

STANDARD ARTICLE OPEN ACCESS

Small Animal Internal Medicine Neurology

Prognostic Utility of F-Waves in Paraplegic Dogs With Absent Pain Perception Secondary to Intervertebral Disc Extrusion

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ABSTRACT

Background: Approximately 50%–60% of paraplegic deep pain negative (DPN) dogs secondary to thoracolumbar intervertebral disc extrusion (TL-IVDE) recover ambulation after surgery. Mean F-wave duration has been associated with injury severity in TL-IVDE in dogs, but the relationship to outcome is unknown.

Objective: Evaluate the prognostic utility of F-waves in DPN dogs secondary to TL-IVDE treated surgically.

Animals: Thirty client-owned, acutely paraplegic DPN dogs secondary to TL-IVDE were managed surgically.

Methods: Multi-center prospective and observational study. F-waves were performed at baseline (within 24 h post-operatively), 2–4 weeks and 3 months post-operatively. Outcome was categorized as successful or unsuccessful, with success defined as independent ambulation at 3 months post-operatively. F-wave variables were compared between dogs with a successful or unsuccessful outcome and over time using generalized estimating equations. Receiver-operating characteristic curves were generated for baseline F-wave variables.

Results: F-waves were well-tolerated in all dogs. Of 30 enrolled dogs, 12 dogs had a successful outcome, 10 dogs were unsuccessful, and 8 dogs were removed from outcome analysis (3 progressive myelomalacia, 1 severe spinal shock, 2 technical error, and 2 unknown outcome). Baseline mean F-wave duration (displayed as median (range)) was longer in unsuccessful dogs (31.7 (11.4–60.8) ms) versus successful dogs (19.6 (10.8–27.3) ms), $p = 0.003$. Mean F-wave duration > 28.5 ms was 70% sensitive (95% confidence interval (CI): 40%–100%) and 100% specific (95% CI: 100%–100%) in predicting an unsuccessful outcome.

Conclusions and Clinical Importance: F-waves performed shortly post-operatively could aid in predicting outcomes in DPN dogs secondary to TL-IVDE treated surgically.

Abbreviations: L4–S3, fourth lumbar to third sacral; PMM, progressive myelomalacia; SCI, spinal cord injury; T3–L3, third thoracic to third lumbar thoracolumbar intervertebral disc extrusion (TL-IVDE).

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1 | Introduction

Recovery from thoracolumbar intervertebral disc extrusion (TL-IVDE) in dogs varies based on the severity of injury. Among deep pain positive (DPP) dogs, the likelihood of recovery of independent ambulation is good to excellent with medical or surgical management [1]. In paraplegic deep pain negative (DPN) dogs, however, recovery of walking declines to approximately 60% for surgically managed dogs [1–3]. Various prognostic indicators have been investigated to try to predict which severely affected dogs managed surgically will recover. These include measurements on magnetic resonance imaging (MRI) [4–15], as well as blood and cerebrospinal fluid biomarkers [16–23]. While these are promising, the loss of pain perception remains the most reliable factor to predict outcome in TL-IVDE. Several electrodiagnostic tests including transcranial magnetic motor evoked potentials and sensory evoked potentials have been variably associated with outcome in dogs with TL-IVDE [24–29]. Importantly, these tests are frequently unable to be recorded once dogs become non-ambulatory [30–33], limiting their utility in acute, severe spinal cord injury (SCI). Accurate, objective, and readily accessible prognostic indicators are needed for dogs with severe TL-IVDE.

F-waves are an electrodiagnostic test that represents compound muscle action potentials following the antidromic (toward the spinal cord from the stimulation point) conduction of a stimulus along motor axons that activate a subset of α -motor neuron cell bodies in the spinal cord [34]. While F-waves are typically utilized to assess peripheral motor axon function, they also provide information on local circuitry within the spinal cord [35, 36]. An imbalance between excitatory and inhibitory input on spinal cord lower motor neurons (LMNs), which is primarily modulated by descending upper motor neurons (UMNs), alters the LMN pool excitability and can be quantified by F-waves [35]. In people, alterations in F-wave variables have been reported in diseases causing UMN dysfunction [35–38]. In dogs with acute TL-IVDE, F-waves, specifically F-wave duration, are positively associated with increasing neurological severity at the time of diagnosis [39]. This suggests that F-waves can identify more severe UMN SCI. However, it is unknown whether F-wave variables can predict clinical outcomes in severely affected dogs with acute TL-IVDE or how F-waves evolve over time after injury.

The aim of this study was to compare F-wave variables between paraplegic DPN dogs secondary to TL-IVDE managed surgically that do or do not recover independent ambulation. Our secondary aim was to describe the longitudinal evolution of F-waves and look for associations with changes in neurologic status during the post-operative recovery period. We hypothesized that more prolonged F-wave duration documented shortly after surgery would predict a lack of recovery of independent ambulation.

2 | Materials and Methods

2.1 | Study Design and Inclusion Criteria

This was a prospective, observational study. Dogs were enrolled at the Purdue University Veterinary Hospital from September 2022 to April 2024 and the North Carolina State University

Veterinary Hospital from September 2023 to April 2024. To be included, dogs had to be diagnosed with acute TL-IVDE with a neurolocalization between the third thoracic and third lumbar (T3–L3) spinal cord segments and undergo decompressive surgery. Presumptive diagnosis of TL-IVDE was based on computed tomography or MRI features and confirmed during surgery. Dogs were required to be paraplegic DPN of the medial and lateral digits of the pelvic limb toes and tail base on the initial neurological assessment (at diagnosis) and at the time of baseline F-wave testing post-operatively. The duration of signs of neurological dysfunction (other than back pain) had to be ≤ 7 days from onset to surgery. Dogs showing signs consistent with spinal shock were not excluded. Dogs with previous surgery for TL-IVDE, concurrent systemic disease associated with a poor prognosis or serial clinical examination findings consistent with progressive myelomalacia (PMM) prior to enrollment were excluded. The study protocol was approved by both Institutional Animal Care and Use Committees (protocols # 2205002270 and #23-262). Owners provided informed consent prior to enrollment.

Using previously reported values for F-wave duration in dogs with TL-IVDE [39] and a 50%–60% recovery rate, power analysis indicated that 18 dogs would result in 80% power with an alpha of 0.05 to detect a difference in mean F-wave duration of ≥ 3.9 ms between dogs with successful or unsuccessful outcomes. Targeted total enrollment was ≥ 24 dogs to account for 10%–15% death or euthanasia due to PMM after enrollment and 10% loss to follow-up.

2.2 | Study Overview and F-Wave Acquisition

M-waves and F-waves were recorded three times: at baseline (within 24 h post-operatively) and at 2–4 weeks and 3 months post-operatively. Dogs were confirmed to still be paraplegic DPN at the time of baseline testing. In addition to electrodiagnostic testing, a complete neurologic examination was performed at baseline, daily during hospitalization, and at the two recheck visits by a board certified neurologist or neurology resident. Dogs were otherwise managed at the discretion of the attending clinician. Basic rehabilitation exercises were performed during hospitalization (i.e., passive range of motion, assisted standing, and walking) with the recommendation to continue at home. Outpatient rehabilitation was not required or standardized as part of the study. Sedation with 1–5 mcg/kg IV dexmedetomidine (Zoetis Inc., Parsippany, NJ), 0.2 mg/kg IV butorphanol (Zoetis Inc., Parsippany, NJ), 2–3 mcg/kg IV fentanyl (Hospira Inc., Lake Forest, IL), or a combination was used as needed to ensure dogs lay calmly during F-wave testing. As all cases were initially DPN, sedation was not necessary in every case at baseline but was utilized at rechecks for DPP dogs. These sedatives have been shown not to interfere with F-wave recordings [24]. Duration and any adverse effects of electrodiagnostic testing were noted, and body temperature was maintained in the normal range.

Using an electrodiagnostic unit (Sierra Summit, Cadwell, Kennewick, WA or Nicolet Viking Quest, Natus Medical, Middleton, WI) and previously reported protocol and settings, motor nerve conduction studies were performed to confirm

normal peripheral nerve function. M-waves were generated for the left sciatic/tibial nerve by stimulating at the greater ischiatic notch and just proximal to the tarsus and caudal to the distal tibia and fibula, with or without additional stimulation at the level of the stifle, and recording from the plantar interosseous muscles [40, 41]. Monopolar needle electrodes and supramaximal stimulation intensity were used.

