

Editorial comments:

Changes to be made by the author(s) regarding the manuscript:

Comment 1

Please expand the Introduction to include all of the following with more citations.

- a) A clear statement of the overall goal of this method
- b) The rationale behind the development and/or use of this technique
- c) The advantages over alternative techniques with applicable references to previous studies
- d) A description of the context of the technique in the wider body of literature
- e) Information to help readers to determine whether the method is appropriate for their application

Response 1

We have revised the Introduction accordingly (page 3, lines 114 - 116 in our revised manuscript).

Comment 2 and 3

Citations? Please number the citations in order. So 5 should be followed by 6, 7, 8. Presently, 6-8 are missing.

Response 2 and 3

By a careless mistake, we have deleted citation numbers (6 - 8) from our revised manuscript. We have added the citation numbers in the latest version of our manuscript.

Comment 4

Presently the protocol steps are lacking connection between the individual sections (from 1-2 and 2-3 and 3-4). Please bring out this clarity. Maybe including a timeline to show at what time which step is performed may help?

Response 4

We have added Supplementary Figure 1 to clarify the connection between the individual sections.

Comment 5

How is the mouse placed in this case?

Response 5

The mouse was laid in a supine position (page 4, lines 141 - 142 in our revised manuscript).

Comment 6

Any specifics of the wire to be used?

Response 6

The relevant information is given in Table of Materials. We have clarified this in the Protocol section (page 4, line 145).

Comment 7

Do you perform surgery to do the same? Also in the figure there seem to be something else present along with the wire, please comment on the same.

Response 7

No surgical or other invasive procedures were involved in the wiring process. The white material seen beneath the wire (Figure 1A) is the surgical tape used to ease the handling of the hindlimbs. We have added this information in Figure 1A of our revised manuscript.

Comment 8

How is this done?

Response 8

We manually adjusted the configuration of aluminum wire (page 4, lines 150 - 152).

Comment 9

Please comment on post anesthesia steps as well. Do you leave the mouse in the single housed cage? Is the mouse able to maintain normal movement?

Also, what is the control in this case?

Response 9

We just returned the mice to their original cages. Yet, we made sure that 3 hours later, they recovered from anesthesia and accessed to food and water as usual (page 4, lines 154 - 157). With regard to the control, we have modified the sentence to clarify the samples used as a reference for statistical analysis (pages 7 - 8, lines 325 - 327).

Comment 10

Please quantify the weights.

Response 10

We tested 36-g, 66-g, and 200-g. We have added this information in our revised manuscript (page 4, line 163).

Comment 11

Where are the steps for analysis of muscle inflammation and atrophy? Please include the step numbers here to bring out clarity.

Response 11

We have added relevant information (page 4, line 166).

Comment 12

Is this the same mouse from step 1?

Response 12

We have clarified that the mice subjected to pressure measurement were not used for histological analyses (page 4, lines 166 - 167 and Supplementary Figure 1).

Comment 13

Is there a specific reason to use different anesthesia in this case?

Response 13

Because pressure measurement involved more invasive procedures (e.g., skin incision and needle insertion) as compared to hindlimb wiring and LCC (page 4, lines 169 - 170).

Comment 14

We cannot have phrases like could be, should be, would be, etc. Please reword in imperative tense.

Response 14

We have revised the text (page 4, line 172).

Comment 15

Before performing the incision, do you shave the fur, do you sterilize the area? Please include all specific details.

Response 15

We depilated with an electric shaver and semi-sterilizing the skin surface with 70% ethanol-soaked absorbent cotton (page 4, lines 175 - 176).

Comment 16

With respect to?

Response 16

We inserted a needle at an obtuse angle (150° - 170°) to the skin surface (page 4, line 179).

Comment 17

Also please include post anesthesia recovery steps.

Response 17

Please refer to Response 9.

Comment 18

Please provide more details on how to monitor the intramuscular pressure. Button clicks in the software etc?

Again how were the controls treated?

Response 18

We compared 33-g, 66-g, and 200-g. There was no control for pressure measurement. We think we have provided sufficient information on pressure monitoring.

Comment 19

Is this mouse the same as in step 2 or 1? Please bring out clarity with respect to the step number. Also, if same, please mention after how many days is this step performed?

Response 19

As mentioned in Response 12, the mice subjected to pressure measurement were not used for histological analyses. With regard to the protocol for 7-day LCC experiments, we describe the details in 3.3 (page 5, lines 201 - 202) and Supplementary Figure 1.

Comment 20

This needs more clarity. Please mention what part of the LCC instrument contains the cylindrical weight, how is the movement performed, where is the mouse placed etc. Maybe mark the position of weights in the instrument shown. Do you perform any button clicks, knob turns etc to bring the weights onto the mouse?

What is the range of weights tested on the mouse?

What is the control in this case?

Response 20

We have added more detailed information in Figure1B.

Comment 21

Dosage?

Response 21

We have described the information (page 5, lines 209 - 210).

Comment 22

How is this done.

Response 22

After depilating the posterior calf surface, we made a skin incision, and dissected gastrocnemius muscles by separating from tibio-fibular bone using a surgical scissor (page 5, lined 212 - 213).

Comment 23

What are appropriate filters in your case.

Response 23

We have provided relevant information (page 6, lines 259 - 261).

Comment 24

How is this done, please provide all the button clicks in the software, graphical user interface, etc.

Response 24

We think we have provided sufficient information.

Comment 25

Please include a one liner title for each figure with all the panels combined.

Please upload each figure individually to your editorial manager account. Please keep the panels together.

Response 25

We have added figure titles (page 7, lines 306 - 307 and 312 - 313; page 8, lines 331 - 332, 343 - 344, and 360).

Comment 26

Please make the borders thicker in between the panels.

Response 26

We have revised Figure 2A accordingly

Comment 27

Data for this ?

Response 27

Statistical analysis was conducted with reference to the 'Day 0' samples (gastrocnemius muscles from mice that were not subjected to immobilization). We have clarified this in the figure legend (page 7 - 8, lines 325 - 327)

Comment 28

Please do not write the discussion in pointwise manner. Please use paragraph style instead.

Please expand the Discussion to explicitly cover the following in detail in 3-6 paragraphs with citations:

- a) Critical steps within the protocol
- b) Any modifications and troubleshooting of the technique
- c) Any limitations of the technique
- d) The significance with respect to existing methods
- e) Any future applications of the technique

Response 28

We have revised the manuscript accordingly.