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Sexual Function

ORIGINAL ARTICLE

Kinematic analysis of penile reflexes in a rat model of spinal cord injury

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The *ex-copula* penile dorsiflexion reflex (PDFR) is an established measure of sexual dysfunction in male rat models of spinal cord injury. Although the PDFR after complete spinal transection is well described, information regarding the more clinically relevant incomplete spinal contusion injury model is limited. This study examined, using two-dimensional (2D) kinematic analysis, the relationship between the PDFR and degree of white matter sparing (WMS). Male Wistar rats received a T9 contusion with varying degrees of impactor forces. Weekly kinematic recordings of the PDFR were made 3–8 weeks postinjury. Sexual reflex components examined included maximum angle of penile dorsiflexion, total penile event duration, and penile ascent speed. *Post hoc* comparison between animals grouped based upon injury severity (moderate–severe: 13.33%–17.15% WMS *vs* moderate: 20.85%–33.50% WMS) indicated PDFR effects. Specifically, the numbers of animals with more moderate contusions having data points above the median in both maximum angle of penile dorsiflexion and penile ascent speed were significantly lower than animals with more severe injuries. Total penile event duration was also affected but only at more chronic time points (6–8 weeks). Thus, 2D kinematic analysis of the PDFR allows for more consistent and quantifiable analysis of the subtle differences that can occur between injury severity groups in the rat contusion model.

INTRODUCTION

Spinal cord injury (SCI) results in widespread multi-system neurological impairment that includes motor, autonomic, and sensory deficits. One deficit, sexual dysfunction, is a high priority quality of life issue for SCI individuals. Scores from quality of life assessments directly correlate with scores from sexual assessment scales, including the Sexual Interest and Satisfaction Scale and the Sexual Adjustment Scale.[1,2] Such deficits in sexual function in the male SCI population include erectile and ejaculatory dysfunction and impaired fertility.[1] After SCI, short-lived erections with insufficient rigidity occur,[3,4] as well as anejaculation or dribbling ejaculation due to dyssynergia of the bulbospongiosus (BSM), ischiocavernosus (ICM), and urethralis muscles and retrograde ejaculation due to improper closure of the bladder neck.[5–7] Despite the high priority level among SCI males, few experimental studies have focused on sexual dysfunction in a relevant preclinical animal model. Currently, a shortage of sensitive measures for human sexual function, as well as in preclinical animal models, contributes to the gap in research.[8]

One measure of sexual function in the animal model is the *ex-copula* penile dorsiflexion reflex (PDFR). In the rat, mechanical retraction of the prepuce may trigger the PDFR, which consists of engorgement of the penile body, penile glans tip cupping, and dorsiflexion of the penile body.[9] Glans engorgement and cupping is due to activation of the BSM (whose motoneuron pool resides in the L6–S1 dorsomedial nucleus), while penile body dorsiflexion is due to

activation of the ICM (whose motoneuron pool resides in the L5–L6 dorsolateral nucleus).[10–13] The PDFR is difficult to elicit in spinally intact male rats[12] due to tonic descending inhibition from supraspinal brainstem centers[14–16] but is easily evoked after spinal transection.[10,12,14]

Disruption of bilateral descending reticulospinal projections reduces the synaptic efficacy of the dorsal nerve of the penis (DNP) onto motoneurons controlling the perineal musculature. This desensitized circuitry allows for a hyperexcitable state of reflex activity.[14,15] After spinal cord lesion, the latency to onset of the PDFR is significantly reduced, with increases in number and intensity of penile dorsiflexion and glans cupping, as compared to noninjured animals.[12,17] Injury-induced alterations in sexual reflex circuit excitability allows for a quantifiable measure of sexual function; however, there is limited knowledge on the state of the circuitry where residual fibers remain traversing the injury (*i.e.*, with a more clinically-relevant contusion injury rather than a complete spinal transection which rarely occurs clinically). Therefore, the purpose of this study is to assess if there is a relationship between the sexual reflex response and injury severity using kinematic analysis of the PDFR to detect potentially subtle changes in the erectile response.

