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Using vertebral movement and intact paraspinal muscles to determine the distribution of intrafusal fiber innervation of muscle spindle afferents in the anesthetized cat

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Abstract

Increasing our knowledge regarding intrafusal fiber distribution and physiology of paraspinal proprioceptors may provide key insights regarding proprioceptive deficits in trunk control associated with low back pain and lead to more effective clinical intervention. The use of vertebral movement as a means to reliably stretch paraspinal muscles would greatly facilitate physiological study of paraspinal muscle proprioceptors where muscle tendon isolation is either very difficult or impossible. The effects of succinylcholine (SCh) on 194 muscle spindle afferents from lumbar longissimus or multifidus muscles in response to computer-controlled, ramp and hold movements of the L₆ vertebra were investigated in anesthetized cats. Paraspinal muscles were stretched by moving the L₆ vertebra 1.5 to 1.7mm in the dorsal-ventral direction. Initial frequency (IF), dynamic difference (DD), their changes (Δ) following SCh injection (100–400 μ g $\cdot$ kg⁻¹), and post-SCh dynamic difference (SChDD) were measured. Muscle spindle intrafusal fiber terminations were classified as primary or secondary fibers as well as bag₁ (b_{1c}), bag₂ (b_{2c}), b₁b_{2c}, or chain (c) fibers. Intrafusal fiber subpopulations were distinguished using logarithmic transformation of SChDD and Δ IF distributions as established by previous investigators. Increases in DD indicate strength of b_{1c} influence while increases in IF indicate strength of b_{2c} influence. Out of 194 afferents, 46.9% of afferents terminated on b_{2c} fibers, 46.4% on b₁b_{2c} fibers, 1% on b_{1c} fibers and 5.7% terminated on c fibers. Based on these intrafusal fiber subpopulation distributions, controlled vertebral movement can effectively substitute for direct tendon stretch and allow further investigation of paraspinal proprioceptors in this anatomically complex body region.

Keywords

Muscle Spindle; Neurophysiology; Paraspinal Muscles; Trunk; Lumbar Spine; Succinylcholine

Introduction

To ensure lumbar stability and prevent injury during dynamic spinal loading, the central nervous system (CNS) must recruit trunk muscles in the proper sequence and to the appropriate contractile level during robust physical challenges in order to optimize global postures and control intervertebral motions. Aberrant control of trunk muscles is thought to be involved in both the etiology of low back pain and the accompanying inability of

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individuals with back pain to adopt optimal postural control strategies (Hodges 1999;Brumagne et al. 2000;Reeves et al. 2009a;2009b;Claeys et al. 2011;Johanson et al. 2011). To make kinesthesia and reflexive adjustments to postural disturbances the CNS relies on muscle spindle feedback from both the trunk and the limbs (Hulliger 1984;Panjabi 1992).

Muscle spindles are mechanoreceptors sensitive to muscle length and its rate of change. Differences between muscle spindles in trunk and limb muscles relative to their structural complexity, organization, and response to changes in muscle length have been previously described (Richmond and Abrahams 1979;Bakker and Richmond 1982;Durbaba et al. 2006;Cao et al. 2009a;2009b). Greater knowledge of the intrafusal fiber distribution within spindles of trunk muscles and physiological characteristics of trunk proprioceptors may provide important functional clues to understanding the departure from optimal posture control strategies that reportedly occurs in individuals experiencing back muscle fatigue and/or low back pain (Brumagne et al. 2008;Claeys et al. 2011;Johanson et al. 2011).

Each mammalian muscle spindle lies in parallel with extrafusal muscle fibers and has a complex complement of three types of intrafusal muscle fibers: nuclear bag₁, nuclear bag₂, and chain (c) fibers (Boyd 1981;Taylor et al. 1999) whose contractile state is controlled by gamma- or fusimotor neurons. Endings from a sensory nerve indirectly contact intrafusal fibers and respond to mechanical deformation caused by active intrafusal contraction or passive lengthening of the in-parallel extrafusal fibers. The bag₁ fiber is primarily responsible for supplying the CNS with information about the rate of muscle shortening and receives primarily dynamic fusimotor supply. The bag₂ and chain fibers mainly convey information about muscle length and receive static fusimotor supply but not exclusively. Thus, muscle spindles acting as proprioceptors can be thought of as conveying to the CNS information about the velocity of joint movement (dynamic effects) and joint position (static effects).

In 1966, Rack and Westbury showed that SCh could be used during ramp and hold stretches to identify responses from dynamic bag₁ fibers having primary afferent terminations. Since then, SCh alone or in conjunction with measurements of conduction velocity and/or vibration have been commonly used (Scott 1990;Taylor et al. 1992a;1992b;Durbaba et al. 2006) as tools for classifying intrafusal fibers into four populations (b₁c, b₁b₂c, b₂c, and c) based upon responses from primary and secondary muscle spindle afferents. Small doses of SCh produce intrafusal contracture of bag₁ fibers (b₁) and sustained tonic afferent discharge, thereby mimicking the effect of dynamic fusimotor stimulation (Gladden 1976;Boyd 1985;Price and Dutia 1987;Price and Dutia 1989). SCh also elicits contraction of bag₂ (b₂) fibers raising the afferent's resting discharge known as bias. SCh does not cause contraction of c intrafusal fibers (Gladden 1976;Boyd 1985). Increases in dynamic responsiveness as indicated by changes in dynamic difference (ΔDD) during repetitive ramp-and-hold stretches is thought to be the best indicator of b₁ fiber influence while changes in initial frequency (ΔIF) are thought to indicate b₂ fiber influence. Despite identification of intrafusal fiber types, relating muscle spindle structure to function continues to present formidable challenges due in large part to the complexity of innervation patterns as well as distribution variability. In the 1990s, Taylor and colleagues (Taylor et al. 1992a;1992b) established an objective method to classify the intrafusal fibers on which an afferent terminates by applying a natural log transformation introduced by Hald (1952) to the frequency distributions of the change in dynamic and static responses (ΔDD and ΔIF , respectively) before and after succinylcholine during repetitive ramp-and-hold stretches. Using this method, they described the relative percentages of intrafusal fiber types in muscles whose tendons they were able to isolate, including limb (Taylor et al. 1992b), jaw

(Taylor et al. 1992a) and a single back muscle whose tendon is long enough to isolate (Durbaba et al. 2006).

