

Sex Differences in Behavioral Strategies: Avoiding Interpretational Pitfalls

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Introduction

Despite ample evidence for sex differences in brain structure and function, our understanding of the neurobiological basis of behavior comes almost exclusively from male animals. As neuroscientists move to comply with recent National Institutes of Health (NIH) mandates that biomedical researchers include both sexes in their studies, the ways we interpret outcomes in classic rodent behavioral models deserve closer scrutiny and more nuanced evaluation. In this mini-review, we highlight recent papers on sex differences in learning, decision-making, and spatial navigation paradigms that underscore the distinctions between cognitive capabilities and behavioral strategies that may confer unique benefits to males and females.

Neuroscientists have studied animal behavior in laboratory settings for more than a century, leading to an ever-increasing understanding of the relationships between structure, physiology, and function in the mammalian brain. In particular, rodent behavioral models have provided key insights into the neural basis of dozens of complex processes, including learning, decision-making, stress coping, aggression, and substance abuse. However, because the vast majority of behavioral neuroscience research has been conducted in male animals (Beery and Zucker, 2011), we inarguably (and regrettably) know much more about the male brain than we do about the female brain. In a recent attempt to rectify this imbalance across biomedical research, the NIH implemented a policy that requires funded researchers to consider sex as a biological variable (SABV) and include subjects of both sexes in all experiments (Clayton and Collins, 2014). Despite some resistance (Fields, 2014; Eliot and Richardson, 2016), this initiative is likely to succeed in illuminating aspects of brain function that are common to both sexes, as well as those that are sexually dimorphic (Eliot and Richardson, 2016). Information of either kind can be useful to basic and translational scientists alike, but it is critical—especially in behavioral research—that we interpret potential sex differences in outcome measures thoughtfully.

When we conduct behavioral experiments, we are, in essence, asking animals to tell us what the situation we've placed them in means to *them*. In many cases, the animal might engage in any number of responses, and it is up to us to determine what each one means to *us*. When males and females differ quantitatively in the outcome measures that we've defined, it's important to consider whether these differences reflect true disparities in, for example, cognitive

ability or emotional state, or whether they signify a qualitative difference in behavioral strategies that may optimally serve the potentially discrete needs of each sex. In this review, we discuss recent studies that highlight this distinction and emphasize the need for thorough, careful behavioral analyses as more neuroscientists begin to incorporate SABV into experimental design.

Sex Differences in Common Behavioral Models

How do animals process information about threatening environments and stimuli? Although it is not necessarily surprising to learn that males and females might behave differently in response to stressful events, a nuanced understanding of how and why these differences exist is only just starting to emerge. A recent paper from Tronson and colleagues (Keiser et al., 2017) nicely demonstrates that after classical context fear conditioning, female mice are more likely than males to show a generalized freezing response in a novel context; that is, females treat new contexts more cautiously than males, pointing to a potential neural mechanism for this difference in strategy. This was true even with previous exposure to the shock-associated context, a finding that appears to help refine the distinction in male mice (i.e., reduced generalization). These behavioral differences were associated with discrete recruitment of major brain regions: in both contexts, whereas hippocampal activity was greater in males, females selectively showed activation of the basal amygdala. These data could suggest that female mice are unable to discriminate meaningful contexts, or they may instead indicate that after a traumatic experience, treating new environments with extra caution is evolutionarily beneficial to females. Examining this sex difference in a more naturalistic setting will be necessary to appropriately test these hypotheses. In the meantime, experimenters using classic Pavlovian approaches should consider that elevated context generalization in female mice may not reflect a cognitive deficit but instead a strategy to reduce risk to the animal's life.

