Dear JoVE Peer Review,

Thank you for your consideration of our manuscript JoVE58983 titled “Activity-Based Training with Spinal Cord Injured Wistar Rats.” We would also like to thank the reviewers and editors for their input and valuable time given toward improving the manuscript. We have read the comments and have revised the manuscript to address the reviewer’s concerns. In response to the reviewers’ suggestions, we have added more detail where necessary, fixed any grammatical errors, and expanded more description within the Representative Results and Discussion sections. Our revisions are discernable through track changes. Here we would like to respond individually to each of the editorial and reviewer’s comments:

*Editorial Comment:* Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues.

Response: The manuscript has been proofread for spelling and grammatical errors.

*Editorial Comment:* 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].”

Response: According to the American Journal of Physiology, Renal Physiology, and the American Physiology Society (APS), republication of figures, such as Figure 4 in our manuscript is allowed without charge or requesting permission so long as a full citation of the source is provided. In the figure legend we have noted that “This figure is republished with author permission.17” https://www.physiology.org/author-info.permissions

*Editorial Comment:* Keywords: Please provide at least 6 keywords or phrases.

Response: We have added “rehabilitation” and “exercise therapy” to our list of keywords.

*Editorial Comment:* Please expand the Summary to briefly describe the applications of this protocol.

Response: We have added a brief statement “Investigators are able to assess training effects on SCI rats using this protocol.” To describe our application of this protocol.

*Editorial Comment:* Please add more details to your protocol steps. There should be enough detail in each step to supplement the actions seen in the video so that viewers can easily replicate the protocol. Please ensure you answer the “how” question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

Response: We have added in more detail to the protocol steps where we thought was necessary, specifically including the following editorial comments:

*Editorial Comment: 1.1:* Please specify the age, gender and type of animals used. How many animals are used?

Response: We have revised step 1.1 (line 90) to say “Transport the rats (we use adult male Wistar rats - ~50 days of age initially; 200-225 grams) to a quiet room that is dedicated for training. Due to time and personnel constraints, we conduct our daily training in groups of twelve animals, three in each subset group (quadrupedal trained, forelimb only trained, non-trained, and home cage) that are randomly assigned after injury but prior to the start of training.”

*Editorial Comment: 1.3:* For how many days and how many times per day are the animals trained? Please specify how to train the animal that does not acclimate well. For instance, start with what training time/speed/duration?

Response: We have revised step 1.3 to include more detail on how many days (7) a week and how many times a day (once) the animals are trained. Additional detail has also been added on what to do when an animal does not acclimate well.

*Editorial Comment: 2.2:* How is the general antibiotic given to the animal?

Response: Originally, we noted that the animals are given antibiotic “s.c.” meaning subcutaneously. We have revised to write out “subcutaneously” rather than using shorthand.

*Editorial Comment: 2.4:* How large is the incision?

Response: We have added in to make an estimated 5 cm incision.

*Editorial Comment: 2.5:* Please describe how to perform laminectomy.

Response: We have added the following to step 2.5 (line 125): “For mid-thoracic contusions, expose the T8/T9 level of spinal cord via removal (with rongeurs) of the overlying T7 vertebral lamina.”

*Editorial Comment: 4.1:* Please specify the anesthesia and dose used.

Response: Anesthesia for overdose is lab-dependent, but we stated the following based upon what we do: “Administer a lethal dose of anesthesia to the animal (at least twice the dosage, based upon weight, that would be used to achieve a surgical depth of anesthesia). Typical anesthesia drugs we use include urethane or a ketamine/ Xylazine mixture.”

*Editorial Comment: 4.2, 4.3:* Please describe how this is done. What is the temperature for storing these tissues?

Response: We have described what we do in both 4.2 and 4.3, although there are many ways to go about saving tissue from perfused animals.

*Editorial Comment:* 4.4: Please specify how the tissues are later processed.

Response: We have revised this step to include how our tissue is prepared for histological analysis.

*Editorial Comment:* 6. In the protocol, please add steps that describe how to collect the data presented in Figures 4 and 5.

Response: Re Figure 4, the use of metabolic cages is not part of the training protocol; it is something our lab did to assess training effects on bladder function and is being used as an example that references the article containing the protocol. Re Figure 5, the collection of kidney tissue is described. The Western Blot protocol is standard and referenced (again, lab-specific and not part of the training video for the current JOVE article).

*Editorial Comment:* 7. Please revise to explain the Representative Results in the context of the technique you have described, e.g., how do these results show the technique, suggestions about how to analyze the outcome, etc. The paragraph text should refer to all of the figures. However, for figures showing the experimental set-up, please reference them in the Protocol. Data from both successful and sub-optimal experiments can be included.

