addiction, which contributed to the discussion of specific roles of each brain region when expressing BDNF and Fos-LI markers involved in this process.

Mood disorder studies

Razzoli et al.[51] reported that the use of overall reactivity to environmental stimuli was a valid depressive-like measure in animal models. The most common potential depressive-like endophenotypes found in our review were the responses to a stressful context: sweet solution preference, the FST and the social avoidance test. Shortly after repeated stress exposure, rats displayed defensive behaviors, anhedonia and body weight loss. Three weeks after repeated SD stress exposure, these behavioral adaptations were still present, including decreased general activity and decreased sociality in a social avoidance test, as well as depressive-like behaviors in the FST, but not anhedonia.[51] Rats exposed to continuous SD daily over the course of 5 weeks displayed inhibition of weight gain, increased adrenal gland weight and increased immobility in the FST. These behaviors were accompanied by downregulated intracellular mitogen-activated protein kinase (MAPK) cascade in the rat hippocampus.[79] The authors interpreted the prolonged immobility in the FST as behavioral despair and suggested that it was a marker of depression.[99] They explained that the involvement of MAPK was one of the BDNF-induced outcomes to affect cell functioning under SD.[100] Miczek et al.[28] highlighted the divergent anhedonic consequences of stress depending on the length and intensity of exposure. In sum, intermittent SD protocols induced escalation of drug abuse behaviors and neuroadaptation, whereas continuous social stress exposure induced blunted responses to sweet rewards and cocaine. Controversial results were reported in the study conducted by Kanarik et al.[80]: the length of chronic stress increased sweet solution intake in HR rats instead of fragmenting or decreasing this behavior. An important unique feature of this particular chronic protocol was that residents were treated with apomorphine immediately before the session, which affected the resident's behavior; and that intruders were not exposed to the resident continuously, which, therefore, did not characterize a de facto chronic exposure.[28]

The results of reduced sweet solution preference in socially defeated animals were interpreted as an analog of anhedonia. Nocjar et al.[74] investigated a possible neural basis for anhedonia using exposure to an intermittent SD protocol. The animals showed a generalized depressive phenotype, as defined by decreased sucrose and sexual preference after 3 weeks of stress discontinuation and apathy-like behavior in the FST one month after stress discontinuation. These depressive-like behaviors were accompanied by diminished levels of orexin in mesocortical regions of the DA reward system and of orexin and dynorphin in the hypothalamus.[74] VTA hypofunction associated with anhedonic behavior had already been reported, but after a more severe continuous SD exposure.[37] The hypothalamus sends an orexin projection to the VTA, which, when stimulated, produces effort and reward motivation behaviors.[101] The lower levels of orexin in the VTA suggested a possible basis for sexual anhedonia.[71,74] Another feature of the role of the VTA in environmental reactivity to stimuli was evaluated by Fanous et al.[77] They used a virus to deplete BDNF levels in this region and, after intermittent SD

exposure, the rats displayed elevated social behaviors, in contrast with rats infused with a control virus. This study suggested that BDNF might be a pro-depressive factor within mesolimbic regions.[77,102] The conditions of the animals used in the study by Nocjar et al.[74] should be analyzed when defining the validity of SD protocols, as the rats were isolated for 28 days before social stress initiation. Social isolation alone is a stress protocol, at least in social cohesive species, such as rats.[103]

Functional disturbances of the noradrenergic system may also be accountable for the development of depressive-like states. Chen et al.[18] evaluated mRNA levels of NET in the LC and terminal regions, and an increased expression in these regions was accompanied by decreased sweet solution preference and intake after chronic stress exposure. These findings support the involvement of corticosteroids together with catecholamines in the development of such depressive-like states, as adrenalectomy and corticosteroid antagonist treatment prevent the development of stress-induced depressive phenotypes.[18]

In addition to the most common measures of stress (i.e., glucocorticoids and catecholamines[104]), Kroes et al.[70] used other markers in a genomic study in the PAG region accompanied of 22 kHz USV in defeated animals. Six hours after repeated SD exposure, animals had changes in several genes, such as those involved in cholinergic transmission, GTPase mediated signal transduction and molecular function of growth factors activity. Twenty-two kHz USV are also seen in rats exposed to a single SD session,[55] and low range USV (around 22 kHz) were postulated as measures of emotional states of fear and anxiety in animal models.[105,106] Burgdorf et al.[55] evaluated these behavioral components of depressive or anxiety-like states along with expression of IFG-I. Other searches for stress-related measures focused on newly described growth factors as consequences of stress-induced phenotypes. Ordway et al.[81] focused on the involvement of BMP7 and catecholamines in the biology of depression. Other molecules of interest are FGF and IGF-I: these growth factor systems were significantly downregulated in the hippocampus of repeatedly defeated rats[67] and in the frontal and parietal cortices of the brain of one-time defeated rats.[55] Searching for new markers, Grunewald et al.[78] investigated the pathway composed of glucocorticoid and Kruppel-like factor 11, also named transforming growth factor β-inducible early gene 2, activated in continuously defeated animals. Olivares et al.[65] found higher levels of CORT and an imbalance of thyroid hormones one month after continuous SD exposures, together with behavioral stress-induced changes. Together, these studies suggested possible new therapeutic targets in the treatment of major depressive disorder, as growth factors, cell signaling pathways and the hormones discussed above may be abnormal in clinical populations.