This latter interpretation is supported by impressive new work by Pellman et al. (2017), who used a 42-day “closed economy” system to examine sex differences in foraging strategies when the foraging environment is risky. Here, male and female rats lived in a two-chamber home cage in which the nesting side was safe, but eating and drinking required traveling to a foraging arena that randomly delivered foot shocks. After two weeks of chamber acclimation without shocks, the authors observed

the change in animals' behavior as they learned that they would have to endure shocks in order to forage. Although both sexes reduced the time they spent in the foraging chamber during the two-week shock period, males compensated by increasing their meal size, whereas meal size decreased in both intact and ovariectomized females, resulting in reduced overall food consumption and arrested weight gain. In the final two weeks, the shocks were terminated so that the authors could observe extinction. Males rapidly increased time spent in the foraging arena, whereas foraging time in females climbed much more slowly. Together, these data could be interpreted as evidence for impairments in cognitive flexibility or extinction learning in females, but they may instead reflect sex differences in strategy. Specifically, they suggest that females will select general safety over metabolic needs, preferring to avoid a potentially risky environment at the cost of blunted weight gain—even when the risk is no longer there. In contrast, males appear to adapt their feeding efficiency in order to maintain steady weight gain. Although the putative evolutionary value of these sexually divergent strategies is difficult to assess in a controlled laboratory setting, it is clear that males and females assess foraging in risky environments differently. The longitudinal design of this study is noteworthy and laudable because it allows unique insight into complex behavioral strategies over time, rather than capturing a brief snapshot of behavior, as is true of most paradigms.

Our lab has also found that females are more likely to engage in active behaviors to avoid potential threats. As we recently reported (Gruene et al., 2015a), a subset of female rats in a cued fear-conditioning paradigm exhibited escape-like “darting” behavior in response to the conditioned stimulus (CS). This paper suggests that measures of freezing alone are insufficient to quantify fear in females. These animals subsequently demonstrated enhanced extinction retention, suggesting that darting may reflect an adaptive mechanism that promotes cognitive flexibility (Maren et al., 2013). One alternative interpretation is that because they are smaller, female rats perceive the conditioning chamber as larger, and therefore the threshold for “predator imminence” (Fanselow and Lester, 1988) is shifted, thus increasing the likelihood of an escape response instead of freezing. However, this explanation is unlikely for a few reasons. First, within a large cohort ($n = 58$) of females, no relationship was found between body weight and darting prevalence (Gruene et al., 2015a; author response available at <https://elifesciences.org/articles/11352>). Second, the observation that animals are more likely to engage

in escape responses in larger spaces has been reported only in environments much larger than our chambers (e.g., a hallway, as in Blanchard et al., 1986). A more recent attempt to observe this phenomenon in standard chambers that differed in size by a factor of approximately three ($\sim 15 \times 23$ cm versus 15×71 cm) failed to find an effect of chamber size on innate fear behavior (Kabitzke and Wiedenmayer, 2011).

Together, these findings support the idea that darting during classical cued fear conditioning is a sexually dimorphic strategy to promote escape. The fact that it both appears only in females and is advantageous for extinction in the long term may seem contradictory to clinical reports that women are more susceptible to posttraumatic stress disorder (Kessler et al., 1995; Breslau et al., 1999). However, resilient and vulnerable individuals can be found in most populations (Yehuda and LeDoux, 2007), and the absence of darting in males does not necessarily mean that they lack their own strategies and mechanisms for improving long-term outcomes. As we also recently reported (Gruene et al., 2015b), successful extinction retrieval in males (but not females) is correlated with a unique morphology in prefrontal amygdala circuitry. Although the incidence of darting was not associated with the estrous cycle, there is evidence that circulating ovarian hormones can influence fear learning and extinction (Cover et al., 2014). The key message of our work is that if only freezing were measured, darters would have been assumed to be cognitively impaired at forming a CS–US (conditioned stimulus–unconditioned stimulus) association. This is clearly not the case, and therefore freezing by itself is likely an insufficient measure of fear learning and responding, especially in female rats. A more comprehensive examination of animals' behavioral repertoires during classical conditioning tasks will be critical as we move to more thoroughly understand how each sex processes threatening stimuli.

Sex differences in risk evaluation can also be observed in models that more explicitly test decision-making. In an elegant set of experiments, Orsini et al. (2016) used a “risky decision task” to examine how male and female rats weigh reward and punishment against each other. In this task, animals chose between receiving a safe, small food reward or a large food reward that was intermittently punished with a shock. The authors then observed changes in animals' choices as they varied the likelihood of the shock. Although both males and females reliably chose the large reward when there was no chance of receiving punishment, females quickly switched to the small reward as shock probability increased.

