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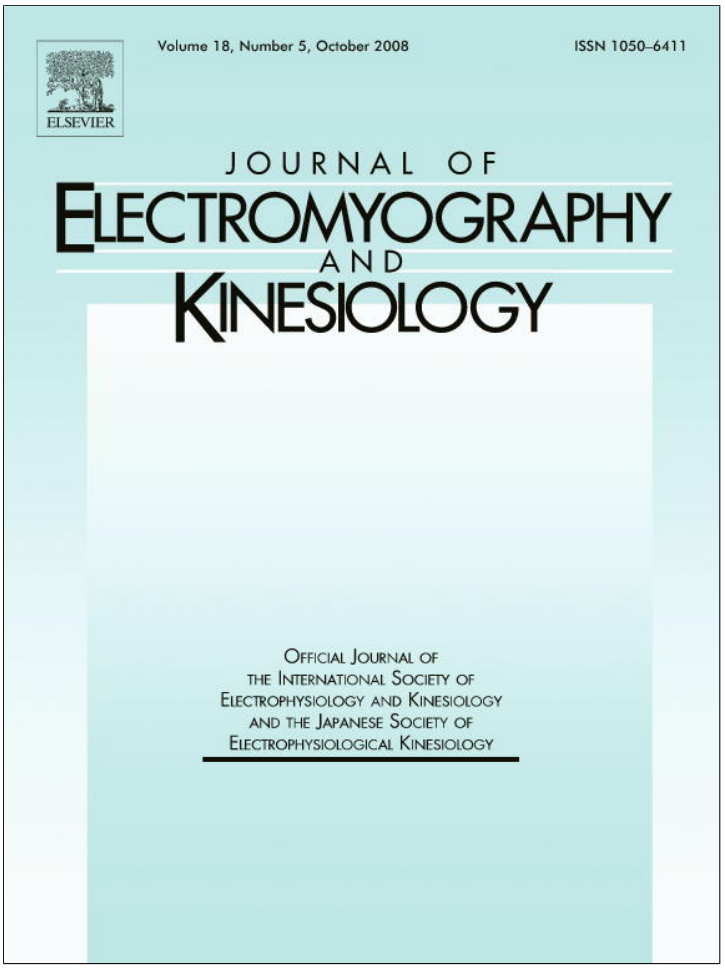

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Effects of disc degeneration on neurophysiological responses during dorsoventral mechanical excitation of the ovine lumbar spine ☆

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Abstract

Mechanisms of spinal manipulation and mobilization include the elicitation of neuromuscular responses, but it is not clear how these responses are affected or altered by disc degeneration. We studied the neurophysiological responses of the normal and degenerated ovine spine subjected to mechanical excitation (varying force amplitude and duration) consistent with spinal manipulative therapy (SMT). Needle electromyographic (EMG) multifidus muscle activation adjacent to the L3 and L4 spinous processes and compound action potentials (CAPs) of the L4 nerve roots were measured during the application of dorsoventral mechanical excitation forces designed to mimic SMT force–time profiles used routinely in clinical practice. The magnitude and percentage of positive EMG responses increased with increasing SMT force magnitude, but not SMT pulse duration, whereas CAP responses were greatest for shorter duration pulses. Disc degeneration was associated with a reduction (20–25%) in positive EMG responses, and a concomitant increase (4.5–10.2%) in CAP responses.
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Keywords: Electromyography; Compound action potentials; Intervertebral disc degeneration; Spinal manipulation; Neurophysiology

1. Introduction

Neuroanatomical research has demonstrated the presence of mechanosensitive and nociceptive afferent fibers in spinal tissues (disc, facet, ligaments, and muscles) (Jiang et al., 1995; McLain, 1994; McLain and Pickar, 1998; Mendel et al., 1992; Roberts et al., 1995), and neurophysiological research has identified the role of such afferent stimulation in pain production (Cavanaugh, 1995; Cavanaugh et al., 1996, 1997) and coordinated neuromuscular stabilization of the spine (Indahl et al., 1995, 1997; Solomonow et al., 1998, 1999, 2000; Stubbs et al., 1998). Active muscular recruitment and reflexes also play a major role in both spinal load and stability (Gardner-Morse and Stokes, 1998; Granata and Marras, 1995), and abnormal load sharing, and repetitive cyclic loading are implicated as mechanical factors involved in the pathomechanisms of musculoskeletal disorders including low back pain (Solomonow, 2004).

