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Vicarious social defeat stress: Bridging the gap between physical and emotional stress

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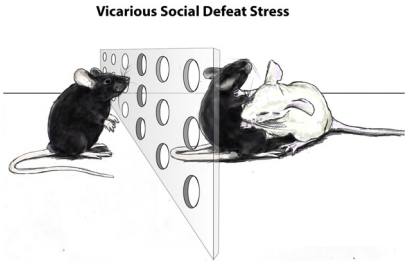
HIGHLIGHTS

- A novel animal model for vicarious defeat stress (VSDS) is described.
- VSDS can model posttraumatic stress, depression, and stress-related disorders.
- This model has high face, construct, and predictive validity.
- VSDS is a tool for studying the neurobiological consequences of emotional stress.

GRAPHICAL ABSTRACT



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ABSTRACT

Background: Animal models capable of differentiating the neurobiological intricacies between physical and emotional stress are scarce. Current models rely primarily on physical stressors (e.g., chronic unpredictable or mild stress, social defeat, learned helplessness), and neglect the impact of psychological stress alone. This is surprising given extensive evidence that a traumatic event needs not be directly experienced to produce enduring perturbations on an individual's health and psychological well-being. Post-traumatic stress disorder (PTSD), a highly debilitating neuropsychiatric disorder characterized by intense fear of trauma-related stimuli, often occurs in individuals that have only witnessed a traumatic event.
New method: By modifying the chronic social defeat stress (CSDS) paradigm to include a witness component (witnessing the social defeat of another mouse), we demonstrate a novel behavioral paradigm capable of inducing a robust behavioral syndrome reminiscent of PTSD in emotionally stressed adult mice.
Results: We describe the vicarious social defeat stress (VSDS) model that is capable of inducing a host of behavioral deficits that include social avoidance and other depressive- and anxiety-like phenotypes in adult male mice. VSDS exposure induces weight loss and spike in serum corticosterone (CORT) levels. A month after stress, these mice retain the social avoidant phenotype and have an increased CORT response when exposed to subsequent stress.
Comparison with existing method(s): The VSDS is a novel paradigm capable of inducing emotional stress by isolating physical stress/confrontation in mice.
Conclusions: The VSDS model can be used to study the short- and long-term neurobiological consequences of exposure to emotional stress in mice.

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1. Introduction

Post-traumatic stress disorder (PTSD) is a trauma-related disorder characterized by the persistent fear of trauma-related stimuli that may emerge after exposure to severe stress (American Psychiatric Association, 2013). This debilitating disorder affects approximately 10% of the population in the United States (Kessler, 2000; Kessler et al., 2005), and carries an economic burden now approximating 85 billion dollars per year (Greenberg et al., 2015). The neurobiology underlying PTSD and related disorders is not well understood (Newport and Nemeroff, 2000), and adding to its complexity is the fact that PTSD can also develop in individuals who simply witness a fearful/traumatic event (Perlman et al., 2011; van Wingen et al., 2011). Unfortunately, there is a considerable gap in our basic understanding of the neurobiological consequences of psychological/emotional stress alone and its influence on mental health of the individual.

Most animal models of stress rely on physical stressors to induce a pathological-like state, but neglect psychological aspects (i.e., involving indirect, non-physical conflict). A witness foot-shock model has been proposed to delineate the biological differences between physical and emotional stress (Van den Berg et al., 1998; Pijlman et al., 2003). In this paradigm, a rat is forced to witness another rat receive unpredictable foot shocks from the safety of an adjacent compartment. In this model, the witness rats showed locomotor hyperactivity and increased sensitivity to saccharin when compared to non-stressed controls, while the foot-shocked rats showed opposite effects (Van den Berg et al., 1998; Pijlman et al., 2003). Given that in this paradigm the witness and foot-shocked rats were housed together, it is possible that differences between experimental groups could be the result of a dominance hierarchy (Warren et al., 2013). Although this paradigm has proven useful in delineating biological effects of witnessing stress, it lacks ethological validity. This highlights the necessity for the continued development of refined animal models of emotional stress and demonstrates the viability of a witness component for the study of psychological stress effects.

