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Interaction of gonadal hormones, dopaminergic system, and epigenetic regulation in the generation of sex differences in substance use disorders: A systematic review

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ABSTRACT

Substance use disorder (SUD) is a chronic condition characterized by pathological drug-taking and seeking behaviors. Remarkably different between males and females, suggesting that drug addiction is a sexually differentiated disorder. The neurobiological bases of sex differences in SUD include sex-specific reward system activation, influenced by interactions between gonadal hormone level changes, dopaminergic reward circuits, and epigenetic modifications of key reward system genes. This systematic review, adhering to PICOS and PRISMA-P 2015 guidelines, highlights the sex-dependent roles of estrogens, progesterone, and testosterone in SUD. In particular, estradiol elevates and progesterone reduces dopaminergic activity in SUD females, whilst testosterone and progesterone augment SUD behavior in males. Finally, SUD is associated with a sex-specific increase in the rate of opioid and monoaminergic gene methylation. The study reveals the need for detailed research on gonadal hormone levels, dopaminergic or reward system activity, and epigenetic landscapes in both sexes for efficient SUD therapy development.

1. Introduction

Drug addiction is a chronic disorder characterized by pathological drug-taking and seeking behaviors despite the adverse consequences. According to the Diagnostic and Statistical Manual of mental disorders 5th edition (DSM-V), substance use disorder (SUD) is diagnosed according to 11 behavioral or psychological criteria, with diagnosis requiring at least two criteria present, and severity increasing (mild, moderate, severe) with more criteria being met (Association, 2013). The addiction process has been described as having three well-defined stages: binge/intoxication, withdrawal/negative affect, and preoccupation/anticipation (craving) (Koob & Moal, 1997). These three stages are influenced by biological and environmental factors, leading to a SUD's development. Biological factors involve variables related to the individual, such as genetics, differences in neurotransmissions and neurocircuitry, or even differences in developmental attributes. The second one is related to the social and cultural systems such as patterns of behavior related with drug use, public policies, economic status, family or even lifestyle but also with the exposition to stress and trauma that the individual might experience in his life patterns of drug use, public policy, economic status, family and support networks, and exposure to stress and trauma (Lazarus, 1993) All these factors can lead to epigenetic changes and impact an individual's vulnerability to SUD.

The neurocircuitry of SUD is deeply linked to the brain reward system; the neural substrate underlying drug addiction. This system consists of dopamine (DA) mesocorticolimbic projections from cell bodies in the ventral tegmental area (VTA) to limbic structures, such as the amygdala, ventral pallidum (VP), hippocampus and nucleus accumbens

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(NAcc) and cortical areas, including the prefrontal (PFC), the orbitofrontal (OFC) and the anterior cingulate cortices (Feltenstein & See, 2008). Although these circuits work in parallel, they may have different roles in the addiction process.

In the first stage – binge/intoxication – drugs of abuse produce a DA increase in the NAcc which seems to be necessary for reward, providing the positive reinforcement and starting the addiction cycle (Koob & Bloom, 1988).

Different projections from the VTA connect with differential areas in the NAcc. Specifically, DAergic neurons localized in the posteromedial VTA selectively project to the ventromedial striatum (medial olfactory tubercle and medial NAcc shell), whereas the anteromedial VTA has few projections to the ventral striatum, and the lateral VTA largely projects to the ventrolateral striatum (NAcc core, lateral shell and lateral tubercle). The rewarding properties of the different drugs can vary depending on the pathway, for example it has been shown that psychostimulants such as cocaine produce a greater response in the ventromedial striatum and nicotine in the posterior VTA (Ikemoto, 2003; Ikemoto, 2005).

Regarding the outputs, the NAcc sends projections to the VP, the medial part of the globus pallidus (GP), and the substantia nigra, as well as the lateral hypothalamus (LH). The main difference between the two subregions of the NAcc is that while the core projects mainly to the LH, the shell projects diffusely throughout the LH as well as to the extended amygdala (Heimer et al., 1991). Due to this anatomical segregation, it has been suggested that the two subregions have different functions: the core is thought to be involved in learning and action selection during goal-directed behavior, whereas the shell appears to be involved in more emotional/motivational value-related responses (Carelli, 2004; Kelley, 2004).

The withdrawal/negative affect stage, the second stage in the addiction cycle, is characterized by elevation of the reward threshold which is correlated with escalation of drug intake (Koob et al., 2014).

The anatomical shifting from the NAcc activation to the dorsal striatum, which is not involved in the acute reinforcement effects, seems to play a key role developing compulsive use in the process of the chronic addiction (Everitt et al., 2008; Hodebourg et al., 2019). In addition to the dorsal striatum, the extended amygdala is the processing system which produces the negative emotional states that act as negative reinforcement mechanisms associated with the development of addiction. All drugs of abuse produce an increase of corticotropin-releasing hormone (CRH), dynorphin and norepinephrine in the extended amygdala during acute withdrawal, contributing to the negative emotional states in abstinence (Delfs et al., 2000; Koob et al., 2014). At the same time, the endogenous anti-stress system, including neuropeptide Y, nociceptin, and endocannabinoids, undergo changes that seem to perpetuate the anxiety-like responses, typical in the withdrawal phase, due to an inability to modulate the brain's response to stress (Gunduz-Cinar et al., 2013; Koob et al., 2014).

The last stage of the addiction cycle – preoccupation/anticipation stage – is defined as a relapse to drug-seeking and drug-taking behaviors following prolonged periods of abstinence and is the most problematic stage for the long-treatments of drug users. Animal models of drug-seeking can be divided in three different groups: drug-induced reinstatement, cue-induced reinstatement, and stress-induced reinstatement (Koob & Volkow, 2016).

The basolateral amygdala (BLA) mediates cue-induced drug seeking through interactions with a number of other brain regions. Evidence has shown an important role for the BLA and the dorsomedial PFC (dmPFC), via glutamatergic and DAergic interactions with the NAcc core (Koya et al., 2006).

Drug seeking behavior that is triggered by stress seems to work with two different neural circuits. The first involves the VTA and its projections to the hypothalamus, NAcc, amygdala, and bed nucleus of the stria terminalis, predominately via noradrenaline. The second involves the central nucleus of the amygdala and projections to the bed nucleus of the stria terminalis using CRH as its major neurotransmitter (Shaham et al., 2000).

Regarding drug-induced reinstatement, different studies suggest that the main areas involved are the dmPFC, the NAcc core and the VTA. With respect to the dmPFC, inactivation has been shown to impair drug-induced seeking of heroin, cocaine and nicotine (Capriles et al., 2003; McFarland & Kalivas, 2001; Rogers et al., 2008). Stimulation of D1 receptors (D1R) in NAcc shell has been related with the cocaine-induced reinstatement attenuating this behaviour, however the same effect could not be found in the NAcc core (Anderson et al., 2003; McFarland & Kalivas, 2001).

Epidemiological data reveals differences between males and females in drug consumption, suggesting a sex-specific mechanism. For example, males show higher rates of substance use or dependence than females and suffer overdose death, but both have the same risk of developing SUD (NIDA, 2022). In contrast, females are more susceptible to craving and relapse, impacting addiction recovery (Bawor et al., 2015; Fox et al., 2014; Hitschfeld et al., 2015; Huang et al., 2021; Kennedy et al., 2013). Moreover, females show a faster progression in becoming substance abusers after their first substance use than males despite the adverse consequences (Ait-Daoud et al., 2019; Bobzean et al., 2014; Greenfield et al., 2010). Finally, females have been reported to engage in larger quantities of substance intake than males (Lau-Barraco et al., 2009).

Estradiol, progesterone, and androgens are thought to contribute to sex differences in SUD because of their sex-dimorphic levels, which change in critical life periods and physiological conditions such as adolescence or menstrual cycle (Corbett et al., 2021; Dazzi et al., 2007; Feltenstein et al., 2011; Joyce et al., 2021; Marceau et al., 2019; Reynolds et al., 2007), together with the sex-specific distribution of their receptors in the brain (Almey et al., 2015; Krentzel et al., 2021). On the other hand, recent evidence suggests that modifications of the epigenetic landscape of the dopaminergic system could be responsible for sex differences in addiction (Ajonijebu et al., 2018; Sasagawa et al., 2017). Therefore, our general hypothesis is that sex differences in SUD are at least in part generated by the interaction of three factors: i) sex-specific changes in the levels of gonadal hormones, ii) sex-specific differences in the activity of dopaminergic reward circuits, and iii) sex-specific epigenetic modifications in key genes involved in the function of the reward circuits. Therefore, this systematic review aims to analyze evidence of the interaction of the dopaminergic system, gonadal hormones, and epigenetics in both sexes, taking into account the different stages of SUD and the different stages of development in human and non-human animals and comparing the findings to the parameters of healthy controls or among different phases of addiction in SUD subjects (e.g., intoxication, abstinence and craving for human studies and acquisition, extinction, and reinstatement for animal studies). Our goal is to identify sex differences in the risk of developing a SUD or during different phases of addiction to help tailor specific interventions in males and females to maximize interventions' effectiveness and minimize relapses.

2. Materials and methods

2.1. Search strategy

Articles were searched following an ascendant two-way approach, that is, all articles resulting from queries in four databases (Web of Science, PubMed, ProQuest (PsycINFO), and SCOPUS) in June 2022 and updated in January 2023, were included. Queries were created combining five main key terms and their respective thesaurus: Substance Use Disorder (related MeSH terms: "substance use disorder" OR "substance consumption" OR "substance abuse" OR "substance dependence"), Sex (related MeSH terms: "gender" OR "sex"), Dopaminergic system (related MeSH terms: "brain reward" OR "dopaminergic system"), Epigenetics (exact MeSH term: "epigenetics") and Hormones (exact MeSH term: "hormones"). The database search was conducted twice by two different researchers.

2.2. Selection criteria and data extraction

Studies were selected according to the PICOS strategy in line with PRISMA-P 2015 guidelines:

- **P**opulation criteria: studies should focus on female and male humans and non-human animals with a SUD diagnosis or protocol of SUD, respectively.
- **I**ntervention criteria: studies should assess "dopaminergic system", "sexual hormones" or "epigenetics markers".
- **C**ontrol criteria: studies should include a group of non-SUD subjects or evaluate different phases of addiction (e.g., intoxication versus withdrawal) or different groups (e.g., adolescent versus adults; pre-menopause versus postmenopause).
- **O**utcome criteria: studies should compare sex differences.
- **S**tudy criteria: empirical studies published from 2002 to 2022 in the scientific literature in Spanish, English, Italian, or French.