F-waves were recorded from the plantar interosseous muscles following stimulation of the left distal tibial nerve just proximal to the tarsus as described previously [42]. The cathode was positioned proximal to the anode for the stimulating electrodes, with the setup otherwise unchanged from the M-waves. Fifteen or 16 consecutive stimulations at supramaximal intensity were applied to produce a series of M-waves followed by F-waves. Tibial nerve length was measured as the distance from the cathode stimulation electrode to the cranial border of the L5 spinous process along the approximate nerve path [42]. Pelvic limb length was measured as the distance from the greater trochanter of the femur to the tip of the fourth digit [43–45].

2.3 | F-Wave Analysis

The following M-wave and F-wave variables were analyzed for each dog (Figure 1):

- a. M-wave variables: Maximum M-wave amplitude was measured from the largest negative to the largest positive peak. For each stimulation, minimum M-wave onset latency was measured as the latency from the stimulus artifact to the initial deflection from baseline. M-wave onset latencies and the distance between stimulation points were used to calculate motor nerve conduction velocity [40].
- b. F-wave duration (ms): Measured for each stimulation from the onset of the initial deflection to the final return to baseline. Mean F-wave duration was calculated as the mean of the individual F-wave durations (15 or 16, depending on the machine). If there was no return to baseline, >100 ms was used (100 ms is the maximum possible duration accommodated by the electrodiagnostic machines) and this trace was excluded from the calculation of mean F-wave duration (since an accurate duration was not possible).
- c. F-wave latency (ms): For each stimulation, minimum F-wave onset latency was measured as the shortest latency from the stimulus artifact to the initial deflection of the F-wave from baseline. Mean F-wave latency was calculated as the mean of the individual F-wave onset latencies.
- d. F-wave conduction velocity (FWCV; m/s): $FWCV = (\text{tibial nerve length} \times 2) / (\text{minimum F-wave latency} - \text{minimum M-wave latency (hock)} - 1)$.
- e. F ratio: $F \text{ ratio} = (\text{minimum F-wave latency} - \text{minimum M-wave latency (hock)} - 1) / 2 \times \text{minimum M-wave latency (hock)}$.
- f. Persistence (%): Percentage of stimulations that produce detectable F-waves, calculated by dividing the number of stimulations that produce an F-wave by the total number of stimulations (0%–100%).

- g. Maximum F-wave amplitude (μV): Measured as the largest amplitude from peak to peak across all stimulations.
- h. F:M ratio: Ratio of maximum F-wave amplitude to maximum M-wave amplitude (μV) $\times 100$.
- i. After-discharge activity: Electrical activity that occurred after F-waves returned to baseline (separated by >2 ms from the F-waves). This was subjectively scored from 0 to 3: 0—No further activity, 1—Mild; sporadic activity, 2—moderate; frequent or semi-continuous activity, 3—Severe; sustained, high-amplitude activity that persisted for the maximum duration accommodated by the machines (100 ms). Mean after-discharge score was calculated as the mean of the scores for each F-wave stimulation.

2.4 | Outcome

Outcome was determined based on neurologic assessment at 3 months post-operatively. A successful outcome was defined as recovery of independent ambulation (ability to take ≥ 50 consecutive unassisted steps at a time). An unsuccessful outcome was defined as being non-ambulatory at 3 months after diagnosis. Dogs that developed PMM were classified as unsuccessful but excluded from outcome analysis due to an inability to record baseline F-waves. Continence and pain perception status were not considered in the definition of success. Dogs that failed to regain pain perception but were able to take ≥ 50 consecutive unassisted steps (i.e., spinal walking) were classified as successful. Dogs with an unknown neurologic status at 3 months were excluded from outcome analysis.

2.5 | Statistical Analysis

Statistical analyses were performed using commercially available software, SAS version 9.4 (Copyright 2024, SAS Institute, Cary, NC, USA) and R version 4.4.1 (R Core Team, 2024, Vienna, Austria). Continuous variables were examined for normality using the Shapiro–Wilk test and reported as median (range). F-wave variables were compared between dogs with a successful or unsuccessful outcome and between time points within outcome groups using generalized estimating equations (GEE). Receiver-operating characteristic (ROC) curve analysis was performed to determine the accuracy of F-wave variables to detect an unsuccessful outcome. p values <0.05 were considered significant.

3 | Results

3.1 | Study Sample and Baseline Clinical Data

Thirty dogs were enrolled. Mean age at diagnosis was 4.6 ± 1.4 years. Mean body weight was 8.6 ± 5.3 kg. There were 14 males (5 sexually intact, 9 neutered) and 16 females (3 sexually intact, 13 neutered). Breeds included Miniature Dachshund ($n=11$), mixed breed dog ($n=8$), French Bulldog ($n=2$), Pomeranian ($n=2$), and 1 each of Cavalier King Charles Spaniel, Maltese dog, Yorkshire Terrier, Shih Tzu, Japanese Chin, Toy Poodle, and American Cocker Spaniel. The presenting complaint

