

Spotlight

Fight, Flight, or Freeze? The Answer May Depend on Your Sex

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Pavlovian fear conditioning is one of the most common and well-understood methods for studying fear learning. However, research is predominantly performed in males. Recently, in a classical Pavlovian fear conditioning paradigm, Gruene and colleagues described an active conditioned fear response ('darting') prevalent in female rats that better maintain an extinction memory.

In 1927, Dr Ivan Pavlov published a series of lectures describing the conditioned response (CR; salivation) of dogs to a conditioned stimulus (CS; a metronome) they had learned signaled the arrival of an unconditioned stimulus (US; meat powder) [1]. Now, almost a century later, learning and memory researchers still employ these same techniques to illustrate associative learning by quantifying either the magnitude or duration of conditioned responding.

Classical Pavlovian fear conditioning, where a conditioned stimulus (e.g., tone) is paired with an aversive unconditioned stimulus (e.g., footshock), is a widely used laboratory model of fear learning, as the underlying physiological circuitry is similar to that involved in pathological conditions of fear and anxiety in humans (e.g., post-traumatic stress disorder, PTSD). When the feared stimulus is known, the response can be reduced through extinction, or exposure therapy, where presentation of the CS in the absence of the aversive event leads to the formation of a new, safe, memory trace and a reduction in fear.

Rodents instinctually become immobile, or freeze, in response to the footshock used in classical conditioning chambers. Freezing is easily quantified as an experimental endpoint, with low levels of freezing indicative of low fear. Outside of conditioning paradigms, impending danger can also elicit an attempt to flee and seek shelter, or even fight, depending on the situation and proximity of a predator [2].

A sexually dimorphic fear response may have advantages in the wild, particularly if females have a nest to protect. For decades animal researchers have noted sex differences in learning tasks, particularly when stress is involved [3]. However, whether these can be attributed to learning or performance differences is difficult to determine. Even in males, performance in fear learning tasks is subject to variation among individuals [4]. Large-scale experiments that allow for the exploration of mechanisms underlying these intra-group patterns of behavior may be the key to elucidating the behavioral and physiological underpinnings of psychiatric illnesses characterized by excessive fear and anxiety. These are important points to consider because, despite widespread exposure to traumatic events, only a portion of those exposed to a trauma will go on to develop the clinical symptoms of PTSD [5,6], and the prevalence in women is twice that in men [7].

Recently, Gruene *et al.* [8] disentangled performance differences from learning impairments between sexes by characterizing an active fear response they refer to as 'darting'. In contrast to the passive response of freezing, darting resembles an attempt to escape and is defined, as the name suggests, by rapid forward movement. The authors carefully demonstrate that, when it occurs, darting is a type of conditioned responding. Using a novel analysis, they quantify this behavior in several ways, including speed (cm/s) and frequency (darts/min), and then bimodally classify rats by their propensity to engage in conditioned darting behavior

at all (darters or non-darters) [8]. By definition, an animal cannot simultaneously freeze and dart; thus, the authors explore Pavlovian fear learning (Figure 1A) in males and females using two qualitatively distinct conditioned responses. Interestingly, the authors found that behavior exhibited during the acquisition phase of classical Pavlovian fear conditioning was a predictor of extinction success.

Overall, the authors found that females were more likely to dart in response to a CS during the acquisition phase of learning than males, with 40% of females and only 10% of males displaying at least 1 dart during CS presentation as a conditioned response (e.g., a response that emerged after CS-US pairing). Importantly, propensity to engage a darting response to the CS did not preclude rats from also freezing in response to the CS. Gruene and colleagues found that both darters and non-darters froze comparably during extinction. However, in females only, darters froze less during retention tests following extinction (without displaying an increased darting response that could explain reduced immobility) (Figure 1B). The small percentage of male rats that did dart in response to the CS did not show the same pattern of responding after extinction as females (Figure 1C) [8].

As suggested by the authors, the enhanced ability to form and retain an extinction memory (CS≠US) is one indicator of cognitive flexibility [i.e., little perseveration of older response patterns (CS=US)]; future research could incorporate other measures of rule change and perseveration that are not centered on the fear response.

