

Sex-driven vulnerability in stress and drug abuse

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Abstract

A growing body of literature shows that a link exists between substance abuse and stress and that the crosstalk of sex hormones with the neuroendocrine system might differently prime vulnerability to drug addiction in male and female subjects. Thus, understanding the neurobiological mechanisms of addiction and the identification of sex-driven determinants in vulnerability to drug abuse may help to better devise and/or implement strategic (pharmacological, behavioural, social) interventions to prevent or face the issue of addiction. Differences between sexes can be found at all stages of life (in both the animal model and human studies) and may account for genetic, epigenetic and environmental/hormonal factors that in turn affect the functionality of the whole organism leading also to a sex-driven differential vulnerability or resilience to non-communicable pathologies. These include the onset and precipitation of stress-related psychiatric disorders as well as “substance-related and addictive disorders” (as defined in the DSM-V). This paper reviews the scientific literature highlighting significant differences in male and female subjects in stress and neuroendocrine function and the implications for sex-dependent differential vulnerability to drug addiction.

Key words

- sex
- stress
- addiction
- hypothalamic-pituitary-adrenal axis
- animal models

“For some traits... the presence or absence of sex differences is sufficiently robust that randomly cycling females can be studied without additional variability being introduced by the estrous cycle” [1]

INTRODUCTION

Understanding the significance of sex differences is becoming a priority not only in clinical research but also in basic science. More than twenty years ago, the US National Institutes of Health (NIH) acknowledged that gender imbalance in clinical research could have been harmful for women’s health, as well as for science, and began requiring women (and minorities) to be included in NIH-funded clinical research. Currently, approximately half of the patients enrolled in (NIH-funded) clinical trials are women [2]. Despite this successful policy, basic science still lags behind [2]. For this reason, starting from January 2016, the US NIH has implemented a policy that expects scientists to take into account sex as a biological variable also in preclinical research [3]. In addition, EU-funded research is also required to add a “gender dimension” under the Horizon 2020 Program (see *Guidance in gender equality in Horizon 2020*, http://ec.europa.eu/research/participants/data/ref/h2020/grants_manual/hi/gender/h2020-hi-guide-gender_en.pdf).

Differences between sexes can be found throughout life (in both animal models and human studies) and

may account for genetic, epigenetic and environmental/hormonal factors. In addition, they might be observed at very different levels, ranging from organismal and systemic to cellular and molecular mechanisms [4]. In this context, individual features (genetic background) and environmental factors (stressful life events) can interact providing the ground for sex-driven liability to non-communicable pathologies, including “substance-related and addictive disorders” (as defined in the DSM-V [5]).

Epidemiological data, widely supported by preclinical research, provide strong evidence for stress being a risk factor for the onset and/or precipitation of many different pathological conditions. Stress is perceived and elaborated differently from male and female subjects and coping strategies towards stress are clearly sex-driven. These differences in stress responses emerge as the result of organizational and activation effects of gonadal hormones as well as of genes on the sex chromosomes [6]. The activation of the neuroendocrine system, particularly the hypothalamic-pituitary-adrenal (HPA) axis, as a result of stress or arousing stimuli, plays also a pivotal role in the neuro-behavioural and psycho-biological processes underlying (the different stages characterizing) drug addiction [1, 7-9]. Sex differences in rates of substance abuse are consistently observed in the general population with regards to rates of substance use, abuse, and dependence. In humans,

men show more drug and alcohol consumption and show higher degree of misuse. By contrast, data obtained from animal studies provide evidence for female sex being overall more vulnerable [1]. Thus, the use of both sexes in animal model might unveil sex differences in the neurobiological mechanisms regardless of cultural factors specific to humans.

A WORKING DEFINITION OF STRESS, HOMEOSTASIS, ALLOSTASIS AND ALLOSTATIC LOAD

Stress is any change of the internal or external milieu affecting the homeostasis. In complex organisms, the central nervous system plays a pivotal role in the stress perception and interpretation by determining what is threatening and potentially harmful [10]. As a consequence, the brain and the body trigger a coordinated set of intercellular signals, physiological as well as behavioural responses leading to individual (sex-specific) strategies to cope with stress. This stress-driven physiological set point, in the short run, is pivotal to face the real or perceived stressor and to maintain homeostasis. However, if stress becomes chronic it can trigger diseases' onset and/or accelerate their progression (including psychiatric disorders) or promote risky changes into lifestyle that might easily precipitate into pathological life-threatening habits (e.g. alcohol or drug users might develop drug addiction) [7]. Thus, stress represents a main risk factor for mental health and comorbid pathological conditions including drug addiction.