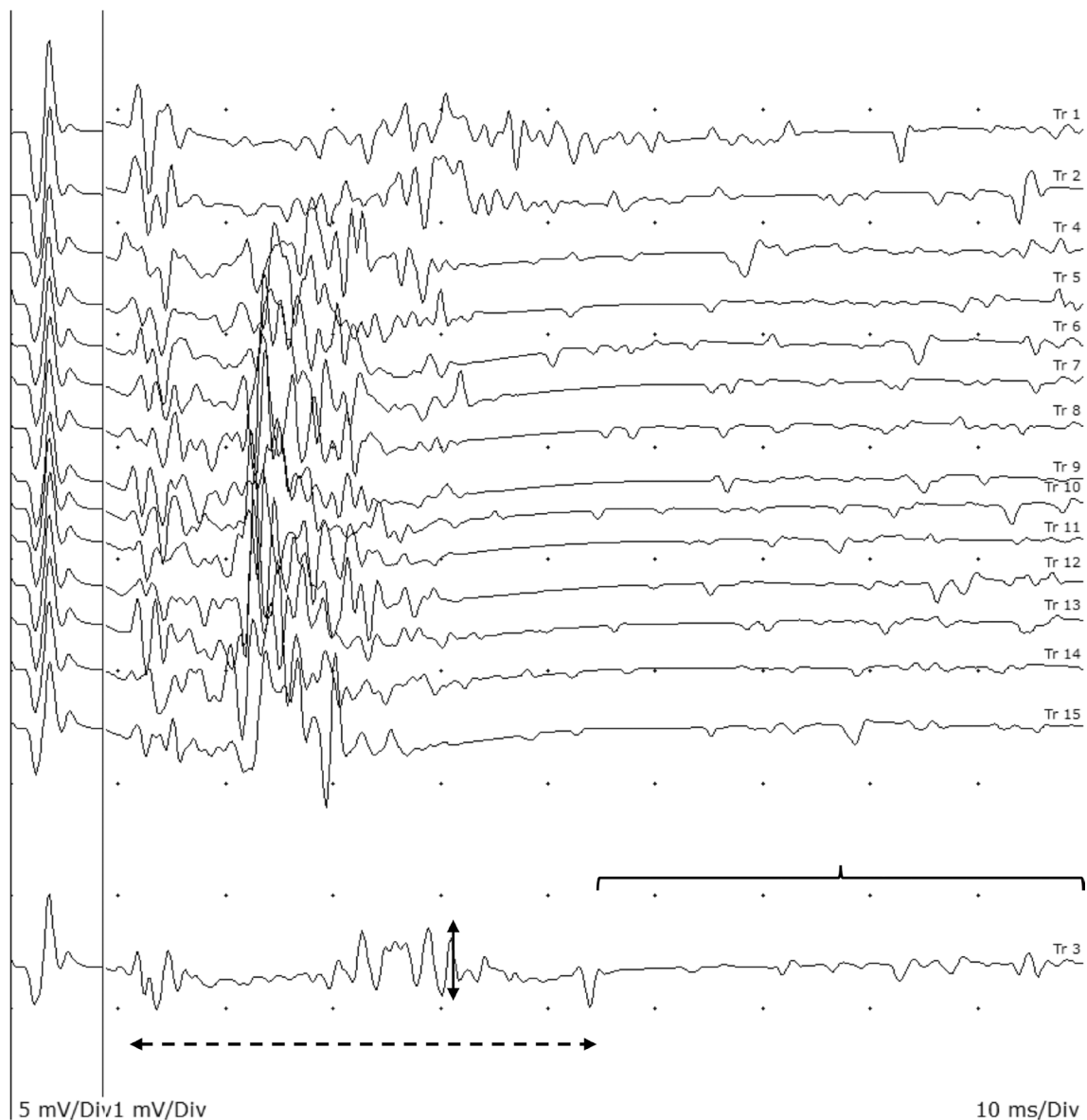


FIGURE 1 | Example F-wave recordings from the initial visit (baseline) in a 3-year-old spayed female Dachshund with L1–L2 IVDE. The dog had an unsuccessful outcome remaining paraplegic deep pain negative. One trace is isolated at the bottom of the figure to distinguish it from the remaining waveforms and to outline specific variables. The dashed double-headed arrow indicates the F-wave duration, the solid double-headed arrow represents the maximum F-wave amplitude, and the horizontal bracket highlights the after-discharge activity.

in all dogs was inability to use their pelvic limbs. Duration of being unable to walk was ≤ 24 h in 13 dogs, 2–3 days in 14 dogs, and 4–7 days in 3 dogs. Compatible with inclusion criteria, all dogs were paraplegic DPN in both pelvic limbs and tail (pre-operatively at diagnosis and post-operatively at baseline testing). The IVDE was between T10–L3 as follows: T10–T11 in 1 dog, T11–T12 in 3 dogs, T12–T13 in 10 dogs, T13–L1 in 8 dogs, L1–L2 in 2 dogs, and L2–L3 in 6 dogs. Hemilaminectomy was performed between T9 and L4, with 14 dogs having a single site and 16 dogs having 2–3 sites.

Clinical signs compatible with spinal shock were noted in 17/30 (56.7%) dogs at the time of baseline testing. This included decreased or absent withdrawal reflex in one or both pelvic limbs in 17/17 (100.0%) dogs, decreased pelvic limb muscle tone in 15/17 (88.2%) dogs, decreased or absent patella reflex in one or

both pelvic limbs in 3/17 (17.6%) dogs, and decreased tail tone in 1/17 (5.9%) dog. Of the 30 dogs, 4 (13.3%) had subjectively severe spinal shock, characterized by absent or severely diminished reflexes and muscle tone in both pelvic limbs.

No dogs had clinical signs compatible with PMM pre-operatively. Three of 30 (10.0%) dogs developed clinical signs of PMM and were euthanized due to presumptive PMM between 2 and 7 days post-operatively; none had confirmation via necropsy. Two of the dogs were suspected to have PMM at the time of baseline testing based on a single examination and characterized by cranial advancement of the cutaneous trunci reflex cutoff compared to the day before, decreased abdominal tone, and patella reflex. One dog had no clinical signs suggestive of PMM at the time of baseline testing and first developed clinical signs of PMM the following day.

3.2 | Follow-Up Clinical Information

Twenty-seven dogs were discharged from the hospital, including 8 dogs that regained pain perception by that time. Two dogs, both with a discharge status of paraplegia DPN, had no further follow-up and an unknown outcome. Twenty-five dogs had information available from the first recheck, including 24 dogs evaluated at the referral hospitals and 1 dog evaluated by the family veterinarian. No dogs demonstrated signs of spinal shock at the first recheck. Twenty-five dogs had information available from the second recheck, including 23 dogs evaluated at the referral hospitals and 2 dogs with follow-up via phone conversation. The owners of the 2 dogs lacking in-person evaluation revealed that one dog was alive but remained paraplegic (with unknown pain perception) and one dog was euthanized at 3 months post-operatively due to lack of any signs of recovery. Five of 25 (20%) dogs participated in outpatient rehabilitation between discharge and the 3 month recheck, with protocols that included laser therapy, underwater treadmill, swimming, and land-based exercises at a frequency of 1–2 sessions per week. Neurologic status at baseline and rechecks among the 25 dogs with follow-up information are summarized in Table 1. By study completion, 11 dogs were DPP (10 of which were ambulatory), 12 dogs were DPN (including 2 spinal walkers) and 2 had an unconfirmed outcome (both presumed to have remained paraplegic DPN). Additionally, 15/25 (60%) dogs exhibited partial (4) or complete (11) urinary incontinence, and 16/21 (76%) dogs in which it could be determined had partial (6) or complete (10) fecal incontinence.

3.3 | F-Waves and Outcome Analysis

Electrodiagnostic testing was performed uneventfully in all dogs with a typical duration of approximately 10 min and with

TABLE 1 | Neurologic status at baseline, first, and second rechecks among the 25 dogs in which follow-up information was available.

Neurologic status (gait, pain perception)	Baseline	First recheck	Second recheck
Normal	0	0	1
Ambulatory paraparesis	0	2	9
Non-ambulatory paraparesis	0	7	1
Paraplegia, DPP	0	1	0
Paraplegia, DPN	25	15	10
Ambulatory paraparesis, DPN (spinal walking)	0	0	2
Unconfirmed status (presumed paraplegia, DPN) ^a	0	0	2

^aUnconfirmed status was presumed to be paraplegia DPN based on owner description of lack of recovery at 3 months post-operatively.

no adverse effects noted. At baseline, F-waves were recorded in 27/30 (90.0%) dogs. No measurable F-waves were detected in the 3 dogs that developed PMM (Figure 2). In 2 other dogs, M-waves were inexplicably diminished, which was attributed to technical or operator error. In 1 dog with severe spinal shock, baseline F-waves were severely diminished to absent, with a persistence of 43%. Two additional dogs where baseline F-waves were recorded had an unknown outcome. Excluding these 8 dogs, the baseline data from the remaining 22 dogs were included in the outcome analysis. Of these 22 dogs, 12/22 (54.5%) dogs had a successful outcome (including 2 spinal walkers) and 10/22 (45.5%) had an unsuccessful outcome. The 5 dogs that underwent outpatient rehabilitation had a successful outcome. Urinary incontinence was present in 3/12 (25%) dogs with a successful outcome and 9/10 (90%) dogs with an unsuccessful outcome. Fecal incontinence was present in 3/12 (25%) dogs with a successful outcome and all dogs with an unsuccessful outcome.

Table 2 compares the baseline F-wave variables stratified by outcome and Figure 3 depicts representative baseline F-waves. In dogs with an unsuccessful outcome, mean F-wave duration was longer, maximum F-wave amplitude was larger, mean after discharge activity score was higher, minimum and mean F-wave latency were shorter, F ratio was smaller, and F:M ratio was larger compared to dogs with a successful outcome ($p < 0.04$). No other significant relationships were identified between other F-wave variables and outcome. Two of 22 (9.1%) dogs exhibited individual F-waves that never returned to baseline, resulting in an F-wave duration of > 100 ms for those stimulations (Figure 4A). While individual F-waves lasting > 100 ms were not included in the calculation, the mean F-wave duration at baseline for these 2 dogs was 54.6 and 32.5 ms. Both dogs had an unsuccessful outcome. Mean after-discharge activity score was $> 2.5/3$ in 5/22 (22.7%) dogs (Figure 4B). Of these 5 dogs, 4 (80.0%) had an unsuccessful outcome.

Among baseline F-wave variables that significantly differed between outcomes, ROC curves were generated (Table 3). Using a cutoff value of 28.5 ms, 70% (95% CI: 40%–100%) of unsuccessful dogs had a mean F-wave duration greater than the cutoff, while 100% (95% CI: 100%–100%) of successful dogs had a mean F-wave duration below the cutoff.