There are some inherent difficulties in comparing males and females, and these caveats should be taken into account when interpreting the present results. Researchers deal with the issue of daily fluctuating estrogens by either surgically removing the ovaries and providing an implant that delivers a controlled amount

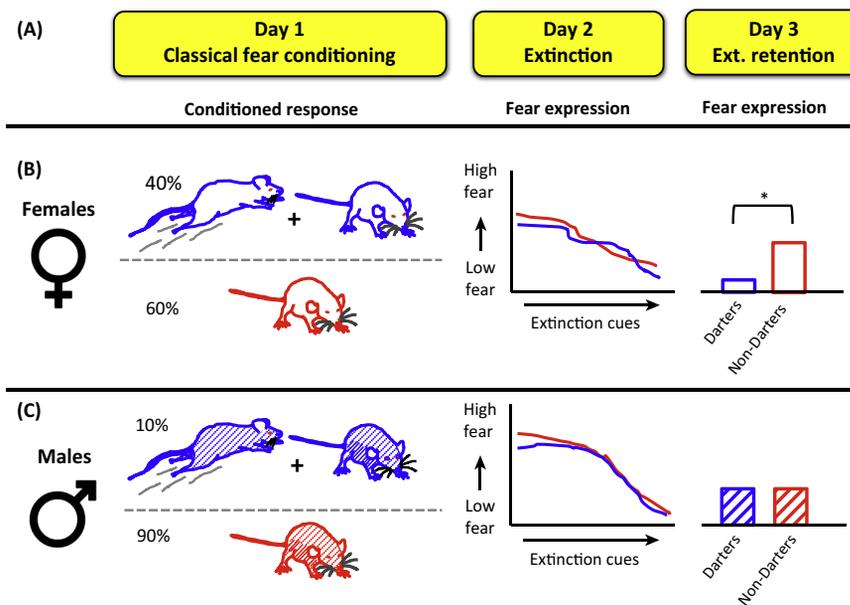


Figure 1. Conditioned Responding during Fear Acquisition Determines Efficacy of Extinction Retention in Female Rats. (A) Experimental timeline: on day 1 male and female rats were fear conditioned in a classical chamber with seven pairings of a tone CS with a footshock US; 24 hours later all rats underwent extinction, where the CS was played repeatedly (20 times) in the absence of the US. The next day, the CS was presented three times to test for retention of the extinction memory. (B) Female rats classified as 'darters' during fear conditioning, displayed both darting and freezing conditioned responses, and were better able to retain the extinction memory (as evidenced by reduced freezing in response to the CS) 24 h after extinction training than 'non-darters' who only froze in response to the CS during fear conditioning. There was not a significant darting CR after extinction in either group of rats. This propensity to dart in response to a CS may reflect underlying processes that result in better extinction learning in females. (C) Only 10% of males tested incorporated darting as a response to the CS and there were no differences in extinction retention between 'darters' and 'non-darters'. Abbreviations: CR, conditioned response; CS, conditioned stimulus; US, unconditioned stimulus.

of hormone or tracking the estrous cycle and either controlling for cyclicity or factoring it into the final analysis. Either method, however, leaves females entering an experiment under very different conditions than the males unless they are also extensively handled or undergo a sham operation or equivalent gonadectomy. Even though Gruene and colleagues show no difference in estrous cycle state between darters and non-darters, this does not eliminate a hormonal interaction on the coping response. Additionally, there is no mention of how their males were treated during the pre-experimental period.

Ascertaining the advantages one fear behavior may have over another is mostly speculative. Where darting may draw attention to the subject, freezing could create an easy target for a predator. In contrast

to active avoidance paradigms, where females do outperform males [3], attempting escape in a classical chamber is futile. Gruene *et al.* suggest that darting as a CR may serve an adaptive purpose and the lack of perseverative darting during extinction is in line with this interpretation.

The mild–moderate shock typically employed as the US (0.4 mA–1.0 mA) in rodent Pavlovian fear conditioning paradigms likely aligns with a moderate perception of threat that does not necessitate the energy expenditure associated with fighting or fleeing, especially in closed conditioning chambers, thereby resulting in a freezing response. Female rats are more sensitive to shock than males [9]; it could be that a portion of females perceive identical shock intensities more aversively than males, thereby lowering the threshold

whereby they begin to engage in active fear responses in an otherwise inescapable situation. Although this may support the overall sex differences in darting as a CR, it does not explain why these female darters express lower fear after extinction. This leaves two possible explanations, both intriguing: (i) the act of engaging an active coping strategy, or an action antagonistic to fear [10], while the threat is present increases likelihood of treatment success in females; or (ii) individual differences in threat responding comprise a behavioral phenotype that includes flexible methods of risk assessment that lead to better extinction learning. As pointed out by the authors, active coping methods reduce fear in settings where control is possible; however, this elegant study by Gruene and colleagues suggests that treatment outcome is predicated by behavioral reactions to initial Pavlovian conditioning. Why, then, is this only the case in females? The neuropsychological mechanism that underlies this sexually divergent response strategy remains unknown. Regardless of the explanation, this research adds to a growing body of work that underscores the relevance of behavioral research in female subjects [11] and highlights the importance of integrating individual variations in behavior into analyses [12,13]. Given the higher prevalence of PTSD in women, incorporating information about a patient's behavioral response to a traumatic event into a tailored treatment plan could lead to better outcomes in a clinical setting. Uncovering the neural and hormonal networks that modulate the relationship between response behavior and treatment outcome as well as investigating longer term results will be exciting next steps.

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