In contrast, males maintained high levels of large-reward choice, even when shock was guaranteed. To rule out the possibility that this effect was the result of greater pain thresholds in males due to their size, the authors recalibrated the intensity of the shock according to each animal's weight, and obtained the same results. Similar to the work described above by Pellman and colleagues (2017), these findings suggest that females will select a behavioral strategy that prioritizes physical safety over metabolic needs. Decision-making work that focuses on the adoption of strategies as the animal learns about outcome contingencies indicates that females take longer to settle on a strategy than males do (van den Bos et al., 2012). Although this delay could be interpreted as a disparity in learning, it is likely that it instead reflects a difference in how males and females use the information presented to them. As Orsini and Setlow (2017) argue in an excellent recent review, it may be more advantageous for males to take a more holistic, swift approach to assessing their situation, whereas females may benefit from a more measured evaluation of each option before settling. They further discuss in detail how this difference applies even in nondangerous scenarios. Here, too, the latter approach in females may indirectly stem from drives related to reproductive success, as mate choice in females is a far more selective process than it is in males (Snoeren and Ågmo, 2014; McCormick et al., 2017). However, it should be noted that this possibility has yet to be directly tested in the lab.

Finally, sexually divergent strategies can be observed in studies of spatial navigation. Although the idea that males and females perform differently in spatially dependent tasks is not new (Gaulin et al., 1990; Galea et al., 1994), recent work has begun to uncover some of the neurobiological mechanisms that determine these differences. Navigation studies are often designed to test whether an animal solves a spatial task using a global, "geometric" strategy or more self-focused or landmark-dependent strategies. Work in both humans and rodents suggests that males prefer the former, whereas females prefer the latter (Blokland et al., 2006; Jones and Healy, 2006). Importantly, this difference appears to depend on circulating ovarian hormones (Korol et al., 2004). More recently, Rodríguez et al. (2013) demonstrated that prepubertal female rats select environment-based strategies in a "snowcone" task and then switch to landmark-guided navigation as adults. They additionally found that ovariectomy caused adult females to adopt a geometric strategy, suggesting that the female preference for nongeometric strategies may develop during puberty in order to aid reproductive success (Jones et al., 2003). Recent work by Yagi et

al. (2016) further defined the significance of strategy distinctions by dividing larger cohorts of both male and female subjects by their strategy preference. They found that males that used a geometric spatial strategy displayed enhanced pattern separation and had greater neurogenesis in the dentate gyrus, but neither of these correlations was observed in females that preferred the same strategy. Therefore, as in other models, the divergence of navigational strategy selection between males and females appears to uniquely serve each sex.

Conclusions

The studies discussed here represent just a small subset of the rapidly growing body of behavioral neuroscience literature that considers SABV. Our primary goal was to emphasize the need to be mindful of outcome interpretations and consider alternative explanations for sex differences in common paradigms. As we have argued previously (Shansky, 2015), doing so may be especially important in stress-related and anxiety-related models, like the elevated plus maze and the forced swim test, which were developed using mostly males and therefore may not tap into the same processes in females (Fernandes, 1999; Kokras et al., 2015). Although the scope of this brief perspective chapter did not encompass stress research, exciting recent work points to novel, sex-dependent mechanisms underlying the impact of stress on cognition and physiology (Laredo et al., 2015; Senst et al., 2016; Grafe et al., 2017). In addition, an excellent review on this topic (Bangasser and Wicks, 2017) can be found in the recent double issue of *Journal of Neuroscience Research* entitled "An Issue Whose Time Has Come: Sex/Gender Influences on Nervous System Function" (*Journal of Neuroscience Research*, 2017). This epic collection of more than 70 reviews and primary research articles should serve as a foundational primer for any neuroscientist interested in sex differences in the brain. For behavioral neuroscientists in particular, it is critical that we be prepared to challenge dogmas about what our models tell us and consider the possibility that even seemingly identical behavioral outcomes in males and females could have discrete underlying mechanisms (De Vries, 2004). This point is especially relevant for those of us whose goal is to inform translational and clinical work: whether or not there are gender disparities in disease prevalence, an understanding of the biological basis of addiction, mental illness, and neurological disorders in each sex will be integral to developing more effective treatments. It is therefore vital that we pay careful attention to behavioral studies and interpret putative sex differences thoughtfully.

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