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Sex differences in behavioral traits related with high sensitivity to the reinforcing effects of cocaine

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ABSTRACT

Cocaine is the most prevalent illegal stimulant drug in Europe among the adult population. Its abuse is characterized by a faster substance abuse disorder (SUD) development than other drugs, with high vulnerability to relapse. However, there does not exist an effective treatment for cocaine dependence. Sex differences have been reported in psychological disorders including SUD. For this reason, it is essential to identify risk factors that predict susceptibility or resilience to cocaine addiction for the development of effective prevention strategies considering sex differences. In the present study, the main objective was to determine more sensitive phenotypes to the conditioned reinforcing effects of cocaine in both sexes. Anxiety-like behavior and the locomotor response to novelty were evaluated in the elevated plus maze, and despair in the tail suspension test, as well as vulnerability traits linked with a high sensitivity to the reinforcing effects of a subthreshold dose of cocaine (1 mg/kg) in the conditioned place preference (CPP) paradigm in male and female mice. Our results indicated that only female mice with high anxiety, low locomotor response to novelty or low despair levels acquired CPP induced by cocaine, while male mice with low anxiety, high locomotor response to novelty or high despair levels presented a higher susceptibility to the rewarding effects of cocaine than others. These sex differences in the results reveal an opposite pattern in males and females on the relationship between anxiety- and depressive-like behaviors and cocaine vulnerability, demonstrating the need to include female mice in preclinical studies.

Cocaine is the most widely consumed illegal stimulant drug in Europe by the adult population, and the second most consumed illegal drug after cannabis [1]. It is one of the substances of abuse with the fastest transition from recreational use to compulsive abuse, due to its capacity to induce plasticity in the mesolimbic dopamine (DA) reward pathway [2], and its addiction presents a persistent and high vulnerability to relapse [3]. Nevertheless, there does not exist an approved specific treatment to cocaine dependence as of yet [4]. Multiple psychological traits have been shown to influence risk for addiction, including impulsivity, novelty and sensation seeking, and stress reactivity [4–6]. Thus, one of the main focuses in addiction research is to characterize behavioral phenotypes that exhibit susceptibility or resilience to initiate the development of this chronic disease [7] in order to pursue a model of prevention based on precision and evidence.

Gender differences have been reported in psychological disorders, including substance use disorder (SUD). Women are twice more likely to be diagnosed with depression [8] and anxiety-related disorders [9] than men. Gender differences have been described in all phases of the addiction development process as well [10–12]. Women transition to a SUD faster than men, they are more reactive to stimuli that trigger relapse, and they have higher rates of relapse [10]. Additionally, up to 75 % of people with a serious mental illness meet the criteria for dual diagnosis, with both a mental disorder and a SUD [13], and the comorbidity is highly prevalent between SUDs and mood and anxiety disorders [14]. However, despite the fact that sexual differences in neural mechanisms underlying addiction have been well established [15], robust behavioral phenotypes related with the development of addiction considering sex differences are still missing.

Thus, the objective of this work was to assess if behavioral traits, such as the locomotor response to novelty or anxiety- and depression-like behaviors, are related with a higher sensitivity to the conditioned reinforcing effects of cocaine. For this purpose, we evaluated the anxiety-like behavior, the locomotor response to novelty and despair behaviors of male and female mice to classify them according to their high or low levels before their exposure to the drug. We used the elevated plus maze (EPM) test to measure anxiety-like behavior and the locomotor response to new environments, and the tail suspension test (TST) to establish despair levels. Afterwards, we evaluated the

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sensitivity to the reinforcing effects of a subthreshold dose of cocaine (1 mg/kg) in the conditioned place preference (CPP) paradigm in adult mice of both sexes, as sex differences are more pronounced with lower doses of drugs [11].

A total of 46 OF1 mice, 25 males and 21 females (Charles River, Barcelona, Spain) participated in this study. The mice arrived at the laboratory at post-natal day (PND) 42 and they were housed in groups of four in plastic cages (25 × 25 × 14.5 cm) under the following conditions: constant temperature (21 °C); a reversed light schedule (white lights on 19:30–07:30); and food and water *ad libitum*, except during tests. The behavioral tests were performed during lights off (9:00 a.m.−3:00 p.m.) for all mice, on PNDs 60–61 for the EPM test, on PNDs 67–68 for the TST and from PND 74 onwards, the CPP was started.

