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RESEARCH HIGHLIGHT

And the best stressed is…? A mechanistic analysis of sex differences in stress-potentiated cocaine-seeking behavior

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The prevalence of cocaine use disorder has increased over the past 10 years. Cocaine is currently second only to opioids as the leading cause of overdose deaths in the United States, and drug relapse remains the foremost obstacle in the treatment of cocaine dependence. Self-reports in cocaine users suggest that women transition to cocaine dependence faster and report more intense craving in response to cocaine-associated cues than men; however, experimental studies demonstrating these gender differences in cocaine craving and relapse are scarce. Evidence from preclinical animal studies indicates that females may be more vulnerable to cocaine craving and relapse, in part due to the influence of ovarian hormones (see [1] for review). Unfortunately, there are currently no FDA-approved medications for cocaine use disorders in men or women.

Stress is a significant factor eliciting drug craving in male and female cocaine users, and it interacts with other triggers, such as drug-associated cues and small amounts of the drug, to increase relapse propensity. Animal studies have offered critical insights into the mechanisms by which stress potentiates cocaine relapse [2], operationalized as reinstatement of drug-seeking behavior, but most of these studies did not consider sex as a biological variable. Thus, the neuroendocrine mechanisms for sex and estrous differences in stress-potentiated cocaine relapse have remained poorly understood; even though some studies report sex or ovarian hormone effects on stress-induced reinstatement of cocaine seeking [3] and stress-potentiated cue-induced reinstatement of cocaine seeking [4].

Research from the laboratory of John Mantsch has revealed that intermittent footshock stress can "set the stage" for cocaine-seeking behavior in male rats by potentiating the effects of a subthreshold priming dose of cocaine, which is mediated by endocannabinoid recruitment and corticosterone (CORT)-dependent disinhibition of pyramidal neurons within the prelimbic (PrL) subregion of the medial prefrontal cortex (mPFC) [5]. In this issue of *Neuropsychopharmacology*, Doncheck et al. report new findings that extend this line of research by providing a mechanistic analysis of the influence of biological sex and estrous phase on stress-potentiated cocaine-seeking behavior [6].

In the study by Doncheck et al. [6], male and female rats received training for intravenous cocaine self-administration and extinction of the drug-reinforced responding. Following extinction, reinstatement of cocaine seeking in response to a subthreshold priming dose of cocaine was assessed after either (a) intermittent footshock, (b) restraint stress, or (c) administration of physiologically relevant concentrations of CORT. The exceptionally thorough approach included multiple footshock intensities and cocaine priming doses. Sex differences in footshock- and restraint-induced ultrasonic vocalizations and defensive behaviors were also measured. Importantly, the estrous phase was monitored daily in female rats throughout the experiment.

Doncheck et al. [6] discovered sex and estrous differences in sensitivity to the combined effects of cocaine and stressors on propensity for drug seeking. Female rats exhibited lower thresholds for cocaine-primed reinstatement than males despite comparable drug intake and behavioral histories. Furthermore, the effects of stress exposure and CORT administration in females peaked during diestrus and proestrus, similar to menstrual cycle-dependent fluctuations in the subjective effects of cocaine and drug craving in female cocaine users [7]. Unexpectedly, female rats failed to show footshock-potentiated cocaine-primed drug seeking unlike males, whereas both sexes exhibited similar restraint stress-induced potentiated drug seeking. Careful behavioral observations indicated that insensitivity of females to the potentiating effects of footshock on reinstatement was not due to reduced nociception or diminished footshock-induced CORT response. Instead, it might be related to sex differences in restraint stress-induced affective states as indicated by distinct ultrasonic vocalization patterns. Thus, not all stressors are equal in their ability to potentiate cocaine-primed drug seeking, and their relative efficacy depends on biological sex. These findings also highlight important considerations for designing and interpreting studies that examine stress as a relapse trigger.

A series of pharmacological and slice electrophysiology experiments next demonstrated that stress-potentiated cocaine-primed reinstatement involved CORT-induced mobilization of the endocannabinoid, 2-arachidonoylglycerol (2-AG), in PrL interneurons. This led to CB1 receptor-dependent disinhibition of layer V pyramidal neurons in female rats, similar to what has been observed in male rats [5]. Thus, the mechanisms that underlie stress-potentiated reinstatement appear to be similar across sexes. This conserved mechanism of action is notable given the extensively documented sex differences in the stress response and the endocannabinoid system [8].

This study adds to a growing body of literature demonstrating that endocannabinoids are effectors of glucocorticoid signaling. This has been previously shown in the context of stress recovery, where CORT increases 2-AG synthesis, thereby disinhibiting mPFC pyramidal neurons to downstream targets that contribute to termination of the stress response [9]. While it is not yet known whether these findings generalize to

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other drugs of abuse, the findings by Doncheck et al. [6]. indicate that CORT-2-AG interactions are not specific to stress recovery but may also orchestrate drug-seeking behavior. Thus, it is tempting to speculate that other forms of mPFC-dependent behavior (e.g., executive functioning, decision making) are also determined by CORT-mediated endocannabinoid recruitment, and thus the endocannabinoid system may be a viable target for mitigating stress-induced deficits in a range of mPFC-dependent behaviors.

Altogether, these findings are significant because they provide a more thorough understanding of the mechanisms underlying stress-potentiated cocaine relapse in both sexes and "set the stage" for future mechanistic studies using this model. Although the identified mechanisms are similar in both sexes, the stressors that are capable of potentiating cocaine-primed cocaine-seeking behavior are different in each sex. This highlights the importance of studying both male and female subjects and provide a valuable foundation for leveraging information about sex differences in cocaine relapse to develop more efficacious strategies for treating cocaine use disorder. Moreover, since vulnerability to cocaine relapse fluctuates according to the estrous cycle in rats, in future studies it will be important to examine whether these estrous cycle effects translate to menstrual cycle effects in women. If these data are consistent in humans, then pharmacological strategies aimed at stabilizing hormonal fluctuations could be an effective adjunct antirelapse strategy in women. Overall, this work will hopefully lead to interventions tailored to the needs of each sex.

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AUTHOR CONTRIBUTIONS

RJM wrote the manuscript and RAF edited the manuscript.

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