MATERIALS AND METHODS

Animal care

All procedures were carried out according to the National Institutes of Health guidelines, and protocols were reviewed and approved by

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the Institutional Animal Use and Care Committee at the University of Louisville, School of Medicine, Louisville, KY, USA. Twelve adult male Wistar rats (approximately 300 g) were individually housed on a standard 12-h light/dark cycle. To ensure a range of incomplete injury extents, the animals were randomized to one of three T9 level contusion severity groups: 150 kilodyne (*n* = 4), 175 kilodyne (*n* = 4), or 215 kilodyne (*n* = 4) injury force performed using an Infinite Horizons Impactor (Precision Systems and Instrumentation, LLC, Fairfax Station, VA, USA).[18] The Basso, Beattie, and Bresnahan (BBB) scale was used to assess overground locomotion preoperatively, postoperatively, and before sacrifice.[19] PDFR kinematic testing was performed once before injury (after handling and habituation to restraint) and at weekly intervals beginning at week 3 postinjury.

Animals also underwent weekly mating behavior testing utilizing a telemetric pressure transducer (PA-C10, Data Science International [DSI], St. Paul, MN, USA) to record penile pressures during awake mating behavior. Three weeks before contusion SCI, animals were implanted with the telemetric pressure transducer into the corpus cavernosum of the penis. Animals underwent PDFR testing both pre- and post-pressure catheter implant. No animals had a PDFR at either time point. Before contusion, the animals underwent several mating behavior tests, and at 3 weeks postcontusion, the animals underwent mating behavior testing weekly. These data are published elsewhere.[20]

Spinal cord injury

The following procedures can be viewed in a recent video journal published by the Hubscher Lab, University of Louisville, Louisville, KY, USA.[20] Animals were anesthetized using an intraperitoneal injection of ketamine (80 mg kg⁻¹, Ketoset®; Fort Dodge Laboratories, Fort Dodge, IA, USA) and xylazine (10 mg kg⁻¹, AnaSed®; Lloyd Laboratories, Shenandoah, IA, USA). Each animal was prepared for surgery, injected subcutaneously with 5 ml of sterile saline solution, and placed in a prone position on a heating pad to maintain a body temperature of 36°C–37°C. The T7–T9 vertebrae were exposed via a rostral/caudal incision, and the T8 lamina was removed to expose the T9 spinal cord. The spinal column was stabilized using spinal clamps attached to the T7 and T9 processes. Incomplete lesions were made using the Infinite Horizon (IH) Impactor Device (Precision Systems and Instrumentation, LLC) followed by closure of the muscle and subcutaneous tissue using 4-0 Ethicon nonabsorbable surgical suture and Michel clips for skin closure.

A topical antibiotic (bacitracin) was placed on the wound immediately after closure. Animals then received another 5 ml of subcutaneous saline solution for hydration, 0.1 ml of Penject® dual penicillin (The Butler Company, Columbus, OH, USA) as a general prophylactic, and 0.3 ml of gentamicin (GentaFuse®; Butler Schein, Dublin, OH, USA) to prevent bladder infection. The gentamicin regimen was continued for 5 days postsurgery, and 0.2 ml of meloxicam (Eloxiject, Henry Schein, Melville, NY, USA) was also given twice a day for 3 days postsurgery for pain management. As per established protocols,[20–23] animals' bladders were emptied three times per day using the Crede procedure until the animals were reflexively voiding without assistance. Animals had a 2-week recovery period before any behavioral testing. At 14 days post-SCI, the BBB locomotor assessment was used as an early assessment of injury severity. PDFR testing was performed weekly for 6 weeks postinjury after completion of the 2-week recovery.

Penile dorsiflexion reflex

Awake animals were placed in a soft cylinder cotton cloth with hindquarter exposure and placed in a dorsal recumbency on a platform.