In order to reduce their stress levels in response to experimental manipulations, mice were handled for 5 min/day on each of the 3 days before the initiation of the experimental procedure. Procedures involving mice and their care were carried out in compliance with national, regional and local laws and regulations, which are in compliance with Directive 2010/63/EU.

Cocaine hydrochloride (Laboratorios Alcaliber, Spain) was diluted in physiological saline (0.9 % NaCl) at a volume of 0.01 mL/g and injected intraperitoneally (i.p.) at a dose of 1 mg/kg [6].

The EPM test is based on the natural aversion of mice to open elevated areas and on the natural spontaneous exploratory behavior that they exhibit in novel environments. The apparatus consisted of two open arms (30 × 5 cm2), two closed arms (30 × 5 cm2), and the junction of the four arms, forming a central platform (5 × 5 cm2). The floor of the maze was made of black plexiglas and the walls of the closed arms were made of clear plexiglas. The open arms had a small edge (0.25 cm) to provide the animals with additional grip. The entire apparatus was elevated 45 cm above floor level. At the beginning of each trial, mice were placed on the central platform of the EPM and were allowed to explore it for 5 min. The behavior displayed by the mice was registered and analyzed by a computerized video-tracking system (Ethovision, Noldus S.A., The Netherlands). The percentage of time spent in the open arms is commonly considered a good measure of the anxiety level of mice, and the distance covered (cm) is regarded as locomotor response scores to novel environments.

In the TST, the mouse is suspended by its tails using adhesive tape to a hook elevated 50 cm above the bench. The behavior displayed was video recorded for 6 min and subsequently analyzed by trained researchers using software (Raton Time 1.0, Fixma, S.L., Valencia, Spain). The time of immobility is considered behavioral despair, in other words, a failure of persistence in escape-directed behavior. It is based on the observation that rodents, after initial escape-oriented movements, develop an immobile posture when placed in an inescapable, stressful situation. In the case of the TST, the stressful situation involves being hung in an uncontrollable fashion by the tail.

The measurements taken into account for the distribution of animals according to their levels of anxiety-like behavior, locomotor response to novelty and despair-like behavior were the percentage of time in the open arms (OA) (% time OA), the distance covered (cm) in the EPM and the total time (sec) spent immobile in the TST, respectively.

For the CPP paradigm, we used sixteen identical Plexiglas boxes with two compartments of equal size (30.7 cm long ×31.5 cm wide × 34.5 cm high) separated by a grey central area (13.8 cm long × 8 cm wide × 34.5 cm high). The compartments had different-colored walls (black vs. white) and distinct floor textures (smooth in the black compartment and rough in the white one). Four infrared light beams in each of the box's compartments and six in the central area allowed the recording of the animals' position and their crossings from one compartment to the other. The equipment was controlled by four IBM PC computers using MONPRE 2Z software (Cibertec, SA, Madrid, Spain). The CPP procedure was unbiased in terms of initial spontaneous preference (for details, see [5,6]. In the first phase (preconditioning/Pre-C), animals were allowed to access two compartments of the apparatus for 15 min (900 s) per day over 3 days, and on day 3, the time spent in each compartment was recorded. In the second phase (conditioning), lasting 4 days, half of mice in each group received the vehicle immediately before being confined for 30 min to one compartment (vehicle-paired compartment) and the other half received it in the other compartment. After an interval of 4 h, they received cocaine (1 mg/kg) immediately before being confined for 30 min to the other compartment (drug-paired compartment). During the third phase (post-conditioning/Post-C), animals were allowed access to three compartments of the apparatus for 15 min (900 s) again, and the time spent by the untreated animal was recorded. The difference in seconds between the time spent in the drug-paired compartment during the Post-C test and the Pre-C phase is a measure of the degree of conditioned reinforcement induced by the drug. Some animals showed a strong unconditioned aversion (less than 27 % of the session time; i.e., 250 s) or preference (more than 73 % of the session time; i.e., 650 s) for a given compartment in the Pre-C session and 4 mice were therefore discarded from the rest of the experimental procedure.