Imaging studies have demonstrated a relationship between muscular degeneration and disc degeneration (Parkkola and Kormano, 1992; Parkkola et al., 1993), and muscular degeneration is purported to further compromise

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spinal stability in patients with back pain and disc degeneration (Parkkola et al., 1993). Changes in neuromuscular reflex responses associated with spinal degeneration may also contribute to limitations in stabilizing control of the paraspinal muscles and thereby influence the risk of injury during activities of daily living (Solomonow et al., 1998). Differences in paraspinal neuromuscular reflex amplitude have also been reported in patients with low back pain, and neuromuscular reflexes are hypothesized to be an indication of the patients' underlying clinical status (Colloca and Keller, 2001).

Mechanisms of spinal manipulation therapy (SMT) and mobilization therapy include the elicitation of neuromuscular responses. Neuromuscular reflex responses to SMT have been observed in asymptomatic subjects (Herzog et al., 1999; Symons et al., 2000), but it is not clear how these responses are affected or altered by disc degeneration. The purpose of this experimental study was to examine the in vivo neurophysiological responses of the normal and degenerated ovine lumbar spine subjected to mechanical excitation (varying force amplitudes and force duration) consistent with SMT. Disc degeneration was established using a validated animal model (Osti et al., 1990). We hypothesized that the neurophysiological response of the spine during mechanical excitation would be reduced in animals with disc degeneration.

2. Materials and methods

2.1. General

Fifteen sheep (mean 47.7 kg, SD 4.9 kg) underwent a survival surgical procedure designed to experimentally model chronic disc degeneration (Osti et al., 1990). Ten sheep (mean 46.5 kg, SD 5.6 kg) served as controls. The sheep were 18–24 months old. Sheep are skeletally mature around 24–30 months at which time they achieve peak bone mass. The research protocol was approved by the Animal Ethics Committees and Institutional Review Board of the Institute of Medical and Veterinary Science (Adelaide, South Australia).

2.2. Disc degeneration model

Under general anesthesia (1 g, Thiopentone; 2.5% Halothane), the lumbar spine was approached via a direct lateral left-side retroperitoneal approach. In each animal, a controlled stab incision was made in the left posterolateral annulus fibrosus midway between the endplates of the L1–L2 disc. Incisions were made with a number-15 scalpel blade directed transversely through the outer aspect of the posterior annulus towards the midline and inserted to the hilt of the scalpel handle (a depth of 5 mm). Fluoroscopic control was used to check the posterior limit of the blade. Care was taken to protect both the spinal cord and the exiting nerve root during the procedure. The incised level was marked by placing a wire suture around the transverse process for future identification (Fig. 1). The wound was closed in layers and the animals received an intramuscular antibiotic injection 2 mL/50 kg (consisting of procaine penicillin 250 mg/mL, streptomycin 250 mg/mL, and procaine HCL 20 mg/mL). Each animal recovered in an air-conditioned indoor facility on a 12-h light/dark cycle for 3 days, and then was transferred to an outdoor facility. The animals were kept in similar paddocks for 20 weeks to allow time for the posterior annular lesion to mature.

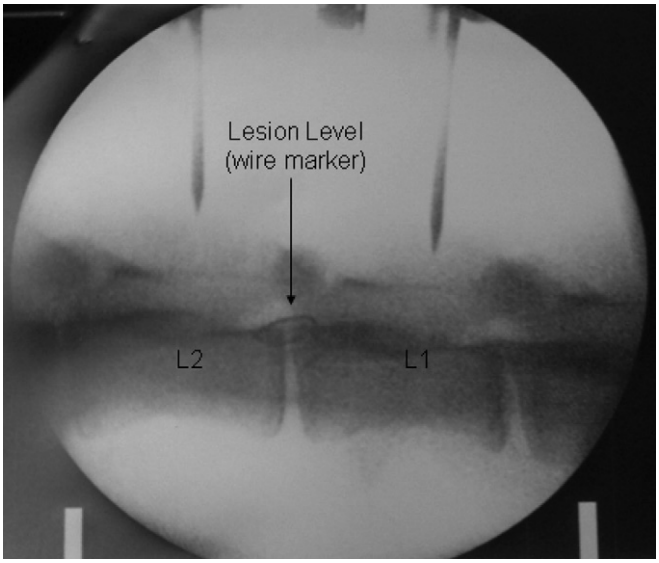

Fig. 1. Fluoroscopic image of the L1–L2 ovine spine showing accelerometer pins and location of lesion marked by a circular wire placed around the transverse process.