The prepuce was then retracted to expose the penis. A set timer was used to record the latency to the onset of the initial PDFR and one subsequent PDFR. Penile dorsiflexion of the penis was required for penile movement to be considered a PDFR response. Glans cupping often accompanies penile dorsiflexion but is not required for PDFR classification. Animals were timed for two PDFR events; if no PDFR event occurred within 20 min, the animal was considered negative for the reflex at that time point.[24] Throughout both PDFR events, the number of dorsiflexions (penile "flips") and glans cupping events was recorded.

PDFR kinematic recording and analysis

Kinematic analysis was performed weekly after a 2-week recovery period postinjury. Permanent markers were used to place point-of-reference dots on the platform for consistent measurements between animals. Before beginning the PDFR test, point-of-reference markings were placed on the skin superior to the prepuce and on the glans penis. PDFR testing was recorded from a sagittal viewpoint using a high-speed video camera (Basler ace acA645gm, Basler Inc., Exton, PA, USA) with a capture rate of 100 frames per s. Video analysis was performed using MaxTRAQ software (Innovision Systems, Columbiaville, MI, USA). Animals were recorded for two PDFR events; if the animal was negative for a PDFR, no event was recorded.

Kinematic analysis of the PDFR using markings on the platform and animal examined three elements: maximum angle of penile dorsiflexion (MAPD), total penile event duration (TPED), and penile ascent speed (PAS). A schematic representation is presented in **Figure 1**. MAPD was measured using marks placed at the base of the prepuce, tip of the glans penis, and permanent relation platform marks. Analysis began at the first penile movement from the rest position. The MAPD was determined to be the maximum calculated angle created by the vectors of (1) the two permanent platform marks (points **e** and **f**; **Figure 1**) which spanned 5 cm in a straight line and (2) the vector created by the marks placed at the glans tip and base of the penis (points **a–c** and **d**; **Figure 1**) during a penile dorsiflexion. TPED was calculated as the time of the full penile dorsiflexion event,
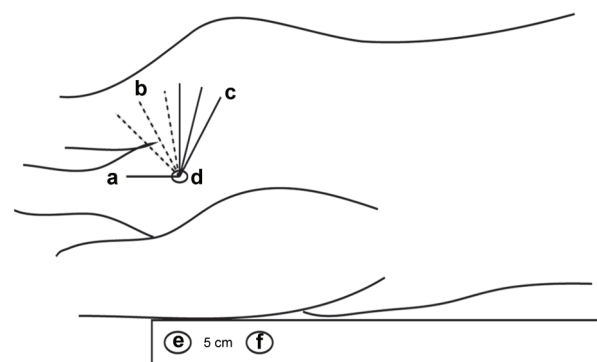

Figure 1: Schematic of PDFR kinematic recording setup. Line **a** represents the penis at rest after prepuce retraction. Line **b** (dashed) represents maximum angles of penile dorsiflexion of animals with a moderate injury. Line **c** (solid) represents maximum angles of penile dorsiflexion of animals with a moderate–severe injury. Point **d** is a representative of the marker placed on the base of the penis during PDFR testing. Points **e** and **f** are representatives of the permanent reference markers on the PDFR testing platform. The angle between the vectors created by points **e/f** and **a/b/c–d** is the measured angle for MAPD. The permanent markers were placed 5 cm apart to allow for distance calibration. This schematic is not drawn to scale. MAPD: maximum angle of penile dorsiflexion; PDFR: penile dorsiflexion reflex.

from first movement from rest position, to return to rest. PAS was determined from the distance (in cm) traveled from first movement from penile rest position until the maximum angle was reached and the time (in s) that it took for this to occur. It is common for the penis to remain at the maximum angle for several frames (*i.e.*, several ms). During PDFR testing where more than one penile dorsiflexion occurred during the two recorded PDFR events, measures were calculated as an average of all individual MAPD, TPED, or PAS measures.