For the distribution of the animals within each sex according to their higher or lower levels of anxiety-like behavior, locomotor response to novelty and despair-like behavior, a cluster analysis of K means was performed (see Fig. 1). For the sex differences, one-way ANOVAs were performed in each behavioral measure. For the CPP data, the time spent in the drug-paired compartment during the Pre- and Post-C tests was analyzed with a mixed ANOVA, with two between variables: Sex (male and female) and Trait (high and low) and one within variable: Days (Pre-C and Post-C); and the delta score was analyzed with an ANOVA, with two between variables Sex and Trait. Post-hoc comparisons were performed with the Bonferroni test. Moreover, analyses of linear regression between measures of behavioral traits and the delta score were performed.

Sex differences were not observed in any of the behavioral measures [anxiety: $F(1,38) = 2{,}612$; $p > 0.05$; locomotor response: $F(1,39) = 0{,}538$; $p > 0.05$; despair: $F(1,39) = 0{,}775$; $p > 0.05$; and conditioned score: $F(1,41) = 0{,}047$; $p > 0.05$]. The following groups were obtained for mice according to each sex and trait: Males, high anxiety, n = 11; low anxiety n = 8; [$F(1,18) = 85.557$; $p < 0.0001$]; high locomotor response, n = 8; low locomotor response, n = 12; [$F(1,20) = 47.225$; $p < 0.0001$]; high despair, n = 9; low despair, n = 11; [$F(1,18) = 56.816$; $p < 0.0001$]; females, high anxiety, n = 8; low anxiety n = 13; [$F(1,19) = 39.113$; $p < 0.0001$]; high locomotor response, n = 11; low locomotor response, n = 10; [$F(1,19) = 28.556$; $p < 0.0001$]; high despair, n = 14; low despair, n = 7; [$F(1,18) = 32.747$; $p < 0.0001$]. The results of male and female mice classified as high or low anxiety in the CPP induced by 1 mg/kg of cocaine are presented in Fig. 2A. The ANOVA for the time spent in the drug-paired compartment during Pre-C and Post-C by mice classified as high or low anxiety significantly revealed the interaction Sex*Trait*Days [$F(1,36) = 9.308$; $p < 0.004$]. The low-anxiety males ($p < 0.026$) and high-anxiety females ($p < 0.001$) significantly increased the time spent in the drug-paired compartment in Post-C with regard to Pre-C. The ANOVA of delta score showed the significant interaction Sex*Trait [$F(1,36) = 9.308$; $p < 0.004$], observing sex differences in high-anxiety mice ($p < 0.021$) and an increase higher in high-anxiety than low-anxiety females ($p < 0.007$). The results of male and female mice classified as high or low motor response in the CPP induced by 1 mg/kg of cocaine are presented in Fig. 2B. The ANOVA for the time spent in the drug-paired compartment during Pre-C and Post-C by mice classified according to their high or low motor response level showed the significant interaction Sex*Trait*Days [$F(1,37) = 4.354$; $p < 0.044$]. The high-response males ($p < 0.036$) and low-response females ($p < 0.025$) significantly increased the time in the drug-paired compartment in Post-C with regard to Pre-C. The ANOVA of the delta score showed the significant interaction Sex*Trait [$F(1,37) = 4.354$; $p < 0.044$]. The results of male and female mice classified as high or low despair in the CPP induced by 1 mg/kg of cocaine are presented in Fig. 2C. The ANOVA for the time spent in the drug-paired compartment during Pre-C- and Post-C by mice classified as high or low despair revealed the significant