2.3. Muscle and nerve root responses

This experimental preparation has been described previously (Colloca et al., 2003, 2004; Keller et al., 2003). Briefly, following animal preparation and anesthesia, four bipolar needle electromyographic (EMG) electrodes (Biopac Systems, Inc., Goleta, CA) were inserted bilaterally into the deepest fascicles of the multifidus muscles adjacent to the L3 and L4 spinous processes, and were used to record electromyographic signals from these tissues (Fig. 2). Spinal nerve roots were isolated using a hemilaminarthrectomy decompression spinal surgery technique (Weiner et al., 1999). Bi-polar, platinum electrodes (PolarProbe, Nicolet, Inc., Madison, WI) were subsequently cradled around the left and right L4 spinal nerve roots adjacent to the spinal cord at the level of the dorsal root ganglia, and were used to record compound action potentials (CAPs) from the nerve roots (Fig. 3) (Colloca et al., 2003). The bipolar electrodes had 10-mm spacing and 64-mm tip length and were shielded and insulated such that the most distal (hooked) end was exposed for recording. CAP electrodes were connected to biopotential amplifiers (ERS100B, Biopac Systems, Inc., Goleta, CA) using a 3-m extension cable and plug (MEC100, Biopac Systems, Inc., Goleta, CA). The amplifier gain setting was 5000 and the amplifier filter settings were 5000 Hz low pass and 10 Hz high pass.

2.4. Mechanical excitation protocol

A custom, computer-controlled mechanical testing apparatus was used to generate mechanical excitation force–time profiles with varying force amplitude and duration (Keller and Colloca, 2006). The apparatus comprised a linear voice coil actuator (model LA25-42, BEI Technologies, Inc., Ashford, Kent, UK) and a programmable, pulse width modulated servo amplifier, voice coil drive controller (model VCA100, BEI Kimko Magnetics, San

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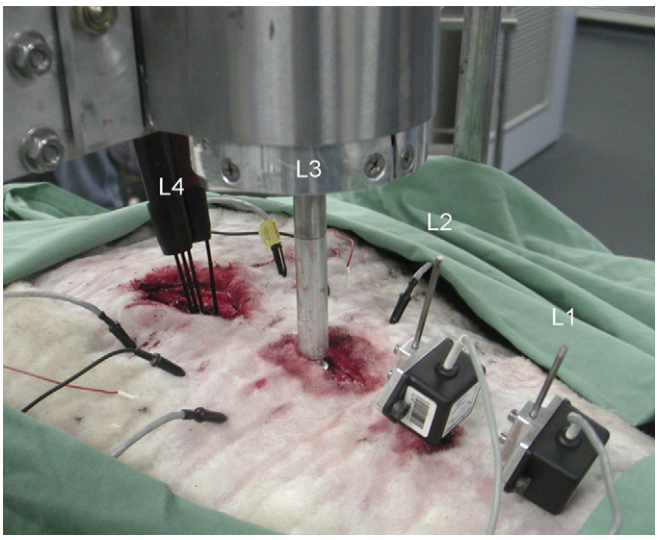

Fig. 2. Computer controlled mechanical testing apparatus positioned over the L3 spinous process. Bipolar platinum electrodes positioned at the L4 spinal nerve roots and EMG electrodes are shown at the level of L3 and L4. Two tri-axial, dynamic accelerometers are located at L1 and L2 to record spinal motions (data presented elsewhere).

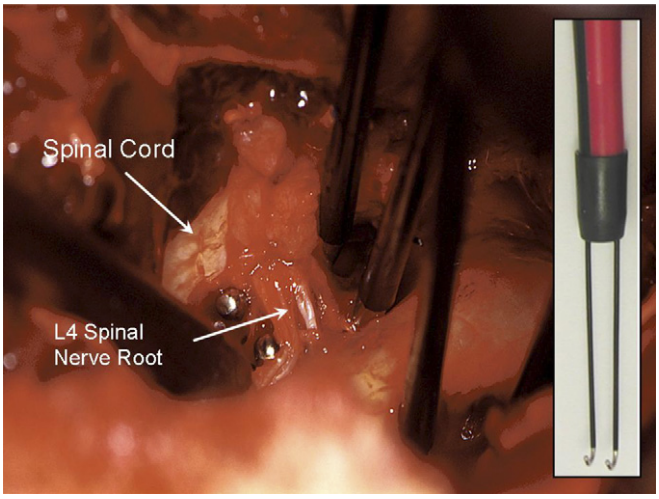

Fig. 3. A bipolar platinum electrode (inset) used to record compound unit action potentials (CAPs) is shown cradling a finely dissected L4 spinal nerve root.