Histology of lesion epicenter

After final PDFR testing, animals were administered a lethal dose of anesthesia and perfused with saline exsanguination fluid immediately followed by a paraformaldehyde/heparin solution and the tissue processed as previously described.[25] Briefly, the T6–T12 spinal cord was removed postperfusion and placed in a 4% paraformaldehyde solution for 2–4 days at 4°C. Twenty-four hours before sectioning, the tissue was moved to a 30% sucrose/phosphate-buffered solution. The tissue was then transversely sectioned at 20-μm thickness on a cryostat and mounted onto slides. The Kluver–Barrera method was then used to stain the tissue to visualize the white and gray matter.

A Nikon E400 microscope (Nikon, Melville, NY, USA) and Spot Advanced software (Diagnostic Instruments, Sterling Heights, MI, USA) were used to capture tissue images for analysis and obtain measurements to quantify lesion extent. As previously described,[23,26] total white matter sparing was assessed based upon intact areas averaged from above and below the level of injury at the lesion epicenter. The left/right white matter sparing was further assessed by subregions in a section from the region having the largest lesion volume: dorsal columns, dorsolateral funiculus, ventrolateral funiculus, and ventromedial funiculus. The central canal, the medial edges of the dorsal horn, and the ventral horn tips were used as landmarks to guide cord divisions.

Statistical analyses

Analysis was performed using Excel (Microsoft Office, Seattle, WA, USA) and SPSS Statistics (IBM, Armonk, NY, USA). The Levene test for inequality was used to determine the equality of variance. PDFR testing was first examined on a weekly basis. Due to the nature of the PDFR testing, animals who were negative at any time point were not included for that specific time point (*i.e.*, if the animal was negative for PDFR at week 3, it would only have two points of data for the early time point). The binomial proportions two-tailed test and the Spearman's rank-order correlation test were used for *post hoc* analyses of the kinematic data.

RESULTS

In this study, the PDFR of 12 animals was examined by kinematic analysis. Before SCI, all animals were tested and 100% exceeded the 20-min PDFR testing cut-off period, and thus, no intact control values are reported. Weekly PDFR kinematic analysis began during the 3rd week postinjury. Postinjury analysis of Infinite Horizon-generated data revealed actual forces and displacements consistent with two clusters of injury severities reflecting moderate and moderate–severe extents (characterization terminology labels used are based upon perceived level of functional deficits), except for one animal outlier per group. WMS and 2-week BBB for those two animals met outlier test criteria, which was used as justification for group re-assignment. Note that *post hoc* WMS was used to determine the extent of injury (typical example provided in **Figure 2**) rather than IH impactor output force values at the time of injury.

The combined distribution of all individual animal kinematic data, including angle, event duration, and speed, is summarized in **Figure 3**. Based upon the median distribution of all data points (see horizontal
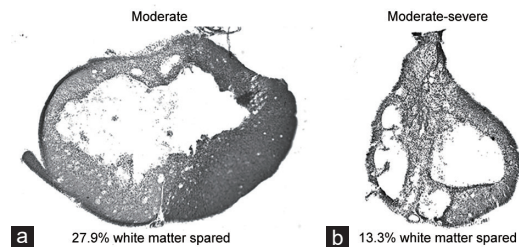

Figure 2: Representative spinal cord segments at the lesion epicenter of (**a**) a moderate injury above 20% white matter sparing (actual value range for current study animals: 20.85%–33.5% WMS) and (**b**) a moderate–severe injury above what would be considered more severe (between 0 and 5%–10% sparing) but below 20% (actual value range for current study animals: 13.33%–17.15% WMS) SCI animal. WMS: white matter sparing; SCI: spinal cord injury.

line in each plot), MAPD, TPED, and PAS were allocated to high and low/no performer groups with respect to median split of the recorded measurements.[25] High performers are thus designated as large MAPD (>71.06°), long TPED (>0.86 s), and high PAS speed (>7.72 cm s^{-1}). In addition, there were observational trends in the data in early weeks (postinjury weeks 3–5) relative to late weeks (postinjury weeks 6–8), resulting in the separation of data based upon time after injury and thus subsequent analysis into 3–5-week and 6–8-week time points. The binomial proportions two-tailed test and the Spearman's rank-order correlation test were used for the analysis of kinematic data after the determination of high and low/no performer status.