These studies were closely connected to the use of continuous SD protocol as an animal model for anxiety-like states and clinical depression, which meets criteria for human psychopathology. This model includes valid measures of behavioral depressive-like symptoms, such as those related to reactivity to environmental stimuli and anhedonia. Decreased locomotion and exploratory behaviors may be interpreted as deficits in motivation; decreased mobility in the FST may be associated with behavioral despair, which in turn is associated with depressive disorders; and decreased sweet solution preference may result from desensitization of reward circuitry, which is analogous to an anhedonic state. All these effects were elicited by chronic SD exposure. The expression of cell signaling molecules associated with BDNF cascade and interactions of DA and BDNF in VTA results from this type of exposure. The importance of these studies about new molecules lies in the need for new treatment targets for clinical depression. Although not more effective than traditional antidepressants, new drugs are gaining acceptance due to significantly better tolerance and fewer side effects.[107,108]

Neurobiological basis of individual vulnerability to stress

Kabbaj et al.[69] discussed several aspects of the neurobiological substrates of individual differences in emotionality and responsiveness to stress and drug addiction. The first refers to the existence of individual specific neurochemical dysfunctions, due to which individuals seek drugs to repair this imbalance[69]; the second is based on the fact that individuals are highly attracted to either the reward and novelty properties of drugs, or the drug ability to help cope with emotional or environmental distress.[109] The second includes personality traits, such as endophenotypes, that mediate the probability that an individual will seek drugs. This view is corroborated by investigations about sensation-seeking and drug-taking behaviors,[109,110] as well as by studies about substance abuse behaviors correlated with mood disorders.[111-113]

Piazza et al.[49] were pioneers in demonstrating the association of an individual's exploratory behavior and drug-taking patterns. Kabbaj et al.[69] further discussed the suggested classification of individuals as HR or LR based on their responsiveness to a mild environmental stress, such as exposure to the OF. The phenotypes of HR rats include less anxious behaviors in the light-dark box apparatus and greater elevated-plus maze (EPM)

than that of LR rats.[114] HR rats have more climbing and less floating in the FST than LR animals.[115] Furthermore, HR rats express less GR in the hippocampus,[116] and both phenotypes have different CORT-releasing patterns. The analysis of drug-taking behaviors and social stress after rats were exposed to chronic SD revealed that the once existing differences in cocaine self-administration tended to be no longer evident: SD induced a delay in cocaine self-administration in HR and a dramatic increase in cocaine self-administration in LR rats.[117] Kabbaj et al.[69] investigated neural signatures, or neural phenotypes, associated with the processes mentioned above. A study using a microarray technique to analyze genes in the hippocampus found an imbalance of a large number of genes involved in neurogenesis after four repeated sessions of SD.[69] The authors suggested that HR animals had a slower rate of cell proliferation, differentiation and transformation than LR rats. These patterns might indicate that there is a link between BDNF, the hippocampus and drug-taking behaviors.[69] The authors focused on individual differences in emotional responsiveness and reactivity to stress.[48] HR rats seem to be more vulnerable to depressive-like symptoms when facing stressful stimuli.[118] This higher vulnerability in HR animals were associated with higher levels of histone acetylation in the hippocampus at baseline and after repeated exposure to SD. HR rats with decreased sweet solution preference also had decreased levels of histone acetylation in the hippocampus.[48] Such cell chromatin modifications are dynamic processes that regulate gene expression without changes in the DNA sequence.[119] Histone acetylation is one of these processes, during which hyperacetylation leads to increased gene expression, while hypoacetylation might be involved in gene silencing.[120] Another study about BDNF and epigenetic regulation of individual vulnerability found that LR individuals have higher levels of hippocampal BDNF after SD exposure than HR rats.[46] This trait may confer stress resilience to LR rats and vulnerability to HR rats. In fact, preventing BDNF signaling in the dentate gyrus of the hippocampus of LR rats leads to SD-induced social avoidance, whereas BDNF activation in the same region in HR animals leads to social approach behaviors.[46] Duclot & Kabbaj[46] discussed the hypothesis that LR rats might have epigenetic tools for the regulation of BDNF expression in the hippocampus, which might confer them resilience to stress, whereas HR animals seem to lack this mechanism.

Kabbaj et al.[69] also found that the phenotypes of HR and LR animals differ in the expression of serotonergic receptors in the hippocampus.[115] In a study about early life stress and subsequent outcomes in adulthood, Gardner et al.[58] focused on the involvement of the serotonergic system and coping with social stress in adult male rats. Regardless of whether submitted to early life stress or normal growth, rats had the same pattern of activation of serotonergic neurons in specific subpopulations of the DR nucleus.[58] This suggests that brainstem monoamine systems may be associated with vulnerability to stress-related psychiatric disorders, specifically in the case of animals exposed to early stress and facing a psychosocial conflict during adulthood.[59,60] The serotonergic system might be involved in inhibitory processes of proactive coping responses, such as aggression[121,122] and escape behaviors[123]; at the same time, it may be associated with facilitation of passive-submissive responses.[124] Using a repeated SD stress protocol, Paul et al.[63] found a shift away from a proactive emotional coping style and towards a reactive emotional coping style during the defeat phase. In unstable social structure periods, adopting a subordinate position may prevent dangerous situations and limit injury and energy wastage.[125] A reactive coping style may be a more flexible and adaptive strategy according to Koolhaas et al.[126] An alternative interpretation of coping phenotypes was presented by Kanarik et al.[80] After the discontinuation of a chronic stress protocol, during the second exposure to the FST, individuals who had more struggling behaviors were non-susceptible to stress and adopted an active coping strategy; inversely, less struggling animals were susceptible to stress and had passive coping responses.[80]