Marcos, CA). The voice coil had a continuous stall force of 84 N and total stroke of 25.4 mm. A 665 N load cell (MLP-150, Transducer Techniques, Temecula, CA) and a 25 mm linear variable displacement transducer (model S1D, Instruments & Control, Inc., Branford, CT) were used to measure the actuator force and displacement signals.

With the animals in a standardized prone-lying position, the bony preeminence of the L3 spinous process was exposed using electrocautery. Using the mechanical testing apparatus, posteroanterior (PA) forces were applied directly to the L3 spinous process via a 12.7 mm-diameter stainless-steel rod equipped with a slotted tip that cradled the exposed bone surface (Fig. 2). To simulate impulsive (very short time duration) and manual SMT force–time profiles, three mechanical pulse durations ($t = 10$, 100, and 200 ms) at a constant PA force (80 N) and three force levels (20 N, 40 N, and 60 N) at constant pulse duration ($t = 100$ ms) were examined. Five mechanical excitation tests were performed for each mechanical intervention. In each case a 10 N preload was applied, and the order in which the mechanical testing protocols were performed was randomly determined. The applied mechanical excitation force–time profiles (PA force amplitude and duration) were chosen to mimick PA spinal manipulations delivered in clinical practice (Herzog et al., 1993, 2001). The 20–80 N maximum PA force amplitudes were chosen to produce maximum displacement responses (4 to 16 mm) consistent with SMT clinical practice.

During the mechanical excitation tests, L3, L4 multifidus EMG and L4 spinal nerve root CAP responses were sampled at 1250 Hz using a 16 channel, 16-bit MP150 data acquisition system and biopotential amplifiers (Biopac Systems, Inc., Goleta, CA). The applied L3 force was recorded at 2500 Hz.

2.5. Pathological examination of intervertebral discs

Following the experimental protocol, the sheep were killed by intravenous injection of 6.5-g pentobarbitone sodium and their lumbar spines were removed *en bloc* by transecting the thoracolumbar junction and the midsacrum. Individual motion segments were isolated by cutting midway through the adjacent vertebral bodies with an electric bandsaw and fixed in 10% buffered formalin for a minimum of 72 h before being decalcified in a solution containing 9.5% nitric acid and 10% edetic acid (EDTA). For histological examination, the spine specimens were cut into six parasagittal slices of equal thickness, and those showing the annulus lesion and a contralateral slice were processed into paraffin wax for histomorphometric examination. Tissue sections were cut at a nominal thickness of 5 μm, stained with hematoxylin and eosin, and independently examined without knowledge of each animal's identity. Intervertebral discs from all animals were graded on a 1–4 scale of degeneration (1 = normal, 2 = mildly degenerated, 3 = moderately degenerated, 4 = severely degenerated) with respect to the overall condition of the disc and morphological characteristics of the annulus fibrosus, nucleus pulposus, vertebral end plates, and subchondral bone (Berlemann et al., 1998).

2.6. Data reduction and analysis

Peak-to-peak EMG amplitude ratios from the filtered signals (peak-to-peak EMG response during the force–time pulse divided by the baseline amplitude) were computed for each mechanical excitation trial. Positive EMG responses were defined as an amplitude ratio >1.5 (Seroussi and Pope, 1987). Spinal nerve root discharge (CAP firing rate) was computed as the instantaneous frequency (IF) for the baseline CAP responses and for the CAP responses recorded during the time duration of the uniform pulse, from which the ratio of pulse/baseline IF was obtained (Sung et al., 2005). Matlab (The Mathworks, Natick, MA) was used to perform all of the above analyses.

Shapiro–Wilks' W test confirmed the normal distribution of all parameters of interest. A repeated measures analysis of variance (ANOVA) was used to determine the effect of SMT pulse duration and amplitude on the neurophysiological responses after each of the five mechanical excitation trials were averaged for each animal. A one-factor ANOVA was performed between normal and degenerated animals and across mechanical test protocols within the normal and degenerated animal groups. Statistical

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significance was set at $p < 0.05$. Microsoft Excel (Microsoft Computer Corporation, Redmond, WA) was used to perform all of the above analyses.