PDFR testing was analyzed at postinjury weeks 3–5 and 6–8 time points. At the early time points (postinjury weeks 3–5 combined), the number of moderate injury animals positive for a PDFR response was significantly fewer than the number of PDFR-positive moderate–severe injury animals ($P < 0.005$). Similarly, at the late time points (postinjury weeks 6–8 combined), the number of PDFR-positive moderate injury animals was significantly fewer than the number of moderate–severe injury animals presenting with a PDFR response ($P < 0.001$; **Figure 4**). There were no significant differences between time points in either injury severity group.

Injury severity affects MAPD, TPED, and PAS

Kinematic analysis of the PDFR allowed for the detection of the MAPD, TPED, and PAS. The MAPD was determined by the maximum angle reached by the angle formed between the vectors of the reference points and the base of the penis to the glans tip (**Figure 1**). Median split was used to determine large (>71.1°) versus small (≤71.1°) angles. The number of moderate injury animals with large angles was significantly fewer than the number of moderate–severe injury animals with large angles at the early time point ($P < 0.05$), as well as at the late time point ($P < 0.001$; **Figure 5a**). There were no differences within injury groups between time points.

TPED was determined by the duration of the penile event from the first framed movement of rest to the point of return to rest. Median split was used to sort durations into long (>0.86 s) and short (≤0.86 s) durations. At the early time point, there were no significant differences between the number of moderate and moderate–severe injury animals with a long or short duration. At the late time point, there were significantly fewer moderate injury animals with long durations as compared to moderate–severe injury animals ($P < 0.01$). There were no differences within injury groups between time points (**Figure 5b**).

PAS was determined by the distance traveled over time by the penile body from rest to the point of MAPD. Median split was used
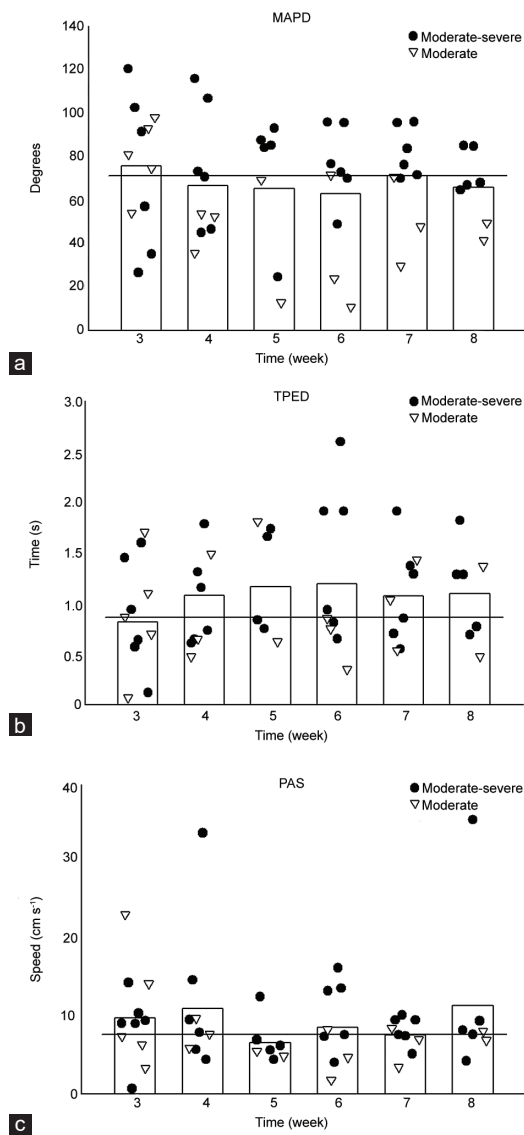
Figure 3: Distribution of individual rat data over 6-week testing periods (3–8 weeks post-SCI). (**a**) MAPD; (**b**) TPED; and (**c**) PAS. The horizontal line represents the median measurement. MAPD: maximum angle of penile dorsiflexion; TPED: total penile event duration; PAS: penile ascent speed; SCI: spinal cord injury.

to determine high (>7.7 cm s⁻¹) versus low (≤7.7 cm s⁻¹) speed. The moderate injury group had significantly fewer animals with high speeds at early time point as compared to the moderate–severe injury group ($P < 0.05$). At the late time point, the number of moderate injury animals presenting with high PAS was significantly fewer than that of moderate–severe injury animals ($P < 0.05$). There were no differences within injury groups between the early and late time points (**Figure 5c**).

