Peer Review File

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Reviewer A

Comment 1: However, they do not compare the results obtained wtih this impactor with commercial ones. In particular, to my knowledge the most widely used impactor is the Infinite Horizon Impactor developed by PSI. This is a major limitation to this study. Indeed, it is hard to say that this new impactor is "better" than those already available if there is no result to compare the different devices.

Reply 1: Thank you for reviewing our manuscript and providing valuable feedback.

The establishment of a standardized and ideal animal model of SCI is essential for studying the pathophysiological mechanisms of SCI and exploring new therapeutic approaches. The ideal SCI model should exhibit the following characteristics: it can simulate injuries similar to clinical SCI; it is controllable, reproducible, and stable; it involves simple and easy-to-learn techniques; and the equipment used to generate the model is simple and quick to produce. The New York University-Multicenter Animal Spinal Cord Injury Study (NYU-MASCIS) device and infinite horizon (IH) impactor are currently widely used and established commercial contusion SCI impactors for the preparation of rodent SCI models. Both devices provide good control of the severity of SCI and allow the use of functional and histological indicators to evaluate the degree of injury in animals.

However, the IH device provides transient striking forces that only simulate the initial impact injury during acute SCI, without the subsequent continuous compression effect, which does not fully simulate the pathological changes of clinical SCI. In addition, it is relatively expensive.

The idea for this study emerged from the finding that when the NYU/MASCIS device was used for SCI modeling, we occasionally found that the modeling was not ideal, such as off-center modeling.

Based on the principle of Allens, we designed a modified impactor. This device consists of a stereotaxic frame, a channel with holes, a pulling rod, a weight (10 g), an impactor tip (weight of 0.2 g, diameter of 2.5 mm), vertebral clamps, and an animal platform. The major difference in the improvement is the replacement of the impactor rod with a weight of concentrated mass and a passive impactor tip of slight mass. Prepositioning the passive impactor tip in the center of the spinal cord surface may reduce the deviation of the drop point. Weights with a lower center of gravity and less friction with the channel may decrease rebound and injury variability. Although the improvements may seem small, for SCI models, small improvements are beneficial, which will facilitate the creation of more accurate SCI models.

In this study, the results confirm that this impactor can model mild, moderate, and severe SCI contusions based on height. We strongly acknowledge the important role played by the IH device in standardizing SCI contusion models. However, the existing literature also contains some studies whereby a portion of the model uses Weight Drop, and further standardization of this part of the model is also necessary. We designed this modified impactor to provide an available alternative.

We do not intend for the impactor to replace a professional IH device for SCI, so we have not yet made a direct comparison with a commercially available impact device. However, we propose that the use of this cost-effective impactor could help more researchers in SCI research while maintaining consistency and reproducibility of injury.

Based on your valuable suggestions, we have made some changes in the introduction and discussion to clarify the improvements of our striker.

Changes in the text: We have modified our text as advised (see Page 4, line 75-85; Page 22-23, line 400-415).

Comment 2: Another limitation is the fact that the authors present BBB results up to 35 days after SCI but only immunohistological results obtained 7DPI. It could have been interesting to present also histological results obtained 35 DPI.

Reply 2: Thank you very much for your valuable comments. In the pre-experiments, we found that at 7 days, behavioral scores reached significant differences in the different SCI groups according to strike height; specifically, HE staining revealed that the spinal cord tissue destruction area increased with increasing strike height. Therefore, we believe that 7 days is the appropriate testing time point. In addition, we are interested in the pathophysiological events that occur during the subacute period, such as demyelination, neuroinflammation, neurophysiology, and trends in the alteration of various types of nerve cells. Based on the 3R (reduction, replacement and improvement) principle of reducing animal usage, we only executed animals at 7 days to detect early indicators instead of assessing chronic histological changes. If necessary, we can provide chronic-phase changes in the SCI model at 5 weeks, which requires an additional time of approximately 2 months.

Changes in the text: No modifications were made at this time.

Reviewer B

Comment 1: First, the new device system (modifications) is poorly characterized. Additionally, the improvements the author's claim their device has compared to the NYC/MASCIS, are not really probed in the paper, and their claims are a bit doubtful- that is in my understanding the main scope of the paper.

Reply 1: Thank you for reviewing our manuscript and providing valuable feedback.

The establishment of a standardized and ideal animal model of SCI is essential for studying the pathophysiological mechanisms of SCI and exploring new therapeutic approaches. The ideal SCI model should exhibit the following characteristics: it can simulate injuries similar to clinical SCI; it is controllable, reproducible, and stable; it involves simple and easy-to-learn techniques; and the equipment used to generate the model is simple and quick to produce. The New York University-Multicenter Animal Spinal Cord Injury Study (NYU-MASCIS) device is currently the most widely used and established commercial contusion SCI impactor for the preparation of rodent SCI models.

The NYU-MASCIS device is currently the most commonly used striker based on the Allens principle with modifications, but there may be some shortcomings in its

practical use. For example, the striking rod may rub against the channel when falling, resulting in reduced striking speed. The striking rod may be off-center, resulting in inconsistent injury. The impactor rod may bounce back after landing on the spinal cord surface, leading to variability in injury. In addition, it is relatively expensive.

The origin of the idea for this study was that when we used the NYU/MASCIS device for SCI modeling, we occasionally found that the modeling was not ideal, such as off-center modeling.