BDNF metabolism was shown to be highly relevant to this topic in several stress-related studies. The investigation of emotional differences seems to be a fruitful avenue for research to understand differences in vulnerability to stress, substance abuse and mood disorders. This may be achieved by either studying behavior directly or combining the behavioral features and molecular differences, both at baseline and in response to stress. These particular differences in reacting to stress may be the key to the development of better pharmacological and therapeutic treatments and to the explanation of failed drug trials.

Conclusion

Studies about stress are filled with data, but most results are contradictory or have to be more carefully discussed. Measures of social stress-induced effects are complex and may rely on the characteristics of stressors, such as duration, intensity and predictability, and of the biological sample, such as time of sampling, rat strain used and sample quality. These differences and similarities should be discussed to derive knowledge about stress physiology and to develop new pharmacological and

therapeutic treatments for stress-induced or aggravated disorders. For instance, studies that claim to have discovered brain areas activated during social stress or as a consequence of it should be analyzed considering that studies about immediate early gene expression may differ in a wide variety of control conditions. Fos proteins do not seem to be good markers of inhibition, which may also be the result of neuronal signaling.[127] Absence of Fos labeling does not necessarily equal lack of involvement. The cumulative process of acquiring evidence may be a reliable way to discuss and critically analyze new findings. Several important points should be addressed when dealing with data provided by SD protocols. Each laboratory may have its own version of procedures, and protocol phases may be either prolonged or even omitted. These discrepancies should be evaluated in accordance with the animal model used and the conditions to be achieved. The analysis of species and strains in animal models revealed that the studies reviewed used few strains of rats. Most of the written protocols used Long-Evans male rats as residents; species variation of stressed animals was high, although still most studies used Sprague-Dawley rats. It is beyond this review to discuss implications of strain variations in the models. However, the exact purpose of each experiment should shed some light in the importance of rat strain.

SD exposure activates cortical and limbic circuits, areas that underlie the processing of emotional stress and reward, which reinforces the role of protocols in the study of addictive behaviors. The short length of activation induced a short-lived adaptation important to the understanding of the physiological and behavioral response to stress. Progressively, the increases in duration or recurrence of stress induced transient or long-term adaptations, referred to as neuroadaptations, and consequent changes in the neuronal paths involved in dealing with stress. Continuous stress exposures induced lasting consequences, such as coping dysfunctions and development of affective disordered states. The SD model may be a relevant method to study stress responses and the development of addictive behaviors. It may also be used as a model of clinical depression and anxiety for the development of therapeutic and pharmacological treatments.

Acknowledgements

We gratefully acknowledge the advice of Klaus Miczek in the translation of the manuscript. We thank Keitiline Viacava and Fernanda Lopes for the insights about the method used in this study. We thank Liziane Bizarro and Marcia Kauer Sant'Anna for the advice about the study as a whole.