The first selection was conducted by a first reviewer (either RS, DG or NL) who checked each article's title, abstract, and keywords. After this pre-selection, a second reviewer read the pre-selected articles in their entirety to confirm the selection. Finally, a third reviewer approved the selection. When a selection discrepancy occurred, it was resolved by discussion between reviewers. Data extraction was performed by a coder – distinct from the reviewers who selected the articles – using a common coding manual. Data were extracted for the following variables: stage of development, sex, type of study, subjects, ethnic group or animal model, sample size, substance of abuse, stage of addiction, risk factors,

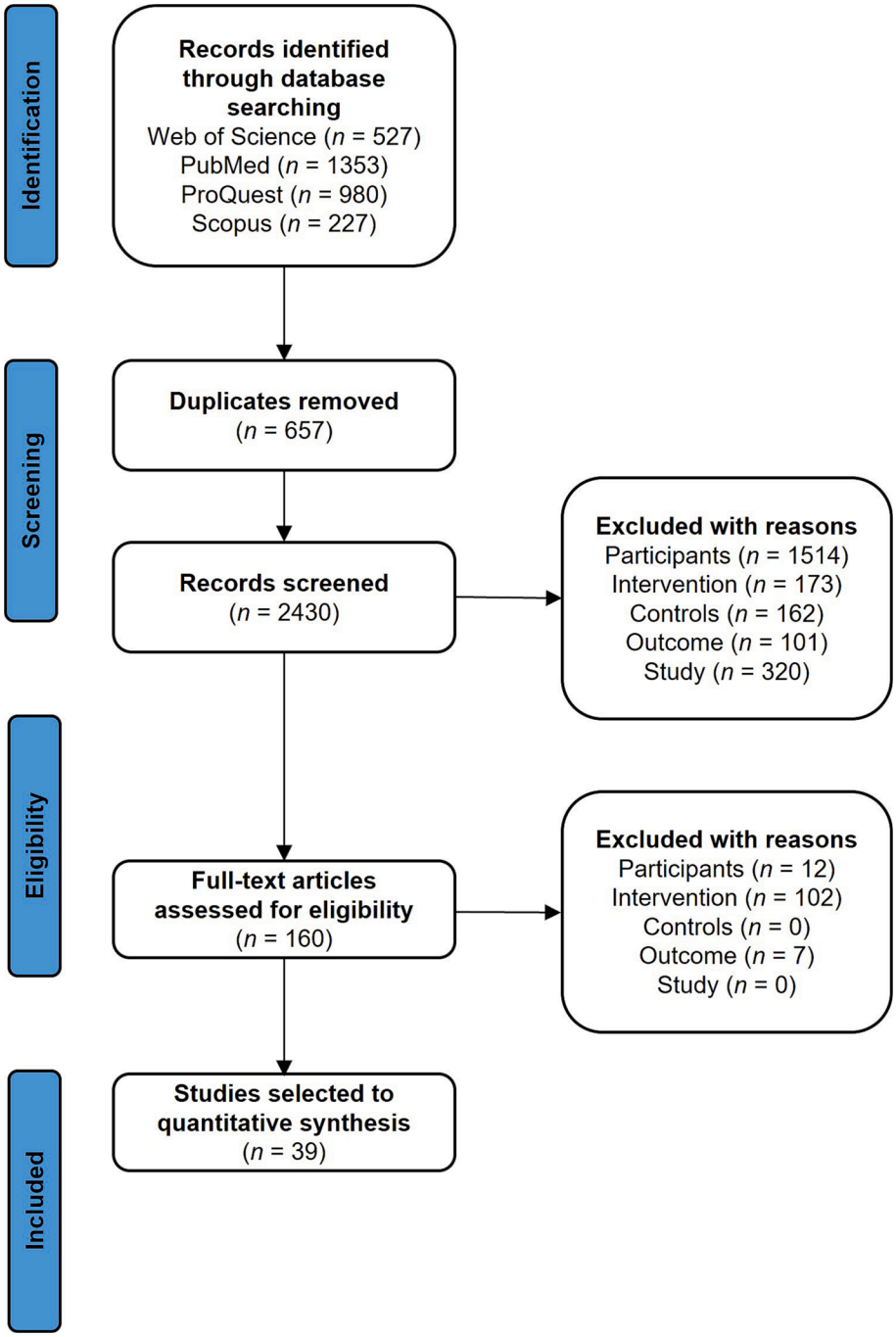

Fig. 1. Flow diagram of study selection after database searches.

measurements of the dopaminergic system/hormones/epigenetic factors, and sex differences results.

3. Results

A total of 3087 records were identified throughout the four databases consulted, 657 of which were immediately removed as duplicates. The remaining 2430 records were then screened, and 2270 were removed with reasons, leaving 160 records selected for full-text reading. Finally, 39 records were selected for this literature review (Fig. 1).

18 of the selected studies were performed with human subjects (Bawor et al., 2014; D'Addario et al., 2017; Evans & Foltin, 2006; Ho et al., 2019; Lenz et al., 2017; McClernon et al., 2008; Milivojevic et al., 2019; Moran-Santa Maria et al., 2014; Mühle et al., 2019; Okita et al., 2016; Philibert et al., 2008; Potenza et al., 2012; Sperling et al., 2010; Stolf et al., 2019; Tiili et al., 2017; Weinland et al., 2021; Wetherill et al., 2015; Wiers et al., 2016) and 21 with non-human animal subjects (Balda et al., 2006; Caine et al., 2004; Calipari et al., 2017; de Siqueira Umpierrez et al., 2022; DePoy et al., 2021; Diaz et al., 2005; Elgueta-Reyes et al., 2022; Forquer et al., 2011; Hammerslag et al., 2019; Hauser et al., 2019; Henricks et al., 2022; Kikusui et al., 2005; Kohtz et al., 2022; Lynch, 2008; Newman et al., 2009; Pisanu et al., 2022; Quigley & Becker, 2021; Quigley et al., 2021; Santoro et al., 2017; Westbrook et al., 2020).

Notably, in human studies, alcohol was the most common substance of abuse studied (D'Addario et al., 2017; Ho et al., 2019; Lenz et al., 2017; Mühle et al., 2019; Philibert et al., 2008; Sperling et al., 2010; Weinland et al., 2021), followed by cocaine or crack (Evans & Foltin, 2006; Milivojevic et al., 2019; Moran-Santa Maria et al., 2014; Potenza et al., 2012; Stolf et al., 2019), whereas the less frequent were nicotine (McClernon et al., 2008; Okita et al., 2016; Tiili et al., 2017), cannabis (Wetherill et al., 2015; Wiers et al., 2016), and opioids (Bawor et al., 2014). In addition, ten studies evaluated subjects during the ongoing addiction phase (Bawor et al., 2014; D'Addario et al., 2017; Evans & Foltin, 2006; Milivojevic et al., 2019; Moran-Santa Maria et al., 2014; Okita et al., 2016; Philibert et al., 2008; Stolf et al., 2019; Tiili et al., 2017; Wiers et al., 2016) whereas eight did it during the abstinence period (Ho et al., 2019; Lenz et al., 2017; McClernon et al., 2008; Mühle et al., 2019; Potenza et al., 2012; Sperling et al., 2010; Weinland et al., 2021; Wetherill et al., 2015). Regarding the age of the subjects, almost all studies were performed only with adults, although one distinguished between younger adult and aged patients (Weinland et al., 2021). Finally, six studies measured aspects of the dopaminergic system (McClernon et al., 2008; Okita et al., 2016; Potenza et al., 2012; Stolf et al., 2019; Wetherill et al., 2015; Wiers et al., 2016), nine evaluated sexual hormones levels (Bawor et al., 2014; Evans & Foltin, 2006; Ho et al., 2019; Lenz et al., 2017; Milivojevic et al., 2019; Moran-Santa Maria et al., 2014; Mühle et al., 2019; Sperling et al., 2010; Weinland et al., 2021), and three epigenetic markers (D'Addario et al., 2017; Philibert et al., 2008; Tiili et al., 2017). In addition, one study reported prenatal androgen levels as an associated risk of developing SUD in adulthood (Lenz et al., 2017), and another studied the risk associated with a stress condition during abstinence (Potenza et al., 2012) (Table 1) (de Siqueira Umpierrez et al., 2022).

In non-human animal studies, the most studied substance of use was cocaine (Balda et al., 2006; Caine et al., 2004; Calipari et al., 2017; de Siqueira Umpierrez et al., 2022; DePoy et al., 2021; Kikusui et al., 2005; Kohtz et al., 2022; Lynch, 2008; Quigley & Becker, 2021; Quigley et al., 2021), followed by alcohol (Forquer et al., 2011; Hammerslag et al., 2019; Hauser et al., 2019; Henricks et al., 2022; Newman et al., 2009; Silva et al., 2009), opioids (Diaz et al., 2005; Santoro et al., 2017), and others such as amphetamine (AMPH) (Pisanu et al., 2022), methamphetamine, methylphenidate, methylenedioxy-methamphetamine (MDMA) and methylenedioxy-α-pyrrolidinohexiophenone (MDPHP) (Elgueta-Reyes et al., 2022; Pisanu et al., 2022; Westbrook et al., 2020). Fourteen studies used a self-administration paradigm (Caine et al., 2004; de Siqueira Umpierrez et al., 2022; DePoy et al., 2021; Hammerslag et al., 2019; Hauser et al., 2019; Henricks et al., 2022; Kohtz et al., 2022; Lynch, 2008; Newman et al., 2009; Pisanu et al., 2022; Quigley & Becker, 2021; Quigley et al., 2021; Silva et al., 2009; Westbrook et al., 2020) and seven used administration by injection or inhalation (Balda et al., 2006; Calipari et al., 2017; Diaz et al., 2005; Elgueta-Reyes et al., 2022; Forquer et al., 2011; Kikusui et al., 2005; Santoro et al., 2017). Regarding the animal's age, three studies evaluated adolescents (Diaz et al., 2005; Lynch, 2008; Pisanu et al., 2022), one assessed adolescents and adults (Westbrook et al., 2020), and all the others studied subjects during adulthood. Finally, aspects of the dopaminergic system were assessed in eleven studies (Balda et al., 2006; de Siqueira Umpierrez et al., 2022; Diaz et al., 2005; Hammerslag et al., 2019; Hauser et al., 2019; Henricks et al., 2022; Kikusui et al., 2005; Newman et al., 2009; Pisanu et al., 2022; Santoro et al., 2017; Westbrook et al., 2020), sex hormone levels in five (Caine et al., 2004; Forquer et al., 2011; Kohtz et al., 2022; Lynch, 2008; Silva et al., 2009), and both dopaminergic system and sex hormones in five (Calipari et al., 2017; DePoy et al., 2021; Elgueta-Reyes et al., 2022; Quigley & Becker, 2021; Quigley et al., 2021). Additionally, some studies evaluated risk factors; for example, five of them reported risk associated with adolescence (Balda et al., 2006; Hammerslag et al., 2019; Hauser et al., 2019; Henricks et al., 2022; Westbrook et al., 2020), two with early infant stress (Kikusui et al., 2005; Newman et al., 2009), one with disruptions in circadian rhythms (DePoy et al., 2021), one with maternal immune activation (Henricks et al., 2022) and, finally, one with early life exposure to sex hormones (Elgueta-Reyes et al., 2022) (Table 2).

