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Viewpoint

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We Know Very Little about the Subjective Effects of Drugs in Females

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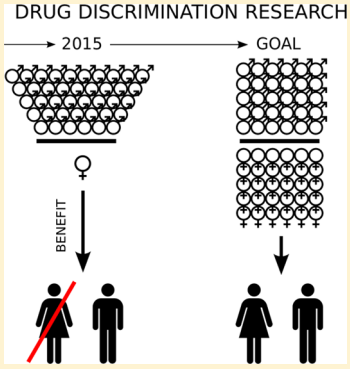
ABSTRACT: Pharmaceutical companies assessing the nervous system effects of candidate therapeutics often use a behavioral assay in rodents that assesses the drug's subjective (internal stimulus) effects. Variants of this so-called "drug discrimination task" have also been widely used by basic scientist for more than 50 years to study the receptor actions of a host of ligands related to disease states and neuropathologies. Notably, most published research with this task has used male rats or mice. This situation is unfortunate and severely limits the utility of the research, given the well-documented differences between women and men on drug efficacy and safety, as well as known sex differences in the neural and behavioral effects of drugs. In this Viewpoint, we highlight the need for basic researchers, as well as pharmaceutical scientists, to include females in drug discrimination studies in a manner that allows detection and interpretation of potential sex differences.



KEYWORDS: *Drug discrimination, interoceptive stimuli, gender differences, sex differences*

If you drink a few glasses of wine at a celebration, you feel different. Later ingestion of ibuprofen alleviates the headache that followed from the excessive celebration. After your morning coffee, you may feel more alert. If you have an extra cup or two, you are likely to feel jittery, have an elevated heartbeat, and, perhaps, experience some nausea. In some individuals, antidepressants such as sertraline (Zoloft) will improve mood, enhance energy and appetite, as well as decrease anxiety and fear. Of course, the use of antidepressants can come with adverse effects that include sleepiness, dizziness, and nausea. These brief examples are meant to demonstrate that ingestion of a drug comes with various desirable and undesirable effects. Many of these effects include alterations in internal states that are perceptible to the individual—feeling tipsy after a few drinks and better mood when adhering to antidepressant medication regimen. These interoceptive or subjective effects of drugs have been of interest to pharmaceutical scientists and basic researchers. Indeed, much effort has gone into developing and refining behavioral methods to study the subjective effects of drugs in rats and mice so they can be used to study the receptor actions of a drug, as well as the commonality and differences between drugs.[1]

MEASURING SUBJECTIVE EFFECTS

At present, the two-lever drug discrimination task is, by far, the most common approach to studying the subjective or interoceptive effects of drugs in rats and mice. In this task, there are two response options available in the experimental chamber; presses on a right or left lever in the chamber displayed in Figure 1. There are two session types intermixed across days: drug and nondrug (vehicle) days. On a drug day, presses on, say, the right lever are followed by a reinforcer according to some experimenter prescribed schedule. In the present example, we list a fixed ratio (FR) 25 which means that after the 25th lever press, there will be brief access to liquid sucrose in the dipper receptacle. Responding on the opposite lever (i.e., left in this example) does not have any programmed consequence on drug days. In the jargon of the field, left lever