Twenty-three dogs and 22 dogs at the first and second rechecks, respectively, underwent repeat electrodiagnostic testing (Table 4). All dogs had recordable F-waves at both timepoints. Follow-up testing was well-tolerated, quick (approximate testing duration of 10 min) and the sedation protocol allowed data acquisition in dogs that regained pain perception. In the unsuccessful dogs, mean F-wave duration was shorter at the first recheck compared to baseline ($p = 0.01$). In the successful group, maximum F-wave amplitude was significantly higher at both rechecks compared to baseline ($p < 0.02$). No other relationships were identified for the F-wave variables over time, stratified by outcome.

4 | Discussion

Among a group of acutely paraplegic DPN dogs secondary to TL-IVDE managed surgically, F-waves were associated with

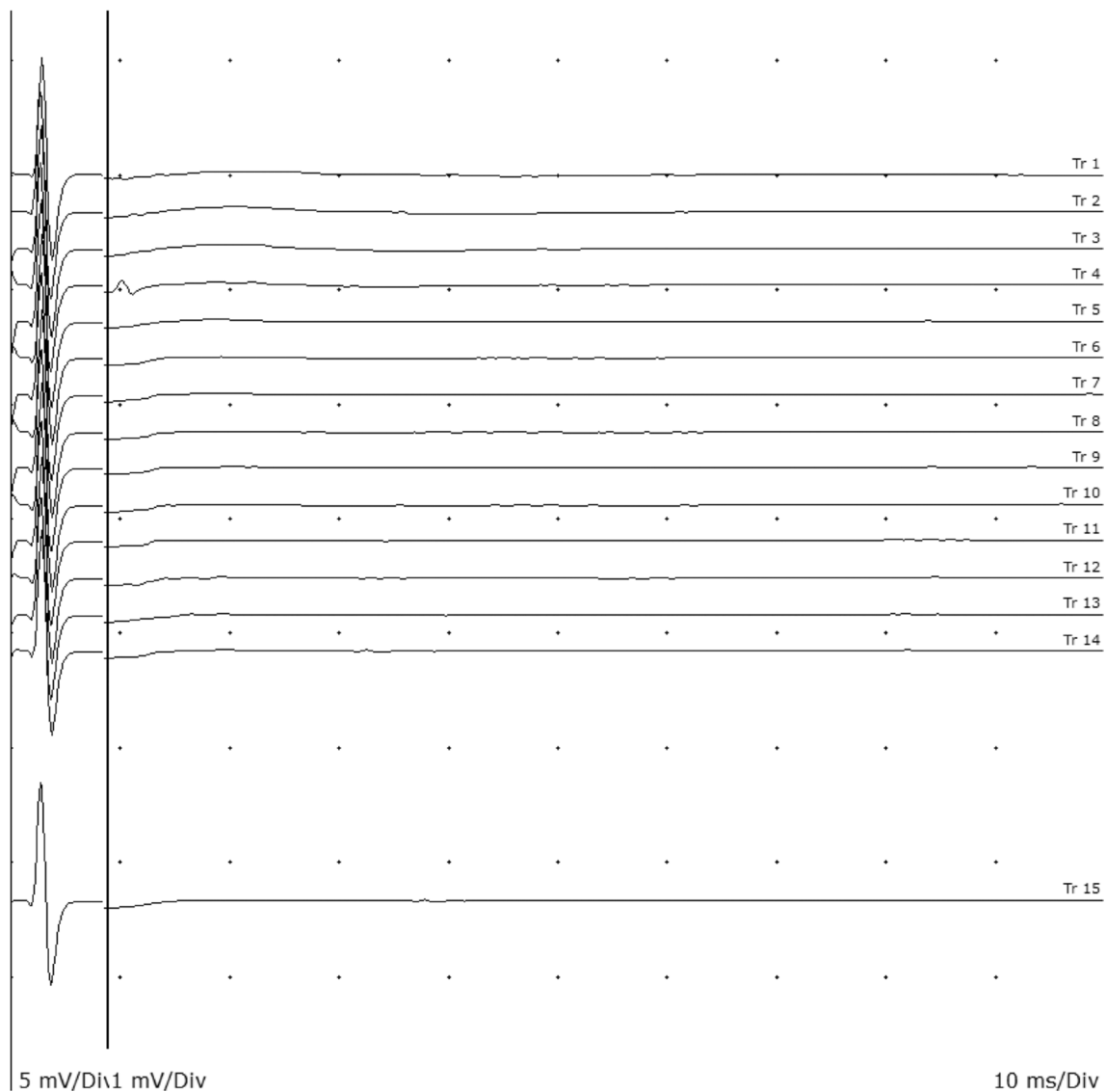


FIGURE 2 | Example F-wave recordings from the initial visit (baseline) in a 4-year-old neutered male Pomeranian who developed progressive myelomalacia secondary to T12–T13 IVDE. At the time of the recording, the patient was paraplegic deep pain negative but had not yet shown clinical signs of progressive myelomalacia. M-waves are present on the left side of the screen, but there are no visible F-waves.

outcome. F-wave testing was well-tolerated and performed without sedation in DPN dogs. Taken together, F-waves could be a useful tool for predicting recovery of walking in dogs with severe TL-IVDE.

F-waves represent compound muscle action potentials resulting from the excitation of a subset of α -motor neurons in the ventral horn of the spinal cord. Since these LMNs are influenced by UMNs, F-waves can be utilized to capture alterations in this relationship and provide information about injury severity. In our study, alterations in F-waves included prolonged duration, high amplitude, short latency, prominent after discharge activity, decreased F ratio, and increased F:M ratio. These derangements in F-wave variables were more pronounced in dogs with poor outcomes, which likely reflected more severe injury. This is in line with a study in dachshunds with acute TL-IVDE [39], where more prolonged mean F-wave duration was associated with greater severity of neurologic dysfunction. Compared to SCI in people, the changes in F-wave variables observed in our

study align better with those seen in the later stages of injury in people with spasticity, where increased F-wave persistence, amplitude, F ratio, and prolonged duration have been reported [35, 46–50]. These changes in people have been attributed to disinhibition of descending supraspinal pathways [35, 46–50]. We speculate that in dogs with acute TL-IVDE, the injury to the UMNs leads to an imbalance of inhibitory and excitatory input to the LMNs, resulting in LMN disinhibition, similar to what is observed in people with spasticity during the subacute to chronic stage of SCI.

There is conflicting data on F-wave latency in people, which is variably reported as prolonged [51] or within normal limits [47, 48]. The reason for the shorter F-wave latency in unsuccessful dogs in our study remains unclear. One possible explanation is limb length. Most dogs were small breeds, but three medium-sized dogs were included and all had a successful outcome. This suggests that latency might be less useful in dogs due to inter-individual variability in limb length.

TABLE 2 | Baseline F-wave variables among the 22 dogs included in the outcome analysis and stratified by successful or unsuccessful outcome at 3 months post-operatively.

F-wave variables	All dogs	Successful	Unsuccessful	<i>p</i>
	(<i>N</i> =22)	(<i>N</i> =12)	(<i>N</i> =10)	
Mean duration (ms)	23.1 (10.8–60.8)	19.6 (10.8–27.3)	31.7 (11.4–60.8)	0.003
Minimum latency (ms)	9.7 (5.7–18.0)	10.6 (8.3–18.0)	9.6 (5.7–12.0)	0.022
Mean latency (ms)	12.2 (7.0–24.0)	14.7 (9.3–24.0)	11.0 (7.0–13.6)	0.013
FWCV (m/s)	79.5 (51.2–211.5)	82.5 (51.2–108.9)	78.1 (68.6–211.5)	0.12
F ratio	6.3 (2.1–26.5)	6.6 (4.2–26.5)	5.6 (2.1–9.7)	0.039
Persistence (%)	100 (66.7–100)	100 (66.7–100)	100 (100–100)	0.16
Maximum amplitude (μV)	880 (330–2380)	590 (330–1400)	1280 (400–2380)	0.028
F:M ratio	9.9 (4.1–33.8)	8.3 (4.3–13.6)	18 (4.1–33.8)	0.005
After-discharge activity	2.0 (0–3)	1.4 (0–2.5)	2.7 (0.4–3)	0.006

Note: F-wave variables are presented as median (range).
Abbreviation: FWCV: F-wave conduction velocity.

