Devon L. Poeta
Brown University
Department of Cognitive, Linguistic & Psychological Sciences
171 Meeting St
Providence, RI 02912

Journal of Visualized Experiments
1 Alewife Center #200
Cambridge, MA 02140

February 5, 2019

We very much appreciate the comments of the editor and reviewers. Both reviewers had positive comments as well as some suggestions for improving the manuscript. We have addressed all comments as indicated below in blue text, and we think our manuscript is much improved.

Editorial comments:

1. Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. We have edited the paper carefully for style, clarity and accuracy.

2. Please obtain explicit copyright permission to reuse any figures from a previous publication. Explicit permission can be expressed in the form of a letter from the editor or a link to the editorial policy that allows re-prints. Please upload this information as a .doc or .docx file to your Editorial Manager account. The Figure must be cited appropriately in the Figure Legend, i.e. "This figure has been modified from [citation]." We have replaced Fig. 1A with our own image, so copyright permission is no longer needed.

3. Please adjust the number of all references. Please number all references in the order of their appearance in the manuscript. We have carefully gone through the text and revised the reference numbers based on order of appearance.

4. JoVE cannot publish manuscripts containing commercial language. This includes company names before an instrument or reagent. Please remove all commercial language from your manuscript and use generic terms instead. All commercial products should be sufficiently referenced in the Table of Materials and Reagents. We have gone through the text and removed all company names.

5. Please add a one-line space between each of your protocol steps. This has been corrected.

6. Step 1.1: Please ensure that all text is written in the imperative tense. This has been corrected.

7. 1.3: Please ensure that all text is written in the imperative tense. This has been corrected.

8. 3.6: Please ensure that all text is written in the imperative tense. This has been corrected.

9. 4.1.2: Please ensure that all text is written in the imperative tense. This has been corrected.

10. 4.1.3: Please ensure that all text is written in the imperative tense. This has been corrected.

11. 4.2.1-4.2.7: Please ensure that all text is written in the imperative tense. These steps have been eliminated.

12. 4.3.1-4.3.7: Please ensure that all text is written in the imperative tense. These steps have been eliminated.

13. 5.1-5.3: Please ensure that all text is written in the imperative tense. This has been corrected.

14. There is a 2.75 page limit for filmable content. Please highlight 2.75 pages or less of the Protocol steps (including headings and spacing) in yellow identifies the essential steps of the protocol for the video, i.e., the steps that should be visualized to tell the most cohesive story of the Protocol. This has been completed.

15. For steps that are done using software, a step-wise description of software usage must be included in the step. Please mention what button is clicked on in the software, or which menu items need to be selected to perform the step. We have added a more step-wise description of how to use the program (Lines 192-220).

16. Please ensure that the references appear as the following: Lastname, F.I., LastName, F.I., LastName, F.I. Article Title. Source. Volume (Issue), FirstPage – LastPage, (YEAR). We have revised all of the references to follow this format.

17. All tables should be uploaded separately to your Editorial Manager account in the form of an .xls or .xlsx file. New .xls files for Tables 1 and 2 have been uploaded.

Reviewers' comments:

Reviewer #1: This manuscript is very well written - it is clear and concise. All the i's are dotted and the t's are crossed. You clearly explain what you are going to do, and then guide the reader through the steps needed to complete the task in an orderly, easy to follow manner. In this regard, I believe this manuscript, as it is currently written, is suitable for publication in JoVE.

What this study lacks is sufficient justification as to why other researchers would ever use these methods. There is mention of PrAnCER being cheaper, yet there is no supporting evidence. There is mention of PrAnCER being more flexible, yet there is no evidence. And most worrisome of all is that there is mention of PrAnCER's semitransparent flooring being better because clear walkways can mask motor deficits - yet there is no evidence.

We have revised the text to clarify our meaning that PrAnCER is a less expensive alternative to commercial systems (Lines 66-68). We did not intend to compare it to other open source systems.

In regard to being cheaper to construct, we have added approximate prices for each item in our Table of Materials.

All references to flexibility have been removed. We have also clarified the statement about 'masking motor deficits', which was intended to reference treadmill gait systems, not transparent flooring (69-73).

