Response to Reviewers

We thank the Science Editor and the reviews for their comments. We have now fully revised the manuscript in accordance with these suggestions.

Science Editor Comments

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

   The manuscript has been reviewed by both authors for errors.

2. What is the concentration of the heparin solution used in step 1.1?

   The heparin concentration has now been added to the text.

3. What antiseptic solution is used in step 2.7?

   The antiseptic solution is now described in step 2.2.3.

4. Step 1.3 is the same as 1.2.

   Step 1.3 is now deleted.

5. Figure 1: A clearer tube would be better for depicting the results.

   We have now changed the picture in Figure 1 to more clearly illustrate the difference between the two tubes.

6. In addition to Figure 1, which shows the visual appearance of the blood sample, the authors should include at least one representative result that demonstrates the effectiveness of their technique and/or confirms sample viability with a quantitative measure. The abstract states, "The goal of this method is to obtain blood samples that are not altered by the acute stress response," so perhaps a representative result that compares data gathered using blood collected with this method to data collected using a sample that was altered by the acute stress response.

   As suggested by the editor, we have now added blood plasma corticosterone measurements to highlight the difference between pre-stress baseline and post-stressor exposure (Figure 2).

Reviewer 1. Comments

Major Concerns:

1. "…the manuscript could have a broader impact if some discussion was given as to how the protocol could be modified for blood collection in mouse."

   Blood collection from the tail vein is possible in the mouse, though sampling from the submandibular vascular bundle is the preferable method. A paragraph has been

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included in the discussion to reflect this and explain why submandibular bleeding is better for the mouse.

Minor Concerns

1. For Keywords, "three 'R's" is listed in the manuscript. I have no idea what that is.

The three 'R's, which refer to the federal requirement to reduce, replace and refine the use of laboratory animals, has been omitted.

2. For Introduction, consider rewording the last sentence to "The goal of this method is to minimize the influence of the acute stress response on obtaining the blood sample." Obviously the sampling procedure is still "stressful".

The last sentence in the introduction has been reworded to better describe the goal of the technique.

3. For Protocol, a heating pad might be an alternative tool for dilating blood vessels when a heated water source is unavailable (e.g. behavioral testing rooms).

This is an excellent suggestion. A heating pad as an alternative to heated water has been included in Step 2.1.1.

4. For Representative Results, figure subnumbering 1A and 1B are indicated in the text but A and B are not indicated on the figure.

Figure 1 is now modified to include the subcharacters to be consistent with the figure legend.

5. For Materials, consider adding product information for collection tubes and EDTA.

The EDTA and collection tube product information is now included in the materials list.

Reviewer 2. Comments

1. Two additional areas should be further addressed in the discussion. The authors discuss the importance of maintaining the rat in an unstressed state. The video will likely show this method at a slightly slower speed to allow the observers to study all the details. However, it is quite likely that this method must be completed relatively rapidly to obtain unstressed blood samples. The authors should describe a timeframe that would be optimal to obtain unstressed samples. Finally, it would also be useful is they could provide examples of corticosterone levels obtained using this procedure within an optimal time frame (compared to trunk blood of unstressed rats) to validate its use for obtaining unstressed blood samples.

An optimal time frame is now included in the discussion. We have also added data (Figure 2) that compare corticosterone levels taken in an "unstressed" state with those following an explicit stressor. These results show that the procedure performed in a "basal" state do not elevate corticosterone as does the stressor exposure.

2. 1.2 and 1.3 appear to be the same, so 1.3 could likely be deleted.

Step 1.3 has been deleted

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Reviewer 3. Comments

1. Are points 1.2 and 1.3 meant to be the same? Or is point 1.3 reaffirming that the rat should be secure after being moved to the edge of the table?

   Step 1.3 is now deleted. This was an accidental duplication on our part.