References

1. Kessler RC, Berglund P, Demler O, Jin R, Koretz D, Merikangas KR, et al. The epidemiology of major depressive disorder: results from the National Comorbidity Survey Replication (NCS-R). JAMA. 2003;289:3095-105.
2. Prince M, Patel V, Saxena S, Maj M, Maselko J, Phillips MR, et al. No health without mental health. Lancet. 2007;370:859-77.
3. Saarni SI, Suvisaari J, Sintonen H, Pirkola S, Koskinen S, Aromaa A, et al. Impact of psychiatric disorders on health-related quality of life: general population survey. Br J Psychiatry. 2007;190:326-32.
4. Ustun TB, Ayuso-Mateos JL, Chatterji S, Mathers C, Murray CJ. Global burden of depressive disorders in the year 2000. Br J Psychiatry. 2004;184:386-92.
5. Mathers CD, Loncar D. Projections of global mortality and burden of disease from 2002 to 2030. PLoS Med. 2006;3:e442.
6. Blanchard RJ, McKittrick CR, Blanchard DC. Animal models of social stress: effects on behavior and brain neurochemical systems. Physiol Behav. 2001;73:261-71.
7. Sapolsky RM. The influence of social hierarchy on primate health. Science. 2005;308:648-52.
8. Von Holst D. The concept of stress and its relevance for animal behavior. Adv Study Behav USA [Internet]. 1998 [cited 2014 Jun 4]. http://agris.fao.org/agris-search/search.do?f=1998/US/US98047.xml;US1997069446
9. Raab A, Dantzer R, Michaud B, Mormede P, Taghzouti K, Simon H, et al. Behavioural, physiological and immunological consequences of social status and aggression in chronically coexisting resident-intruder dyads of male rats. Physiol Behav. 1986;36:223-8.
10. Koolhaas JM, Schuurman T, Wiepkema PR. The organization of intraspecific agonistic behaviour in the rat. Prog Neurobiol. 1980;15:247-68.
11. Miczek KA. A new test for aggression in rats without aversive stimulation: differential effects of d-amphetamine and cocaine. Psychopharmacology (Berl). 1979;60:253-9.
12. Albonetti ME, Farabollini F. Social stress by repeated defeat: effects on social behaviour and emotionality. Behav Brain Res. 1994;62:187-93.
13. Fuchs E, Flügge G. Social stress in tree shrews: effects on physiology, brain function, and behavior of subordinate individuals. Pharmacol Biochem Behav. 2002;73:247-58.
14. Keeney AJ, Hogg S, Marsden CA. Alterations in core body temperature, locomotor activity, and corticosterone following acute and repeated social defeat of male NMRI mice. Physiol Behav. 2001;74:177-84.
15. Marini F, Pozzato C, Andreetta V, Jansson B, Arban R, Domenici E, et al. Single exposure to social defeat increases corticotropin-releasing factor and glucocorticoid receptor mRNA expression in rat hippocampus. Brain Res. 2006;1067:25-35.
16. Sapolsky RM. Why zebras don't get ulcers. New York: W.H. Freeman and Co; 2004.
17. McEwen BS. Central effects of stress hormones in health and disease: Understanding the protective and damaging effects of stress and stress mediators. Eur J Pharmacol. 2008;583:174-85.
18. Chen P, Fan Y, Li Y, Sun Z, Bissette G, Zhu MY. Chronic social defeat up-regulates expression of norepinephrine transporter in rat brains. Neurochem Int. 2012;60:9-20.
19. De Kloet ER, Vreugdenhil E, Oitzl MS, Joëls M. Brain corticosteroid receptor balance in health and disease. Endocr Rev. 1998;19:269-301.
20. Kovács KJ, Földes A, Sawchenko PE. Glucocorticoid negative feedback selectively targets vasopressin transcription in parvocellular neurosecretory neurons. J Neurosci. 2000;20:3843-52.
21. Pace TW, Spencer RL. Disruption of mineralocorticoid receptor function increases corticosterone responding to a mild, but not moderate, psychological stressor. Am J Physiol Endocrinol Metab. 2005;288:E1082-8.
22. Bartolomucci A, Palanza P, Costoli T, Savani E, Laviola G, Parmigiani S, et al. Chronic psychosocial stress persistently alters autonomic function and physical activity in mice. Physiol Behav. 2003;80:57-67.
23. Razzoli M, Carboni L, Guidi A, Gerrard P, Arban R. Social defeat-induced contextual conditioning differentially imprints behavioral and adrenal reactivity: a time-course study in the rat. Physiol Behav. 2007;92:734-40.
24. Bartolomucci A, Palanza P, Gaspani L, Limiroli E, Panerai AE, Ceresini G, et al. Social status in mice: behavioral, endocrine and immune changes are context dependent. Physiol Behav. 2001;73:401-10.