MAPD and TPED are inversely correlated with WMS
MAPD and TPED were compared against percent WMS using the Spearman's rank correlation test. MAPD was significantly correlated inversely with percent WMS ($\rho = -0.507$; $P = 0.010$), where with increasing WMS, there were smaller MAPD (**Figure 6a**). Similarly, TPED was significantly correlated inversely with percent WMS ($\rho = -0.410$; $P = 0.042$), where with increasing WMS, there were shorter durations of the TPED (**Figure 6b**).
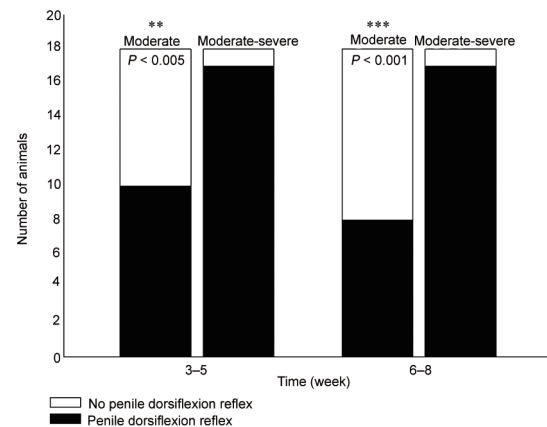
Figure 4: Penile dorsiflexion reflex occurrence in moderate- versus moderate–severe-injured animals at early and late time points (18 measures in total, one per week for a 3-week period, in 6 rats per group). The number positive for the PDFR in animals with moderate injury severities was significantly fewer than those with moderate–severe injuries at the early time point (**$P < 0.005$). At the late time point, the number with a positive PDFR test was significantly fewer for moderate than moderate–severe groups of animals (***$P < 0.001$). PDFR: penile dorsiflexion reflex.

DISCUSSION
During the PDFR, penile glans cupping and penile dorsiflexion are directly related to copulatory behavior, where glans cupping ensures proper seminal plug placement against the female's cervix and penile dorsiflexion allows for intromission.[11,26,27] The PDFR is difficult to elicit in the intact rat as it is under tonic descending brainstem inhibition. If the PDFR response does occur in the intact rat, the penile dorsiflexions and glans cupping are less extreme as what is seen in the spinally lesioned rat.[9,12] Supraspinal tonic bilateral descending inhibition originates in the nucleus paragigantocellularis (nPGl) of the medullary reticular formation and travels through the reticulospinal pathway within the lateral funiculus in the rat,[26] where these projections eventually reach the motoneuron pools in the lumbosacral cord controlling the perineal musculature, specifically the BSM (L5–L6) and the ISM (L6–S1).[14–16] Disruption of these descending projections allows for a hypersensitivity of the reflex circuitry responsible for the PDFR. In this study, moderate injury animals with a percent WMS between 20.85% and 33.50% had significantly fewer instances of PDFR than moderate–severe injury animals (13.33%–17.15% WMS) at both early and late time points. This difference is consistent with the mediolateral and dorsoventral distribution of reticulospinal fibers,[26] whereby a bilateral contusion injury with a central core lesioned area and differential rim sparing infringes to different degrees upon the lateral funiculus (**Figure 2**).