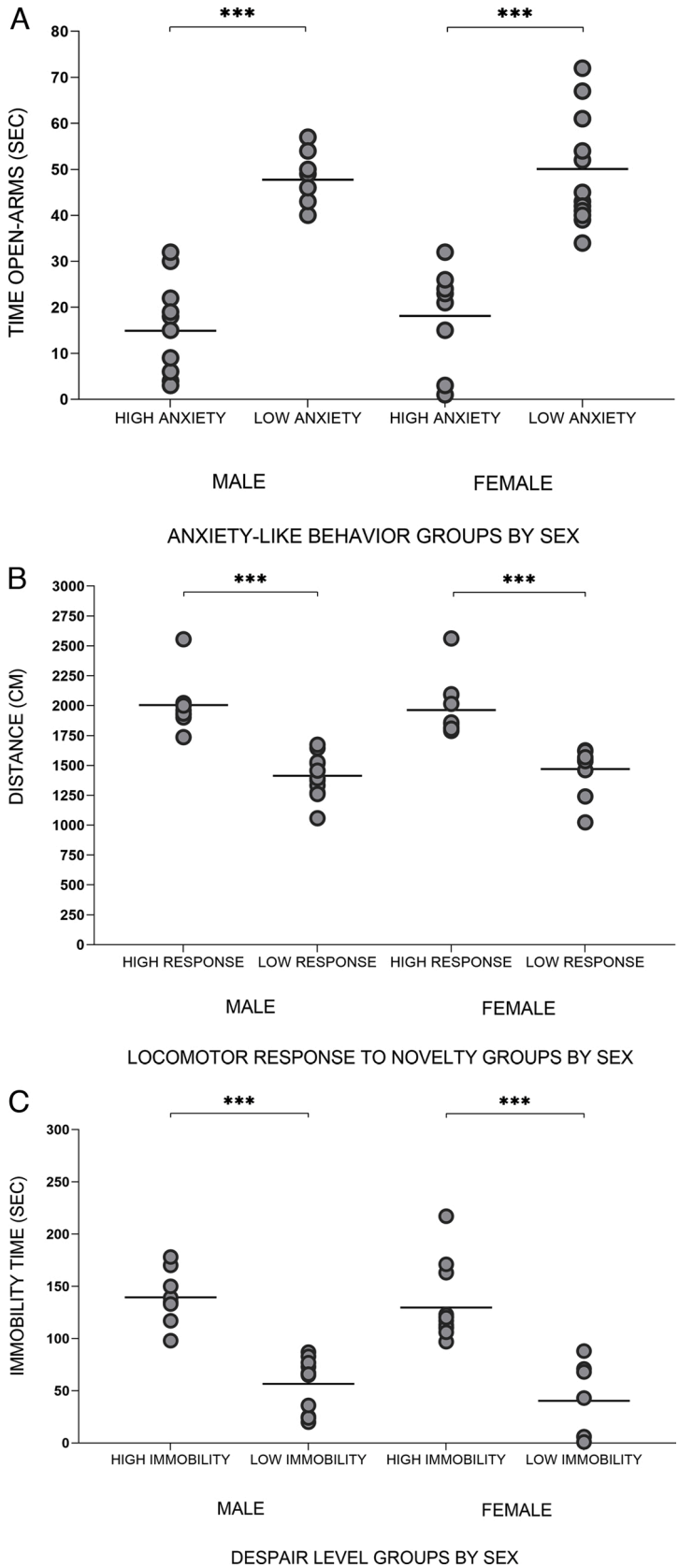

Fig. 1. K-means cluster analysis. A Classification as high anxiety or low anxiety for mice groups of both sexes. ***$p < 0.0001$ vs high anxiety for male and female groups. B Classification as high response or low response for mice groups of both sexes. ***$p < 0.0001$ vs high response for male and female groups. C Classification as high or low immobility for mice groups of both sexes. ***$p < 0.0001$ vs high immobility for male and female groups.