A very simplified model of how stress is perceived by an organism, elaborated and translated into (more or less efficient physiological and behavioural) coping strategies involves the activation of the HPA axis. More in detail, hypothalamic corticotropin-releasing factor (CRF) neurons within the paraventricular nucleus (PVN) release CRF into the hypophyseal portal circulation leading to the release of adrenocorticotrophic hormone (ACTH) into the general circulation, eventually leading to the synthesis and release of the glucocorticoid hormones (GC – cortisol in humans and corticosterone in rodents) from the adrenal gland cortex. The primary function of GC is to make energy storage available (catabolic action) to increase fuel in order to promote survival abilities of an organism during stress. However, under basal conditions, they coordinate daily activities and sleep-related events through circadian (and ultradian) secretory bursts [11, 12]. The action of GC hormones on central and peripheral targets is mediated by mineralocorticoid receptors (MR) and glucocorticoid receptors (GR). In the brain, both these receptors are widely expressed in the limbic system (including hippocampus, hypothalamus and pituitary, lateral septum and amygdala) a brain circuit involved in the modulation of emotions and cognition and in the elaboration of stress-related behavioural responses. In addition, GR and MR are also highly expressed in the pre-frontal cortex, a brain region involved in the inhibitory as well as in the emotional control of behaviour [13-16].

Responsible for much of the effects of basal and low-stress levels of GC at the onset of the stressor (*i.e.* the permissive effects) are MR, whereas GR largely medi-

ates the effects of high-stress levels of GC, facilitating the re-establishment of homeostasis (negative feedback) when GC stress levels prevail [8, 14, 17]. These receptors are also involved in the feedback mechanisms that tightly regulate GC secretion and the functionality of the HPA axis, preventing the axis from the deleterious effects of its overshooting. In fact, alterations in the effectiveness of negative feedback by GC have profound implications for the activity of the HPA axis and regulation of stress responses, such that aberrant action can increase the vulnerability of the individual to stress-induced disorders or diseases [14].

During chronic stressful conditions, prolonged activation of the HPA axis and the consequent hyper-exposure to GC hormones might lead to a syndromal state that in 1936 Selye described as the “general adaptation syndrome” [18]. Thus, GC have both protective and damaging effects on the brain and the body. In the short run they are essential for adaptation, maintenance of homeostasis, and survival (allostasis = maintaining stability through changes). Yet, over longer time intervals, they impose a cost (allostatic load) that can accelerate disease processes or participate to pathological changes that may include – among many others – the onset and precipitation of different psychiatric conditions and even promote addictive processes [7, 8, 19-22].

SEX-DEPENDENT VULNERABILITY TO STRESS

Epidemiological data provide strong evidence for stress being a risk factor for the onset and/or precipitation of many different pathologies. To this regard, many psychiatric disorders are accompanied by a hyper- or hypo-activity of the HPA axis, as well as by disturbances in the temporal pattern of GC secretion [14, 17]. Worth noticing, as also reported by the WHO (www.who.int/mental_health/prevention/genderwomen/en/), the overall rates of psychiatric disorders are almost identical for men and women. However, prominent sex differences exist in different psychiatric pathologies. As an example, the incidence of major depressive disorder is almost two-fold greater in women than in men [23, 24]. Thus, investigating sex-specific mechanisms underlying mental health, as well as sex-dependent determinants of resilience/vulnerability to stress, should be a priority for clinical and preclinical science.

Efficient (physiological and behavioural) coping strategies involve the activation of a tightly regulated set of signalling pathways where sex differences can be found at all levels. Male and female subjects show substantial differences within the HPA axis physiology that include different aspects ranging from its function, organization and morphology (see also [25] and references therein). Studies dating back to the '60s already reported differences in the basal activity of the HPA axis with female rats (holding mature ovaries) showing a facilitation in the diurnal excursions of corticosterone levels due to circadian rhythm [26]. Successively Kant *et al.* provided evidence for plasma corticosterone levels showing a faster increase in females than in males rats following stressors of different nature [27]. This is mainly due to a suppression of the HPA axis in males by

activational testosterone after puberty. At a molecular level, studies have provided evidence for gonadal hormones affecting sex differences in stress responsivity through the modulation of neurotransmitter systems including serotonin (5-HT) norepinephrine and CRF receptor expression and internalization. In addition, behavioural sex-differences have been observed in response to stress with female subjects being characterized by more passive coping strategies (e.g. increased immobility in the forced-swim and tail-suspension tests compared to males) (see [6, 25] and references therein for complete reviews).