The results were separated between human and non-human animal studies and were summarized, taking into account only the relevant results to the review (i.e., dopaminergic system, sexual hormones, and epigenetics results).

3.1. Sex differences in the dopaminergic system in human studies

Sex differences in the dopaminergic system of SUD subjects were reported at different phases of addiction.

3.1.1. Sex differences in the dopaminergic system in human studies during the intoxication phase.

Three studies assessed the implication of sex differences in the dopaminergic system during this phase.

In the first study, Okita and colleagues showed increased availability of dopamine D2-type receptors, measured by binding potential (non displaceable) (BPND), in the midbrain of female compared to male nicotine smokers, with no sex differences between non-smoker groups. Additionally, striatal BPND (caudate and putamen) values were positively correlated with midbrain BPND in both sexes for the non-smokers groups and female smokers but not in male smokers (Okita et al., 2016). In the second study, Stolf and colleagues approached genetic variation in D2-type receptor (D2R) as a possible risk factor for crack cocaine addiction. The results showed that, in females, the presence of the D2R-T allele and concomitant absence of the D4-type receptor (D4R)-7R allele were associated with the risk for crack cocaine addiction. In contrast, no influence of D2R and D4R variants was observed in males regarding crack cocaine addiction (Stolf et al., 2019). Beyond the dopamine receptors, in the third study, Wiers and colleagues evaluated dopamine activity by blocking dopamine (DA) transporters with methylphenidate. The results showed an increase in whole-brain metabolism in both cannabis-dependent females and female controls but not in male groups. Moreover, cannabis-dependent females showed blunted methylphenidate challenge-induced responses in the cerebellum, medial frontal gyrus, pons, and in a cluster encompassing the hippocampus, thalamus, and midbrain, whereas there were no differences in cannabis-dependent males (Wiers et al., 2016).

Table 1
Summary of main characteristics and findings in human studies.

ARTICLE ID	SAMPLE SIZE	SAMPLE AGE	RISK FACTOR	ETHNICITY	SUD DIAGNOSIS CRITERIA	SUBSTANCE OF ADDICTION	ADDICTION PHASE	MEASUREMENT	TECHNIQUE	RESULTS
Bawor, 2014	1014	Adults	No	European	Opioid dependence according to DSM-IV criteria	Opioid	Current consumption	Testosterone (T) plasma levels	Enzyme-Linked Immunosorbent Assay (ELISA)	• **T levels:** ♀ < ♂ of all groups; SUD + methadone ♂ < no-SUD ♂
D'Addario, 2017	1823	Adults	No	Scandinavian	Alcohol dependence according to DSM-IV criteria	Alcohol	Current consumption	DNA methylation patterns in the prodynorphin gene (PDYN) promoter	Bisulfite PyroSeQuencing (PSQ)	• **Methylation of PDYN gene values:** SUD ♂ > no-SUD ♂ on CpG 4, CpG 7 sites and in the average of the 7 CpG sites SUD ♀ > no-SUD ♀ on CpG 4 site
Evans, 2006	21	Adults	No	African American (90 %), Hispanic (10 %)	Cocaine dependence according to DSM-IV criteria	Cocaine	Current consumption	Estradiol (E2) and progesterone (P4) plasma levels	ChemiLuminescent ImmunoAssay (CLIA)	• **E2 levels:** SUD ♀ > SUD ♂ during follicular phase • **P4 administration:** SUD ♀ = SUD ♂ progesterone levels; ↓ positive subjective effects of cocaine only in SUD ♀
Ho, 2019	88	Adults	No	No specified	Alcohol dependence according to DSM-IV-TR criteria	Alcohol	Abstinence (within the next 15 days after admission)	Estrogens (17-β-estradiol, estrone), progesterone (P4), testosterone (T) plasma levels	Gas Chromatography/Mass spectrometry (GC/MS) and Enzyme-Linked Immunosorbent Assay (ELISA)	• **P4 levels:** SUD ♂ > no-SUD ♂; (+) correlated with craving intensity and daily ethanol intake (in the past) only in SUD ♂ • **T levels:** (+) correlated with propensity to drink under temptations only in SUD ♂
Lenz, 2017	440	Adults	Prenatal androgens	No specified	Alcohol dependence according to DSM-V and ICI-10 criteria	Alcohol	Abstinence (24–72 h of abstinence)	5 alpha-dihydrotestosterone (DHT), testosterone (T) plasma levels	Enzyme-Linked Immunosorbent Assay (ELISA)	• **DHT levels:** SUD ♂ > no-SUD ♂ and SUD ♀ • **Bioavailable DHT/T ratio:** SUD ♂ > no-SUD ♂ and SUD ♀ • **Prenatal androgens levels:** (+) correlated with risk for alcohol dependence only in ♂
McClernon, 2008	30	Adults	No	Caucasian	Fagerström Test for Nicotine Dependence (FTND)	Nicotine	Abstinence (24 h of abstinence)	Striatal reactivity to smoking clues	functional Magnetic Resonance Imaging (fMRI)	• **Striatal reactivity to smoking clues:** SUD ♀ > SUD ♂
Milivojevic, 2019	46	Adults	No	African American (74 %) Caucasian (22 %) Hispanic (4 %)	Cocaine dependence according to DSM-IV criteria	Cocaine	Current consumption	Pregnenolone (Preg), allopregnanolone (AlloP), progesterone (P4), testosterone (T), androstanediol, DHEA plasma levels	Gas Chromatography/Mass spectrometry (GC/MS)	• **T and androstanediol levels:** SUD ♀ < SUD ♂ • **P4 administration:** ↑ P4, Preg and AlloP • **Preg and androstanediol levels:** (-) correlated with years of cocaine use
Moran-Santa Maria, 2014	119	Adults	No	Caucasian (41 %)	Cocaine dependence according to DSM-IV criteria	Cocaine	Current consumption	Dehydroepiandrosterone (DHEA) plasma level	Enzyme-Linked Immunosorbent Assay (ELISA)	• **DHEA levels:** SUD ♀ = SUD ♂

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Table 1 (*continued*)

ARTICLE ID	SAMPLE SIZE	SAMPLE AGE	RISK FACTOR	ETHNICITY	SUD DIAGNOSIS CRITERIA	SUBSTANCE OF ADDICTION	ADDICTION PHASE	MEASUREMENT	TECHNIQUE	RESULTS
Mühle, 2019	440	Adults	No	Caucasian	Alcohol dependence according to DSM-V and ICI-10 criteria	Alcohol	Abstinence (24–72 h of abstinence)	Estradiol (E2), testosterone (T) plasma level and single nucleotide polymorphisms (SNPs) of the estrogen receptor 1 gene (ESR1)	Enzyme-Linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR)	• **E2 levels:** ↓ during withdrawal in SUD ♀ and SUD ♂ • **E2/T ratio:** SUD ♀ < no-SUD ♀; SUD ♂ = no-SUD ♂ • **ESR1 SNPs:** (rs6557171) ↑ risk for alcohol dependence in ♀ and ♂, (rs2982683) ↑ risk for alcohol dependence only in ♂
Okita, 2016	37	Adults	No	No specified	FTND	Nicotine	Current consumption	Dopamine D2-type receptor availability (BNDP) in striatum and midbrain	Positron Emission Tomography (PET)	• **Midbrain BPND values:** SUD ♀ > SUD ♂; no-SUD ♀ = no-SUD ♂, (+) correlated with BPND values in caudate nucleus and putamen in SUD ♀ but no in SUD ♂
Philibert, 2008	191	Adults	No	White (93 %) African American (4 %) Hispanic (1,5 %) Other (1,5 %)	Alcohol dependence according to DSM-IV criteria	Alcohol and nicotine	Current consumption	Variable NucleoTide Repeat (VNTR) polymorphism and methylation quantification of the Monoamine Oxidase A (MAOA) gene	Polymerase Chain Reaction (PCR) and bisulfite assay coupled to MassARRAY™ mass spectrometer	• **Methylation of MAOA gene values:** ↑ methylation status was significantly associated with SUD symptoms (in both nicotine and alcohol dependence), but only in ♀
Potenza, 2012	66	Adults	Stress	Caucasian (50 %)	Cocaine dependence according to DSM-IV criteria	Cocaine	Abstinence (after 2 weeks of abstinence)	Corticostriatal-limbic tract reactivity	functional Magnetic Resonance Imaging (fMRI)	• **Corticostriatal-limbic reactivity:** ↑ in SUD ○ and SUD ♂, ↑ in stress condition only in SUD ♀
Sperling, 2010	500	Adults	No	No specified	Alcohol dependence according to DSM-III-R criteria	Alcohol	Abstinence (21 days of abstinence)	Testosterone (T) plasma levels	ElectroChemiLuminescence (ECL)	• **T levels:** SUD ♂ > no-SUD ♂ and SUD ♀
Stolf, 2019	1077	Adults	No	Caucasian	Crack –cocaine dependence according to DSM-IV-TR criteria	Crack-cocaine	Current consumption	SNPs of D2R and D4R genes	Polymerase Chain Reaction (PCR)	• **D2R and D4R SNPs:** presence of D2R-T allele and concomitant absence of D4R-7R allele ↑ risk for crack-cocaine dependence, only in ♀
Tiili, 2017	1260	Adults	No	Russian	FTND	Nicotine	Current consumption	Methylation quantification of MAO genes	Bisulfite PyroSeQuencing (PSQ)	• **Methylation of MAOA gene values:** ♀ > ♂ on the CpG sites SUD ♂ and SUD ♀ > former SUD, ↑ methylation values of the CpG sites were associated to SUD phenotypes in ♀ • **Methylation of MAOB gene values:** ↑ methylation values of the CpG sites were associated to SUD phenotypes in both ♀ and ♂