25. Meerlo P, Overkamp GJ, Daan S, Van Den Hoofdakker RH, Koolhaas JM. Changes in behaviour and body weight following a single or double social defeat in rats. Stress. 1996;1:21-32.
26. Padgett DA, Glaser R. How stress influences the immune response. Trends Immunol. 2003;24:444-8.
27. Sgoifo A, Costoli T, Meerlo P, Buwalda B, Pico'-Alfonso MA, De Boer S, et al. Individual differences in cardiovascular response to social challenge. Neurosci Biobehav Rev. 2005;29:59-66.
28. Miczek KA, Yap JJ, Covington HE 3rd. Social stress, therapeutics and drug abuse: preclinical models of escalated and depressed intake. Pharmacol Ther. 2008;120:102-28.
29. Badiani A, Rajabi H, Nencini P, Stewart J. Modulation of food intake by the κ opioid U-50,488H: evidence for an effect on satiation. Behav Brain Res. 200129;118:179-86.
30. Vanderschuren LJ, Kalivas PW. Alterations in dopaminergic and glutamatergic transmission in the induction and expression of behavioral sensitization: a critical review of preclinical studies. Psychopharmacology (Berl). 2000;151:99-120.
31. Covington HE 3rd, Miczek KA. Repeated social-defeat stress, cocaine or morphine. Effects on behavioral sensitization and intravenous cocaine self-administration "binges". Psychopharmacology (Berl). 2001;158:388-98.
32. Nikulina EM, Covington HE 3rd, Ganschow L, Hammer RP Jr, Miczek KA. Long-term behavioral and neuronal cross-sensitization to amphetamine induced by repeated brief social defeat stress: Fos in the ventral tegmental area and amygdala. Neuroscience. 2004;123:857-65.
33. Robinson TE, Berridge KC. The psychology and neurobiology of addiction: an incentive–sensitization view. Addiction. 2000;95:S91-117.
34. Tidey JW, Miczek KA. Social defeat stress selectively alters mesocorticolimbic dopamine release: an in vivo microdialysis study. Brain Res. 1996;721:140-9.
35. Yerkes RM, Dodson JD. The relation of strength of stimulus to rapidity of habit-formation. J Comp Neurol Psychol. 1908;18:459-82.
36. Diamond DM, Campbell AM, Park CR, Halonen J, Zoladz PR. The temporal dynamics model of emotional memory processing: a synthesis on the neurobiological basis of stress-induced amnesia, flashbulb and traumatic memories, and the Yerkes-Dodson law. Neural Plast. 2007;2007:60803.
37. Miczek KA, Nikulina EM, Shimamoto A, Covington HE 3rd. Escalated or suppressed cocaine reward, tegmental BDNF and accumbal dopamine due to episodic vs. continuous social stress in rats. J Neurosci. 2011;31:9848-57.
38. Wang J, Fanous S, Terwilliger EF, Bass CE, Hammer RP Jr, Nikulina EM. BDNF overexpression in the ventral tegmental area prolongs social defeat stress-induced cross-sensitization to amphetamine and increases ΔFosB expression in mesocorticolimbic regions of rats. Neuropsychopharmacology. 2013;38:2286-96.
39. Nikulina EM, Lacagnina MJ, Fanous S, Wang J, Hammer RP Jr. Intermittent social defeat stress enhances mesocorticolimbic ΔFosB/BDNF co-expression and persistently activates corticotegmental neurons: implication for vulnerability to psychostimulants. Neuroscience. 2012;212:38-48.
40. Koolhaas JM, Meerlo P, de Boer SF, Strubbe JH, Bohus B. The temporal dynamics of the stress response. Neurosci Biobehav Rev. 1997;21:775-82.
41. Rygula R, Abumaria N, Domenici E, Hiemke C, Fuchs E. Effects of fluoxetine on behavioral deficits evoked by chronic social stress in rats. Behav Brain Res. 2006;174:188-92.
42. Von Frijtag JC, Kamal A, Reijmers LG, Schrama LH, van den Bos R, Spruijt BM. Chronic imipramine treatment partially reverses the long-term changes of hippocampal synaptic plasticity in socially stressed rats. Neurosci Lett. 2001;309:153-6.
43. Kendler KS, Karkowski-Shuman L. Stressful life events and genetic liability to major depression: genetic control of exposure to the environment? Psychol Med. 1997;27:539-47.
44. Koolhaas JM, de Boer SF, Buwalda B, van Reenen K. Individual variation in coping with stress: a multidimensional approach of ultimate and proximate mechanisms. Brain Behav Evol. 2007;70:218-26.
45. Pawlak CR, Ho YJ, Schwarting RK. Animal models of human psychopathology based on individual differences in novelty-seeking and anxiety. Neurosci Biobehav Rev. 2008;32:1544-68.
46. Duclot F, Kabbaj M. Individual differences in novelty seeking predict subsequent vulnerability to social defeat through a differential epigenetic regulation of brain-derived neurotrophic factor expression. J Neurosci. 2013;33:11048-60.
47. Harro J. Inter-individual differences in neurobiology as vulnerability factors for affective disorders: Implications for psychopharmacology. Pharmacol Ther. 2010;125:402-2.
48. Hollis F, Duclot F, Gunjan A, Kabbaj M. Individual differences in the effect of social defeat on anhedonia and histone acetylation in the rat hippocampus. Horm Behav. 2011;59:331-7.
49. Piazza PV, Deminière JM, Le Moal M, Simon H. Factors that predict individual vulnerability to amphetamine self-administration. Science. 1989;245:1511-3.
50. Duclot F, Hollis F, Darcy MJ, Kabbaj M. Individual differences in novelty-seeking behavior in rats as a model for psychosocial stress-related mood disorders. Physiol Behav. 2011;104:296-305.
51. Razzoli M, Carboni L, Arban R. Alterations of behavioral and endocrinological reactivity induced by 3 brief social defeats in rats: relevance to human psychopathology. Psychoneuroendocrinology. 2009;34:1405-16.
52. Bourke CH, Raees MQ, Malviya S, Bradburn CA, Binder EB, Neigh GN. Glucocorticoid sensitizers Bag1 and Ppid are regulated by adolescent stress in a sex-dependent manner. Psychoneuroendocrinology. 2013;38:84-93.
53. Green MK, Rani CS, Joshi A, Soto-Piña AE, Martinez PA, Frazer A, et al. Prenatal stress induces long term stress vulnerability, compromising stress response systems in the brain and impairing extinction of conditioned fear after adult stress. Neuroscience. 2011;192:438-51.
54. Kamal A, Van der Harst JE, Kapteijn CM, Baars AJ, Spruijt BM, Ramakers GM. Announced reward counteracts the effects of chronic social stress on anticipatory behavior and hippocampal synaptic plasticity in rats. Exp Brain Res. 2010;201:641-51.
55. Burgdorf J, Kroes RA, Beinfeld MC, Panksepp J, Moskal JR. Uncovering the molecular basis of positive affect using rough-and-tumble play in rats: a role for insulin-like growth factor I. Neuroscience. 2010;168:769-77.
56. Fekete ÉM, Zhao Y, Li C, Sabino V, Vale WW, Zorrilla EP. Social defeat stress activates medial amygdala cells that express type 2 corticotropin-releasing factor receptor mRNA. Neuroscience. 2009;162:5-13.
57. Funk D, Li Z, Lê AD. Effects of environmental and pharmacological stressors on c-fos and corticotropin-releasing factor mRNA in rat brain: Relationship to the reinstatement of alcohol seeking. Neuroscience. 2006;138:235-43.
58. Gardner KL, Thrivikraman KV, Lightman SL, Plotsky PM, Lowry CA. Early life experience alters behavior during social defeat: focus on serotonergic systems. Neuroscience. 2005;136:181-91.
59. Gardner KL, Hale MW, Oldfield S, Lightman SL, Plotsky PM, Lowry CA. Adverse experience during early life and adulthood interact to elevate tph2 mRNA expression in serotonergic neurons within the dorsal raphe nucleus. Neuroscience. 2009;163:991-1001.
60. Gardner KL, Hale MW, Lightman SL, Plotsky PM, Lowry CA. Adverse early life experience and social stress during adulthood interact to increase serotonin transporter mRNA expression. Brain Res. 2009;1305:47-63.
61. Green MK, Devine DP. Nociceptin/orphanin FQ and NOP receptor gene regulation after acute or repeated social defeat stress. Neuropeptides. 2009;43:507-14.
62. Kavushansky A, Ben-Shachar D, Richter-Levin G, Klein E. Physical stress differs from psychosocial stress in the pattern and time-course of behavioral responses, serum corticosterone and expression of plasticity-related genes in the rat. Stress. 2009;12:412-25.
63. Paul ED, Hale MW, Lukkes JL, Valentine MJ, Sarchet DM, Lowry CA. Repeated social defeat increases reactive emotional coping behavior and alters functional responses in serotonergic neurons in the rat dorsal raphe nucleus. Physiol Behav. 2011;104:272-82.
64. Carboni L, Piubelli C, Pozzato C, Astner H, Arban R, Righetti PG, et al. Proteomic analysis of rat hippocampus after repeated psychosocial stress. Neuroscience. 2006;137:1237-46.
65. Olivares EL, Silva-Almeida C, Pestana FM, Sonoda-Côrtes R, Araujo IG, Rodrigues NC, et al. Social stress-induced hypothyroidism is attenuated by antidepressant treatment in rats. Neuropharmacology. 2012;62:446-56.
66. Furay AR, McDevitt RA, Miczek KA, Neumaier JF. 5-HT1B mRNA expression after chronic social stress. Behav Brain Res. 2011;224:350-7.
67. Turner CA, Calvo N, Frost DO, Akil H, Watson SJ. The fibroblast growth factor system is downregulated following social defeat. Neurosci Lett. 2008;430:147-50.
68. Hueston CM, Barnum CJ, Eberle JA, Ferraioli FJ, Buck HM, Deak T. Stress-dependent changes in neuroinflammatory markers observed after common laboratory stressors are not seen