3. Results

3.1. Histological analysis

The macroscopic and microscopic features of the discs in this study closely resemble those described in a previous ovine study of rim lesions (Osti et al., 1990). The degenerated model discs were consistently at a stage of moderate to advanced degeneration compared to the normal discs. Macroscopically there was unequivocal evidence of the annular incision in the incised disc with extension of the lesion to involve the central nucleus pulposus in all cases (Fig. 4). The lesion resulted in substantial loss of height due to breakdown of disc matrix. Microscopically all injured discs showed advanced repair of the most peripheral annular fibers or in some cases, more organized collagenised scar tissue. In most cases, there was radial and circumferential extension of the initial annular lesion with secondary displacement of the nucleus towards the anterior aspect, resulting in prominent inversion of the posterior annular fibers from their usual concave orientation. Also, in most cases, the nucleus showed substantial migration with early clefting of the matrix in some cases.

Table 1 summarizes the overall histological grading of the L1–L2 discs and grading with respect to the annulus fibrosus (AF), nucleus pulposus (NP), vertebral end plates (EP), and subchondral bone (SCB) (Berlemann et al., 1998). In all normal animals, the AF was graded as 1 whereas the degenerated group scored 3.30 (SD = 0.48). The NP averaged 1.40 (SD = 0.52) for the normal group compared to the degenerated group score of 2.60 (SD = 0.52). Vertebral body endplate and subchondral bone differences were less remarkable among the normal and degenerated groups. All normal group L1–L2 discs were graded as 1 whereas the mean score of the degenerated group was 3.10 (SD = 0.57). With the exception of 2 animals who were graded as 2, all of the incised discs were generally graded either as 3 (moderately) or 4 (severely) degenerated.

3.2. Neurophysiologic responses

Positive multifidus muscle EMG responses were observed for all SMT mechanical excitation pulse protocols (Fig. 5). The percentage of positive EMG responses increased with increasing mechanical excitation force magnitude. With the exception of the 10 ms pulse protocol, there was a 100% EMG response rate for forces 60 N and higher. Mechanical excitation pulse duration (constant force amplitude, 80 N) did not appreciably affect the EMG responses (all were close to 100% in the normal disc group). With the exception of the 60 N, 100 ms mechanical excitation protocol, animals in the degenerated disc group showed a significant ($p < 0.05$) 20–25% reduction in positive EMG responses. Compound action potential IF responses were greatest for the 10 ms, constant force pulse protocol and were least for the 100 ms, constant force protocol (Fig. 6). Within group differences were found for both the constant force (10 ms vs. 100 ms vs. 200 ms) and constant pulse duration protocols (20 N vs. 60 N, 40 N vs. 60 N). Compared to the normal disc group, CAP IF responses showed a non-significant trend for slightly greater (4.5–10.2%) in the degenerated disc group.

4. Discussion

In this study, an established animal model of disc degeneration (Osti et al., 1990) was used that produces a clinically relevant healing response that is well established after 12 weeks (Gries et al., 2000). Analogous to disc degeneration in humans (Vernon-Roberts and Pirie, 1977), the healing response in the ovine spine was associated with annular disruption, nuclear migration and granulation tissue forma-

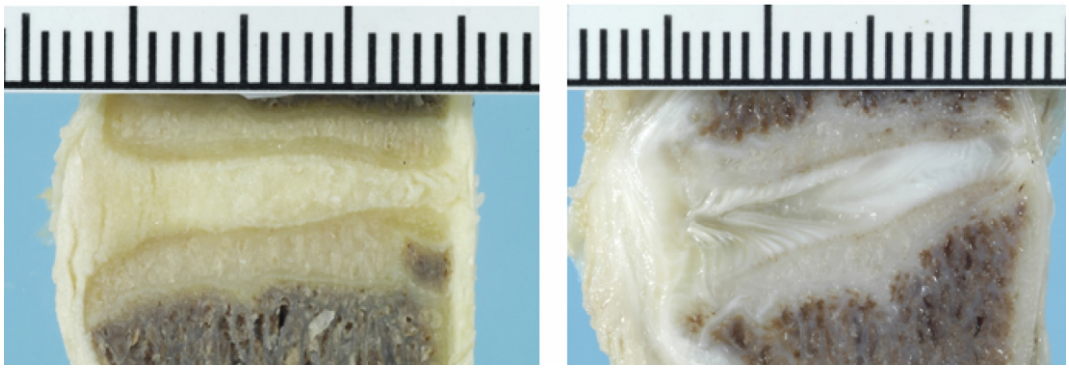

Fig. 4. Low power mid sagittal photomicrographs of ovine L1–L2 lumbar intervertebral discs. A normal disc serving as a control in the current study is shown on the left disc showing the normal arrangement of the annulus fibrosus and the central nucleus pulposus. The injured disc (right) is shown 20 weeks following annular incision and is characterized by extensive disruption of the anterior annulus, anterior migration of the nucleus pulposus, and medial contraction of the posterior annulus fibers. Markedly thickened repair tissue is present in the vicinity of the initial annular incision. Small transverse fissures and irregular thickening of the calcified zone is observed at the vertebral body endplate in the injured disc as well.