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Table 1 (*continued*)

ARTICLE ID	SAMPLE SIZE	SAMPLE AGE	RISK FACTOR	ETHNICITY	SUD DIAGNOSIS CRITERIA	SUBSTANCE OF ADDICTION	ADDICTION PHASE	MEASUREMENT	TECHNIQUE	RESULTS
Weinland, 2021	440	Adults	No	Caucasian	Alcohol dependence according to DSM-IV criteria	Alcohol	Abstinence (24–72 h of abstinence)	Progesterone (P4) plasma levels	Enzyme-Linked Immunosorbent Assay (ELISA)	• **P4 levels:** SUD ♂ > no-SUD ♂, postmenopausal SUD ♀ < premenopausal SUD ♀,(-) correlated with craving intensity in SUD ♀
Wetherill, 2015	44	Adults	No	African American (74 %)	Cannabis dependence according to DSM-IV criteria	Cannabis	Abstinence (24 h of abstinence)	Neuronal reactivity in rewarding structures to cannabis cues	functional Magnetic Resonance Imaging (fMRI)	• **Brain reactivity during withdrawal:** (+) correlated with bilateral insula reactivity and (-) correlated with lateral orbitofrontal cortex reactivity in SUD ♀, (+) correlated with the striatum reactivity in SUD ♂
Wiers, 2016	48	Adults	No	No specified	Marijuana Dependency Questionnaire (MDQ) based in DSM-IV criteria	Cannabis	Current consumption	Increasing Dopamine (DA) activity blocking DA transporters by methylphenidate challenge	Positron Emission Tomography (PET)	• **Methylphenidate challenge:** ↑ whole-brain metabolism only in SUD and no-SUD ♀, ↓ responses in cerebellum, medial frontal gyrus, pons, hippocampus, thalamus, and midbrain only in SUD ♀

3.1.2. Sex differences in the dopaminergic system in human studies during the abstinence and craving period

Three studies evaluated the sex differences in the dopaminergic system during this phase. In the first study, McClernon and colleagues demonstrated sex-dependent patterns of brain reactivity to visual smoking cues in nicotine-dependent subjects. Female nicotine smokers showed increased striatal reactivity to visual smoking cues compared to male nicotine smokers. In contrast, male nicotine smokers showed increased reactivity in the left hippocampus and the orbitofrontal cortex (McClernon et al., 2008). In the second study, Potenza and colleagues evaluated corticostriatal-limbic tract reactivity during cocaine craving. The results showed that cocaine-dependent subjects showed increased corticostriatal-limbic reactivity, regardless of sex. However, sex differences only emerged when a stress condition was added; cocaine-dependent females showed increased corticostriatal-limbic activation during the stress condition, whereas no differences were found in cocaine-dependent males in all conditions (Potenza et al., 2012). Finally, in the third study, Wetherill and colleagues assessed neural responses to cannabis cues in cannabis-dependent groups, demonstrating that both males and females showed greater neural responses than the healthy population in reward-related brain regions, including the striatum, hippocampus/amygdala, insula, anterior cingulate cortex, and lateral orbitofrontal cortex. However, sex differences emerged when correlations between brain responses to cannabis cues and cannabis-craving symptoms were analyzed. In females, the results showed a positive correlation in the bilateral insula and a negative correlation in the left lateral orbitofrontal cortex, whereas males showed only a positive correlation in the striatum (Wetherill et al., 2015).

3.2. Sex differences in gonadal hormones from human studies

3.2.1. Sex differences in gonadal hormones in human studies during the intoxication phase

Four studies reported sex differences between SUD groups during the ongoing consumption phase in testosterone, estradiol, and progesterone levels. Bawor and colleagues demonstrated that testosterone was higher in both opioid-dependent males and control subjects than in female groups. Interestingly, male subjects treated with methadone showed lower testosterone levels than control males, whereas methadone treatment did not affect testosterone levels in female subjects compared to control females, and methadone dose was inversely associated with testosterone levels only in males (Bawor et al., 2014). Similarly, males have been shown to display higher testosterone and androstenedione levels (testosterone precursor which is involved in the production of testosterone and estrogen) than females in cocaine-dependent subjects (Milivojevic et al., 2019). In contrast, in cocaine-smoking subjects, females showed higher estradiol levels during the follicular phase than males. Indeed, Evans and colleagues showed that cocaine's subjective positive effects increased during the follicular phase compared to the luteal phase (Evans & Foltin, 2006). Additionally, two studies reported the effects of exogenous progesterone administration on SUD subjects. Milivojevic and colleagues demonstrated that exogenous progesterone administration increased GABAergic neurosteroids (NAS) pregnanolone and allopregnanolone levels in both sexes, results particularly of interest as lower pregnenolone and androstanediol (GABAergic NAS precursors) levels were associated with years of cocaine use in these subjects (Milivojevic et al., 2019). Additionally, Evans and colleagues found no differences in progesterone levels between females and males administered with exogenous progesterone, neither during the follicular phase in females in comparison with males administered with placebo (Evans & Foltin, 2006). However, progesterone administration attenuated the positive subjective effects of cocaine only in females. In contrast, when dehydroepiandrosterone (DHEA) levels, an endogenous sex steroid hormone precursor, was evaluated in cocaine-dependent and control groups, no differences were found, regardless of sex (Moran-Santa Maria et al., 2014).

Table 2
Summary of main characteristics and findings in non-human studies.

ARTICLE ID	SAMPLE SIZE	SAMPLE AGE	RISK FACTOR	SPECIES & STRAIN	SUD MODEL	SUBSTANCE OF ADDICTION	ADDICTION PHASE	MEASUREMENT	TECHNIQUE	RESULTS
Balda, 2006	No specified	Adults	Adolescent consumption	Mouse (nNOS KO and WT, progeny of SV129 and C57BL/6)	Injections	Cocaine	Drug-CPP, extinction and reinstate	Inhibition of Dopamine (DA) release	Knock-out (KO) model lacking the neuronal Nitric Oxide Synthase (nNOS) gene	• **Cocaine-CPP**: adolescent WT groups acquire and maintain CPP whereas adult WT groups reinstate CPP. Adolescent nNOS KO groups acquire but no maintain CPP whereas adult nNOS KO ♂ no reinstate CPP • **Cocaine-CPP magnitude**: adolescent WT ♀ = adolescent WT ♂, whereas adult WT ♀ > adult WT ♂
Caine, 2003	120	Adults	No	Rat (Sprague-Dawley)	Self-Administration paradigm	Cocaine	Consumption	Hormonal deprivation, hormonal replace treatment, and plasma estradiol (E2) and testosterone (T) concentrations	Gonadectomy, subcutaneous implantation of steroid treatments, and Enzyme-linked immunosorbent assay (ELISA)	• **Cocaine consumption**: control ♀ > OVX ♀ during the 5 days immediately preceding acquisition of stable cocaine self-administration • **E2 levels**: control ♀ and OVX + estradiol ♀ > OVX ♀ • **T levels**: control ♂ and OKX + testosterone ♂ > OKX ♂
Calipari, 2017	No specified	Adults	No	Mouse (C57BL/6J and TH-BAC-Cre)	Injections	Cocaine	Drug-CPP	Estradiol (E2) and progesterone (P4) plasma levels, and dopaminergic function in Ventral Tegmental Area (VTA) and Nucleus Accumbens (NAc)	Enzyme-linked immunosorbent assay (ELISA), single-unit in vivo electrophysiology, fast-scan cyclic voltammetry (FSCV), Ca2 + transients through VTA and the Nac, and treatment with CNO and quinpirole	• **E2 levels**: estrus ♀ > diestrus ♀ • **Cocaine-CPP**: estrus ♀ > diestrus ♀ and ♂ • **Dopaminergic function in VTA and Nac**: estrus ♀ > diestrus ♀ and ♂ • **VTA dopamine neuron firing**: ↑ by CNO treatment in diestrus ♀
DePoy, 2021	No specified	Adults	Disruptions in circadian rhythms	Mouse (Npas2 mutant and C57BL/6J)	Self-Administration paradigm	Cocaine	Drug-CPP	Hormonal deprivation, and D1R expression in Nucleus Accumbens (NAc) and DorsoLateral Striatum (DLS)	Ovariectomy, and RNAscope in situ hybridization (ISH) followed by ImmunoHistoChemistry (IHC) for ΔFosB	• **Cocaine-CPP**: Npas2 groups > Control groups in the light (inactive) phase; Npas2 ♀ > Npas2 ♂ during the dark (active) phase

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Table 2 (*continued*)