I use automated gait analysis all the time. If you want me to use PrAnCER, prove to me that it is cheaper than AGATHA. Show me what I'm missing by using a clear walkway. Take a known injury model (not your haloperidol model presented here) and show that with a clear walkway PrAnCER shows exactly what CatWalk shows. Then put the vellum on the glass and have PrAnCER show me what I've been missing all

these years. But be careful… animal body axis is a huge aspect of rodent gait. There is going to be a trade-off. Show me that the loss of body axis info is worth it to have a semi-transparent walkway.

In our introduction, we expanded upon why using semi-transparent flooring is advantageous, citing previous work that demonstrates the effects of treadmills systems and clear flooring on natural behavior (lines 69-73). We acknowledge that the loss of body axis information is a trade-off with our system. This limitation and ways to address it are covered in the discussion (352-355).

By having a variable measuring device AND a variable injury model I don't know where the reported changes are coming from. In this regard, I believe this study, as it is currently constructed, is unsuitable for publication. Of course, editors and other reviewers often disagree with me, so below are some minor comments.

Although we appreciate the reviewers concern, our goal in this study was to demonstrate that our system could identify gait changes in a model in which gait changes would be expected, and we have done this. As is evident in Figure 6, in Experiment 1 a high dose haloperidol significantly increased stride length and maximum contact area compared to the saline condition. In Experiment 2, haloperidol significantly increased stance duration and maximum paw contact area. Finally, when comparing the haloperidol treated conditions from both experiments, the high dose haloperidol increased base of support, maximum contact area and interlimb distance compared to the low dose condition.

Ln35 - very awkward sentence, please re-write. This sentence has been rephrased.

Ln47-this is a red flag in my eyes. Is this unexpected finding a result of the injury model or an error with PrAnCER? We have provided an explanation for why this finding occurred (Line 47).

Ln68- Noldus is incorporated in Wageningen, The Netherlands. Their US sales office is in Leesburg Deleted all company names so this no longer applies.   We have removed all company names from the text so this is no longer applicable.

Ln71- if this is your big selling point please expand. We have expanded on this point (lines 68-73).

Ln78 - "computationally expensive algorithms"??? I'm not sure what this means. Does PrAnCER save me a few teraflops? This wording has been deleted.

Ln132 - tunnel feels like the wrong word, corridor or walkway seem more accurate. We have replaced "tunnel" with "walkway" (Line 134). The rest of the text reflects this change as well.

Ln286- the biggest reason to use automated gait analysis over manual is the labor involved. Please include how many man hours were saved by using PrAnCER. We added a sentence estimating the approximate time it takes to run one video through PrAnCER analysis vs. the time it takes to manually score the same video (Lines 297-301).

Ln324- I find this a bit misleading. You tested the accuracy of manual vs automated classification of the recorded videos. You didn't test if PrAnCER was mis-recording paw prints in the first place. We have rephrased this sentence to reflect what we actually tested (Lines 310-312).

Ln344 - NO! You did not show that a translucent floor eliminates anything. This sentence has been rephrased (Line 330-331).

Ln356 - maze? What maze? The word 'maze' has been replaced with 'walkway' (Line 341).

Figures - I know you want to differ yourself from CatWalk, but they are the industry leader, and a vast majority of gait analysis researchers have adopted their color assignments. Please stick to RF-cyan, RH-magenta, LF - yellow, LH-green. We have revised all applicable figures with these color assignments.

Reviewer #2:

Manuscript Summary:

The document proposes a system and a protocol to perform gait analysis of rodents while walking free in a custom made tunnel. The system consists of a camera that records the foot prints of the rodent, then an automatic image segmentation method is used to identify the coordinates of the foot on each step and then measure important parameters such as length of step and speed. The system is validated using a Parkinson model using a small number of rodents.

Major Concerns:

The system may be useful for people doing research using rodent models, especially because the current options are costly. My concern is that the authors presents an open source system, but I do not see where I can download the code of the software in order to be able to reproduce the results they present. I will recommend that the code and detailed instructions of how to build the system are shared in a webpage or repository, otherwise the proposed system makes no sense.

We provide a link to the GitHub website where you can download the code on Line 222. Also included on this website are instructions on how to build together the walkway apparatus.