DEPARTMENT OF PSYCHOLOGY

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UNIVERSITY OF CALIFORNIA-LOS ANGELES

405 HILGARD AVENUE

LOS ANGELES, CALIFORNIA 90095-1563

Alisha DSouza, Ph.D.  
Senior Review Editor  
[JoVE](http://www.jove.com/)  
617.674.1888

Dear Dr. DSouza

We appreciate the rapid and thorough reviews of our manuscript entitled, “Stress-enhanced fear learning, a robust rodent model of post-traumatic stress disorder”. We thank you for the opportunity to submit a revised version of the paper. The comments were very helpful and we have incorporated all the suggestions into our revision; changes are indicated in red.

Sincerely,

Michael S. Fanselow, PhD

Staglin Family Chair in Psychology,

Director Staglin Music Festival Center for Brain & Behavioral Health

UCLA Distinguished Professor,

Chair of Learning & Behavior,

Director UCLA Behavioral Testing Core,

Co-Director UCLA ICLM.

**Reviewers' comments:**  
  
Reviewer #1:  
  
Manuscript Summary:  
The authors lucidly describe stress-enhanced fear learning (SEFL), which arguably is the most applicable rodent model of PTSD. The introduction, protocol, results, and discussion sections are all outstandingly written, and the figures convincingly support the SEFL model. This article will likely impact the preclinical PTSD research as well as the general field of fear learning and memory.  
  
**We appreciate the comment and feedback.**  
  
  
Reviewer #2:  
  
In the manuscript by Rajbhandari et al, the authors detail a stress-enhanced fear learning protocol for use in rats and mice. Overall, the protocol is thorough in detail with clear description of expected outcomes. Just a few comments to help readers interested in adopting the approach and to enhance readability:  
1. In the DSM-5, PTSD is no longer classified as an anxiety disorder. It is now under "Trauma and Stressor-Related Disorders".

**Changed “anxiety disorder” to “psychiatric disorder” to reflect DSM classification in Long Abstract and Introduction.**

2. Some justification and discussion regarding the requirement for single housing, which is a stressor in itself, would be helpful.

**Added statement to Protocol: “Single housing is advised as group housing produces variability due to interactions between animals in the home cage, particularly following stress exposure.”**

3. Please include instruction in the Protocol for when animals should be transported to the fear conditioning room on SEFL days. For instance, is it "one round's worth of animals" or all animals, followed by an acclimation period? If it is the latter, where are the animals placed for acclimation?

**Added statement to Protocol: “Only bring one round’s worth of animals to the experiment room at a time.”**

4. Given the multiple statistical comparisons in the Representative Results section, are t-tests the most appropriate statistical choice?

**We have revised our Representative Results section to use one-way ANOVAs rather than t-tests, although the statistical results are the same. ANOVAs are typically applicable for experiments using this method, which often involve addition of another independent variables (e.g. drug treatment). We argue that corrections for multiple statistical comparisons are not necessary because we are making comparisons between only 2 independent groups on each measure, and as each measure is collected at a different time point and for different durations it would be inappropriate to combine all measures into a single analysis.**

5. There seems to be a small detail that is likely a simple typo. The Discussion states that mice require 2-sec foot shocks, but the Protocol (3.2.4.1) states 1-sec for mice.

**For mouse experiments, the traumatic stressor consists of 10 1-sec, 1-mA footshocks while the mild stressor consists of a single 2-sec, 1-mA footshock. The portion of the protocol referenced in this comment (3.2.4.1) describes the 1-sec shocks used for the traumatic stressor, while the Discussion is referring to the 2-sec shock used as the mild stressor, as described in the Protocol (3.4.3.1).**   
  
  
  
Reviewer #3:  
  
This is a clearly written methods paper outlining the procedure to induce stress-enhanced fear learning (SEFL), a model of post-traumatic stress disorder. Animals (rats or mice) are exposed to a traumatic event (15 unsignaled electric footshocks in Context A). The following day, they are exposed to a single unsignaled footshock in a second different context (B). On the third day, contextual fear memory is measured in Context B. Animals exposed to the trauma show enhanced levels of freezing on the test day. The senior author (Fanselow) has published multiple papers using this technique starting in 2005 (Rau, DeCola and Fanselow, 2005).  
One benefit of this protocol is that it recapitulates certain aspects of PTSD, namely a long-term sensitization of fear learning caused by an acute stressor. One of the strengths of this manuscript is the authors insistence that Context A and B must be sufficiently different to reduce baseline generalization between these two contexts. Indeed, even the method of transportation to the testing chambers must be different on days 1 and 2. I have only a few minor suggestions for improvement:  
  
1. The authors could add a sentence summarizing a few of the findings from their 2009 paper (Rau and Faneslow, 2009). Namely, that pre-exposure to 1 shock does not lead to SEFL, but 4 or 15 shocks do.  
**Added statement to Introduction: “In contrast, pre-exposure to a single footshock does not produce SEFL (Rau and Fanselow, 2009).”**

2. The authors note that extinction in context A does not mitigate SEFL. They could also add that a hallmark of PTSD is that it is resistant to exposure therapy (extinction), thus strengthening the link between SEFL and PTSD.

**Added statement to Discussion: “As a hallmark of PTSD is resistance to extinction (in the form of exposure therapy), this further strengthens the link between SEFL and PTSD (Craske et al, 2008).”**