following acute social defeat of the Sprague Dawley rat. Physiol Behav. 2011;104:187-98.
69. Kabbaj M, Evans S, Watson SJ, Akil H. The search for the neurobiological basis of vulnerability to drug abuse: using microarrays to investigate the role of stress and individual differences. Neuropharmacology. 2004;47:111-22.
70. Kroes RA, Burgdorf J, Otto NJ, Panksepp J, Moskal JR. Social defeat, a paradigm of depression in rats that elicits 22-khz vocalizations, preferentially activates the cholinergic signaling pathway in the periaqueductal gray. Behav Brain Res. 2007;182:290-300.
71. Niikura S, Yokoyama O, Komatsu K, Yotsuyanagi S, Mizuno T, Namiki M. A causative factor of copulatory disorder in rats following social stress. J Urol. 2002;168:843-9.
72. Burke AR, Watt MJ, Forster GL. Adolescent social defeat increases adult amphetamine conditioned place preference and alters D2 dopamine receptor expression. Neuroscience. 2011;197:269-79.
73. Buwalda B, Stubbendorff C, Zickert N, Koolhaas JM. Adolescent social stress does not necessarily lead to a compromised adaptive capacity during adulthood: a study on the consequences of social stress in rats. Neuroscience. 2013;249:258-70.
74. Nocjar C, Zhang J, Feng P, Panksepp J. The social defeat animal model of depression shows diminished levels of orexin in mesocortical regions of the dopamine system, and of dynorphin and orexin in the hypothalamus. Neuroscience. 2012;218:138-53.
75. Covington HE 3rd, Miczek KA. Intense cocaine self-administration after episodic social defeat stress, but not after aggressive behavior: dissociation from corticosterone activation. Psychopharmacology (Berl). 2005;183:331-40.
76. Fanous S, Hammer RP Jr, Nikulina EM. Short- and long-term effects of intermittent social defeat stress on brain-derived neurotrophic factor expression in mesocorticolimbic brain regions. Neuroscience. 2010;167:598-607.
77. Fanous S, Terwilliger EF, Hammer RP Jr, Nikulina EM. Viral depletion of VTA BDNF in rats modulates social behavior, consequences of intermittent social defeat stress, and long-term weight regulation. Neurosci Lett. 2011;502:192-6.
78. Grunewald M, Johnson S, Lu D, Wang Z, Lomberk G, Albert PR, et al. Mechanistic role for a novel glucocorticoid-klf11 (tieg2) protein pathway in stress-induced monoamine oxidase a expression. J Biol Chem. 2012;287:24195-206.
79. Iio W, Matsukawa N, Tsukahara T, Kohari D, Toyoda A. Effects of chronic social defeat stress on MAP kinase cascade. Neurosci Lett. 2011;504:281-4.
80. Kanarik M, Alttoa A, Matrov D, Kõiv K, Sharp T, Panksepp J, et al. Brain responses to chronic social defeat stress: effects on regional oxidative metabolism as a function of a hedonic trait, and gene expression in susceptible and resilient rats. Eur Neuropsychopharmacol. 2011;21:92-107.
81. Ordway GA, Szebeni A, Chandley MJ, Stockmeier CA, Xiang L, Newton SS, et al. Low gene expression of bone morphogenetic protein 7 in brainstem astrocytes in major depression. Int J Neuropsychopharmacol. 2012;15:855-68.
82. Kieran N, Ou XM, Iyo AH. Chronic social defeat downregulates the 5-HT1A receptor but not Freud-1 or NUDR in the rat prefrontal cortex. Neurosci Lett. 2010;469:380-4.
83. Tornatzky W, Miczek KA. Long-term impairment of autonomic circadian rhythms after brief intermittent social stress. Physiol Behav. 1993;53:983-93.
84. Tornatzky W, Miczek KA. Behavioral and autonomic responses to intermittent social stress: differential protection by clonidine and metoprolol. Psychopharmacology (Berl). 1994;116:346-56.
85. Marini F, Pozzato C, Andreetta V, Jansson B, Arban R, Domenici E, et al. Single exposure to social defeat increases corticotropin-releasing factor and glucocorticoid receptor mRNA expression in rat hippocampus. Brain Res. 2006;1067:25-35.
86. Potter E, Sutton S, Donaldson C, Chen R, Perrin M, Lewis K, et al. Distribution of corticotropin-releasing factor receptor mRNA expression in the rat brain and pituitary. Proc Natl Acad Sci U S A. 1994;91:8777-81.
87. Zorrilla EP, Koob GF. The therapeutic potential of CRF1 antagonists for anxiety. Expert Opin Investig Drugs. 2004;13:799-828.
88. Luiten PGM, Koolhaas JM, de Boer S, Koopmans SJ. The cortico-medial amygdala in the central nervous system organization of agonistic behavior. Brain Res. 1985;332:283-97.
89. Blanchard DC, Canteras NS, Markham CM, Pentkowski NS, Blanchard RJ. Lesions of structures showing FOS expression to cat presentation: Effects on responsivity to a Cat, Cat odor, and nonpredator threat. Neurosci Biobehav Rev. 2005;29:1243-53.