ARTICLE ID	SAMPLE SIZE	SAMPLE AGE	RISK FACTOR	SPECIES & STRAIN	SUD MODEL	SUBSTANCE OF ADDICTION	ADDICTION PHASE	MEASUREMENT	TECHNIQUE	RESULTS
										whereas Npas2 + OVX ♀ = Npas2 ♂ during the dark (active) phase • **ΔFosB expression in D1 + neurons of NAc and DLS**: Npas2 ♀ > Control ♀ and Npas2 ♂ = Control ♂
de Siqueira Umpierrez, 2022	85	Adults	No	Rat (Wistar)	Self-Administration paradigm	Cocaine	Consumption	Brain dopamine depletion by neonatal 6-Hydroxydopamine (6-OHDA) administration	6-OHDA intraperitoneal injection on postnatal day 4.	• **Cocaine consumption**: control ♀ > 6-OHDA ♂ and Control ♂; 6-OHDA ♀ = 6-OHDA ♂ and Control ♂, and 6-OHDA groups < control groups
Diaz, 2005	24	Adolescents	No	Mouse (Swiss-Webster albino)	Injections	Morphine	Withdrawal (induced 60 min after last consumption by naloxone)	Dopamine (DA) and 3,4-DihydrOxyPhenylAcetic ACid (DOPAC) levels in striatum, frontal cortex and hippocampus Implications of D1R and D2R	High Performance Liquid Cromatography (HPLC)- coupled to an electrochemical detector (BAS LC-4C) Administration of D1 or D2 agonist	• **DA and DOPAC in striatum**: ♀ > ♂ (only during withdrawal) • **Administration of D1 and D2 agonist**: ↓ withdrawal signs in both ♀ and ♂
Elgueta-Reyes, 2022	305	Adults	Early life exposure to sex hormones	Rat (Sprague Dawley)	Injections	Amphetamine (AMPH) or methylphenidate (MDP)	Drug-CPP	Testosterone propionate (TP) or estradiol valerate (EV) treatment; and extracellular DA levels in Nucleus Accumbens (NAc), DA Transporter (DAT) mRNA expression in ventral tegmental area (VTA) and substantia nigra (SN), and DA reuptake in the striatum	In Vivo Brain Microdialysis, Real-time Polymerase Chain Reaction (PCR), and in vivo fast-scan cyclic voltammetry	• **AMPH-CPP**: control ♂ = TP and EV ♂; control ♀ > TP and EV ♀ • **MDP-CPP**: control ♂ = TP and EV ♂; control ♀ = TP and EV ♀ • **DA levels in Nac:** (after AMPH) control ♂ = TP and EV ♂; control ♀ > TP and EV ♀; (after MDP) control ♂ > EV ♂; TP ♀ > control ♀ > EV ♀ • **DAT mRNA**: (VTA) control ♂ > TP ♂; control ♀ > EV ♀; (SN) EV ♂ > control ♂, TP ♂ and EV ♀ • **DA reuptake**: control ♂ < TP and EV ♂; control ♀ < TP and EV ♀
Forquer, 2011	No specified	Adults	No	Mouse (WSR and WSP lines from HS/Ibg mice)	Inhalation	Alcohol	Chronic consumption and withdrawal (evaluated at	Testosterone (T) and estradiol (E2) plasma levels, and oestrous cycle	Enzyme-linked immunosorbent assay (ELISA) and Radioimmunoassay (RIA)	• **T levels**: ↓ in WSP ♂ after chronic ethanol exposure, ↓ in WSP and WSR ♂ during withdrawal, ↑ in

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Table 2 (*continued*)

ARTICLE ID	SAMPLE SIZE	SAMPLE AGE	RISK FACTOR	SPECIES & STRAIN	SUD MODEL	SUBSTANCE OF ADDICTION	ADDICTION PHASE	MEASUREMENT	TECHNIQUE	RESULTS
							4, 8, 12, 16 and 24 h after last consumption)			WSP ♀ after chronic ethanol exposure, and ↑ in WSP and WSR ♀ during withdrawal • **E2 levels**: ↓ in WSP and WSR ♀ and ♂ during withdrawal • **Estrous cycle**: disrupted in WSP ♀ after chronic ethanol exposure
Hammerslag, 2019	178	Adults	Adolescent impulsivity	Rat (Sprague-Dawley)	Self-Administration paradigm	Alcohol	Consumption	D2R expression in prelimbic PreFrontal Cortex (PFC)	Western blot (WB)	• **Total alcohol consumption**: ♀ > ♂ • **Alcohol consumption across blocks**: ↑ only in ♂ • **D2R expression**: ↓ only in ♀ with higher levels of impulsive choice
Hauser, 2019	192	Adults	Adolescent consumption (AIE)	Rat (Wistar)	Self-Administration paradigm	Alcohol	Consumption	D1R, D2R, D3R and D5R gene expression in posterior Ventral Tegmental Area (VTA)	Quantitative Polymerase Chain Reaction (PCR)	• **Alcohol consumption**: exposed-AIE ♂ > control ♂, exposed-AIE ♀ > control ♀, and control ♀ > control ♂ • **D3R gene expression**: exposed-AIE ♂ < exposed-AIE ♀ and control ♀ and ♂
Henricks, 2022	No specified	Adults	Maternal immune activation (MIA) and adolescent exposure (AE)	Rat (Sprague-Dawley)	Self-Administration paradigm	Alcohol	Consumption	Cortical-striatal-hippocampal local field potentials (LFPs)	LFPs oscillation analysis	• **Alcohol consumption**: overall ♀ > ♂; MIA + AE ♀ > all other ♀ and ♂ groups; • **LFPs**: MIA + AE ♀ ≠ control ♀, MIA ♀ and AE ♀; MIA + AE ♂ ≠ control ♂ and AE ♂
Kohtz, 2022	62	Adults	No	Rat (Sprague-Dawley)	Self-Administration paradigm and behavioral economics (BE) protocol	Cocaine	Consumption	Testosterone (T) progesterone (P4) and estradiol (E2) plasma levels, estrous cycle, hormonal deprivation, hormonal replacement with E2 and P4	Enzyme-linked immunosorbent assay (ELISA), cytology, ovariectomy (OVX) and subcutaneous injection	• **Cocaine demand (motivation)**: overall ♀ > ♂, but proestrus and OVX ♀ = ♂; higher P4 levels < higher E2 levels in ♀
Kikusui, 2005	94	Adults	Early infant stress	Mouse (Carworth Farms White)	Injections	Cocaine	Consumption	Positive DopAmine Transporter (DAT) cells in the Nucleus Accumbens	ImmunoHistoChemistry (IHC)	• **DAT cells**: ♀ > ♂ regardless of maternal separation

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ARTICLE ID	SAMPLE SIZE	SAMPLE AGE	RISK FACTOR	SPECIES & STRAIN	SUD MODEL	SUBSTANCE OF ADDICTION	ADDICTION PHASE	MEASUREMENT	TECHNIQUE	RESULTS
Lynch, 2007	39	Adolescents	No	Rat (Sprague-Dawley)	Self-Administration paradigm	Cocaine	Consumption	Estradiol (E2), progesterone (P4), and testosterone (T) plasma levels	Radioimmunoassay (RIA)	• **E2 levels**: ↑ ♀ during estrus phase • **Cocaine consumption**: infusions ↑ in ♀ during estrus phase • **Self-administration** is acquired more readily and in a greater percentage by ♀ than ♂
Newman, 2009	96	Adults	Early infant stress	Macaque (Rhesus)	Self-Administration paradigm	Alcohol	Consumption	SNPs (−111 G/T) in the 5′UTR of D1R gene	Polymerase Chain Reaction (PCR)	• **Alcohol consumption**: maternally deprived G/T ♂ > all the other groups, principally, versus no-maternally deprived G/G ♀
Pisanu, 2022	84	Adolescents	No	Rat (Sprague-Dawley)	Self-Administration paradigm	Methylenedioxymethamphetamine (MDMA) and methylenedioxy-α-pyrrolidinohexiophenone (MDPHP)	Consumption	Firing rate of rostral ventral tegmental area (rVTA) dopaminergic neurons	Electrophysiological patch-clamp recordings	• **MDMA and MDPHP consumption**: ♀ = ♂ • **Firing rate of rVTA DA neurons**: (after MDMA or MDPHP) only increased in ♂
Quigley, 2021a	51	Adults	No	Rat (Sprague-Dawley)	Self-Administration paradigm	Cocaine	Drug-conditioning, extinction and reinstate	GPER1 activation by GPER1 selective agonist G1 administration intra-Dorsolateral Striatum (DLS)	Bilateral cannula	• **G1 treatment**: ↑ motivation to cocaine self-administration and drug-induced reinstatement only in ♀
Quigley, 2021b	62	Adults	No	Rat (Sprague-Dawley)	Self-Administration paradigm	Cocaine	Drug-CPP	Estrogen Receptor (ER) α, ERβ, and GPER1 expression in dorsal striatum	Quantitative Polymerase Chain Reaction (PCR)	• **ERs expression in the dorsal striatum**: ♀ = ♂ for ERs; GPER1 mRNA > ERα and ERβ mRNA • **Cocaine-CPP**: ↓ only in ♂ treated wit ICI or G1, ↑ only in ♂ treated with G15
Santoro, 2017	48	Adults	No	Rat (Sprague-Dawley)	Injections	Morphine	Withdrawal (spontaneous during the next 2 days after last consumption)	Metabolism in striatal structures	18FDG and micro Positron Emission Tomography (micro PET)	• **Caudate/putamen metabolism**: ♀ < ♂ only during withdrawal, ↑ in ♂ treated with methadone • **Ventral striatum metabolism**: ↑ in both ♀ and ♂ buprenorphine treated • **Globus pallidus metabolism**: buprenorphine-treated ♀ >

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Table 2 (*continued*)

ARTICLE ID	SAMPLE SIZE	SAMPLE AGE	RISK FACTOR	SPECIES & STRAIN	SUD MODEL	SUBSTANCE OF ADDICTION	ADDICTION PHASE	MEASUREMENT	TECHNIQUE	RESULTS
										buprenorphine-treated ♂
Silva, 2009	No specified	Adults	No	Rat (Wistar)	Self-Administration paradigm	Alcohol	Withdrawal (after 2 weeks of withdrawn and during the next 2 weeks)	Estradiol (E2), progesterone (P4), and testosterone (T) plasma levels	Enzyme-linked immunosorbent assay (ELISA)	• **T levels**: ↓ in ♂ after 6 months of alcohol consumption, was not reversed by withdrawal • **E2 and P4 levels**: no variations reported in ♀
Westbrook, 2020	165	Adolescents and adults	Adolescent onset consumption	Rat (Sprague-Dawley)	Self-Administration paradigm	Methamphetamine	Consumption	D1R expression in PreFrontal Cortex (PFC) and Nucleus Accumbens (NAc)	Western blot (WB)	• **Methamphetamine consumption**: ↑ in adolescent-onset groups • **Methamphetamine infusions**: adolescent-onset ♀ > all other groups • **D1R expression**: No differences