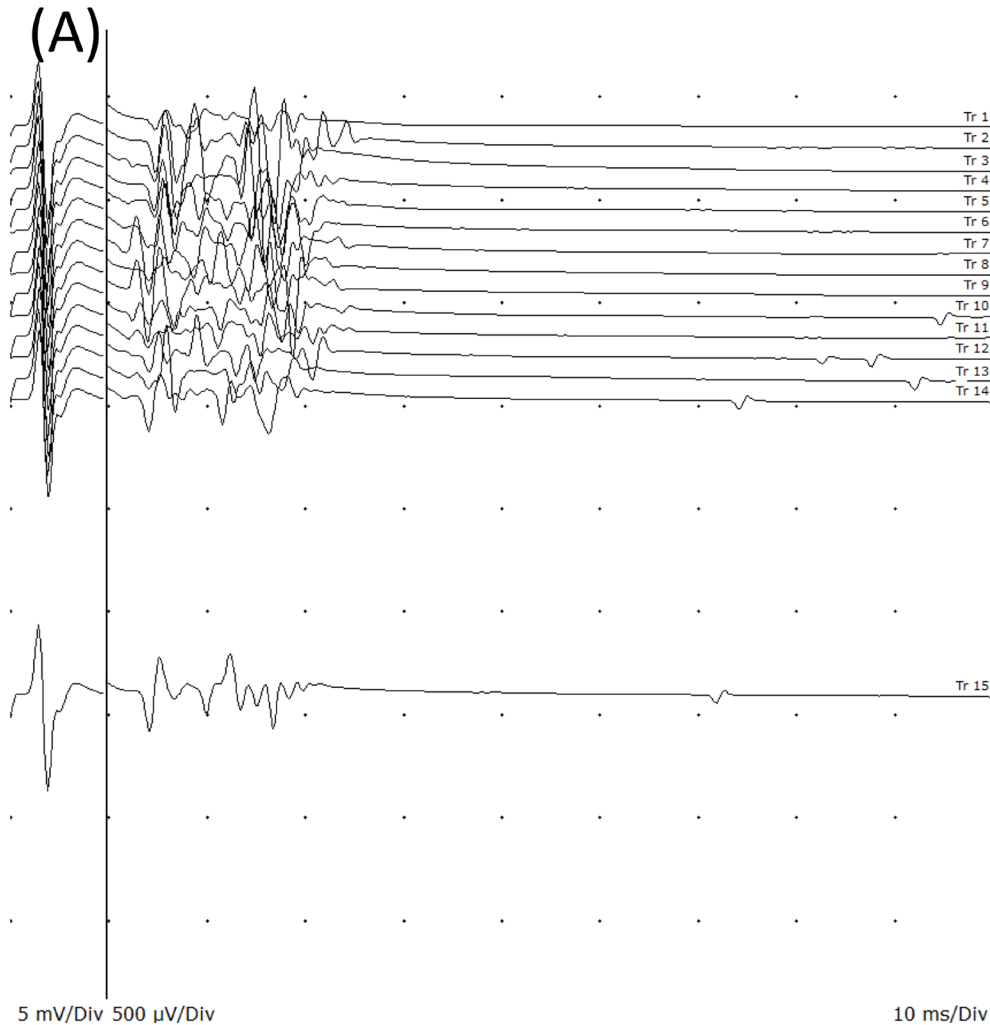


FIGURE 3 | Example F-wave recordings from the initial visit (baseline). (A) A 3-year-old neutered male Maltese dog with T12–T13 IVDE. The dog had a successful outcome and was deep pain positive. (B) A 4-year-old intact male mixed breed dog with T12–T13 IVDE. The dog had a successful outcome but remained deep pain negative (spinal walker). (C) A 3-year-old spayed female Dachshund with L1–L2 IVDE. The patient had an unsuccessful outcome remaining paraplegic deep pain negative. In example C compared to A and B, note the longer F-wave duration, higher F-wave amplitude, shorter F-wave latency, and prominent after-discharge activity.

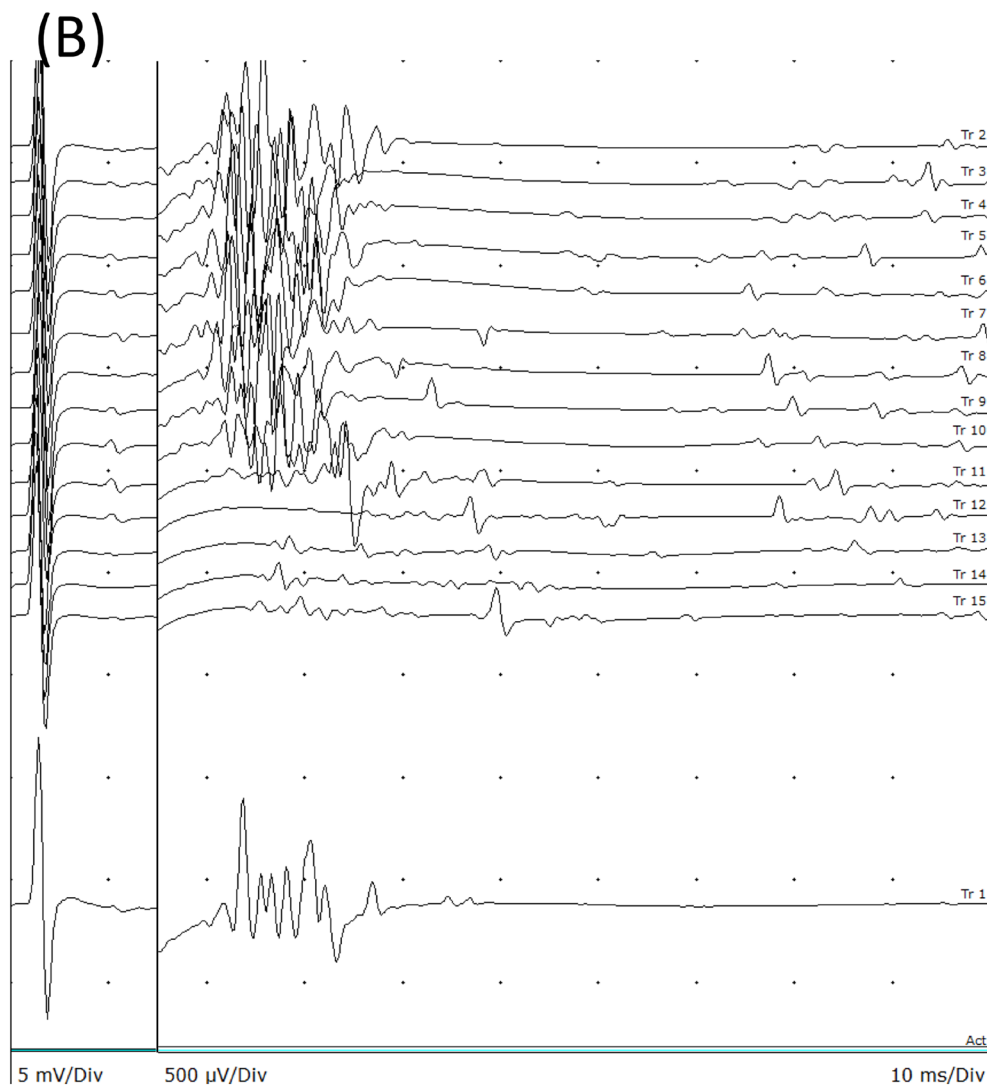


FIGURE 3 | (Continued)

F-waves performed shortly post-operatively in dogs with severe injury due to acute TL-IVDE demonstrated multiple variables significantly associated with outcome. Longer mean F-wave duration, larger maximum F-wave amplitude, higher mean after-discharge activity score, shorter minimum and mean F-wave latency, smaller F ratio, and higher F:M ratio were each associated with an unsuccessful outcome. Based on the ROC curve analysis, F-wave duration was determined to be the most useful in predicting outcome (i.e., the 95% CI for the AUC did not straddle 0.5 suggesting this variable was effective in differentiating between outcomes and it provided the best combination of sensitivity and specificity). Using a cutoff of 28.5 ms, mean F-wave duration was very specific, with only unsuccessful dogs having durations greater than this value. This was reinforced by unsuccessful outcomes noted in both dogs with extremely prolonged F-waves (> 100 ms). However, sensitivity was relatively low, with 30% of unsuccessful dogs having a mean F-wave duration less than the cutoff value. This suggests that markedly prolonged F-wave duration could be useful in predicting a negative outcome in severely affected dogs with TL-IVDE but that a shorter F-wave duration is less helpful. Even with this limitation, F-waves are likely to be more useful in establishing prognosis in acute,

severe TL-IVDE than has been suggested for motor and sensory evoked potentials [24–29]. Multiple clinical, blood, cerebrospinal fluid, and imaging biomarkers have high sensitivity and specificity to predict recovery in dogs with IVDE but there are limitations [14, 16, 19, 20, 22, 52–54]. With further development, F-waves performed peri-operatively might be an additional tool utilized in combination with other prognostic assessments to reliably predict recovery of walking for paraplegic DPN dogs secondary to acute TL-IVDE.