Based on the principle of Allens, we design a modified impactor. This device consists of a stereotaxic frame, a channel with holes, a pulling rod, a weight (10 g), an impactor tip (weight of 0.2 g, diameter of 2.5 mm), vertebral clamps, and an animal platform. The major difference in the improvement is the replacement of the impactor rod with a weight of concentrated mass and a passive impactor tip of slight mass. Prepositioning the passive impactor tip in the center of the spinal cord surface may reduce the deviation of the drop point. Weights with a lower center of gravity and less friction with the channel may decrease rebound and injury variability. The impactor tip was placed on the dorsal surface of the spinal cord, and the spinal cord was then compressed by dropping the weight from different heights to establish a contusion SCI model. Although the improvements may seem small, for SCI models, small improvements are beneficial, which will facilitate the creation of more accurate SCI models.

In this study, our main objective was to investigate whether a graded SCI contusion model could be established using a modified impactor. The results confirm that this impactor can model mild, moderate, and severe SCI contusions based on height. We strongly acknowledge the important role played by the NYU/MASCIS device in standardizing SCI contusion models. However, the existing literature also contains some studies whereby a portion of the model uses Weight Drop, and further standardization of this part of the model is also necessary. We designed this modified impactor to provide an available alternative.

It is worth mentioning that the materials used for the modified impactor are easily available, the production process is relatively simple, and the cost-benefit ratio is high, making it convenient for general laboratories to use and fabricate the impactor. This means that a lack of financial ability to acquire sophisticated instruments does not become an obstacle in the pursuit of science.

We do not intend to replace a professional IH device for SCI, so we have not yet made a direct comparison with a commercially available impact device. However, we propose that the use of this cost-effective impactor could help more researchers in SCI research while maintaining consistency and reproducibility of injury.

Based on your valuable suggestions, we have made some changes in the introduction, discussion and conclusion to clarify the improvements of our striker.

Changes in the text: We have modified our text as advised (see Page 4, line 75-85; Page 22-23, line 400-415; Page 28, line 520-524).

Comment 2: Second, I do believe the field is moving towards the use of more controlled impactor systems. Thus, parameters like force, displacement, compression

depth, and impact velocity can be recorded, pre-set in the end accounted for in order to discriminate variability, create more standardized injuries and predict future outcomes. The author's new system is lacking control for all of those variables.

Reply 2: We very much agree with your opinion about recording parameters related to the strikers and thank you for your valuable comments. The measurement of damage parameters, such as force, velocity, and displacement of the striker, can yield varying degrees of damage with corresponding consistency and reproducibility, thus providing a platform with which to assess the effectiveness of neurotherapeutic strategies. Therefore, this impactor still requires further improvement and validation. The determination of damage parameters can be achieved using relevant sensors, which is the next direction of our research. We state this limitation in the discussion.

Changes in the text: We have modified the text as advised (see Page 27-28, line 509-514).

Reviewer C

Comment: A modified impactor induced injury strength-dependent motor impairment in SCI rats. These results are not new. This paper lacks superiority of the modified impactor compared with previous impactors. Deviation of BBB scores were smaller than previous methods? Especially in Abstract and Conclusion, you should emphasize the superiority of the modified impactor.

Reply: Thank you for reviewing our manuscript and providing valuable suggestions.

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The NYU-MASCIS device is currently the most commonly used striker based on the Allens principle with modifications, but there may be some shortcomings in its practical use. For example, the striking rod may rub against the channel when falling, resulting in reduced striking speed. The striking rod may be off-center, resulting in inconsistent injury. The impactor rod may bounce back after landing on the spinal cord surface, leading to variability in injury. In addition, it is relatively expensive.

The origin of the idea for this study was that when we used the NYU/MASCIS device for SCI modeling, we occasionally found that the modeling was not ideal, such as off-center modeling.

Based on the principle of Allens, we design a modified impactor. This device consists of a stereotaxic frame, a channel with holes, a pulling rod, a weight (10 g), an impactor tip (weight of 0.2 g, diameter of 2.5 mm), vertebral clamps, and an animal platform. The major difference in the improvement is the replacement of the impactor rod with a weight of concentrated mass and a passive impactor tip of slight mass. Prepositioning the passive impactor tip in the center of the spinal cord surface may reduce the deviation of the drop point. Weights with a lower center of gravity and less

friction with the channel may decrease rebound and injury variability. Although the improvements may seem small, for SCI models, small improvements are beneficial, which will facilitate the creation of more accurate SCI models. Furthermore, the materials used for the modified impactor are easily available, the production process is relatively simple, and the cost-benefit ratio is high. This means that a lack of financial ability to acquire sophisticated instruments does not become an obstacle in the pursuit of science.

In this study, our main objective was to investigate whether a graded SCI contusion model could be established using a modified impactor. The results confirm that this impactor can model mild, moderate, and severe SCI contusions according to height. The existing literature includes some studies that utilize Weight Drop in their model, and further standardization of this part of the model is necessary. We designed this modified impactor to provide an available alternative.

The selected modeling parameters may vary for different SCI contusion devices, so the degree of damage and behavioral scores cannot be compared. In this study, the use of different strike heights yielded differential behavioral scores, and the BBB scores showed a tendency to recover over time, confirming that the striker is indeed feasible.

Following your valuable suggestions, we have added a section on the improvement of the striker to the abstract and conclusions. Due to word limitations, not much text was added to the abstract.

Changes in the text: We have modified the text as advised (see Page2, line27-28; Page 3, line 49-51; Page 4, line 75-85; Page 28, line 520-524).