3.2.2. *Sex differences in gonadal hormones in human studies during the abstinence and craving phase*

Five reports studied the implication of sex differences on gonadal hormones during this phase. Sperling and colleagues demonstrated that after the withdrawal phase (21 days) levels of testosterone were higher in alcohol-dependent males than in control males and alcohol-dependent females (Sperling et al., 2010). Furthermore, Ho and colleagues reported that total testosterone levels and propensity to drink under temptations were increased in alcohol-dependent males but not in females in early abstinence (15.5 days average) (Ho, 2019). Moreover, Lenz and colleagues extended this data showing that the dihydrotestosterone level and the bioavailable dihydrotestosterone/bioavailable testosterone ratio were significantly higher in alcohol-dependent males compared to the male controls and alcohol-dependent females (Lenz et al., 2017). In addition, a positive correlation was found between prenatal androgen activity and an elevated risk for alcohol dependence during adulthood only in males (Lenz et al., 2017). On the other hand, estradiol levels were not different between alcohol-dependent subjects at early abstinence and control subjects, although estradiol levels were observed to decrease during abstinence to levels different from those of controls in both sexes. Additionally, the estradiol-to-testosterone ratio was lower in alcohol-dependent females during early withdrawal compared to female controls, whereas no differences were detected in males. The study analyzed ESR1 (estrogen receptor α) single nucleotide polymorphisms (SNP) and the risk for alcohol dependence. Results displayed a significantly increased risk for alcohol dependence associated with the rs6557171 variation in the minor allele in both the total cohort and the female subgroup and with the rs2982683 variation in the male subgroup (Mühle et al., 2019). Moreover, in postmenopausal alcohol-dependent females, higher baseline progesterone levels correlated significantly with lower craving symptoms. The role of progesterone on craving was reinforced by reports of significantly higher craving levels in postmenopausal alcohol-dependent females versus premenopausal alcohol-dependent females with higher progesterone levels (Weinland et al., 2021). Additionally, high craving and former high daily ethanol intake positively correlated with progesterone levels in alcohol-dependent males, and they showed higher progesterone levels than healthy male controls (Ho et al., 2019).

3.3. *Sex differences in epigenetics in human studies*

Two studies exploring the possible involvement of DNA methylation addressed the Monoamine Oxidase A (MAOA) genes. One focused on nicotine (Tiili et al., 2017), and the other on the nicotine and alcohol effects (Philibert et al., 2008). Both studies showed that the MAOA gene appeared to be methylated at very variable levels among the different CpG islands, but, consistently, methylation level was higher (10.4 %) in females and lower (0.5 %) in males. Further, female and male smokers showed increased methylation of the MAOA gene versus former smokers. In contrast, Philibert et al. (2008) reported a higher level of methylation of MAO genes in alcohol-dependent females compared to control females, but no significant differences between males.

A third study assessed the effects of alcohol on the methylation of the prodynorphin (PDYN) gene promoter. The results showed a significant increase in DNA methylation of CpG 4, CpG 7 sites, and the overall average methylation of all 7 reported sites in alcohol-dependent males compared with controls. Additionally, when comparing alcohol-dependent females with controls, an increase in CpG 3 DNA methylation was found (D'Addario et al., 2017).

3.4. *Sex differences in the dopaminergic system in non-human studies*

The analysis of sex differences in the dopaminergic system in non-human animal SUD models revealed several differences at molecular, structural, and genetic levels.

3.4.1. Sex differences in the dopaminergic system in non-human studies during the acquisition phase

One study assessed the implication of the dopaminergic system in cocaine addiction using a model of brain dopamine depletion caused by the administration of 6-hydroxydopamine (6-OHDA) to neonatal female and male rats and subsequent cocaine self-administration in adulthood. The authors demonstrated that control females consumed more cocaine than the males in both conditions, control and 6-OHDA-treated. Furthermore, 6-OHDA-treated females did not show differences in cocaine consumption compared to both male groups, and 6-OHDA groups consumed less cocaine than control groups, regardless of sex (de Siqueira Umpierrez et al., 2022).

Three studies (Elgueta-Reyes et al., 2022; Henricks et al., 2022; Kohtz et al., 2022) were interested in measuring neuronal activity in dopaminergic structures. One study found that overall, females consumed more alcohol than males, increased further in both sexes in the maternal immune activation plus adolescent exposure to alcohol consumption condition (MIA + AE). Moreover, MIA + AE groups showed specific sex differences of cortical-striatal-hippocampal local field potentials (LFPs) compared to their controls in both sexes, specifically that MIA + AE LFPs were different from MIA and AE in female groups; however, in males, MIA + AE LFPs were different only from the AE group (Henricks et al., 2022).

In the second study, Pisanu and colleagues measured the firing rate of rostral ventral tegmental area (rVTA) dopaminergic neurons under the slice perfusion with Phenethylamine 2-chloro-4,5-methylenedioxy-methamphetamine (2-Cl-4,5-MDMA) and synthetic cathinone 3,4-methylenedioxy-α-pyrrolidinohexanophenone (3,4-MDPHP). The results showed that both drugs can stimulate VTA dopaminergic signaling due to an increase in the firing rate in this region, however this effect was found just in males. They also showed that abuse potential for these 2 drugs was very low due to the absence of differences between the groups in self-administration (Pisanu et al., 2022).

Lastly, Elgueta-Reyes and colleagues measured DA levels in nucleus accumbens (NAc) after AMPH consumption in two different experimental groups: early life exposure (postnatal day 1) to testosterone propionate (TP) or estradiol valerate (EV). They found differences between the female groups, showing that increased DA levels in control compared with TP and EV groups. No differences were found between the male groups. Regarding methylphenidate (MDP) consumption, increased DA levels were found in control males but only in comparison with the male TP group, whereas in control females, the increase was found in comparison only with the EV female group. Additionally, DA Transporter (DAT) mRNA expression in the ventral tegmental area (VTA) and substantia nigra (SN) and DA reuptake in the striatum were measured. Results of DAT mRNA expression showed sex differences in control males with an increase of DAT mRNA expression in the VTA compared with the TP male group. In contrast, control females showed increased expression only compared to the EV female group. On the other hand, the male EV group showed increased expression compared with control males, TP males, and EV females in the SN. Finally, DA reuptake in the striatum decreased in both sexes' control groups compared with TP and EV groups. Behavioral results from conditioned place preference (CPP) tests showed that the controls and groups exposed to TP or EV in both sexes were all conditioned in MDP-CPP, but only male groups and the female control group were conditioned in the AMPH-CPP (Elgueta-Reyes et al., 2022).

Two studies evaluated the role of dopamine receptors on consumption, including adolescent consumption or adolescent impulsivity as a risk factor for developing an addiction. In Hauser et al. (2019), rats were exposed to the adolescent intermittent ethanol paradigm and tested for its effects in adulthood. The study evaluated its effects on the reinforcing properties of ethanol (0 to 200 mg) self-administration into the posterior ventral tegmental area (pVTA) and on the expression of dopaminergic factors within the pVTA. Results found that AIE males increased ethanol infusions at 75, 100, 150, and 200 mg compared to control males, whereas AIE females increased ethanol infusions only at 75 and 100 mg compared to control females. No sex differences were reported between AIE groups at the different ethanol concentrations, but control females increased 150 mg ethanol infusions compared with control males. Additionally, the authors evaluated mRNA expression of dopamine receptor *D1R*, *D2R*, *D3R,* and *D5R* genes in the posterior ventral tegmental area (pVTA). The results showed a reduced expression of the *D3R* gene only in AIE males, but no effects were reported in other dopamine receptor genes or groups. Furthermore, Hammerslag and colleagues assessed *D2R* gene expression in prelimbic prefrontal cortex and the impact of inhibitory control (impulsive action) and impulsive decision-making (impulsive choice) during adolescence or adulthood on alcohol drinking behavior. The results showed that ethanol consumption was higher in females, but drinking increased across blocks of test sessions in males only, and prelimbic *D2R* gene expression was reduced in adolescent and adult females with higher levels of impulsive choice, but not in males (Hammerslag et al., 2019). Moreover, two other studies evaluated the impact of early infant stress: Kikusui et al. (2005) measured the effect of early maternal separation on positive cells of dopamine transporter (DAT) after ten days of intraperitoneal cocaine administration in adult mice. The results showed sex differences in the number of DAT immunoreactive sites in the nucleus accumbens, with greater immunoreactivity in females than in males, regardless of maternal separation experience. The second study, by Newman and colleagues, assessed both the influence of genetic variation (−111 G/T SNP) in the 5′UTR of the *D1R* gene and exposure to early infant stress on alcohol consumption in rhesus macaques. The results showed that maternally deprived males, heterozygous for the T allele, consumed significantly more ethanol than all the other groups, principally in comparison with females no-maternally deprived and with the G/G variation gene (Newman et al., 2009). Finally, Westbrook and colleagues evaluated *D1R* gene expression in the prefrontal cortex (PFC) and nucleus accumbens (NAc) in female and male rats with adolescent-onset or adult-onset methamphetamine self-administration. Subjects received seven days of daily 2-h self-administration sessions followed by 14 days of daily 'long access' (LgA) sessions of six hours. The results showed that during LgA, adolescent-onset groups increased methamphetamine intake more rapidly than adult-onset groups, with adolescent-onset females administering the most infusions, but no differences in *D1R* gene expression were reported in PFC or NAc between any comparisons (Westbrook et al., 2020).