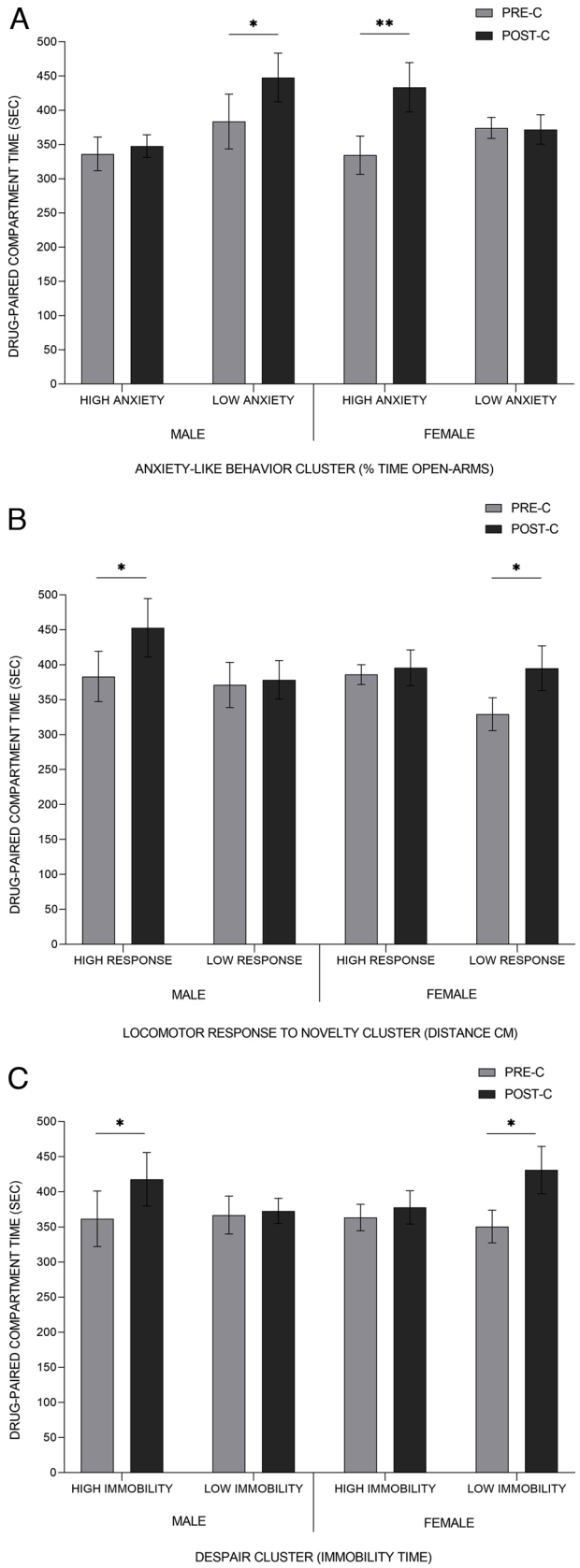

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Fig. 2. Conditioned place preference induced by 1 mg/kg of cocaine in male and female mice. Bars represent the mean (±SEM) time spent in the drug-paired compartment before conditioning sessions (gray) and after conditioning sessions (black). A Conditioned place preference for anxiety-like behavior groups. *$p < 0.05$ for male mice group with low anxiety vs Pre-C. **$p < 0.01$ for female mice group with high anxiety vs Pre-C. B Conditioned place preference for locomotor response to novelty groups. *$p < 0.05$ for male mice group with high response and female mice group with low response vs Pre-C. C Conditioned place preference for despair groups. *$p < 0.05$ for male mice group with high immobility and female mice group with low immobility vs Pre-C.

interaction Sex*Trait*Days [$F(1,37) = 4.604$; $p < 0.039$]. The high-despair males ($p < 0.05$) and low-despair females ($p < 0.016$) significantly increased the time spent in the drug-paired compartment in Post-C with regard to Pre-C. The ANOVA of the delta score showed the significant interaction Sex*Trait [$F(1,36) = 6.019$; $p < 0.019$], observing sex differences in low-despair mice ($p < 0.034$). Significant linear regressions between locomotor response and CPP score [$F(1,19) = 4.573$; $p < 0.045$; $t = 2.138$; $p < 0.046$] and between anxiety and Post-C score [$F(1,18) = 9.824$; $p < 0.006$; $t = 3.134$; $p < 0.006$] were observed in males; while only a significant linear regression between anxiety and CPP score was detected in females [$F(1,18) = 4.109$; $p < 0.05$; $t=-2.027$; $p < 0.05$].

To our knowledge, this is the first study evaluating the relation of traits such as anxiety and the locomotor response in a novel environment and the despair in a stressful situation with the sensitivity to the conditioned rewarding effects of cocaine in male and female mice. Our results have shown sex differences in behavioral phenotypes of mice that acquired CPP induced by a subthreshold dose of cocaine (1 mg/kg). Thus, male mice with low anxiety-like behavior, high locomotor response to novelty or high despair levels presented a higher susceptibility to the rewarding effects of cocaine than those with high anxiety-like behavior, low locomotor response to novelty or low despair levels. In contrast, the female mice that showed higher sensitivity to the rewarding effects of the drug were those with high anxiety-like behavior, low locomotor response to novelty or low despair levels.