90. Dayas CV, Buller KM, Day TA. Neuroendocrine responses to an emotional stressor: evidence for involvement of the medial but not the central amygdala. Eur J Neurosci. 1999;11:2312-22.
91. Blandino P Jr, Barnum CJ, Solomon LG, Larish Y, Lankow BS, Deak T. Gene expression changes in the hypothalamus provide evidence for regionally-selective changes in IL-1 and microglial markers after acute stress. Brain Behav Immun. 2009;23:958-68.
92. Johnson JD, Campisi J, Sharkey CM, Kennedy SL, Nickerson M, Greenwood BN, et al. Catecholamines mediate stress-induced increases in peripheral and central inflammatory cytokines. Neuroscience. 2005;135:1295-307.
93. O'Connor KA, Johnson JD, Hansen MK, Wieseler Frank JL, Maksimova E, Watkins LR, et al. Peripheral and central proinflammatory cytokine response to a severe acute stressor. Brain Res. 2003;991:123-32.
94. Covington HE 3rd, Kikusui T, Goodhue J, Nikulina EM, Hammer RP Jr, Miczek KA. Brief social defeat stress: long lasting effects on cocaine taking during a binge and zif268 mRNA expression in the amygdala and prefrontal cortex. Neuropsychopharmacology. 2005;30:310-21.
95. Nestler EJ, Kelz MB, Chen J. ΔFosB: a molecular mediator of long-term neural and behavioral plasticity. Brain Res. 1999;835:10-7.
96. Geisler S, Zahm DS. Afferents of the ventral tegmental area in the rat-anatomical substratum for integrative functions. J Comp Neurol. 2005;490:270-94.
97. Kalivas PW, Duffy P, Latimer LG. Neurochemical and behavioral effects of corticotropin-releasing factor in the ventral tegmental area of the rat. J Pharmacol Exp Ther. 1987;242:757-63.
98. Fanselow MS, Dong HW. Are the dorsal and ventral hippocampus functionally distinct structures? Neuron. 2010;65:7-19.
99. Castagné V, Moser P, Roux S, Porsolt RD. Rodent models of depression: forced swim and tail suspension behavioral despair tests in rats and mice. Current Protocols in Neuroscience [Internet]. 2011 Apr 1 [cited 2014 Jul 28]. http://onlinelibrary.wiley.com/doi/10.1002/0471142301.ns0810as55/abstract
100. Chang L, Karin M. Mammalian MAP kinase signalling cascades. Nature. 2001;410:37-40.
101. Muschamp JW, Dominguez JM, Sato SM, Shen RY, Hull EM. A role for hypocretin (orexin) in male sexual behavior. J Neurosci. 2007;27:2837-45.
102. Nestler EJ, Carlezon WA Jr. The mesolimbic dopamine reward circuit in depression. Biol Psychiatry. 2006;59:1151-9.
103. Brain P, Benton D. The interpretation of physiological correlates of differential housing in laboratory rats. Life Sci. 1979;24:99-115.
104. McEwen BS. Allostasis and allostatic load: implications for neuropsychopharmacology. Neuropsychopharmacology. 2000;22:108-24.
105. Sánchez C. Stress-induced vocalisation in adult animals. A valid model of anxiety? Eur J Pharmacol. 2003;463:133-43.
106. Miczek KA, Weerts EM, Vivian JA, Barros HM. Aggression, anxiety and vocalizations in animals: GABAA and 5-HT anxiolytics. Psychopharmacology (Berl). 1995;121:38-56.
107. Montgomery SA, Baldwin DS, Blier P, Fineberg NA, Kasper S, Lader M, et al. Which antidepressants have demonstrated superior efficacy? A review of the evidence. Int Clin Psychopharmacol. 2007;22:323-9.
108. Papakostas GI. Tolerability of modern antidepressants. J Clin Psychiatry. 2008;69:8-13.
109. Cloninger CR, Sigvardsson S, Przybeck TR, Svrakic DM. Personality antecedents of alcoholism in a national area probability sample. Eur Arch Psychiatry Clin Neurosci. 1995;245:239-44.
110. Cloninger CR, Sigvardsson S, Bohman M. Childhood personality predicts alcohol abuse in young adults. Alcohol Clin Exp Res. 1988;12:494-505.
111. Franken IH, Hendriks VM. Screening and diagnosis of anxiety and mood disorders in substance abuse patients. Am J Addict. 2001;10:30-9.
112. Kell MJ. Opiate dependence, comorbidity and seasonality of birth. J Addict Dis. 1995;14:19-34.
113. Milby JB, Sims MK, Khuder S, Schumacher JE, Huggins N, McLellan AT, et al. Psychiatric comorbidity: prevalence in methadone maintenance treatment. Am J Drug Alcohol Abuse. 1996;22:95-107.
114. Dellu F, Piazza PV, Mayo W, Le Moal M, Simon H. Novelty-seeking in rats-biobehavioral characteristics and possible relationship with the sensation-seeking trait in man. Neuropsychobiology. 1996;34:136-45.
115. Jama A, Cecchi M, Calvo N, Watson SJ, Akil H. Inter-individual differences in novelty-seeking behavior in rats predict differential responses to desipramine in the forced swim test. Psychopharmacology (Berl). 2008;198:333-40.