3.4.2. Sex differences in the dopaminergic system in non-human studies during the withdrawal and reinstatement phase

Three reports have investigated the role of sex differences in the dopaminergic system during the withdrawal phase. In the first study, Díaz and colleagues measured dopamine (DA) and dihydroxyphenylacetic-acid (DOPAC) brain levels during the dependence phase and naloxone (NAL)-precipitated withdrawal in mice injected with morphine, showing an increase of DA and DOPAC levels in the striatum of females compared with males during withdrawal. Additionally, in order to study the role of D1R and D2R, mice were treated with specific D1R (SCH 23390) or D2R (raclopride) antagonists before NAL administration. The results displayed that both antagonists decreased withdrawal symptoms in both sexes (Diaz et al., 2005). In a second study, differences in regional brain glucose metabolism were measured by Santoro and colleagues, using micro-positron emission tomography (microPET) in combination with 18F-fluorodeoxyglucose (18FDG) during morphine withdrawal, followed by methadone or buprenorphine treatments. The results demonstrated that, only during withdrawal, females exhibited decreased metabolism in the caudate/putamen compared with males. In addition, methadone treatment increased caudate/putamen metabolism in males, whereas buprenorphine treatment increased ventral striatum metabolism in both females and males. Finally, buprenorphine-treated females showed increased metabolism in the globus pallidus compared to buprenorphine-treated

males (Santoro et al., 2017). Furthermore, Balda et al. (2006) evaluated possible differences in the acquisition, expression, maintenance, and reinstatement conditioned place preference (CPP) for cocaine injections from adolescence through to adulthood in wild-type (WT) and neuronal nitric oxide synthase (nNOS) knockout mice. The results demonstrated that adolescent WT mice acquired and maintained cocaine-CPP, and upon extinction, CPP was reinstated in adulthood after a cocaine injection. However, while adolescent nNOS KO male mice acquired cocaine-CPP it was neither maintained nor reinstated by cocaine injection. Moreover, no sex differences were found in adolescent groups, but sex differences were observed in adult mice; the magnitude of cocaine-CPP in adult WT males was lower than in females, and cocaine-CPP was reinstated during adulthood in nNOS KO females, with no differences compared with adult WT females (Balda et al., 2006).

3.5. Sex differences in gonadal hormones in non-human studies

3.5.1. Sex differences in gonadal hormones in non-human studies during the acquisition phase

Four studies analyzed the role of sex hormones during ongoing drug consumption in SUD models, either focusing on measurements of hormonal levels or using gonadectomy models. Lynch and colleagues showed that when higher levels of estradiol are reached during the estrus phase, adolescent female rats displayed increased cocaine infusions and acquired cocaine self-administration behavior more readily and a greater percentage of the sample met the acquisition criteria versus males (Lynch, 2008). In the second study, Caine and colleagues trained rats in cocaine self-administration, before and after gonadectomy, and in gonadectomized groups before and during chronic treatment with estradiol or testosterone. The results showed that during the five days immediately prior to the acquisition of stable cocaine self-administration, drug intake was significantly lower in ovariectomized females compared with intact females (with higher estradiol levels), and differences were reported in males or treated groups. Indeed, testosterone was lower in gonadectomized males than in control and testosterone-treated males (Caine et al., 2004). In the third study (Kohtz et al., 2022), cocaine demand flexibility (motivation to consume) was measured using a behavioral economics (BE) protocol in intact males and females, and in ovariectomized females treated with estradiol (E2) or progesterone (Pr). The results showed that, overall, females were more motivated to consume cocaine than males, but when females were in proestrus or ovariectomized, no differences were found. Among female groups, cocaine demand increased when E2 levels were higher (during estrous or by E2 administration) and decreased when Pr levels were higher (during proestrus or by Pr administration). Lastly, in the fourth work, Calipari and colleagues assessed the effect of sex hormones on the dopaminergic system and their implications for drug consumption. The authors evaluated cocaine-conditioned place preference (CPP), ventral tegmental area (VTA) dopamine neuron activity, and dopamine transporter (DAT) activity in female mice in estrous and diestrus, in comparison with male mice. The results showed that estrus female mice exhibit significantly higher estradiol levels and elevated CPP for cocaine than diestrus females. Moreover, in comparison with diestrus females and males, estrous females showed increased basal putative VTA dopamine neuronal activity, enhanced cocaine actions on the VTA-NAc reward pathway, increased affinity for cocaine to bind directly to DAT and inhibit its uptake function and exhibited enhanced VTA and NAc responses to cocaine-associated cues. Finally, clozapine-N-oxide (CNO) treatment enhanced VTA dopamine neuron firing and increased cocaine reward processing in diestrus females (Calipari et al., 2017).

One study evaluated the relationship between estrogen receptors and the striatal function of cocaine addiction. In this study, mRNA expression of estrogen receptor (ER)α, ERβ, and GPER1 in the dorsolateral striatum (DSL) were measured in rats treated with ICI (ERα/ERβ antagonist; GPER1 agonist), G1 (GPER1 selective agonist) or G15 (GPER1 selective antagonist) by administration into DLS in order to assess how they modulate preference for cocaine-rewarding stimuli. The results displayed no sex differences in relative ERs mRNA expression in the dorsal striatum, but GPER1 mRNA expression was greater than ERα and ERβ mRNA in both sexes. Interestingly, GPER1 activation, via ICI or G1 administration, attenuated conditioned place preference (CPP) for 10 mg/kg cocaine, whereas GPER1 inhibition, via G15, enhanced CPP for 5 mg/kg cocaine dose only in males (Quigley et al., 2021).

3.5.2. Sex differences in gonadal hormones in non-human studies during the withdrawal and reinstatement phase

Four studies assessed the implication of sex hormones during these phases. In the first study, Silva and colleagues showed that six months of alcohol consumption (self-administration) provoked a significant decrease in testosterone levels in male rats, which was not reversed by withdrawal. In contrast, no differences in estrogen and progesterone concentrations were reported in females (Silva et al., 2009). In the second report, Forquer et al. (2011) measured testosterone and 17β-estradiol levels after chronic exposure to ethanol and during withdrawal in both sexes of Withdrawal Seizure-Resistant (WSR) and -Prone (WSP) selected lines of mice. The results demonstrated that testosterone was lower in WSP mice after chronic ethanol exposure in male groups and transiently reduced during the withdrawal period in both WSR and WSP lines. In contrast, during withdrawal, testosterone levels increased (hyperandrogenemia) in WSP females and WSR and WSP mice. Additionally, chronic ethanol exposure disrupted the normal estrous cycle in WSP mice, and estrogen levels were modestly reduced during withdrawal in both WSR and WSP lines, although predominantly in males (Forquer et al., 2011). In the third study, Quigley and Becker (2021) trained both female and male rats to self-administer cocaine and, midway through the test, administered a G-protein coupled estrogen receptor 1 (GPER1) selective agonist, G1 into the dorsolateral striatum (DLS), in order to evaluate the role of GPER1 activation in cocaine motivation. In addition, after extinction, the effect of intra-DLS GPER1 activation on drug-induced reinstatement was examined. The results showed that activation of GPER1 improved motivation for cocaine self-administration, resulting in greater drug-induced reinstatement only in females (Quigley & Becker, 2021). Lastly, in the fourth report, performed by DePoy and colleagues, a circadian dysregulation of the dopaminergic system during cocaine-conditioned place preference (CPP) was evaluated. Neuronal PAS domain protein 2 (NPAS2) is a circadian transcription factor profusely expressed in reward-related brain regions and participating in reward regulation. In order to measure NPAS2's impact on cocaine-CPP, intravenous cocaine self-administration was measured in WT and Npas2 mutant mice at different times of the day. The results showed that, during the light (inactive) phase, cocaine-CPP increased in all Npas2 mutant groups, regardless of sex. However, sex differences were evident during the dark (active) phase, with only Npas2 mutation females increasing cocaine-CPP and reinstatement, but, interestingly, sex differences were abolished by ovariectomy. Moreover, after dark phase self-administration, ΔFosB expression was increased in D1R positive neurons in the nucleus accumbens core (NAcc) and the dorsolateral striatum (DLS) in Npas2 mutant females compared with WT females, with no differences reported in male groups (DePoy et al., 2021).

4. Discussion

4.1. Sex differences in SUD and dopamine system

In human studies, an increase in the rewarding effect of cocaine is described in females during the follicular phase (phase of the menstrual cycle where estrogens are at high levels) compared with the luteal phase (phase of the menstrual cycle where estrogen are at low levels) (Evans & Foltin, 2006). These data have been confirmed in non-human models, showing vulnerability to drug consumption associated with higher estrogen levels (estrus phase) during the estrous cycle in adult and

adolescent females (Calipari et al., 2017; Kohtz et al., 2022; Lynch, 2008). In addition, non-human females also display an increase in the reward effect, resulting in an improvement of the conditioned place preference (CPP) or its reinstatement (Balda et al., 2006; Calipari et al., 2017; Elgueta-Reyes et al., 2022; Quigley & Becker, 2021) or an increase of substance intake, regardless of substance (Caine et al., 2004; Calipari et al., 2017; de Siqueira Umpierrez et al., 2022; Hammerslag et al., 2019; Hauser et al., 2019; Westbrook et al., 2020). Data clearly highlights a close link between estrogens and increased dopamine activity in reward structures (Calipari et al., 2017; Kikusui et al., 2005). Indeed, human studies demonstrated an increase in dopamine D2-type receptor (D2R) gene expression in reward structures in SUD females compared with SUD males (Okita et al., 2016) and an increase in brain dopamine activity in females, regardless of SUD condition (Wiers et al., 2016). However, in contrast, a decrease in substance conditioning occurs when estrogen activity was pharmacologically increased in non-human male animals, and when an antagonist of estrogen receptors is administered, an increase is seen in substance conditioning (Quigley et al., 2021).