The categorization of rodents as high- or low-responder depending on their novelty-induced motor activity has been often used to investigate sensitivity to the rewarding properties of psychostimulants [6]. In agreement with the present results, we had previously observed that the male mice considered as high responders and low-responder females, exhibited a higher sensitivity to the conditioned rewarding effects of this subthreshold dose of cocaine [6]. It is important to note that the novelty-induced motor activity was evaluated with a different apparatus (actimeter) from the one in the present study observing similar results. Nevertheless, few studies have assessed other personality traits that increase an individual's vulnerability to develop a SUD [7]. Conversely to our findings, female mice with a depressive-like state, given their long immobility duration in the TST, displayed a stronger CPP induced by cocaine [16]; and male mice that exhibited an anxious phenotype in the EPM were more prone to search for cocaine in the CPP paradigm [17]. However, these studies used an effective and high dose of cocaine (10 or 20 mg/kg, respectively), while in our study, a subthreshold cocaine dose was employed to assess the sensitivity to the conditioned reinforcing effects of cocaine. Additionally, female mice with a depressive-like state simultaneously presented anxiety-like behaviors [16]; similarly, the females with high anxiety-like behavior in our study also presented more sensitivity to the rewarding effects of the drug.

Only a small percentage of men and women who sample drugs will develop a SUD. Currently, non-pharmacological factors in psychoactive drug abuse and addiction are being progressively considered more relevant to understand individual differences in the development of a SUD [18]. The results of this study highlight the relationship between anxiety- and depressive-like behaviors and cocaine vulnerability in both male and female subjects. An increased sensitivity to drugs of abuse has been related to a higher vulnerability to developing substance abuse

disorders [19]. Previous studies have revealed that subjects presenting a higher sensitivity to the rewarding effects of cocaine have an increased risk to develop a SUD [5,6]. Thus, the identification of the risk factors that predict susceptibility to cocaine addiction is useful for the development of effective prevention strategies for SUD. Nonetheless, the main contribution of this study is the demonstration of sex differences in the behavioral traits associated with a vulnerability to the development of an addiction, revealing the need to include female rodents in preclinical research.

Ethical statement

The experimental protocol has been approved by an Institutional Review Committee for the use of animal subjects. All procedures involving mice and their care were conducted in accordance with national (BOE-2013-1337) and EU (Directive 2010-63EU) guidelines regulating animal research and were approved by the ethics committee of the University of Valencia. All the efforts were made to minimize animal suffering and to reduce the number of animals used.

Author contributions

Sergio Pujante-Gil: methodology, validation, investigation, formal analysis, writing and editing; Carmen Manzanedo: conceptualization, methodology, validation, investigation, formal analysis, writing-review and editing; M.Carmen Arenas: conceptualization, methodology, validation, formal analysis, writing-review, visualization, supervision, project administration and funding acquisition.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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References