Response: Figures 1, 2 and 3 are referenced in the Protocol as they deal with training of the animals, the topic of the video. Figures 4 and 5 are examples of how training and exercise can benefit function (in the case of spinal cord injury, we show benefits to the urinary system, although there are many other benefits – locomotion, cardiovascular function, etc…). The intent is the have this video show how training is done as different labs use it or will want to use it for different purposes (our benefits to the urinary system is just an example based on what we’re studying; it’s not meant for others to do as part of the protocol; they will apply training/exercise for their own purposes and whatever condition they are studying and will remove tissues relevant for their system of interest).

*Editorial Comment: 8*. Figure 4: Please change “ml” to “mL”. Please label the different panels and describe them in the figure legend. Please define the \* and # symbols as well as error bars in the figure legend. Please move the discussion of the figure to the Representative Results section. Please note that Figure 4 shows that after 9 weeks of LT training, total urine volume seems to be higher than baseline if baseline refers to the Pre-SCI value. Please check and clarify.

Response: Changes have been made to Figure 4 and the legend as requested. Yes, urine volume is significantly improved but remains significantly above pre-injury baseline. This is now pointed out in the legend and the discussion of it referenced in the paper.

*Editorial Comment: 9*. Figure 5: Please provide a figure with higher resolution. Please define error bars.

Response: Higher resolution figure now provided, and error bars now defined in legend.

*Editorial Comment: 10*. Discussion: Please describe any future applications of the technique.

Response: Lines 316-319 have been revised to describe our intended future application of this technique.

*Editorial Comment: 11*. Reference #12: Please spell out the journal name.

Response: The journal has been spelled out.

*Editorial Comment:* On line 90-91, the description of the pre-contusion acclimation training makes it sound like it very easy to do and naïve animals are quick to learn it. If that is the case, fine, but our experience has shown that animals should be eased into the harness in the beginning, then later, we introduce them while in the harness to the weight support mechanism. Only then do we turn on the treadmill and have them walk. If there are similar experiences, perhaps that authors can provide more detail so as not to give the impression of it being fairly easy to do.

Response: Edits have been made to reflect the different procedures used during acclimation, including clarifying that this is done post and not pre-injury.

*Editorial Comment:* Around line 146, the training details should include whether the set up allows any forward/backward movement on the treadmill belt. It seems the animals are fixed in one spot? If so, please address this as it has some implications for training.

Response: A few edits have been made for clarity but as this is a video journal, all details will be evident in the accompanying video.

*Editorial Comment:* Around line 157, "more severely injured rats" implies (rightly so) that there may be some variability in recovery after contusion. Do the authors have some criteria for removing animals from the study, i.e. those who may not appear injured or appear too injured?

Response: Wording fixed. All rats in the separate groups receive the same contusion impact forces; random assignment just prior to training accounts for potential variability in spontaneous recovery. Our injury inclusion/exclusion criteria are study-specific and is not relevant to the training video.

*Editorial Comment:* I assumed there would be a video given this is JOVE, if there is one available, it may be helpful to include the training of rats, especially how the manual assistance is provided.

Response: The written manuscript protocol is submitted prior to videotaping (video on how the training is done).

*Editorial Comment:* 1.3 - The final sentence "reduce the time and increase training by only 10 min/day" needs to be clarified. It is not immediately clear how and by how much.

Response: We have revised the steps to include more detail on how to train animals that are not initially compliant.

*Editorial Comment:* 2.4 - There is a typo at the end of the first line "and ith a #10"

Response: The typo has been corrected to say “with a #10…”

*Editorial Comment:* 3.6.1 - This explanation should include a corresponding figure for clarification

Response: It is our belief that the main purpose of submitting to JoVE is to demonstrate this aspect of our training protocol via a video demonstration. Therefore, a figure would be redundant and not as helpful.

*Editorial Comment:* Include a discussion on how the body weight support system cannot be controlled precisely by this system and how this could affect locomotor performance.

Response: The following edits have been added to address this point: The amount of body weight support needed varies from animal to animal and changes as training progresses. The spring support system gives enough assistance to keep the animal positioned for a proper gait. Further support is provided as needed by the trainer. A key element of LT is functionally appropriate paw placement for stepping and interlimb coordination that is promoted by the trainer and is independent of the support system.

*Editorial Comment:* In the discussion of the influence of afferent information on functional recovery, how skin afferents activated by the straps holding the hip could affect spinal circuits?

Response: Edits made to emphasize the non-trained control group that wears a training jacket.