Three dogs had no measurable F-waves at baseline, all of which developed presumptive PMM. The lack of F-waves indicated that the dysfunction from PMM had progressed to impact the neuronal cell bodies of the L6–S1 spinal cord segments. However, one dog did not exhibit clinical signs consistent with PMM until the day after F-wave testing, and the other two dogs still had intact withdrawal reflexes at the time of testing. This observation raises the potential to explore F-waves as an objective marker to detect PMM in the early stages. This should be interpreted cautiously, given the small number of dogs and the lack of histopathologic confirmation of PMM or which spinal cord segments exhibited malacia. Similar to recovery, several biomarkers have

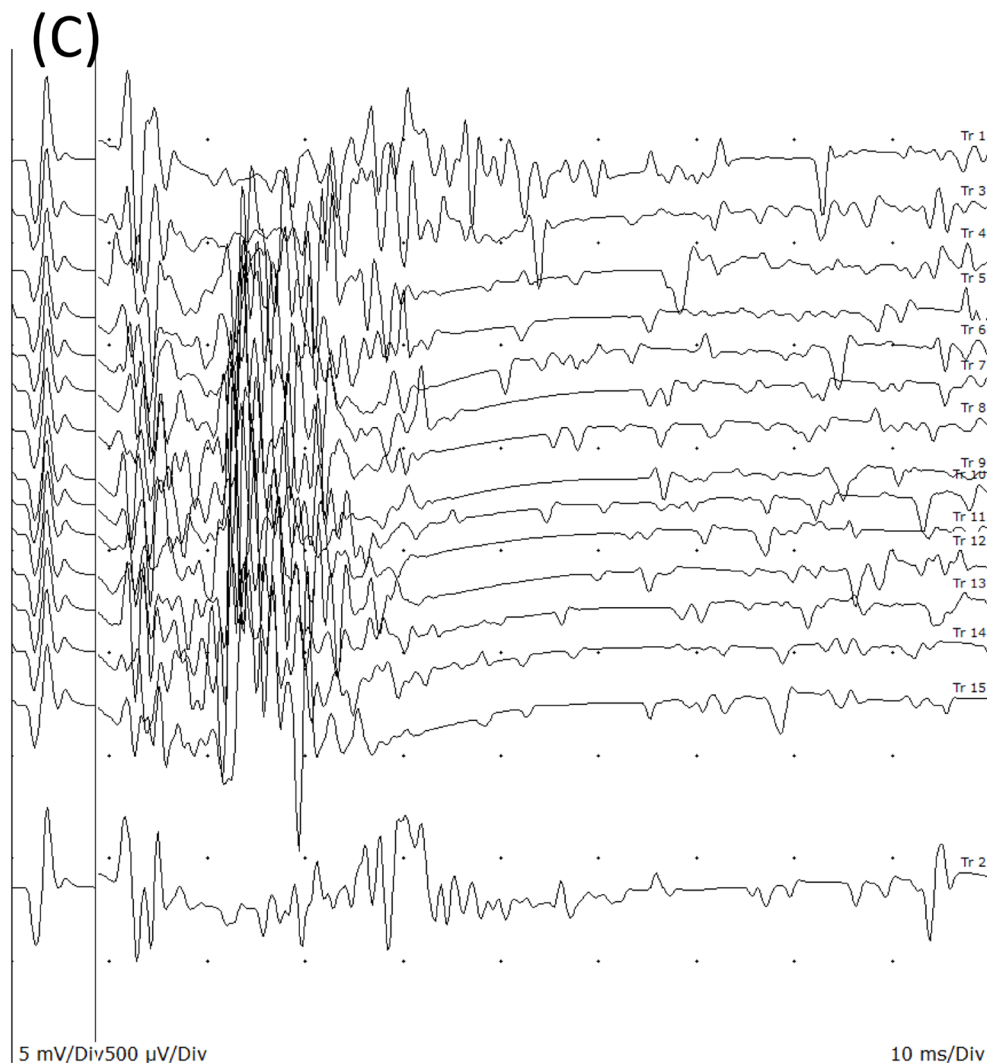


FIGURE 3 | (Continued)

been proposed for predicting PMM in dogs with IVDE [10–14, 23]. F-wave testing might serve as an additional method for identifying the imminent development of PMM in these dogs.

Spinal shock was common, affecting 17/30 (56.7%) dogs in this study. F-waves with 100% persistence were recordable in 16/17 dogs, including 3 of 4 dogs with subjectively severe spinal shock. F-waves have previously been reported to be recordable in all dogs with acute TL-IVDE of varying severity, but the presence of spinal shock was not mentioned [39]. Studies in people with acute SCI are conflicting but commonly indicate absent or reduced F-waves with spinal shock [37, 55, 56]. People also typically experience more severe, prolonged spinal shock lasting up to 6 weeks [37, 57] while in dogs it usually resolves within hours to several days [58–61]. One theory to explain this difference is that in dogs compared to people, the corticospinal tract is less well developed and the LMNs are instead primarily indirectly influenced by UMNs of the corticospinal and other motor tracts through interneurons [62]. Consistent with this, in people with cerebrovascular accidents in which the corticospinal tracts are affected resulting in flaccid hemiparesis, F-waves are substantially reduced in the affected limb [47]. The F-wave changes documented in our study highlight species differences between

dogs and people and might enhance understanding of mechanisms underlying spinal shock in dogs. Additionally, our results might be influenced by the timing of F-wave testing. While the frequency of spinal shock was within the reported range of 6%–65% in dogs with acute T3–L3 myelopathy [59–61, 63], increasing duration of clinical signs has been associated with decreased risk of spinal shock [61]. If F-wave testing was performed at presentation, we would anticipate encountering more dogs with severe spinal shock. As such, it is possible that severe spinal shock could pose a barrier to measuring F-waves pre-operatively in some dogs or that repeat testing might be needed as spinal shock resolves to predict the likelihood of recovery.

Two dogs developed spinal walking by the 3-month recheck and were classified as having a successful outcome. The physiology underlying spinal walking is complex, multifactorial, and yet to be fully elucidated [64–66]. It is thought to result from interactions between intra-spinal circuitry (central pattern generator) and proprioceptive feedback after injury, with conflicting evidence to support a component of supraspinal input. While our study prioritized ambulation as the primary outcome measure, it is uncertain whether classifying the spinal walking dogs as successful was appropriate and the small number prevented

analysis as an independent outcome group. While these dogs were distinct from the rest of the successful group, remaining DPN and largely incontinent, they were clearly able to walk unassisted, which could be considered an important, practical recovery benchmark for some owners. F-waves are purely a motor phenomenon [34] and, in the case of SCI, reflect motor neuron pool excitability [38, 67]. F-waves might therefore provide information about the state of the LMNs and motor recovery but be unable to differentiate whether or not pain perception will be recovered. Confirmation of this suspicion and more nuanced determination of how success should be classified would require a larger number of dogs, particularly more who regain ambulation despite remaining DPN.

