Biological Psychiatry: Celebrating 50 Years

Sex and Hormonal Status Influence the Persistence of Addiction in Animal Models

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In this issue of *Biological Psychiatry*, Nicolas *et al.* (1) add to a growing literature demonstrating the importance of sex and gonadal hormones in the development of substance use disorders. Their study focused on the incubation of cocaine craving in male versus female rats tested at different estrous cycle phases. Overall, they showed that estrous cyclicity significantly altered cocaine craving (or seeking) after a drug-free period. From a translational perspective, these incubation of craving and self-administration procedures model several key features of human substance use. Specifically, animals self-administered cocaine under an intermittent-access schedule, which induces the binge–abstinence patterns of cocaine use that are often reported by individuals with cocaine and other stimulant use disorders. In addition, the incubation of craving procedure is relevant to drug relapse in that the animals reencounter the drug-taking environment and have access to cues associated with the drug after a period of forced abstinence. One novel aspect of the incubation of craving procedure is that it does not implement an extinction period to decrease drug-maintained operant responding prior to assessing cue-induced relapse behavior. Research using extinction and reinstatement methodologies is vital to understanding the role that classically conditioned cues play within the drug-taking environment and the particular salience in relapse. The forced abstinence model used here, however, allows conclusions to be drawn about circumstances under which drug use abruptly ceases and resumes at a later timepoint (e.g., after incarceration or inpatient programs), which affords additional ecological validity. The primary finding of the present study was increased responding after protracted abstinence during the estrus phase relative to nonestrus phases among females. These data add to previous research showing that endogenous (and exogenous) gonadal hormones can influence craving (or seeking) behavior; similar effects have been shown during various stages of drug use, including initiation, maintenance, and relapse. They are also in agreement with clinical data wherein women have reported increased drug craving during abstinence while in the follicular phase, i.e., when estrogen is high relative to progesterone.

Despite another recent report by Kawa and Robinson (2) showing sex differences in incentive sensitization after intermittent access to cocaine, Nicolas *et al.* (1) report no significant sex differences in their first experiment. This is important for at least two reasons. First, the lack of a sex differences is still important data for the field of sex differences research (3). For many decades, no conclusions could be drawn regarding the presence or absence of sex differences because female subjects were seldom included in preclinical studies. Critically, sex differences in drug use often emerge at transitional periods, such as the acquisition, escalation, or relapse of drug taking, as in this report (4). By including female subjects, the field can generate a more complete understanding of the complex problem that substance use disorder represents. Sex differences data, for example, becomes critical when developing treatment strategies that may show differential sensitivity between men and women. Second, while Nicolas *et al.* (1) and other recent preclinical studies (5,6) have not reported overt differences between males and females, these studies did find estrous cycle–dependent changes in self-administration behavior. Furthermore, the link between estrogen and dopamine is well established, with multiple studies showing that estrogen enhances psychostimulant-mediated dopamine release. The present findings by Nicolas *et al.* (1) are notable in that drug seeking after forced abstinence was moderated by hormonal state. Kerstetter *et al.* (7) similarly found that animals in the estrus phase emitted more lever presses during an extinction period and subsequent tests of reinstatement after cocaine self-administration.

The inclusion of freely cycling animals represents another notable strength of the Nicolas *et al.* (1) study. Experiments employing ovariectomy and the replacement of hormones exogenously have many advantages and certainly have furthered our knowledge of the link between substance use and gonadal hormones (4). However, allowing freely cycling animals to self-administer provides some offsetting strengths. The use of freely cycling subjects provides an improvement in ecological validity to the extent that fluctuations in hormonal status may be relevant to the success or failure among women beginning or maintaining abstinence, for example. In addition, collecting vaginal cells and tracking the estrous cycle is a relatively noninvasive procedure that is easily implemented and can be used more readily than surgical removal of the ovaries and subsequent implantation or injection of hormones. Thus, it is comparatively easier for laboratories to contribute to this body of work by collecting estrous cyclicity data that have great potential upside at a relatively low cost.

Studies that include estrous cycle tracking within addiction science tend to provide mixed findings regarding the proestrus phase of the estrous cycle. Some studies have compared the estrus phase to all other (nonestrus) phases; others have reported estrus, proestrus, metestrus, and diestrus (often combining metestrus and diestrus). Clearly, an emphasis on estrus is justified, but some reports suggest proestrus may decrease drug-relevant behaviors. Several preclinical studies have shown that exogenous progesterone reduces cocaine intake and seeking in animals. These findings are bolstered by

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clinical reports showing that the subjective effects of cocaine are reduced during the luteal phase in women [when progesterone is high relative to estradiol; for review, see Anker and Carroll (8)]. Furthermore, additional preclinical research has reported robust decreases in heroin self-administration during the proestrus phase among freely cycling females (9). The current report by Nicolas *et al.* (1) indicates no differences in responding between metestrus, diestrus, or proestrus, which were subsequently combined for analysis relative to estrus. Discrepancies in detecting proestrus effects may be related to methodology and timing of vaginal cell collection/assessment. These findings emphasize a need for further research and new statistical methods to elucidate the contribution of estrous cycle to drug abuse. For example, we recently showed that multilevel modeling and behavioral economic demand for cocaine and remifentanil were sensitive to detecting significant differences between estrous phases in drug demand among female subjects (6). Furthermore, the fact that cocaine (a psychostimulant) and opioids (heroin and remifentanil) may be interacting with gonadal hormones differently represents a gap in our knowledge. To date, most of the sex differences research has focused on cocaine, and far fewer studies have examined opioids (or other drug classes). New research on the interaction of gonadal hormones and opioids may aid in the development of treatments by leveraging progestins to reduce opioid use disorder.

It is important to address and highlight the contribution of the report by Nicolas *et al.* (1) and similar research in the larger biomedical community regarding the inclusion of female subjects in preclinical research. Historically, drug use research (and most preclinical research) selected male animals because scientists were concerned that cycling sex hormones in female subjects might negatively impact the reliability of data. However, following the National Institutes of Health guidance and the requirement that all studies receiving National Institutes of Health grants consider sex as a biological variable beginning in 2016, researchers had to face this new challenge and opportunity. For a sex differences researcher, the advantages of including males and females in a study are clear, given that sex is a straightforward and clinically relevant independent variable to examine. Multiple meta-analyses have concluded that females are no more variable than males, and this includes drug use research (10). Importantly, the inclusion of males and females in an experiment does not necessarily require doubling sample size but can be partially addressed by statistical methods powered to adequately detect sex effects. Finally, there has been a steady increase in publications looking at the relationship between sex differences and drug abuse. A PubMed search of those terms, "sex differences AND drug abuse," reveals a positive upward trend, with 178, 178, 338, 431, and 456 citations for the years 1997, 2002, 2007, 2012, and 2017, respectively. These are encouraging data that suggest that a more nuanced understanding of this topic is emerging, but we are far from done. The treatment of substance use disorder hinges on not only the understanding of sex differences but also the complex interaction that sex has with other variables including developmental, (epi)genetic, and sociocultural factors. We, as a society, are at a unique cultural intersection as our understanding and assumptions regarding gender are changing amidst the most significant drug abuse/overdose crisis in human history. Consequently, it may be more important than ever for sex differences researchers to continue this important work.

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