[1] European Monitoring Centre for Drugs and Drug Addiction, European Drug Report: Trends and Developments, 2020, https://doi.org/10.2810/123451.
[2] A.H. Ashok, Y. Mizuno, N.D. Volkow, O.D. Howes, Association of stimulant use with dopaminergic alterations in users of cocaine, amphetamine, or methamphetamine a systematic review and meta-analysis, JAMA Psychiatry 74 (2017) 511–519, https://doi.org/10.1001/jamapsychiatry.2017.0135.
[3] M.R. Farrell, H. Schoch, S.V. Mahler, Modeling cocaine relapse in rodents: behavioral considerations and circuit mechanisms, Prog. Neuropsychopharmacol. Biol. Psychiatry 87 (2018) 33–47, https://doi.org/10.1016/j.pnpbp.2018.01.002.
[4] N.D. Volkow, M. Boyle, Neuroscience of addiction: relevance to prevention and treatment, Am. J. Psychiatry 175 (2018) 729–740, https://doi.org/10.1176/appi.ajp.2018.17101174.
[5] A. Vidal-Infer, M.C. Arenas, M. Daza-Losada, M.A. Aguilar, J. Miñarro, M. Rodríguez-Arias, High novelty-seeking predicts greater sensitivity to the conditioned rewarding effects of cocaine, Pharmacol. Biochem. Behav. 102 (2012) 124–132 (Accessed May 1, 2021), https://reader.elsevier.com/reader/sd/pii/S0091305712001098?token=B7785A79D09BBDB2FB0CE5727E8FD18E50906C6F99B5993EFDBE8B46BF8A21DD668115A19DFF3C2F7AC2645A06184566&originRegion=eu-west-1&originCreation=20210501195126.
[6] M.C. Arenas, M. Daza-Losada, A. Vidal-Infer, M.A. Aguilar, J. Miñarro, M. Rodríguez-Arias, Capacity of novelty-induced locomotor activity and the hole-board test to predict sensitivity to the conditioned rewarding effects of cocaine, Physiol. Behav. 133 (2014) 152–160, https://doi.org/10.1016/j.physbeh.2014.05.028.
[7] A.M. Belcher, N.D. Volkow, F.G. Moeller, S. Ferré, Personality traits and vulnerability or resilience to substance use disorders, Trends Cogn. Sci. 18 (2014) 211–217, https://doi.org/10.1016/j.tics.2014.01.010.
[8] R.S. Eid, A.R. Gobinath, L.A.M. Galea, Sex differences in depression: insights from clinical and preclinical studies, Prog. Neurobiol. 176 (2019) 86–102, https://doi.org/10.1016/j.pneurobio.2019.01.006.
[9] L.Y. Maeng, M.R. Milad, Sex differences in anxiety disorders: interactions between fear, stress, and gonadal hormones, Horm. Behav. 76 (2015) 106–117, https://doi.org/10.1016/j.yhbeh.2015.04.002.
[10] S.A.M. Bobzean, A.K. DeNobrega, L.I. Perrotti, Sex differences in the neurobiology of drug addiction, Exp. Neurol. 259 (2014) 64–74, https://doi.org/10.1016/j.expneurol.2014.01.022.
[11] M.E. Roth, K.P. Cosgrove, M.E. Carroll, Sex differences in the vulnerability to drug abuse: a review of preclinical studies, Neurosci. Biobehav. Rev. 28 (2004) 533–546, https://doi.org/10.1016/j.neubiorev.2004.08.001.
[12] J.B. Becker, G.F. Koob, Sex Differences in Animal Models: Focus on Addiction (n. d.), 2020, https://doi.org/10.1124/pr.115.011163.
[13] C. Fantuzzi, R. Mezzina, Dual diagnosis: a systematic review of the organization of community health services, Int. J. Soc. Psychiatry 66 (2020) 300–310, https://doi.org/10.1177/0020764019899975.
[14] H.M.X. Lai, M. Cleary, T. Sitharthan, G.E. Hunt, Prevalence of comorbid substance use, anxiety and mood disorders in epidemiological surveys, 1990–2014: a systematic review and meta-analysis, Drug Alcohol Depend. 154 (2015) 1–13, https://doi.org/10.1016/j.drugalcdep.2015.05.031.
[15] J.B. Becker, E. Chartoff, Sex differences in neural mechanisms mediating reward and addiction, Neuropsychopharmacology 44 (2019) 166–183, https://doi.org/10.1038/s41386-018-0125-6.
[16] V. Rappeneau, A.L. Morel, M. El Yacoubi, J.M. Vaugeois, L. Denoroy, A. Berod, Enhanced cocaine-associated contextual learning in female h/rouen mice selectively bred for depressive-like behaviors: molecular and neuronal correlates, Int. J. Neuropsychopharmacol. 18 (2015) 1–12, https://doi.org/10.1093/ijnp/pyv022.
[17] D. Ladrón de Guevara-Miranda, F.J. Pavón, A. Serrano, P. Rivera, G. Estivill-Torrús, J. Suárez, F. Rodríguez de Fonseca, L.J. Santín, E. Castilla-Ortega, Cocaine-conditioned place preference is predicted by previous anxiety-like behavior and is related to an increased number of neurons in the basolateral amygdala, Behav. Brain Res. 298 (2016) 35–43, https://doi.org/10.1016/j.bbr.2015.10.048.
[18] S.H. Ahmed, A. Badiani, K.A. Miczek, C.P. Müller, Non-pharmacological factors that determine drug use and addiction, Neurosci. Biobehav. Rev. 110 (2020) 3–27, https://doi.org/10.1016/j.neubiorev.2018.08.015.
[19] K.E. Yoest, J.A. Quigley, J.B. Becker, Rapid effects of ovarian hormones in dorsal striatum and nucleus accumbens, Horm. Behav. 104 (2018) 119–129, https://doi.org/10.1016/j.yhbeh.2018.04.002.