Adult response to stress are the result of the individual genetic asset and the pre- and/or post-natal environment. Thus, stressful events experienced during prenatal life and the early postnatal periods until adolescence might profoundly impact the developing systems leading to a life-long vulnerability to diseases [28, 29]. Patchev and colleagues first described how the gonadal hormones might shape the development of the HPA axis leading to a male or female neuroendocrine phenotype. More in detail, they provide evidence that the exposure to the hormonal milieu is tightly regulated leading to a sex-driven expression of glucocorticoid receptors (MR and GR) as well as of CRF within the PVN [30]. A great body of evidence has clearly shown that exposure to stressful experiences during sensitive ontogenetic phases might greatly affect the final outcome of developmental trajectories possibly leading to a mismatch between the gonadal sex and brain sex [28, 29]. This, might in turn disrupt the sexually dimorphic brain, eventually changing sex-driven responses to stress and coping strategies, leading to sex-driven risk in disease onset [6].

Studies on animal models of prenatal stress have clearly shown that HPA axis alterations may vary according to gender and to the nature or the intensity of the stressor, setting the stage for a life-long vulnerability [29, 31-38]. A hyperdrive of the HPA axis is observed in both male and female rats exposed to prenatal stress and this is associated to increased behavioral responses in both sexes as a result of exposure to novel stimuli and to increased anxiety-like behavior only in adult males (reviewed in [29, 38]). Very recently, Luoni *et al.* found that exposure to prenatal restraint stress produces significant changes in BDNF expression, a neurotrophin acting as a neuroendocrine effector leading to plastic changes in response to stress. This, results in a selective reduction in the hippocampus of female rats. Moreover, female subjects showed a reduced ability to cope with acute stress at adulthood [37]. Interestingly, exposure to prenatal stress might lead also to a selective sex-dependent vulnerability to drug addiction. To this regard, Thomas *et al.* found that, under basal conditions, female rats showed a greater ability to acquire cocaine self-administration within a certain number of test sessions when compared to males. By contrast, prenatal stress led to a significant increase in the proportion of male offspring reaching the criterion and this was comparable to that of females. Furthermore, these authors provide also evidence for prenatal stress resulting in sex-specific risk factor for different aspects of substance abuse [39].

Differently from early ontogenetic phases, adolescence is a critical time characterized by a strong rearrangement of neurochemical systems underlying brain excitability. During this time, organisms are still vulnerable to stress but, at the same time, they are already able to respond to it. During adolescence, the neuroendocrine system undergoes a main rearrangement towards the adult phenotype and this is often paralleled by main changes in the sexual hormones levels leading to the onset of puberty [40]. Significant modifications in the psychosocial behavioural patterns occur during this time that also include an increase in novelty seeking, possibly priming the initiation to drug use/abuse (see later). Likewise, in humans, adolescence might results in a critical temporal window for the emergence of psychopathology. However, given the prominent plasticity characterizing this time, it could also represent an important critical time for early intervention [41]. Studies in rodents have shown that new cells are added to the hypothalamus and amygdala in a sex-specific manner during puberty and gonadectomy before reaching sexual maturity prevents this effect [42]. These sex-driven differences in the number of newly added cells are paralleled by similar differences in adult brain volumes, suggesting that the changes programmed during puberty are long lasting [6].

Overall, the mechanisms by which sex differences in stress responsivity emerge and promote sex-driven disease risk (including substance-related and addictive disorders) are very complex and might rely upon a dynamic crosstalk between environmental factors and dynamic fluctuation of sexual hormones throughout pre- and post-natal development (see *Figure 1*). As recently reviewed by Bale and Epperson [6], animal models and human studies suggest that male subjects might be at greater risk for behavioural or neurodevelopmental disorders (e.g. schizophrenia, autism spectrum disorder and attention deficit hyperactivity disorder) in association with prenatal stress. By contrast, females might be more vulnerable to the onset of affective disorders (e.g. depression) as the result of stressful experiences occurring during early post-natal and peri-pubertal phases [6].