Intensity scoring, where glans engorgement and penile dorsiflexion were qualitatively categorized based upon cup intensity and penile angle observation, has previously been used as a method of scoring the PDFR.[15,27,28] In this study, two-dimensional (2D) kinematic analysis was used as a method to gather a more robust quantification of PDFR intensity. The moderate injury group had significantly fewer animals with large MAPD at both early and late time points, suggesting overall less intense penile dorsiflexion. The duration of the penile dorsiflexion from first movement from rest, to maximum angle, back to rest, demonstrates the sensitivity of the perineal musculature motoneuron pool. In the current study, the moderate injury group had significantly fewer animals with long event durations at the late (more chronic post-SCI) time point. As TPED is defined as the duration from the
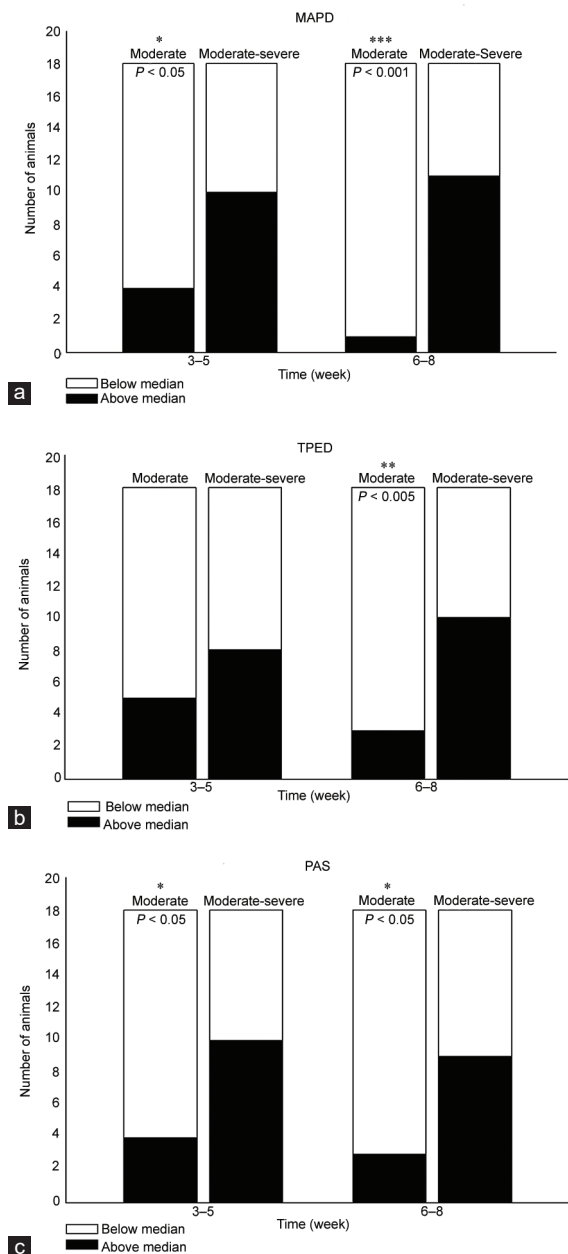
Figure 5: Injury severity affects MAPD, TPED, and PAS high and low performers at early and late time points (18 measures in total, one per week for a 3-week period, in 6 rats per group). (**a**) The number of moderate injury animal measurements with a large MAPD was significantly fewer than the number of moderate-severe injury animal measurements with a large MAPD at both early ($^*P < 0.05$) and late ($^{***}P < 0.001$) time points. (**b**) The number of moderate injury animal measurements with a long TPED was significantly fewer than that of the moderate-severe injury animal measurements at late time points ($^{**}P < 0.005$). (**c**) The number of moderate injury animal measurements with a high speed of penile ascent was significantly fewer than the number of moderate–severe injury animal measurements with a high speed of penile ascent at both early ($^*P < 0.05$) and late ($^*P < 0.05$) time points. MAPD: maximum angle of penile dorsiflexion; TPED: total penile event duration; PAS: penile ascent speed.

first penile movement from rest to the return of the penile body to rest, TPED measurement includes the duration at which the penis remains at the maximum angle reached. During the PDFR, pulsatile contractions of both the BSM and ICM allow for "elongated" dorsiflexions to occur,
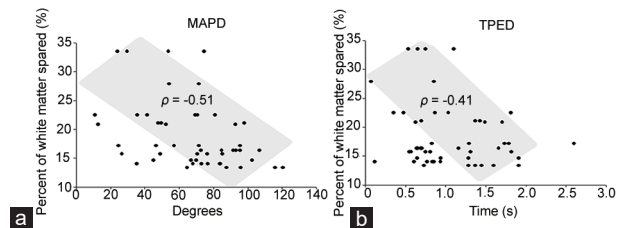