4.2. Sex differences in SUD and gonadal hormones

The impact of estrogen on dopamine activity and motivation to reward has been related to estradiol binding to membrane estrogen receptors in reward structures, especially in the striatum, and its sex-dependent action is postulated to be due to an unequal distribution of the receptors between males and females (Almey et al., 2015; Krentzel et al., 2021; Morissette et al., 2008). Supporting these findings, Martel et al. (2017) reported that elevated estrogens in the context of decreased progesterone levels were associated with a higher risk of drinking and binge drinking (Martel et al., 2017). Notably, adolescence, a time when females begin to produce estrogens at high levels due to having reached gonadal maturity, is the most common time to initiate substance consumption in humans, and adolescent females are more vulnerable to substance consumption compared with adolescent males. It has been suggested that estradiol promotes DA release and therefore enhances the reinforcing effect of the most addictive drugs (Becker et al., 2012; Kuhn et al., 2010), and therefore sex differences in estradiol can lead to sex differences in drug abuse at this stage. Another critical period is perimenopause when the female gonads reach senescence. Females identified as excessive drinkers are more likely to transition to non-excessive drinking across all menopausal transition stages (early/late peri- and postmenopause) versus pre-menopausal females (3–5 times more likely). An increase in likelihood of transitioning from non-excessive drinking to excessive drinking relative to premenopausal females was also seen, but the increased risk was smaller (50–100 % increase) and only seen in early peri- and postmenopausal stages (Peltier et al., 2020). This could be because gonadal senescence reduces the effect of estrogens on facilitating substance use. However, the decreased production by the postmenopausal ovary of progesterone, which attenuates the rewarding effect of different substances, may partially compensate for or complicate these changes. It is worth mentioning that the estrogens have been described as a protective factor in postmenopausal females after treatment with hormone therapies, which cause an increase in the levels of estrogens, such as estrone and estradiol, and in 2-hydroxylation estrogen metabolism, and was associated with lower total alcohol consumption compared with never/former hormone therapy users (Playdon et al., 2018).

On the other hand, progesterone seems to be relevant in SUD phases, although it may have opposing roles in females and males with SUD. Progesterone administration attenuates female cocaine-related reward during the follicular phase (Evans & Foltin, 2006). Higher progesterone levels are associated with low withdrawal symptoms in pre and postmenopausal females (Weinland et al., 2021), highlighting an important role of progesterone in fostering a lower risk of relapse in females. In contrast, progesterone is increased in alcohol-dependent males (Weinland et al., 2021), and higher levels of progesterone were associated with greater craving intensity during withdrawal in males (Ho et al., 2019). The impact of progesterone in the SUD phases seems to be related to its ability to interact with gamma-aminobutyric acid (GABA) receptors, specifically with $GABA_A$ receptors. This evidence is of utmost relevance for the SUD since GABA can decrease anxiety and inhibit substance reward effects (Stephens et al., 2017). This leads to a decrease in the rewarding effect of the substance and the withdrawal symptoms and an improvement of the treatment outcome decreasing the risk of relapse, although only in females. In males, instead, the opposite effects of progesterone on substance dependence and craving symptomatology during withdrawal are possibly mediated by allopregnanolone (AlloP), a progesterone metabolite. This metabolite, known for its neuroprotective action and modulation of GABA neurotransmission (Diviccaro et al., 2022), was found to play a different role in the brain of female and male subjects. Sex differences in AlloP brain sensitivity have been demonstrated, with female mice showing a greater potentiation of GABAergic neurotransmission by AlloP than males (Kelley et al., 2011).

In some ways mirroring the role of estrogens in SUD in females, androgens have a more prevalent role in SUD in males. Androgens are increased in males with SUD compared with females with SUD or males without SUD during the dependence and abstinence phases (Bawor et al., 2014; Milivojevic et al., 2019; Sperling et al., 2010). However, a decrease in testosterone levels after chronic alcohol exposure has been observed in alcohol-dependent male rodents compared with healthy controls (Forquer et al., 2011; Silva et al., 2009). Care should be taken in interpreting these results, as the reduction in testosterone seen by Forquer et al. (2011) the was specific to Withdrawal Seizure-Prone (WSP) mice but not in Withdrawal Seizure resistant mice,'wild-type' mice were not assessed, and Silva et al. (2009) report a decrease in solid diet intake during chronic alcohol exposure, an event that has been previously demonstrated to be associated with a decrease in testosterone levels in male rats (Govic et al., 2008). Supporting the evidence that testosterone facilitates alcohol consumption, a study conducted in 2010 with males with no alcohol dependence and ranked in low, average, and high testosterone levels demonstrate that males with high testosterone levels consume significantly more alcohol than males with normal and low testosterone levels (Haring et al., 2010). Moreover, higher testosterone levels in males are associated with an elevated drinking rate under temptations during the abstinence period (Ho et al., 2019). These data suggest a crucial role for testosterone in the reward system of males, and the results of our research show that, during abstinence, SUD males display enhanced reactivity of reward structures (Potenza et al., 2012; Wetherill et al., 2015). Moreover, intranasal administration of testosterone in male rats increases dopamine levels in the nucleus accumbens (de Souza Silva et al., 2009), and elevated prenatal testosterone in male children is associated with enhanced response to positive compared with negatively valenced cues in the caudate, putamen and nucleus accumbens nuclei (Lombardo et al., 2012), findings that can be related with an enhancement in reactivity of reward structures. Higher levels of prenatal androgens are also associated with an increased risk of later developing alcohol dependence in males (Lenz et al., 2017). The involvement of testosterone in substance abuse increases during the abstinence period. Notably, when methadone is administered to opioid-dependent subjects, testosterone levels decrease and are inversely associated with methadone dose, but only in males (Bawor et al., 2014), whereas testosterone seems not to impact opioid function in females. These findings are relevant to treatment of opioid dependence, since females need higher doses of methadone than males, and higher estradiol levels were associated with higher methadone doses (Chiang et al., 2017). Moreover, opioid-dependent young females (<18 years) show a higher failure ratio in methadone maintenance therapy compared to males and females of other ages (Heydari et al., 2019), which can be associated with their higher levels of estrogens. The fact that testosterone levels are not affected by methadone therapy may be one of the factors to be taken into account to improve the outcomes of methadone therapy in opioid-dependent females. Beyond the different involvement and action of

estradiol, testosterone, and progesterone in females and males, lower levels of pregnenolone and androstenediol are significantly associated with more years of cocaine dependence in both sexes (Milivojevic et al., 2019). Both pregnenolone and androstenediol are modulators of $GABA_A$ receptors; androstenediol is a positive allosteric modulator that potentiates GABAergic response, and pregnenolone is a precursor of neuroactive steroids (Reddy, 2010). Higher levels of both steroids can enhance GABAergic response.

4.3. Sex differences in SUD and epigenetics

Taken together, about the research suggests a clear, albeit complex sex-dependent role of gonadal hormones in SUD, and at least some of these differences are related to differential epigenetic modulation of the reward system. Methylation of the prodynorphin (*PDYN)* gene is higher in alcohol-dependent subjects than in healthy populations and higher in alcohol-dependent males than in alcohol-dependent females (D'Addario et al., 2017). *PDYN* gene encodes the opioid peptide dynorphin, which binds to kappa opioid receptors (KOPr), forming the KOPr/dynorphin system implicated in mood, cognition, and the processing of reward (Schwarzer, 2009). Moreover, dysregulations in the KOPr/dynorphin system have been described as a consequence of alcohol dependence, and it has been proposed as a pharmacological target for alcohol dependence treatments (Shippenberg et al., 2007; Walker et al., 2012). An increase in methylation appears to decrease *PDYN* gene expression, and interestingly, higher levels of *PDYN* gene expression in the nucleus accumbens of mice are associated with a decrease in sensitivity to the rewarding effects of drugs (Gieryk et al., 2010; Maiya et al., 2009). This increase in the *PDYN* gene methylation is mediated by alcohol consumption in both sexes; however, there also seems to be a modulating role of testosterone. Androgens have been reported to regulate the expression of opioid receptor kappa 1 (*OPRK1)*, a dynorphin receptor that contains androgen receptor binding sites. *OPRK1* gene expression is upregulated after androgen deprivation by orchiectomy (Makino et al., 2022), demonstrating an androgen-dependent modulation on the KOPr/dynorphin system. Taken together, this evidence points out testosterone as a possible target for alcohol dependence treatments in SUD males. Finally, increased methylation of the monoamine oxidase A (*MAOA)* gene is significantly associated with both nicotine and alcohol dependence symptoms, but only in females (Philibert et al., 2008), and females generally show an increased *MAOA* methylation rate than males. Additionally, nicotine-dependent subjects show increased levels of monoamine oxidase B *(MAOB)* gene methylation in both sexes compared with the healthy population (Tiili et al., 2017). Alcohol and nicotine consumption modify *MAOA* and *MAOB* gene methylation, but sex differences in methylation rate may be the result of the action of non-androgens. Estrogens and progesterone, for example, are able to modulate the *MAOA* and *MAOB* gene expression. Indeed, administration of isoflavones (phytoestrogens) inhibits the MAO activity *in vitro* (da Silva Schmitz et al., 2019), and ovariectomy as well as treatment with estradiol, progesterone, or both, modifies MAOA and MAOB mRNA expression in the dorsal raphe nucleus and hypothalamus of female macaques (Gundlah et al., 2002).

5. Conclusion

Sex-dependent roles for estrogens, progesterone, and testosterone have been highlighted in SUD. Taking into account the results of the studies analyzed, we can speculate on the possible involvement of estradiol in the female dopaminergic reward system, the role of which has been determined in both human and animal studies. Indeed, under elevated estrogen levels, there is an increase in the reward effects of substances and substance intake in females, which can be linked to an increased dopaminergic activity in the reward system. Moreover, increased levels of progesterone were associated with decreased withdrawal symptoms in SUD females, which can be postulated to be associated with a decrease in DA activity in SUD females. On the other hand, testosterone is increased during SUD, and the treatment for withdrawal symptoms rescued it only in males, suggesting an inhibitory effect of DA activity on testosterone levels.

Finally, the present review includes some instances of a role of testosterone in female subjects and estrogens/progesterone in male subjects, highlighting the error of not conducting matching measurement in both sexes need to perform more studies measuring all variables. For example, it would be advisable to study in depth the therapeutic role of testosterone in SUD females and progesterone in SUD males, since, generally, the role of testosterone is analyzed only in SUD males and that of progesterone only in SUD females. It is also relevant to mention the lack of studies that simultaneously measure sex hormones and dopaminergic system activity in the SUD population of both sexes. Thus, sexual hormone levels, dopaminergic or reward system activity, and epigenetic landscapes should be evaluated in both female and male subjects for a better understanding of the mechanisms involved in SUD and improving the efficiency of therapies in both men and females.

Author contributions

RS, DG, and NL performed the search in the databases, the selection of the papers, and the analysis of the results. DG and NL prepared the figures for publication. RS, DG, and NL wrote the first draft of the manuscript. All the authors contributed to the final version of the manuscript and approved the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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