MECHANISMS AND SUBSTRATES OF STRESS-RELATED VULNERABILITY TO DRUG ADDICTION

Drug use and drug abuse are distinct phenomena and, in humans, most drug users will never develop addiction [7]. Clinical and animal experimental studies have shown that a clear distinction can be done between “vulnerable” and “resistant” individuals, the former showing a prominent aptitude towards drug addiction [8]. Epidemiological and preclinical data have provided evidence that the activation of neuroendocrine system upon stress and its crosstalk with sex hormones might differently prime vulnerability to drug addiction in male and female subjects [2, 43-45]. Thus, understanding the neurobiological mechanisms of addiction and identifying individual and sex-driven determinants in vulnerability to drug abuse has become a main target in this field of research [1, 8]. In this context, the role played by environmental and socio-economic factors should

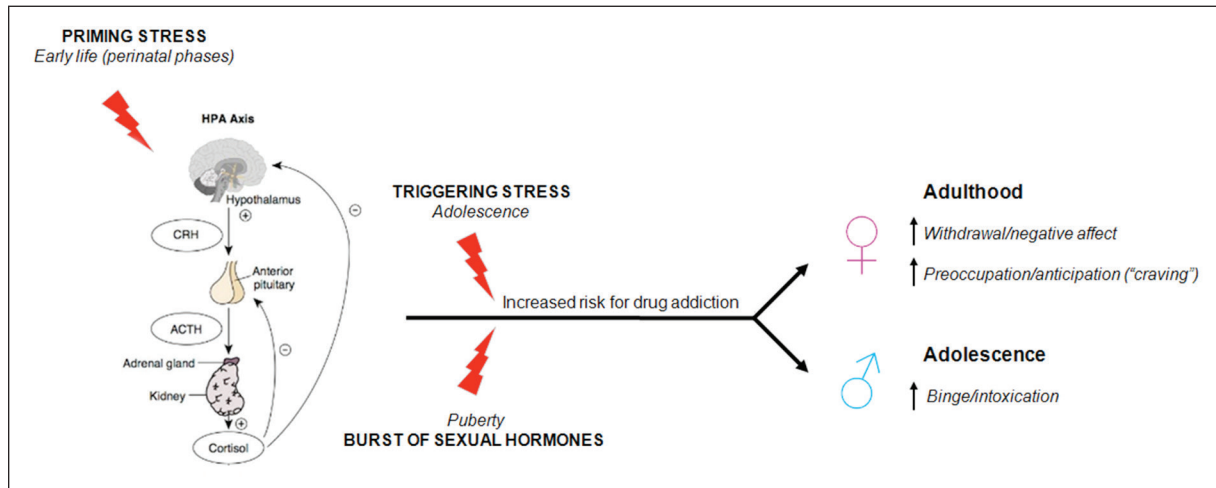


Figure 1

Stress experienced during early life phases (pre- and/or post-natal life) might prime the developing HPA axis setting the stage for long-term vulnerability. A second stressful hit in combination with puberty-induced burst of sexual hormones might lead to sex-biased vulnerability to drug addiction onset and/or relapse.

be also carefully considered as a main interactor with individual features setting the ground for vulnerability.

The concept of sensation seeking, as originally defined by Zuckerman [46], refers to “the need for varied, novel, and complex sensations and experiences and the willingness to take physical and social risks for the sake of such experience” and has been considered as an endophenotype of drug addiction [47, 48]. Epidemiological data and studies performed on animal models have shown that certain individuals actively seek for stimuli or situations characterized by arousing/stressful features (sensation seekers) that lead to the activation of the HPA axis and consequently to the secretion of GC hormones. Sensation seeking – in humans – is a personality trait characterized by a defined developmental pattern showing a peak at adolescence and declining with age (inverted “U” shape). During adolescence, the brain and the neuroendocrine system undergo a main rearrangement towards the adult phenotype and this is often – though not always – paralleled by main changes in the sexual hormones milieu leading to the onset of puberty. Significant modifications in the psychosocial behavioural patterns occur during this time that also include an increase in the novelty seeking [46]; as an obvious consequence adolescence is a critical age very prone to the initiation of nicotine, alcohol and recreational drug consumption [40] or to pathological gambling [49, 50]. Interestingly, the presence of personality traits (in healthy adults), such as sensation seeking, have been related with changes in the HPA axis function [51]. More in detail, high degree of disinhibition and novelty seeking consistently show an inverse relationship with cortisol levels across studies (also assessed by means of the dexamethasone/CRH suppression test) [51-54] with young males showing a stronger association than females [55]. Thus, it is possible to hypothesize that individuals showing reckless and risky behaviours might be characterized by higher threshold for physiological arousal [51]. Accordingly, low salivary cortisol levels

have been observed in children with oppositional behaviour and conduct disorder [56-58] suggesting that some behavioural traits might share common neurobiological mechanisms.