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Table 1
Grading of histological changes in the L1–L2 lumbar discs of the normal and 15 degenerated model specimens examined (AF, annulus fibrosus; NP, nucleus pulposus; EP, vertebral endplate; SCB, subchondral bone) (Berlemann et al., 1998)

Animal	AF		NP		EP		SCB		Grade	
	Normal	Lesion	Normal	Lesion	Normal	Lesion	Normal	Lesion	Normal	Lesion
1	1	3	1	2	1	1	1	1	1	3
2	1	3	1	2	1	1	1	1	1	3
3	1	3	2	3	1	1	1	1	1	3
4	1	3	1	2	1	1	1	1	1	3
5	1	4	1	3	1	2	1	1	1	4
6	1	4	1	3	1	1	1	1	1	4
7	1	3	2	3	2	1	1	1	1	2
8	1	3	2	2	1	1	1	1	1	3
9	1	3	2	3	1	1	1	1	1	3
10	1	4	1	3	1	2	1	2	1	3
11		4		2		3		2		3
12		3		2		1		1		3
13		3		2		1		1		3
14		4		3		1		1		4
15		2		3		1		1		2
Mean	1.00	3.30	1.40	2.60	1.10	1.20	1.00	1.10	1.00	3.10
SD	0.00	0.48	0.52	0.52	0.32	0.42	0.00	0.32	0.00	0.57

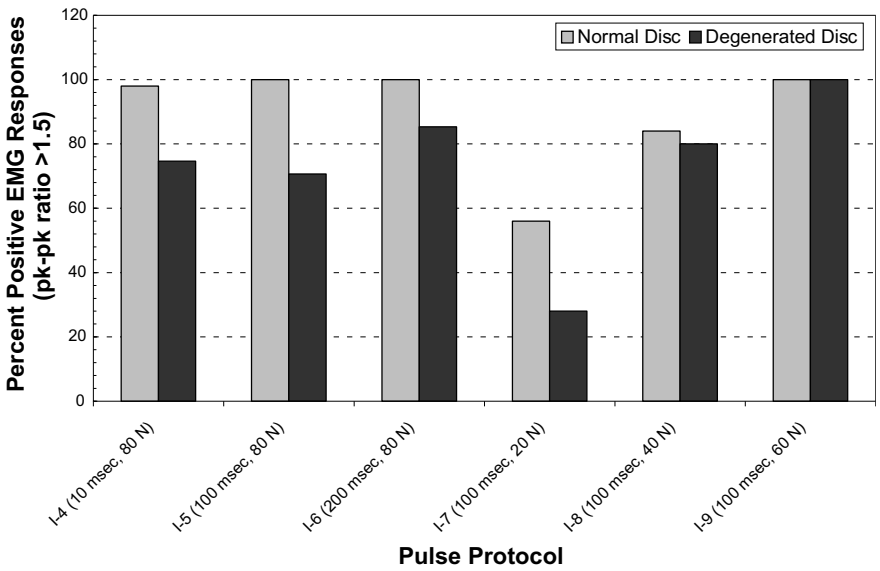

Fig. 5. Summary of the multifidus muscle EMG responses obtained for the constant force (80 N) protocols (10 ms, 100 ms, and 200 ms pulse duration) and constant pulse duration (100 ms) protocols (20 N, 40 N, 60 N, and 80 N). Bars indicated the percentage of positive EMG responses (any occurrence for bilateral L3 and L4 electrodes) for the normal disc group (gray) and degenerated disc group (black).

tion in the outer annular region of the ovine disc. Previous investigations have also demonstrated biomechanical and biochemical similarities between sheep and human intervertebral discs (Reid et al., 2002).

This is the first study reporting changes in neuromuscular responses to SMT among normal and degenerated specimens. The decrease in neuromuscular reflex activity and increased nerve root activity that we observed in the degenerated disc group may therefore be characteristic of and acting synonymously with the pathomechanisms of disc degeneration from the chronic lesion induced. The result of this animal experimental study provide a better understanding of the neurophysiologic responses elicited during posteroanterior mechanical excitation of the normal and degenerated lumbar spine, and represents an important first step in validating chiropractic theories.

