Biological Psychiatry: Celebrating 50 Years

Adapting Social Defeat Stress for Female Mice Using Species-Typical Interfemale Aggression

Ryan W. Logan

In recent years, there has been momentum to develop innovative experimental approaches that better investigate and incorporate species-typical and sex-specific behaviors in the study of psychiatric disorders. With the advent of novel approaches comes the responsibility of careful validation, evaluation, and recalibration of behavioral assays for animal studies, with the hope of improving translation from preclinical research to human disease (1). As a field, we have also focused on studying the brain and behavior primarily of male animals (2), which is especially problematic given that prevalence rates of the most common psychiatric disorders of depression and anxiety in women are more than twice that in men, and that our ultimate goal is to understand human disease. Improving our knowledge of the range of normal and abnormal behaviors in rodents may aid in the quest for assessing their translational relevance in the study of psychiatric disorders and comorbid diseases. Many approaches have been developed to expose mice and rats to various stressors with differing intensities and durations in an effort to model human depression and anxiety. There are many variations of these stress-induced rodent models of anxiety- and depression-related phenotypes. Chronic social defeat stress (CSDS) has become more widely used as an approach to induce a range of physiological and behavioral phenotypes with high face, predictive, and construct validity to human major depressive disorder (3).

CSDS has evolved to promote high-throughput behavioral screening in mice while also maintaining the reliability and robustness of depression-like behaviors across laboratories (3,4). The standard model of CSDS subjects C57BL/6J (B6) male mice to repeated daily bouts (10 days of 5–10 minutes) of attacks by a larger CD1 male mouse, followed by cohousing of the defeated B6 male with the aggressor CD1 male. A perforated transparent divider during cohousing subjects the defeated mouse to continuous psychosocial stress via sensory contact with the aggressor mouse. CSDS has largely been used to investigate the impact of stress on various physiological and behavioral phenotypes in male rodents, particularly male B6 mice. In male B6 mice, CSDS consistently leads to increased anxiety-related behaviors and reduced social interaction. Until recently, attempts to adapt CSDS to female mice of any strain have been limited. For example, applying male odorants to female B6 mice (5) or chemogenetically activating the ventrolateral subdivision of the ventromedial hypothalamus of aggressor male mice (6) promotes aggressive behaviors between male and female mice. A potential advantage of these approaches is the relative control over the nature and intensity of the male aggression when the intention is to directly compare the consequences of repeated defeat in male and female mice. A downside is that these manipulations elicit atypical aggression between sexes.

A major challenge for adapting CSDS to female mice has been implementing experimental scenarios where interfemale aggression is species typical and consistently intense across repeated daily episodes. Species-typical interfemale aggression is usually restricted to pregnancy or immediately postpartum. In this issue of *Biological Psychiatry*, Newman *et al.* (7) begin to overcome this challenge by developing a defeat model for female B6 mice using outbred Swiss Webster (CFW) female mice as the aggressors. When compared with ovariectomized or multiparous CFW female mice, intact nulliparous CFW females housed with castrated CFW males displayed highly aggressive behaviors toward B6 females over repeated daily episodes across 10 days, similar to the duration used for the CSDS model in male B6 mice. Consistent with the effects found in defeated male mice (8), circulating corticosterone levels were similar between acute and chronic defeat, suggesting that the hypothalamic-pituitary-adrenal axis does not habituate to the repeated exposure to SDS. In contrast to defeated male mice (3), CSDS had no overall effect on anxiety-related behaviors, although this may be due to differences in the behavioral assays used between studies—i.e., the elevated plus maze (3) and the light/dark box (7).

