Novel Techniques for the Evaluation of Physical Activity in a Large Animal Intervertebral Disc Degeneration Model

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Introduction

Intervertebral disc degeneration (IDD) is a progressive, age-related condition that leads to structural and mechanical failure of the disc. This deterioration is commonly associated with low back pain (LBP). Therefore, pain is the most clinically significant characteristic of IDD, and the ideal animal model should recapitulate pain and functional impairment in addition to structural and mechanical alterations to the disc. Our group previously developed a large animal goat model of IDD that effectively recapitulates the structural and mechanical changes that occur with degeneration[1]; however, intervertebral disc degeneration in sheep or goats does not result in clinically perceptible pain, even at very advanced stages. Various methods currently exist to evaluate activity and pain in small animal models, including: the LABORAS platform, which measures vibration/force for position and behavior tracking[2]; hindpaw withdrawal in response to mechanical (von Frey Test) and thermal (Hargreaves Test) hyperalgesia signifying increased pain sensitivity[3]; and the Rotarod Test, which uses a rotating rod to measure balance and activity endurance[3]. These techniques are not readily translatable to large animal models. An objective tool to assess functional change that is consequent to painful degeneration would be invaluable to evaluation of therapeutics in preclinical animal models. The objective of this study was to develop and validate two novel techniques for quantifying physical activity in an established caprine model of disc degeneration.

Methods

Two male large frame goats, ~2 years of age, were housed together in a 3-sided barn. IDD was induced at 4 lumbar levels per animal via intradiscal injection of 1U chondroitinase ABC. Our previous work showed that this insult results in moderate to severe degeneration of the disc after 12 weeks, as assessed via MRI, disc height, and histology[1]. Over this 12-week period, two methods of activity monitoring were investigated. *Overhead Video-Based Motion Tracking*: A GoPro HERO4 camera recording in SuperView mode was mounted to the barn ceiling to capture live images of the entire pen. Video was recorded for 1 hour per day when humans were not present to capture unprovoked activity. Motion was tracked for one goat in MATLAB using the DLTdv5 texture tracking program[4], which tracks a manually selected monochromatic texture region of interest—in this case the goat's body (Figure 1A). The center of this region for each video frame was then output to Excel as x-y coordinates mapped to the resolution of the video. Frames were grouped into 1-second increments, and the average x-y position was rounded to the nearest whole number. The distance formula was used to calculate change in position between each 1-second increment, and these values were summed over the hour-long video to yield total activity. Activity was monitored during 2 pre-operative weeks to establish baseline activity and from 1-12 weeks following induction of disc degeneration.

Daily activity measurements were binned into two-week periods for analysis. Differences between time points were assessed via unpaired Student's *t*-tests compared to pre-op activity. To test for inter-observer reliability of the video tracking software, pre-op videos were tracked by two observers, and the activity levels were compared via unpaired Student's *t*-test. *Step-Count Quantification using a Custom Wearable Device*: Step count was also measured on a daily basis in a separate goat to characterize activity. A custom built wearable device[5] consisting of a sensor board with gyroscope, accelerometer, and magnetometer; microcontroller; radio; data logger; and lithium polymer battery was attached to the right forelimb proximal to the carpus (Figure 1B). A neodymium magnet was attached distal to the carpus. Discrete steps were identified by local maxima in the magnetic field strength, which occurred with carpal flexion during ambulation. Data from the device was uploaded to a computer each day over a period of 4 weeks prior to surgery, and for 12 weeks following surgical induction of disc degeneration. MATLAB was used to count the number of steps in a 30-minute window each day. Prolonged periods of elevated magnetic field strength—indicating that the goat was lying down—were excluded from the analysis. As with the video tracking data, activity was binned into two-week periods for analysis. Differences between time points were assessed via unpaired Student's *t*-tests compared to pre-op activity.

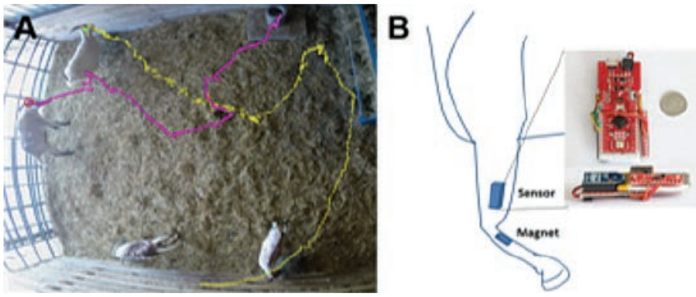

Figure 1. (A) Overhead tracking of goat locomotion from video footage via the DLTdv5MATLAB code. **(B)** Schematic illustrating placement of the custom wearable device on the goat forelimb.

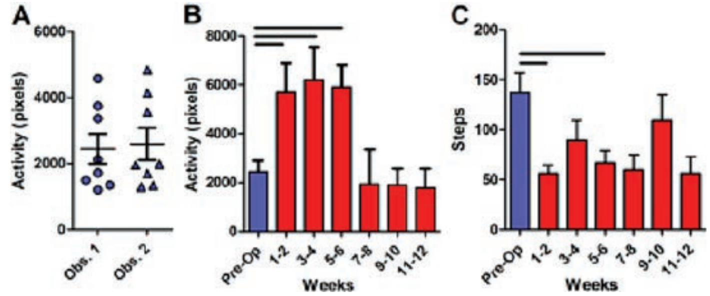

Figure 2. (A) Inter-observer video tracking. **(B)** Video tracking and **(C)** Custom wearable device tracking of daily activity pre-op and 1-12 weeks post-op in two separate goats.

Results

Overhead Video-Based Motion Tracking

No significant difference in pre-op activity level was found between observers (Figure 1A). There was a significant increase ($p < 0.05$) in activity from 1-6 weeks post-operative compared to pre-op baseline, followed by a return to baseline activity from 7-12 weeks post-op (Figure 2B).

Step-Count Quantification using a Custom Wearable Device

A significant reduction ($p < 0.05$) in activity 1-2 and 5-6 weeks post-op was observed compared to the pre-op baseline, with 7-12 weeks post-op also trending towards decreased activity ($p = 0.0614$ at 11-12 weeks) (Figure 2C).

Discussion

We developed two novel, independent methods for quantifying large animal activity in a model of lumbar disc degeneration and demonstrated that both methods are able to detect changes in activity over time. While activity levels differed between the two goats immediately post-surgery, both tracking methods show a long-term trend towards returning to or below baseline. Ongoing work will further validate these methodologies to explore and optimize relationships between disc degeneration and functional parameters in large animals. Concurrently we are assessing biomarker signatures such as serum inflammatory markers and immunohistochemistry for nociceptive nerve fibers. Recently, NIH leaders called for improved transparency and reproducibility in animal models[6,7]. Our activity monitor methodologies described here combined with competent physical examination will offer a platform for improved *in vivo* assessment when using large animal models. Other applications for the wearable device include tracking limb movement during augmentation of orthopedic hardware in fragility fractures or tracking three-dimensional head and neck kinematics in future work involving goats undergoing cervical total disc replacement.

Conclusions

Use of these novel activity monitoring techniques in large animal models of musculoskeletal disease will enhance the clinical relevance of these models by improving scientific rigor and model fidelity resulting in a more predictable translation to human clinical use.

References

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