Force–time profiles (amplitude, duration) of spinal manipulation therapy, SMT, are known to vary according to the region of the spine being manipulated and the skill and proficiency of the clinician (Triano, 2000; Triano et al., 2002). In this study, therefore, the applied mechanical excitation force–time profiles were chosen to closely

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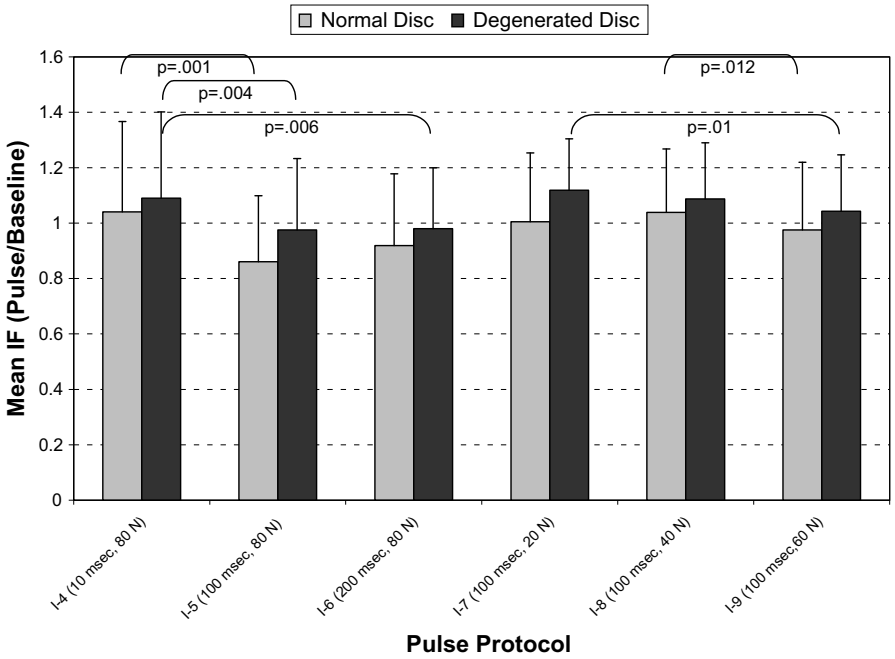

Fig. 6. Instantaneous frequency (IF) response for the constant force (80 N) protocols (10 ms, 100 ms, and 200 ms pulse duration) and constant pulse duration (100 ms) protocols (20 N, 40 N, 60 N, and 80 N). Bars indicated mean (+SD) for the normal disc group (gray) and degenerated disc group (black). *P*-values for significant within group (I-4 vs. I-5, etc.) differences are indicated. No across group differences were observed (e.g. normal vs. degenerated disc). The IF values obtained during the application of the uniform mechanical stimulus pulse are normalized with respect to the IF values obtained prior to the mechanical stimulus. Values greater than 1 indicate increased nerve CAP response frequency during the application of the mechanical stimulus.

resemble SMTs delivered in clinical practice. Specifically, the 10 ms thrusts mimicked mechanical force manually assisted adjusting (MFMA) instruments (Keller et al., 1999), while the 100 ms and 200 ms pulse durations more closely resembled high velocity low amplitude (HVLA) SMTs (Herzog et al., 1993, 2001). Maximum loads imparted to the ovine spine were approximately 17% of the mean animal body weight, which is lower (about threefold) than the maximum loads commonly delivered among clinicians practicing SMT (Herzog et al., 1993, 2001). However, the quasi-static DV stiffness of the anesthetized ovine spine (4.3 N/mm) is also lower (about three- to fourfold) in comparison to the quasi-static PA stiffness of the resting human L3 spine (Keller and Colloca, 2006). Therefore, the peak PA forces imposed on the ovine spine resulted in peak PA displacements comparable to clinical practice.

In data from the current study presented elsewhere (Colloca et al., in review), animals with degenerated discs showed significantly decreased DV displacement and L2-L1 intersegmental accelerations. However, statistically significant disc degeneration-related changes in segmental and intersegmental kinematics were not observed for the shorter duration (10 ms) mechanical excitation pulse protocol, which seems to reflect the fact that impulsive loading produces a more variable kinematic response. In addition, increasing pulse duration from 100 to 200 ms did not appreciably change the amount of DV displacement at the segmental contact point (L3) or adjacent segment motion at L1–L2. This suggests that spinal manipulation treatment strategies that employ shorter duration SMTs (100 ms) are biomechanically more efficient since appreciably less energy is delivered to the spine. The neuromuscular and neurophysiological differences observed during different mechanical excitation force–time profiles are further evidence of changes in somatosensory inputs to varying SMT speeds and forces during SMT. Indeed, the different neurophysiological responses among normal and degenerated animals are novel findings in this area.

