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Short communication

High-speed video analysis improves the accuracy of spinal cord compression measurement in a mouse contusion model

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HIGHLIGHTS

- Biomechanical parameters in SCI contusion models need to be better assessed.
- Video analysis improved the assessment of impactor-spinal cord time-to-contact.
- Precise time-to-contact provided more accurate spinal cord compression measurements.

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ABSTRACT

Background: Animal models of spinal cord injuries aim to utilize controlled and reproducible conditions. However, a literature review reveals that mouse contusion studies using equivalent protocols may show large disparities in the observed impact force vs. cord compression relationship. The overall purpose of this study was to investigate possible sources of bias in these measurements. The specific objective was to improve spinal cord compression measurements using a video-based setup to detect the impactor-spinal cord time-to-contact.
New method: A force-controlled 30 kDyn unilateral contusion at C4 vertebral level was performed in six mice with the Infinite Horizon impactor (IH). High-speed video was used to determine the time-to-contact between the impactor tip and the spinal cord and to compute the related displacement of the tip into the tissue: the spinal cord compression and the compression ratio.
Results & comparison with existing method(s): Delayed time-to-contact detection with the IH device led to an underestimation of the cord compression. Compression values indicated by the IH were 64% lower than those based on video analysis (0.33 mm vs. 0.88 mm). Consequently, the mean compression ratio derived from the device was underestimated when compared to the value derived from video analysis (22% vs. 61%).
Conclusions: Default time-to-contact detection from the IH led to significant errors in spinal cord compression assessment. Accordingly, this may explain some of the reported data discrepancies in the literature. The proposed setup could be implemented by users of contusion devices to improve the quantative description of the primary injury inflicted to the spinal cord.

1. Introduction

Experimental animal Spinal Cord Injury (SCI) models aim to reproduce clinical features of human SCI and to control all parameters of the trauma in order to obtain consistent and reproducible injuries. Most human SCIs are caused by blunt trauma induced by disk or bone displacement into the medullar canal (Sekhon and

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Fehlings, 2001). In animal studies, the most common way to reproduce this type of injury is the contusion model (Sharif-Alhoseini et al., 2017) which consists of a dynamic compression of the spinal cord.

Three commercially-available devices are proposed for performing spinal cord contusion in rodent models. The *NYU-MACSIS Impactor* (Gruner, 1992) drops a weight from a pre-defined distance onto the exposed spinal cord while the *ESCID (Ohio State University)* (Jakeman et al., 2009) and the *Infinite Horizon (*IH, *Precision Systems and Instrumentation)* (Scheff et al., 2003) impactors drive a cylindrical tip onto the exposed spinal cord until a user-define displacement (ESCID) or force value (IH) is reached. The ESCID and the IH then estimate the undefined force or displacement imposed on the spinal cord, respectively. The ESCID and IH devices were independently reported to produce reliable and reproducible lesions (Sharif-Alhoseini et al., 2017). However, replication studies of rodent SCI models using both devices alternately have shown great variability in both the injury morphologies and the corresponding neurological effects (Steward et al., 2012). Moreover, similar protocols have led to large differences between force/displacement outcomes. For example, Myers et al. (2014) performed contusions at T9/T10 on C57Bl/6 female adult mice using the IH device with a force input of 50 kDyn and obtained a mean displacement of 0.59 mm whereas Ma et al. (2001) performed contusions on the same animal model with the ESCID device and only required a displacement input of 0.3 mm in order to obtain a mean force of 50 kDyn. Furthermore, Engesser-Cesar et al. (2005) reported significant differences in displacement outcomes for the same force set point value when using two identical IH devices. Potential experimental factors explaining these differences are not identified in the related paper but the lack of sensitivity of the force sensor at the point-of-contact is probably the most important source of variability in this context.

Several factors could account for this lack of consistency in force/displacement outcomes. Failure of the vertebral stabilization system has been frequently mentioned in this regard (Sharif-Alhoseini et al., 2017). The ESCID and IH devices also use different force thresholds to detect contact between impactor tip and the cord (Jakeman et al., 2009; Scheff et al., 2003). In addition, the impactor tip geometry or positioning might also introduce biases. According to Basso et al. (2006), a variation of 0.1 mm in impactor displacement or 10 kDyn in the applied force may lead to important differences in behavioral outcomes. Therefore, there is a crucial need to identify and report these sources of bias and define sustainable mechanical criteria to achieve reproducible injuries.

The main objective of this work was to propose an video-based system to detect impactor-spinal cord time-to-contact and thus, to improve the accuracy of the spinal cord compression measurement. This system was designed to also allow qualitative assessment of the vertebral clamping system effectiveness.