As for the mechanisms, animal models have greatly contributed to unveil the neurobiological and behavioural correlates underlying the influence of stress on drug addiction. In fact, laboratory conditions allow controlling for the “history” and the “experiences” of each subject (that can be manipulated on purpose); moreover, they allow reliable measure of the outcome of stress on drug abuse since all individuals have an equal access to drugs under identical environmental conditions [9]. Studies dating back to almost forty years ago showed that electric shock might hold positive reinforcing properties in squirrel monkeys leading to self-stimulation upon lever pressure. In rats, intense handling (a mild form of stress) may induce place preference - a behaviour often observed during experimental protocols of drug addiction - while certain individuals self-stimulate aversive brain regions resulting in a behavioural and autonomic profile comparable to that observed in conditions of physiological stress [59-61]. In this context, Piazza *et al.*, in a seminal work, provided insights into the physiological role of GC in the biology of sensation seeking and the mechanisms relating individual susceptibility to (psychostimulant) drug addiction [62]. In fact, they showed that the activation of the HPA axis and the consequent GC secretion underlies the appetitive properties of stressful and stimulating experience and that in vulnerable subjects (HR rats) they may potentiate the reinforcing properties of drugs of abuse. Thus, the higher sensitivity of certain individuals to the reinforcing effects of GC (secreted during stressful events) might be a biological basis of the phenomenon described as sensation seeking, setting the ground for vulnerability to drug addiction [62]. Dopaminergic neurons in the mesocorticolimbic system are the main substrate for the rewarding effects of different drugs of



abuse and are characterized by elevated expression of GR [63]. A number of studies using transgenic models have further shed light on the role played by GC and GR in the mechanisms underlying vulnerability to drug addiction. Deroche-Gamonet and colleagues showed that disruption of the GR gene in the central nervous system results in decreased motivation to cocaine self-administration and in a suppressed psychomotor sensitization [64]. In addition, they further provide evidence for GR being specifically involved in the modulation of cocaine-induced reinforcing effects [65]. Interestingly, Saal *et al.* [66] provided evidence for GC mediating the stress-induced changes in synaptic plasticity similar to those observed following cocaine self-administration within the dopaminergic reward circuit (see also the review by [67]). Thus, it is possible that GC may prime the brain reward circuit for the subsequent actions of drugs of abuse; this might be achieved by triggering crucial synaptic changes acting synergistically with drugs of abuse [8].

“STRESSING” SEX DIFFERENCES IN VULNERABILITY TO DRUG ADDICTION: FOCUS ON ANIMAL MODELS

Preclinical research is providing ever-increasing evidence for sex differences being found at very different levels, ranging from systemic to cellular and molecular mechanisms of target models [4]. Therefore, it is not surprising that sex differences can be found in vulnerability to drug addiction. Such sex-dependent differences, in human subjects, are enriched through the course of life by cultural aspects, leading to the observed gender-dependent differences in behaviour and habits [68].

Drug addiction can be viewed as a progressive hedonic-homeostatic dysregulation eventually leading to main allostatic changes in the brain reward and stress systems and can be defined by the stages through which users progress: 1) binge/intoxication; 2) withdrawal/negative affect; and 3) preoccupation/anticipation [1, 7]. Studies on animal models taking into account all these phases have provided evidence for female subjects (mostly rats) being overall more vulnerable than males; this finding is even more intriguing if considering that drugs of abuse are not identical with regard to their impact on the addiction cycle. More in detail, female rats show a faster learning of drugs self-administration as well as a faster dose escalation in addition to a greater motivational withdrawal. Moreover, animal models specifically focusing on “craving” (stage 3 of addiction) have provided evidence for a more prominent relapse in female subjects. The groundwork for such differences might rely both on sex specific organizational effects (taking place mainly during development, *e.g.* neonatal rats treated with estradiol show the male phenotype), and activational effects of gonadal hormones (*e.g.* adult female subjects undergoing ovariectomy are mostly comparable to the male phenotype) [30, 69]. In this regard, brain regions such as the dorsal striatum, prefrontal cortex, nucleus accumbens, and medial extended amygdala, that play pivotal roles in the different stages of drug addiction, show a prominent sexual dimorphism and contain gonadal hormone receptors possibly being

involved in mediating, at least in part, sex-dependent vulnerability to drug addiction [70].