116. Dellu F, Mayo W, Vallée M, Maccari S, Piazza PV, Le Moal M, et al. Behavioral reactivity to novelty during youth as a predictive factor of stress-induced corticosterone secretion in the elderly—a life-span study in rats. Psychoneuroendocrinology. 1996;21:441-53.
117. Kabbaj M, Akil H. Individual differences in novelty-seeking behavior in rats: a c-fos study. Neuroscience. 2001;106:535-45.
118. Taghzouti K, Lamarque S, Kharouby M, Simon H. Interindividual differences in active and passive behaviors in the forced-swimming test: implications for animal models of psychopathology. Biol Psychiatry. 1999;45:750-8.
119. Crosio C, Heitz E, Allis CD, Borrelli E, Sassone-Corsi P. Chromatin remodeling and neuronal response: multiple signaling pathways induce specific histone H3 modifications and early gene expression in hippocampal neurons. J Cell Sci. 2003;116:4905-14.
120. Ito K, Adcock IM. Histone acetylation and histone deacetylation. Mol Biotechnol. 2002;20:99-106.
121. Miczek KA, Mos J, Olivier B. Brain 5-HT and inhibition of aggressive behavior in animals: 5-HIAA and receptor subtypes. Psychopharmacol Bull. 1989;25:399-403.
122. Stein DJ, Miczek KA, Lucion AB, de Almeida RM. Aggression-reducing effects of F15599, a novel selective 5-HT1A receptor agonist, after microinjection into the ventral orbital prefrontal cortex, but not in infralimbic cortex in male mice. Psychopharmacology (Berl). 2013;230:375-87.
123. Graeff FG. Serotonin, the periaqueductal gray and panic. Neurosci Biobehav Rev. 2004;28:239-59.
124. Chung KK, Martinez M, Herbert J. Central serotonin depletion modulates the behavioural, endocrine and physiological responses to repeated social stress and subsequent c-fos expression in the brains of male rats. Neuroscience. 1999;92:613-25.
125. Korte SM, Koolhaas JM, Wingfield JC, McEwen BS. The Darwinian concept of stress: benefits of allostasis and costs of allostatic load and the trade-offs in health and disease. Neurosci Biobehav Rev. 2005;29:3-38.
126. Koolhaas JM, Korte SM, de Boer SF, Van Der Vegt BJ, Van Reenen CG, Hopster H, et al. Coping styles in animals: current status in behavior and stress-physiology. Neurosci Biobehav Rev. 1999;23:925-35.
127. Robertson HA, Peterson MR, Murphy K, Robertson GS. D1-dopamine receptor agonists selectively activate striatal c-fos independent of rotational behaviour. Brain Res. 1989;503:346-9.

Correspondence:
Mailton Vasconcelos, Instituto de Psicologia
Universidade Federal do Rio Grande do Sul
Rua Ramiro Barcelos, 2600, room 105
90035-003 - Porto Alegre, RS - Brazil
E-mail: mailton.vasc@gmail.com