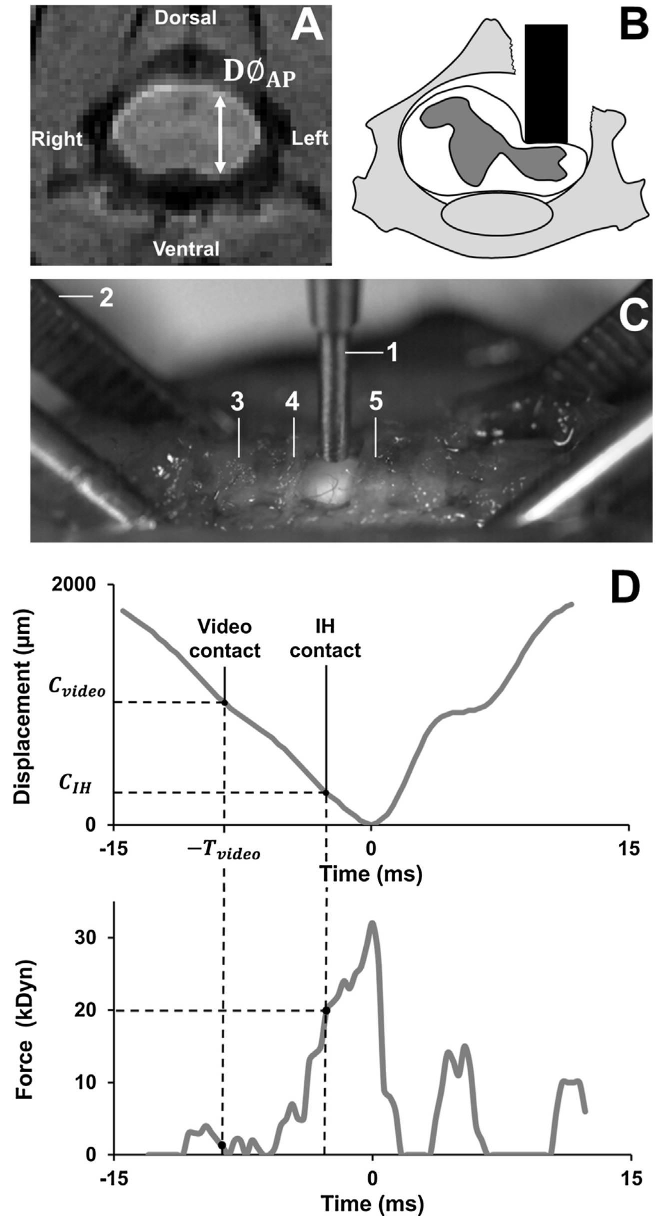

Fig. 1. Representative MRI acquisition (Callot et al., 2007) for antero-posterior diameter ($DØ_{AP}$) measurement of C4 spinal cord (A). Diagram of the contused spinal cord with representive laminectomy extent (B). Representative frame of a Photron SA3 acquisition 1 – impacting tip. 2 – vertebral clamp. 3 – C3 lamina. 4 – partial C4 lamina. 5 – C5 lamina. (C). Representative Infinite Horizon tip displacement acquisition $Disp_{IH}(t)$ and load acquisition $F_{IH}(t)$ (D).

2. Materials and methods

Experiments were performed on a total of six C57BL/6 mice using a protocol approved by our institutional ethics board "Comité d'Ethique Marseille (N°14)" (Approval number 18–26072012).

2.1. Morphological measurements

Anatomical magnetic resonance acquisitions of Callot et al. (2007) on female 17–24 weeks old C57BL/6 mice (n = 9) were retrospectively used to evaluate the mean lateral anteroposterior diameter of the spinal cord ($DØ_{AP}$ in mm). Transverse images at the C4 vertebral level were acquired at 11.75T (Brucker, Ettlingen, Germany) with in-plane spatial resolution of 0.1 × 0.1 mm. Measurements were performed manually along the dorsoventral axis of the cord, on the left side and where the diameter is maximal (Fig. 1A). Measurements were made using ImageJ (Schneider et al., 2012) and repeated three times to evaluate intra-operator repeatability. Maximal right-left diameter was also evaluated.

2.2. Experimental model

The spinal cord contusion was performed using the Infinite Horizon impactor device (IH-0400, version 5.0, Precision Systems & Instrumentation, Lexington, KY, USA) equipped with a IH custom-made 0.6 mm diameter impacting tip. Before the spinal cord impact, mice (Female, 17–30 weeks old, 20.1–23.5 g) were anesthetized and a midline longitudinal skin incision was performed over the upper cervical area. Vertebral muscles were dissected to reach the