Comparing data from the first recheck to baseline values stratified by outcome, F-wave duration was significantly shorter in the unsuccessful group and amplitude was significantly increased in the successful group. Additionally, after discharge activity scores

in the unsuccessful group were less pronounced at the rechecks, though the differences were not significant. These longitudinal changes in F-waves suggest a trend away from the marked disinhibition noted immediately post-operatively, but the generally wide ranges for variables at the rechecks also demonstrated the broad variability in recovery trajectory after severe TL-IVDE. In dogs with chronic (>3 months) deficits secondary to prior acute, severe TL SCI including many with TL-IVDE, F-waves have been reported but variables other than latency and F ratio were not described making direct comparisons challenging [24]. However, H-reflexes were altered in those dogs compared to healthy controls and electrical activity after the initial F-waves or H-reflexes (also termed after-discharges) was also described in nearly 90% of dogs [24]. In people and dogs in the chronic stages, changes in H-reflexes (e.g., increased H:M ratio, lowered H-reflex threshold) have been associated with increased motor neuron pool excitability and more severe spasticity [24, 68–70]. It is suspected that after-discharge activity might provide similar information on the

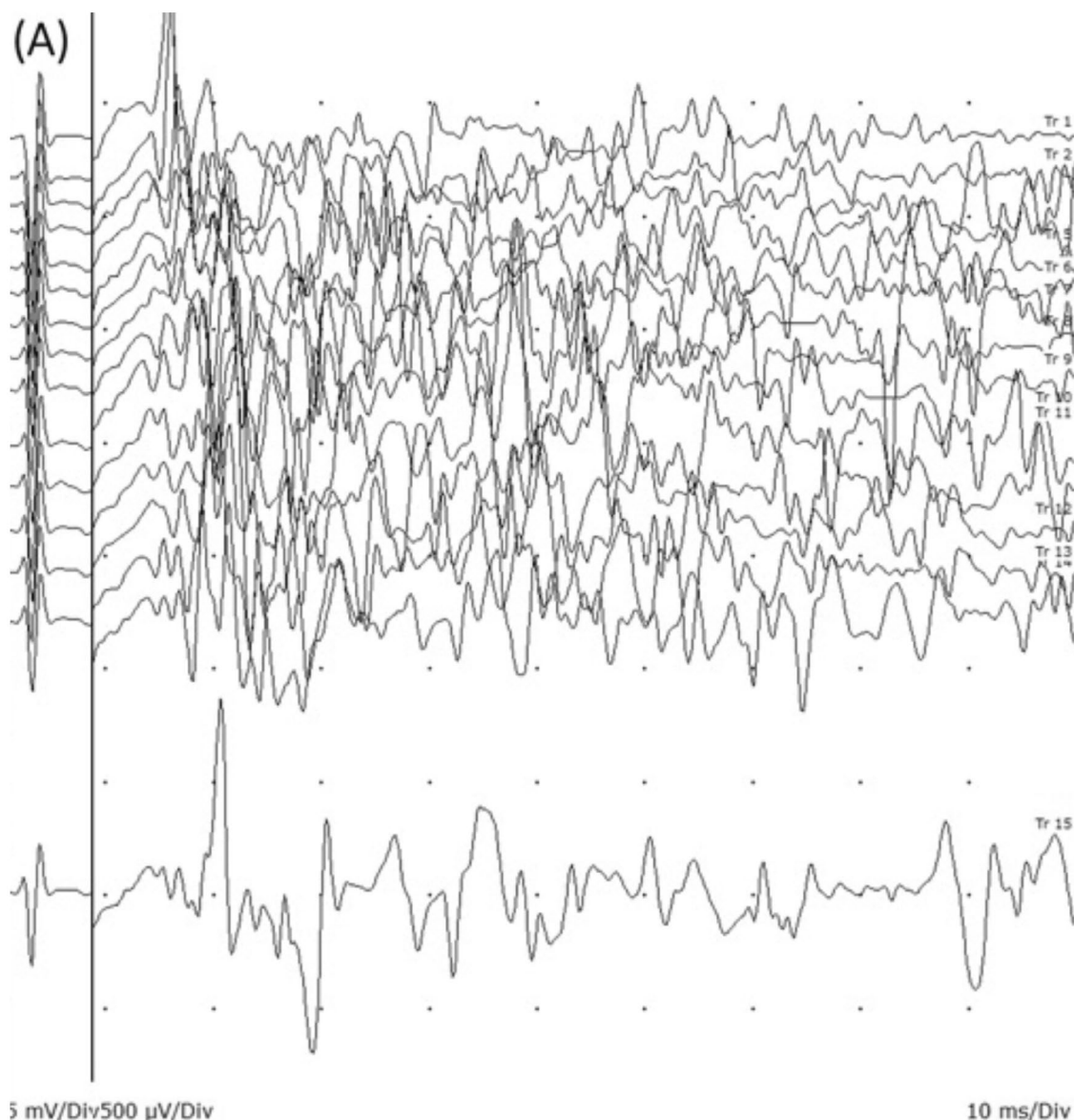


FIGURE 4 | Example F-wave recordings from the initial visit (baseline) in two dogs with an unsuccessful outcome. (A) A 4-year-old female French Bulldog exhibiting severely prolonged (> 100 ms) F-wave duration that did not return to the baseline during the recording limit of 100 ms. (B) A 5-year-old spayed female French Bulldog exhibiting severe after-discharge activity (score > 2.5/3).

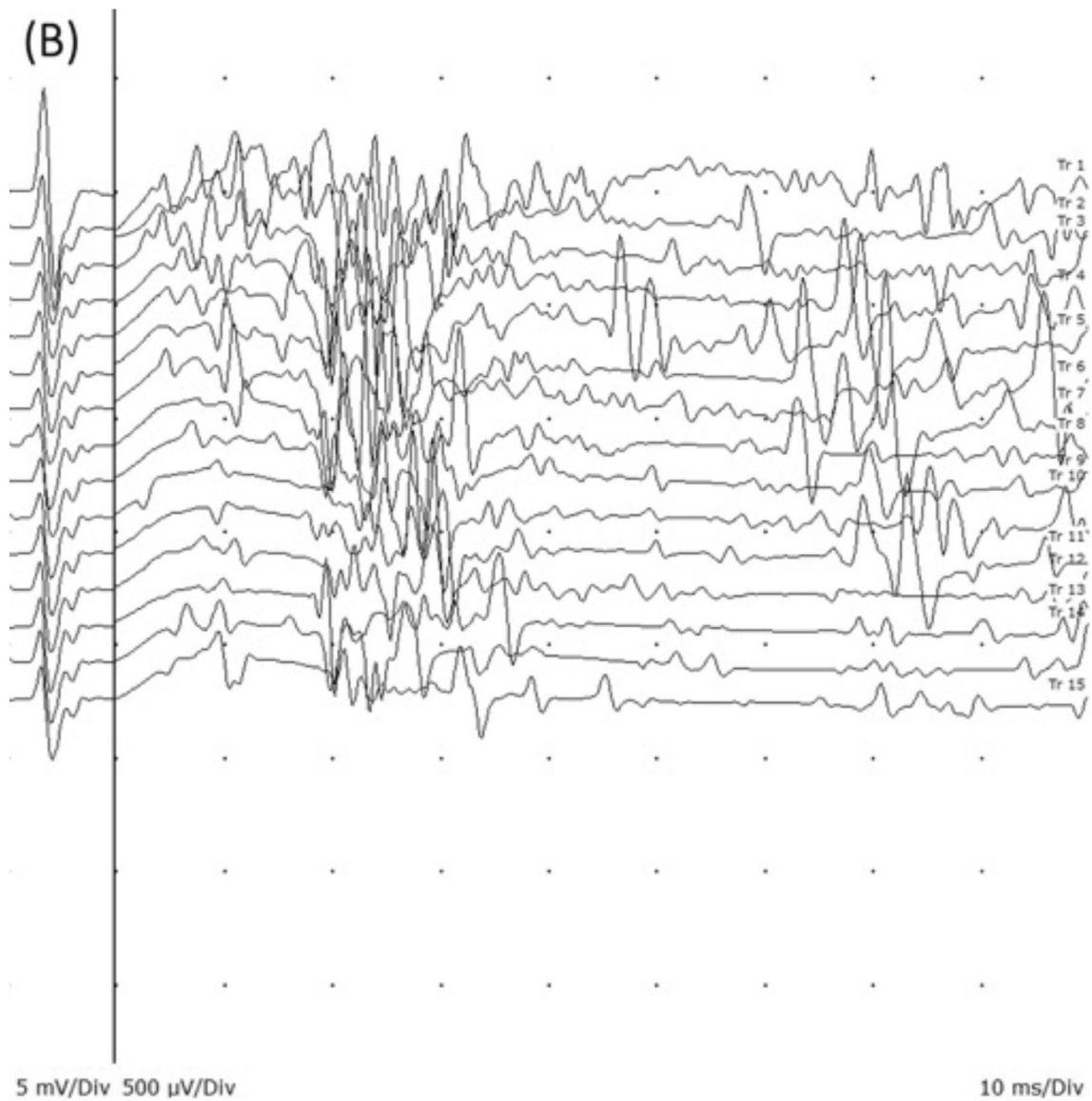


FIGURE 4 | (Continued)

TABLE 3 | Summary of receiver-operating characteristic (ROC) curve analysis.