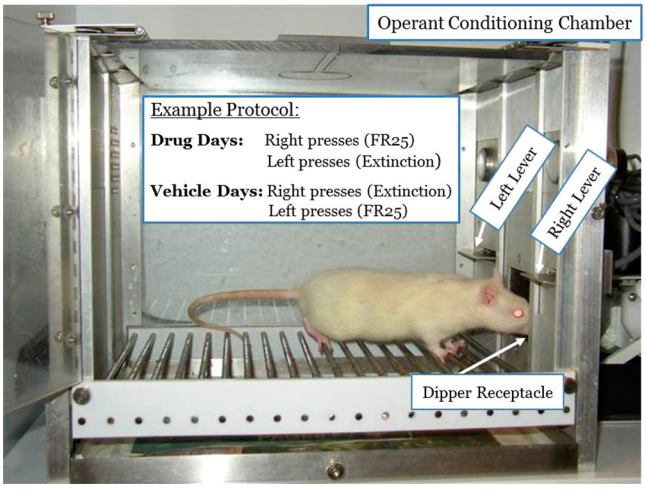


Figure 1. Image displaying an experimental chamber configured to support a two-lever drug discrimination study. The chamber includes a lever to the right and a lever to the left of a dipper receptacle. The dipper includes an arm with a 0.1 mL cup on the end. When the arm is raised, the rat will have access to the fluid that is in the dipper well for a prescribed amount of time. This fluid (e.g., sucrose) is used to maintain drug-state appropriate lever pressing on the experimenter set reinforcement schedule; a fixed ratio (FR) 25 in this example.

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Table 1. List of Drug Discrimination Studies in Rats and Mice Utilizing Males and Females

author(s)	strain	training drug	sex difference	test ligand(s)	sex difference	DOI/PMID
rats						
Heinsbroek at al., 1987	Wistar	pentobarbital	no	progesterone	yes	DOI: 10.1016/0091-3057(87)90455-2
Beun, 1992	Wistar	LHRH	yes			DOI: 10.1016/0306-4530(92)90077-K
Heyser at al., 1994	Sprague−Dawley	cocaine	no			DOI: 10.1007/BF02244870
Craft and Stratmann, 1996	Sprague−Dawley	cocaine	no	D-amphetamine	no	DOI: 10.1016/0376-8716(96)01259-8
				morphine	no	
				BW373U86	no	
				U69,593	no	
Craft, Kalivas, and Stratmann, 1996	Sprague−Dawley	morphine	yes	fentanyl	no	PMID: 11224471
				buprenorphine	yes	
				BW37U86	no	
				U69,593	no	
Craft et al., 1998	Sprague−Dawley	U69,593	yes	bremazocine	yes	DOI: 10.1016/S0091-3057(98)00124-5
				ethylketazocine	no	
Craft, Morgan, Bernal, 1998	Sprague−Dawley	morphine	no			PMID: 10065924
Craft, Heideman, Bartok, 1999	Sprague−Dawley	morphine	yes	fentanyl	no	DOI: 10.1016/S0376-8716(98)00112-4
				buprenorphine	no	
				nalbuphine	no	
Jung et al., 1999	Long-Evans hooded	pentylenetetrazol	yes			PMID: 10525074
Anderson and Haaren, 2000	Wistar	cocaine	no			DOI: 10.1016/S0091-3057(99)00256-7
Jung et al., 2000	Long-Evans hooded	pentylenetetrazol	yes	nicotine[a]	yes	PMID: 10823403
Jung et al., 2000	Long-Evans hooded	*meta*-chlorophenylpiperazine	yes			DOI: 10.1007/s002139900353
Krivsky et al., 2006	Sprague−Dawley	morphine	yes	morphine[b]	yes	DOI: 10.1097/00008877-200605000-00007
Kohut et al., 2009	Sprague−Dawley	cocaine	no	GBR12909	no[c]	DOI: 10.1007/s00213-008-1368-4
				SKF38393	no[c]	
				quinpirole	no[c]	
Broadbear, Tunstall, and Beringer, 2011	Sprague−Dawley	MDMA	yes	carbetocin	no	DOI: 10.2174/1874941001104010010
				atosiban	no	
		amphetamine	yes	carbetocin	no	
				atosiban	yes	
Harper, Langen, and Schenk, 2014	Norway hooded	MDMA	no			DOI: 10.1016/j.pbb.2013.11.011
		D-amphetamine	no	MDMA	no	
mice						
Shannon et al., 2005	DBA/2J	pregnanolone	no	allopregnanolone	no	DOI: 10.1124/jpet.104.082644.droxy-5
				alloTHDOC	no	
				androsterone	no	
				epiallopregnanolone	no	
				midazolam	yes	
				zolpidem	no	
				ethanol	no	
				MK-801	no	
				SR57727A	no	
				CPBG	no	

[a]Substitution testing during ethanol withdrawal. [b]Intracebroventricular administration. [c]No direct statistical comparison between males and females was conducted. (DOI) Digital Object Identifier. (PMID) PubMed Identification Number.

pressing is under extinction. On vehicle days, the availability of the sucrose reinforcer switches levers. In our example, left lever presses would now be reinforced with sucrose on an FR25; right lever pressing would be under extinction.