Newman *et al.* (7) also assessed the effect of CSDS on social interaction in a novel open-field arena and familiar home cage environments. Measuring the duration and nature of social interaction behavior between unfamiliar CD1 male mice and defeated B6 male mice has become the standard for several laboratories to distinguish whether defeated mice are categorized as "resilient" or "susceptible." Only susceptible mice are socially avoidant and have reduced sucrose preference—a measure of anhedonia-related behavior—while both susceptible and resilient mice have increased anxiety-related behaviors. Notably, Newman *et al.* (7) reported that CSDS only had reduced social interaction behavior between unfamiliar female CFW mice and defeated female B6 mice if the environment was familiar—i.e., home cage. However, CSDS had no apparent effect on social interaction behavior in a novel open-field arena. Further, attack frequency by the aggressor CFW mouse was inversely correlated with social avoidance, suggesting that the intensity of interfemale aggression is related to the severity of depressive-related phenotypes, and should be considered for assessing interindividual variation of neurobiological and behavioral differences related to stress. Interestingly, social avoidance in the home cage was reversed 24 hours after an acute administration of ketamine, a rapidly

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acting antidepressant, suggesting that the interfemale CSDS model has some predictive validity.

The fact that defeated females perceive unfamiliar nonaggressive B6 females as threatening, despite being introduced in their own familiar environment and being different from the aggressor CFW females, suggests that threat processing in defeated females may become maladaptive. Overgeneralization of a perceived threat to nonthreatening stimuli or the inability to differentiate between threatening stimuli has links to human depression and anxiety disorders. In support of this, Newman *et al.* (7) show that defeated female mice become wary of novel objects and report a marked increase in vigilance-related behaviors during the social interaction test. Possibly, hypervigilance may lead to behavioral instability in environments that are perceived as threatening and may contribute to disorganized behavior due to the intrusion of attentional bias to threat. Nest building is a species-typical behavior thought to be goal directed, requiring both motor planning and cognitive planning. Interestingly, Newman *et al.* (7) used nest building, a species-typical behavior thought to be goal directed and require motor and cognitive planning, to demonstrate that defeated females build nests that are incomplete, disorganized, and messy compared with those built by unstressed female mice.

To begin to understand the potential neural circuits involved in stress-induced hypervigilance and social avoidance behaviors in female mice, Newman *et al.* (7) spatially mapped the neuronal activation marker c-Fos across the brain, focusing on sexually dimorphic regions involved in social, defensive, and threat-processing behaviors. While acute defeat activated c-Fos in the ventral lateral septum, chronic defeat activated c-Fos in the medial amygdala, the hypothalamic paraventricular nucleus, and the ventromedial hypothalamus. Social stress has also been shown to activate c-Fos in these brain regions in male rodents, and therefore, further investigation should focus on differentiating the roles of these circuits in mediating the effects of CSDS on sex-specific vigilance and social avoidance behavior. We and others have shown in human postmortem brains that there are both shared and opposing changes in molecular pathways in the same brain regions of men and women with major depressive disorder (9,10). Therefore, there is the potential for overlapping neural circuits, cell types, and even molecular pathways to be involved in the development and manifestation of clinical depression depending on the sex of the individual—divergent alterations to shared pathways could lead to sex-specific maladaptive behaviors.

Novel approaches that appreciate and incorporate species-typical and sex-specific behaviors have the hope of improving translation from preclinical research to human disease. Newman *et al.* (7) innovate a widely used chronic stress model for female mice by leveraging scenarios to experimentally control species-typical interfemale aggression. Future studies could assess whether interfemale CSDS generates bimodal distributions of defeated mice based on vigilance and social avoidance behaviors for investigating the underlying mechanisms contributing to susceptibility and resilience in a sex-specific manner. In addition, whether this CSDS model highly recapitulates the molecular and cellular alterations found in the human brains of women with major depressive disorder remains to be explored. These initial steps begin to overcome the challenge of developing ethologically relevant chronic stress models in female mice that can be used in parallel with well-validated approaches in male mice.

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Article Information

From the Translational Neuroscience Program, Department of Psychiatry, University of Pittsburgh School of Medicine, and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, Pennsylvania, and the Center for Systems Neurogenetics of Addiction, The Jackson Laboratory, Bar Harbor, Maine.

Address correspondence to Ryan W. Logan, Ph.D., Translational Neuroscience Program, Department of Psychiatry, University of Pittsburgh School of Medicine, 450 Technology Drive, Office 224, Pittsburgh, PA 15219; E-mail: loganrw@upmc.edu.

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