Variables	Cut-off	Sensitivity (CI)	Specificity (CI)	AUC (CI)
Mean duration (ms)	28.5	0.70 (0.40–1.0)	1.0 (1.0–1.0)	0.83 (0.60–1.0)
Minimum latency (ms)	12.2	1.0 (1.0–1.0)	0.42 (0.17–0.67)	0.70 (0.47–0.93)
Mean latency (ms)	14.9	1.0 (1.0–1.0)	0.50 (0.25–0.75)	0.64 (0.39–0.89)
F ratio	5.3	5.0 (0.20–0.80)	0.83 (0.58–1.0)	0.70 (0.47–0.93)
Max amplitude (μ V)	810	0.80 (0.50–1.0)	0.67 (0.42–0.92)	0.77 (0.55–0.98)
F:M ratio	15	0.60 (0.30–0.90)	1.0 (1.0–1.0)	0.74 (0.49–1.0)
After-discharge activity	2.2	0.70 (0.40–1.0)	0.92 (0.75–1.0)	0.83 (0.64–0.83)

Note: For each of the baseline F-wave variables associated with outcome in the 22 dogs included in outcome analysis, the cutoff values predicted an unsuccessful outcome with the given sensitivity and specificity.

Abbreviations: AUC: Area under the curve; CI: 95% confidence intervals.

excitability of local spinal cord circuitry post-injury. Interestingly, the initial disinhibition and marked excitability noted in some dogs of this study was not necessarily maintained through the

sub-acute into chronic stages of injury and greater disinhibition of LMNs (quantified by F-waves) in the acute setting was associated with an unsuccessful outcome. In contrast, markers of increased

TABLE 4 | F-wave variables at recheck timepoints, stratified by outcome at 3 months.

F wave variables	First recheck (N = 23)			Second recheck (N = 22)		
	Successful (N = 12)	Unsuccessful (N = 11)	p	Successful (N = 12)	Unsuccessful (N = 10)	p
Mean duration (ms)	15.4 (8.6–25.6)	18.5 (12.2–37.4)	0.096	18.1 (6.7–44.0)	19.2 (14.8–43.4)	0.57
Minimum latency (ms)	9.0 (7.8–17.2)	8.7 (6.8–12.0)	0.055	9.2 (5.7–18.7)	11.0 (4.6–15.2)	0.96
Mean latency (ms)	11.6 (8.7–20.0)	9.4 (7.3–13.3)	0.071	11.4 (8.8–20.1)	12.8 (4.9–18.3)	0.58
FWCV (m/s)	93.7 (17.6–142.0)	106.6 (57.9–204.3)	0.12	85.9 (57.9–405.3)	78.6 (51.8–360.9)	0.44
F ratio	6.8 (4.1–22.2)	5.6 (3.6–11.0)	0.06	5.5 (2.7–24.3)	7.7 (1.5–17.0)	0.67
Persistence (%)	100 (53.3–100)	100 (100–100)	0.3	100 (100–100)	100 (93.8–100)	0.29
Max amplitude (μV)	1620 (290–2900)	1350 (400–4500)	0.72	1180 (540–3400)	980 (520–2800)	0.44
F:M ratio	11.9 (1.8–96.3)	13.7 (4.5–62.5)	0.92	10.6 (5.1–24.8)	13.8 (4.5–93.3)	0.29
After-discharge activity	0.9 (0–2.1)	1.7 (0.1–2.3)	0.12	1.1 (0–3.0)	1.0 (0.1–3.0)	1.0

Note: F-wave variables are presented as median (range).
Abbreviation: FWCV: F-wave conduction velocity.

motor neuron pool excitability in dogs in the chronic setting were associated with higher gait scores and suggested to contribute to motor recovery (i.e., spinal walking). This supports distinct influences on the character of motor neuron pool excitability and disinhibition during different stages of recovery and that some LMNs might enter a more quiescent (i.e., less excitable or even inhibited state) that, in some dogs, transitions to a return of excitability in the chronic phase.

Limitations include small sample size and limited follow-up with outcome determined at 3 months post-operatively. In DPN dogs with TL-IVDE managed surgically, average time to recover ambulation ranges from 21 to 47 days [2, 66, 71–73], but a proportion take more than 3 months to walk unassisted [66, 74, 75]. In addition, in dogs permanently DPN, average time to develop spinal walking ranges from about 2.5–9 months [66, 76]. It is possible that some dogs were misclassified as unsuccessful who might have regained walking after 3 months. Other factors that might have impacted our results include the aforementioned limitation of including spinal walkers in the successful group, the lack of consideration of other features of outcome (e.g., continence) in our definition, the lack of standardization of post-operative rehabilitation, and the lack of evaluator blinding (to outcome) when analyzing F-wave data. Differentiating between the end of F-waves and the start of after-discharge activity was also challenging in some dogs. While we utilized a 2 ms gap to distinguish between the two, it is possible that severe after discharge activity occasionally represented continued, severely prolonged F-waves. However, both prolonged F-waves and severe after discharge activity likely indicate LMN disinhibition and were associated with outcome, mitigating the impact of this potential misclassification. F-waves were only acquired on one side, which prevented determining whether F-wave variables varied left to right or were associated with the lateralization of the compressive or surgical side. While clinical asymmetry can occur and side differences in pelvic limb muscle activity have been demonstrated using surface electromyography during the

post-operative recovery period for TL-IVDE [77], we exclusively enrolled paraplegic DPN dogs, which might minimize left–right differences, at least at baseline. This study also exclusively enrolled dogs with TL-IVDE and a neurolocalization of T3–L3. As such, the ability to document F-waves in dogs with other neurolocalizations, particularly L4–S3, is unknown, as is its prognostic utility in those scenarios. It is also unclear if post-operative F-wave acquisition is comparable to testing performed pre-operatively. While obtaining pre-operative data is perhaps the most clinically relevant timepoint prognostically, many owners continue to inquire about their dog's prognosis in the early post-operative days, suggesting a role for post-operative acquisition. Similarly, the predictive value of F-waves in medically managed TL-IVDE or with other causes of acute SCI is also unknown, which impacts the generalizability of our results. Further investigation of F-waves in larger and more varied cohorts, including pre-operative data acquisition, is necessary to determine the practical utility and validate it as a prognostic tool.

Alterations in F-wave variables, particularly prolonged mean F-wave duration, appear to be a useful negative prognostic indicator associated with lack of recovery of walking at 3 months. In DPN dogs with TL-IVDE managed surgically, performing F-waves shortly post-operatively could assist clinical decision-making.

Acknowledgments

The authors thank Brittany Laflen, RVT, VTS (Neurology), Department of Veterinary Clinical Sciences, College of Veterinary Medicine, Purdue University, West Lafayette, IN, United States, for her assistance with obtaining clinical information for cases included in the study. The preliminary results were presented as an oral presentation at the 2024 American College of Veterinary Internal Medicine (ACVIM) Forum, Minneapolis, Minnesota, June 6, 2024.

Disclosure

Authors declare no off-label use of antimicrobials.

Ethics Statement

Approved by the Institutional Animal Care and Use Committee at Purdue University (protocol # 2205002270) and North Carolina State University (protocol #23-262). Authors declare human ethics approval was not needed.

Conflicts of Interest

The authors declare no conflicts of interest.

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