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Over sessions, responding eventually comes to track the drug state with presses becoming nearly exclusive to the reinforced lever. In our example, that would be the right lever on drug days and the left lever on vehicle days. This lever discrimination, once established, provides an opportunity to study behavior controlled by the subjective effects of the training drug. A discussion of proper testing protocols and their utility are beyond the goal of this Viewpoint. For further information, we refer the reader to an edited volume on drug discrimination by Glennon and Young.[1] For our purposes, suffice it to say that one can ask about the similarity of drugs in replacement or substitution tests—are the subjective effects of the antidepressant sertraline similar to or different from those of fluoxetine (Prozac)? Combining substitution testing with antagonist tests will allow one to refine answers to questions regarding receptor action—will a 5-HTc (serotonin) receptor antagonist block responding prompted by sertraline and does a similar antagonism pattern occur with fluoxetine?

FEMALE RATS AND MICE MOSTLY IGNORED

In May of 2014, Janine Clayton and Francis Collins of the United States National Institutes of Health (NIH) published a Comment in the journal *Nature* describing changes in policy regarding preclinical research and the inclusion of females.[2] The historical reasons for mostly ignoring female rats and mice in biomedical research and the impetuses for changes in policies of the United States Food and Drug Administration (FDA) and the NIH for including women in clinical trials has been widely discussed in publications (e.g., refs 2 and 3). The foundational work discovering drug targets and therapeutic approaches for these clinical trials relies heavily on basic science using mice and rats. Yet, in most areas of study, including neuroscience and pharmacology, basic preclinical investigations have not caught up with clinical trials that now include a nearly equal portion of women and men.[2,3] Among the reasons that this situation needs to be remediated is the 100s to 1000s of documented sex differences in gene expression of mice and rats in different tissues that can affect the pharmacokinetics and pharmacodynamics of drugs.[4,5]

What about the drug discrimination literature? How glaring is the deficiency in this critical area of the study? While recently working on a manuscript, we searched the nicotine drug discrimination literature for sex differences research. We could not find a single published paper in rats or mice that used nicotine as the training drug. Further, we could only find one sex differences paper that examined nicotine as the substitution ligand (see Jung et al., 2000 in Table 1). This gap in the nicotine drug discrimination literature was much larger than we expected, and it prompted us to wonder whether this situation was endemic to the field. The answer is shown in Table 1. In an area of study with 1000s upon 1000s of publications, we were only able to find 17 papers explicitly investigating sex differences in the discriminative stimulus (subjective) effects of a drug. The search in Google Scholar in late December 2014 used "drug discrimination", "sex difference", "rat", and "lever"; rat was replaced with "mice" in another search. In case a paper had not used the phrase "sex difference", we conducted another search using "drug discrimination", "female rat", and "lever"; female rat was replaced with "female mice" in a follow-up search.

SEX MATTERS

Although sociocultural and environmental factors may produce some sex differences, the biological root of others cannot be denied.[2,3] These biological mechanisms include the gonadal hormones impact on the organization of the physiology early in development, changes across the lifespan as circulating gonadal hormones shift with age, and the differential expression of X and Y genes in males and females. As shown in other research areas examining diseases and neuropathologies,[2] sex can matter in the subjective effects of drugs (see Table 1). Future drug discrimination research will need to include females in a scientifically responsible manner. For example, studies will need to be designed with sufficient statistical power and then the appropriate analytical tools need to be applied to detect any potential sex differences (cf. ref. 3). Further, we would caution over interpretation of isolated findings; whether they indicate the presence or absence of a sex difference. Replication within and across laboratories should serve as a benchmark for careful conclusions here. Related, replicated sex differences, or the absence of a difference, should not be generalized too far beyond the experimental conditions in which they were initially demonstrated. Not following such practices may mean missing an effect that could be important to the advancement of biomedical science and the improvement of health for women and men.

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Notes

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