It is important to point out that the animals examined in this study were anesthetized, which may have altered the mechanical responses (force–displacement) slightly. Most likely any effects of muscle tone on the force–displacement response of the spine were minimal, however, as the ligamentous spine is a highly damped structure (Keller et al., 2002). Recent work conducted by the authors (Keller et al., 2006) indicates that, while sustained supramaximal muscle stimulation increases the PA stiffness (force–displacement) of the ovine spine up to twofold, the effects of low amplitude muscle stimulation were much less dramatic (less than 5% increase in PA stiffness). Therefore, the absence of muscle tone should have a minimal effect on the neuromechanical responses reported in this study.

Previous work has observed abrupt changes in neural discharge from mechanoreceptive afferents occurring as the duration of the mechanical input approached those typically used in clinically applied SMT (25–200 ms) (Sung et al., 2005). All of the mechanical excitation force–time profiles examined in the current study were found to elicit

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positive EMG responses, which were dependent on mechanical excitation force amplitude, but not on the duration of mechanical excitation. Time-varying mechanical forces delivered during SMT are thought to alter sensory input from primary afferents innervating the paraspinal tissues, thereby having specific actions on central neural mechanisms regulating paraspinal muscles, spinal cord reflexes, and ultimately, spinal biomechanics (Pickar, 2002). Previous research has also determined that SMT atypically activated Group III afferents, evoked high-frequency discharge in both muscle spindle and GTO afferents, which is not observed simultaneously during passive movements (Pickar and Wheeler, 2001). Such sensory input elicited during SMT is thought to contribute to the mechanism of SMT's therapeutic effect (Colloca et al., 2004; Pickar, 2002).

Mechanical excitation force–time profiles were found to affect the discharge of neurons through the spinal nerve roots. During mechanical excitation, mimicking HVLA and MFMA spinal manipulation, we found an abrupt change in the CAP IF, which most likely indicates stimulation of the mechanosensitive afferents in the paraspinal tissues of the spine. Interestingly, the CAP IF response was greatest for the shorter duration SMTs (10 ms, MFMA) and was least for the longer duration SMTs (100 and 200 ms, HVLA). These findings are similar to Sung et al. (2005) who found increasing IF discharge of primary afferents to shorter duration SMTs (25 ms). MFMA-type SMT has been suggested to evoke an unusual or distinctive pattern of somatosensory input to the central nervous system (Sung et al., 2005). Although not explicitly confirmed in the current study, it is likely that the enhanced CAP IF responses are related to the time-varying SMT force–time profiles administered, and deserves further attention.

Indeed, we chose to measure L4 spinal nerve root responses during mechanical excitations being performed at the level above. The L4 level was selected to minimize any destabilizing effect that the necessary decompressive surgery to expose the nerves would have on the biomechanical experiments being conducted cephalad. The polysegmental innervation of the lumbar spine (Bogduk, 1983) and the coupled motion responses in adjacent vertebrae are most likely responsible for the CAP results that were able to be obtained.

While it is unknown just how the presence or magnitude of neuromuscular responses during SMTs relates clinically, preliminary evidence has demonstrated increasing peak-to-peak neuromuscular response thresholds among patients with worsening symptom frequency (Colloca and Keller, 2001). In patients, CAP responses have been found to be markedly decreased on the side of radiculopathy (Colloca et al., 2004). Other research has demonstrated improvement in EMG output following SMT (Keller and Colloca, 2000; Lehman and McGill, 1999). Our finding of decreased EMG responses among animals with degenerated discs likely reflects a disturbance in the neuromuscular reflex control of the stabilizing trunk muscles characteristic of the lesion. These findings are consistent with the pathomechanisms of spinal disorders and underlying mechanisms of SMT. Further research should aim to target the relationships between degenerated discs and neuromuscular responses.

5. Conclusions

Muscular responses were found to increase with increasing mechanical excitation force magnitude, but not force duration, whereas the opposite was observed for compound action potential responses. EMG responses were significantly reduced in the presence of intervertebral disc degeneration, and there was a concomitant significant increase in spinal nerve root discharge in animals with surgically induced disc degeneration. These findings indicate that the neuromuscular stabilizing system of the spine is compromised in the presence of intervertebral disc degeneration.

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