The chronic social defeat stress (CSDS) paradigm is an effective, ethologically relevant, and reliable model for inducing a depressive-like phenotype, PTSD-, and mood- and anxiety-related symptomology in rodents (Berton et al., 2006; Golden et al., 2011; Huhman, 2006; Nestler and Hyman, 2010). This paradigm has strong construct, face, and predictive validity, since repeated, but not acute, antidepressant treatment reverses CSDS-induced deficits (Berton et al., 2006; Nestler and Hyman, 2010; Warren et al., 2013). In mice, CSDS involves an adult male C57BL/6J mouse being forced to intrude upon the home cage of an aggressive CD-1 male mouse. The intruder is quickly overpowered and adopts a submissive posture characterized by rearing, vocalization, and escape-like behaviors. Socially defeated mice display lasting deficits in the elevated plus-maze and forced swim test, behavioral measures of mood dysregulation (Berton et al., 2006; Krishnan et al., 2007). However, the CSDS paradigm alone is not capable of discriminating between the emotional and physical aspects of stress. This limitation can be overcome by the addition of a witness component (i.e., the emotionally-stressed mouse) to the CSDS paradigm. The result is a novel vicarious social defeat stress (VSDS) paradigm, which elicits a robust social avoidant phenotype and other mood-related behavioral deficits. This improved model offers ethological relevance, strong face validity, and easy testability (Warren et al., 2013). The VSDS paradigm also offers a model that removes the confounding influence of physical injury from studies assessing the role of stress on immune and inflammatory systems (Hodes et al., 2014). The VSDS paradigm can help bridge the gap in our understanding of psychological stress by simultaneously eliciting behavioral abnormalities in both physically and strictly emotionally stressed mice. VSDS not only provides an unparalleled method for studying the behavioral consequences of physical and emotional stress, but also potentially provide valuable insight into the cellular and molecular mechanisms underlying them.

2. Methods

All experimental procedures described here are in compliance with the National Institutes of Health *Guide for the Care and Use of Laboratory Animals* (National Institution of Health, 2011) and with approval of the Institutional Animal Care and Use Committee at Florida State University.

2.1. Animals

Eight week-old male C57BL/6J mice (Jackson Laboratory) and CD-1 retired breeders (Charles River) were used in this study. Animals were housed in a vivarium at 23–25 °C on a 12 h light/dark cycle (lights on between 7:00 A.M. and 7:00 P.M.). C57BL/6J mice (four per cage) and CD-1 mice (one per cage) were housed in clear polypropylene boxes containing wood shavings.

2.2. Materials

2.2.1. Social defeat

- Clear polypropylene mice breeding cages (23.5 cm × 45.5 cm × 15 cm)
- Clear polypropylene mice cages (29.5 cm × 18.5 cm × 13 cm)
- Wood shaving bedding
- Water bottles
- Cage cards
- Animal feed (Standard Mouse Chow)
- Paired steel-wire tops
- Clear perforated Plexiglas dividers (45.5 cm × 0.5 cm × 14 cm)

2.2.2. Social interaction test

- Video tracking hardware and software (EthovisionXT; Noldus).
- Social interaction arena custom made from opaque white Plexiglas (40 cm × 40 cm × 40 cm).
- Wire mesh cage (one per social interaction arena)—large enough to hold a CD-1 and allow for their snout and paw to fit though the space between wires is needed. See Appendix A, Fig. G.
- 50% Ethanol for cleaning.

3. Procedure

3.1. 10 Days before defeat—Ordering mice

Eight week-old male C57BL/6J mice (The Jackson Laboratory) arrive and are housed no more than five to a cage. In our experiments, group size per condition is usually 10. However, it is important to have 3–5 extra mice in the unlikely event of losing PS mice to attrition. CD-1 retired breeder mice (Charles River) should be between 4 and 6 months of age and single housed upon arrival. (Note: not all of the CD-1 mice will meet the aggressive behavior threshold required (see screening process below). At least 10 C57 screeners should be ordered per 50 aggressors at the same age as the experimental C57. All mice should be allowed to habituate to the living colony for one week.

3.2. <3–5 Days before defeat—Screening process

Retired breeder CD-1 (aggressors) mice are screened using non-experimental C57 screeners. The screening process involves placing a screener mouse into the aggressor's home cage once a day for