A different, complementary and more subtle way for gonadal hormones to set the ground for sex-dependent vulnerability to drug addiction is through a differential activation of the HPA axis in male and female subjects upon stress. Male and female rodents are characterized by different stress responses that are mediated both by adult levels of gonadal hormones as well as by developmental programming of the HPA axis [25]. The participation/activation of the neuroendocrine system, and particularly of the HPA axis, has been observed, to a greater or lesser extent, in almost all stages of drug addiction [1, 7]. As an example, Torres *et al.* have recently reported that stress triggers the initiation of nicotine intake in female rats [71] that also show increased corticosterone and ACTH levels during nicotine withdrawal [71, 72]. There is evidence for female subjects to show enhanced drug-, cue-, and stress-induced reinstatement in animal models of alcohol and drug seeking [73, 74]. This latter piece of data is consistently observed across different species and might resemble the persistent pre-occupation/anticipation (craving) stage of the addiction cycle [1].

When compared to males, female subjects show higher basal and stress-induced GC secretion and a higher stress response in the hypothalamic PVN (see [25] and references therein for a detailed review). Moreover, estradiol can enhance the stress response during the estrous cycle in rats and monkeys, while progesterone exert an opposite effect [1]. Thus, sex differences in the stress response may well participate in the observed different sex-dependent vulnerability to drug addiction.

Very recently, Becker and Koob have dedicated an extensive review to the neurobiological basis of sex differences in preclinical animal models of drug abuse [1]. Worth to notice, these authors use the framework of drug addiction to highlight the importance of taking into account sex differences in basic science addressing the fact that there is still an unmet need to improve experimental designs by the inclusion of both sexes in preclinical research [2]. In particular, animal models (of drug addiction) should distinguish four main research domains *i.e.* 1) qualitative, 2) quantitative, 3) population, and 4) mechanistic. “Qualitative differences” are represented by apparent sexually dimorphic traits. “Quantitative differences” rely on the magnitude of the response (*e.g.* female rats show a higher locomotion following psychostimulant drugs administration and a greater behavioural sensitization when compared to males [75]). Sex differences in the incidence or distribution of individual traits are referred to as “population differences”. As an example, Perry *et al.* were able to identify a subpopulation of female rats more prone to drug addiction than males though the behaviours showed during cocaine consumption were the same for males and females [76]. Within this domain (“population differences”) the environment (*e.g.* stressful experiences) may play a prominent role. In fact, it may shape individual developmental trajectories in a sex-driven direction and this may in turn affect the distribution of a certain trait, including vulnerability to drug addic-

tion, within a population [37, 77, 78]. To this regard, Thomas *et al.* found that under basal conditions female rats showed a greater ability to acquire cocaine self-administration within a certain number of test sessions (three) when compared to males. By contrast, prenatal stress led to a significant increase in the proportion of male offspring reaching the criterion and this was comparable to that of females thus resulting in a change in the distribution of the population [39]. As for the last domain (mechanism), a trait that may look alike for female and male subjects might be mediated by different sex-dependent neural/molecular mechanisms. A prominent example for this domain is provided by the CRF receptor 1 (CRFR1). This receptor is a primary ligand for CRF that is secreted from the hypothalamus and initiates the signalling cascade triggering HPA axis activation upon stress but also under conditions of acute drug withdrawal [79, 80]. Corticotropin-releasing-factor receptor 1 is expressed in different brain regions, playing a main role in the reward and reinforcement properties as well as in the craving and aversive effects of drugs of abuse [81]. In this context, it is interesting to note that there is evidence for sex-driven differential activation in CRFR1 coupling to G-proteins and β -arrestin, that might lead to an increased vulnerability of females to the effects of acute stress and a reduced capacity to cope with chronic stress [82]. Likewise, sex differences in CRFR1 deficient mice have been reported following morphine withdrawal in a paradigm of conditioned place aversion [80]. It is interesting to note that at least in three of the four domains taken into account by Becker and Koob differences in systems and pathways mediating stress response appear to play a main role.

Sex differences in rates of substance abuse are consis-

tently observed in the general population with regards to rates of substance use, abuse, and dependence. In humans, men show more drug and alcohol consumption and show higher degree of misuse. However, the abuse of drugs in women is quickly approaching that of men suggesting that the distance between females' and males' "cultural niches", that greatly contribute to shape human behaviour and human (cultural) habits, are progressively reducing. In this context, the use of both sexes in animal model hold the great potential to unveil sex differences in the neurobiological mechanisms regardless of cultural factors. As stated by Becker and Koob, "A better understanding of the ways males and females can differ will help scientists design experiments to characterize better the presence or absence of sex differences in new phenomena that they are investigating" [1].

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Conflict of interest statement

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