Figure 6: (**a**) MAPD across all six weeks of analysis is inversely correlated with the percent of white matter spared (moderate $n = 6$; moderate–severe $n = 6$; $\rho = -0.507$). (**b**) TPED across all 6 weeks of analysis is inversely correlated with the percent of white matter spared (moderate $n = 6$; moderate–severe $n = 6$; $\rho = -0.410$). Shaded area indicates negative trend in correlation, where Spearman's rank coefficient is represented by ρ. MAPD: maximum angle of penile dorsiflexion; TPED: total penile event duration.

which is atypical of copulatory behavior.[10,29] Therefore, a long PDFR duration suggests a more severe sexual deficit phenotype, which is more prominently seen in the moderate–severe injury group and consistent with a larger injury extent. Greater intensities of the PDFR as measured by MAPD and TPED in the moderate–severe injury group are likely due to increased hypersensitivity of the perineal motoneurons, where the ICM and BSM of moderate–severe injury animals have an increased contraction in response to initiation of the PDFR as compared to that of the moderate injury animals.

Penile dorsiflexion speed has previously yet to be examined as a measure of sensitivity in the PDFR. The PAS is the measurement of distance (cm) over time (s) with which the penile body moves toward MAPD, or alternatively, PAS is representative of pulsatile contractions of the ICM.[10] Penile dorsiflexion speed measurements are yet another measure of intensity, though examining the speed with which the ICM can propel penile dorsiflexion allows an additional examination of ICM motoneuron hypersensitivity after SCI. Here, we see that numbers of moderate injury animals with high PAS are significantly fewer than that of moderate–severe injury animals at both early and late time points, suggesting an increased hypersensitivity of the ICM motoneurons in the moderate–severe injury group. Although we expected to see differences between injury severity groups at both time points in all measured parameters, PDFR duration differences were only seen at late time points. This is likely due to recovery that is still ongoing at early time points and is likely complete by late time points.[30]

The reticulospinal tract travels through the dorsolateral quadrant of the rat spinal cord at the level of injury in this study (T9) and therefore receives significant insult with contusion injury.[26] Overall, the differences between intensity measurements in the PDFR between injury severity groups are likely due to the residual reticulospinal projections traversing the lesion epicenter and remaining reticulospinal pathways in the rim of the dorsolateral cord, where more percent WMS is indicative of increased spared descending bilateral tonic inhibition onto the circuitry responsible for sexual reflexes. Indeed, both MAPD and TPED are inversely correlated with WMS, where increases in percent WMS led to decreases in angle size and duration, supporting the idea for a discreet pathway for supraspinal control over the coordination of perineal musculature.

Utilizing 2D kinematic analysis of the PDFR allows for a more consistent and quantitative measure of PDFR intensity, allowing for the detection of more subtle differences occurring between injury severity groups than observational approaches. These differences between >20% and <20% detected with PDFR are consistent with telemetry mating results from the same group of animals,[20] which showed significantly

lower intracavernosal pressures and intromission durations (relative to preinjury) in animals with more severe levels of injury (below 20% of WMS). However, kinematic analysis of the PDFR was able to detect more subtle differences between moderate and moderate–severe injury groups that were not detectable through the telemetric intracavernosal pressure measurements during sexual mating behavior. Thus, utility of this novel method for examining sexual dysfunction after SCI provides a further quantitative outcome for testing future therapeutic targets, one that could be used to detect even small differences between injury severities.

AUTHOR CONTRIBUTIONS

CJS carried out behavioral testing, kinematic recording, statistical analysis, study design, and manuscript draft. CJS and SSV shared the task of kinematic data analysis. CHH participated in study design and coordination and helped to draft the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

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