Neurophysiological study of muscle spindles in the trunk musculature in particular has been hampered in large part due to the inherent technical difficulties encountered while investigating this complex anatomical region. Compared to limb muscles, the tendons of many paraspinal muscles are often very short or poorly defined thereby making them difficult if not impossible to isolate in situ. Consequently, the use of direct tendon stretch in which the actual muscle length changes can be clearly defined is severely limited for investigating physiological characteristics and intrafusal fiber distributions in the vast majority of trunk muscles. In an attempt to overcome this anatomical barrier, it would be advantageous if paraspinal muscles could be left intact and controlled vertebral movements used to consistently stretch muscles attached to the vertebra being moved. In the present study we used computer-controlled vertebral movements of the intact lumbar spine before and after succinylcholine (SCh) injection in a feline preparation with an intact lumbar spine to determine the distribution of intrafusal terminations from a large sample of longissimus and multifidus muscle afferents. Although this method has the inherent limitation of not being able to clearly define actual muscle length changes, it has been shown to produce consistent and reliable after-effects in multifidus and longissimus muscle spindles in the intact lumbar spine (Ge et al. 2005; Pickar et al. 2007; Cao et al. 2009b).

The purpose of this study was two-fold. First, it was to determine the distribution of a large number of paraspinal intrafusal fibers using controlled movements of a lumbar vertebra; and second, it was to compare these findings using this novel method of muscle stretch with those that previously used direct muscle tendon stretch (Taylor et al. 1992a; 1992b; Durbaba et al. 2006). Substituting vertebral movement in place of direct tendon stretch to study paraspinal muscle spindles would be a valuable tool allowing the investigation of a majority of back muscles having too short or inaccessible muscle tendons. Greater understanding of trunk proprioceptive feedback could provide key insights into proprioceptive deficits associated with low back pain and perhaps lead to more successful clinical interventions for both acute and chronic low back pain.

Methods

General

Data were obtained from 194 deeply anesthetized adult cats over the course of several studies investigating paraspinal muscle spindle afferent responses to controlled spinal loading of the intact lumbar spine. Cats were of either sex (male, n=146; female, n=48) and weighed between 2.3 and 6.0kg. All experiments were reviewed and approved by the Institution's Animal Use and Care Committee and conducted in accordance with the Guiding Principles in the Care and Use of Animals approved by the American Physiological Society. Only one afferent was investigated per cat because following the spinal loading protocols, deeper tissues needed to be surgically exposed to confirm that the source of afferent activity was in the lumbar multifidus or longissimus muscles. The compromise in lumbar tissue integrity prohibited investigation of additional afferents. Calibrated nylon monofilaments (Stoelting, Ill) were applied to the exposed muscles to verify that the most sensitive portion of the afferent's receptive field was in fact located within either paraspinal muscle. General procedures have been described in detail previously (Cao et al. 2009a; Ge et al. 2005; Pickar 1999; Pickar et al. 2007). Briefly anesthesia was induced with 100% O₂/5% isoflurane or halothane and maintained with 100% O₂/3% isoflurane or halothane. Catheters were placed in the common carotid and external jugular vein to monitor blood pressure and introduce fluids. The trachea was intubated and the cat ventilated mechanically. Anesthetic was then maintained with pentobarbital (35mg kg⁻¹, i.v.) and the gaseous anesthetic

withdrawn. Deep anesthesia was identified by absence of a withdrawal reflex to noxious pinching of the toe pad, mean arterial pressures less than 120mmHg and the absence of a pressor response to surgical manipulation. Additional pentobarbital (5mg kg^{-1}) was administered as needed. As in similar studies (Taylor et al. 1992a;1992b;Durbaba et al. 2006), it was assumed that fusimotor activity would be suppressed by pentobarbital anesthesia. Arterial pH, PCO_2 , and PO_2 were monitored every 60-90 minutes using an i-Stat pH/blood gas analyzer (i-Stat Corp, East Windsor, NJ) to maintain normal physiological ranges (pH 7.32-7.43, PCO_2 , 32-37mmHg; PO_2 >85mmHg). Arterial pH and PCO_2 was corrected by infusing sodium bicarbonate and/or adjusting the ventilator, while PO_2 was maintained by bleeding 100% O_2 into the intake line of the ventilator. Animals were euthanized at the end of the experiment by an intravenous overdose of pentobarbital.

To record afferent nerve activity from the L_6 dorsal roots, a bilateral L_{4-5} laminectomy was performed exposing the L_{5-6} spinal cord as previously described (Pickar 1999). Due to the small laminectomy, we could not rotate the spinal cord to expose and cut the L_6 ventral roots; therefore they remained intact. The musculature on the right side of the spinal column (multifidus, longissimus and iliocostalis muscles) remained intact except for any attachments to the posterior portions of the L_{4-5} vertebrae and for small slit incisions (3mm) on either side of the L_6 spinous process for forceps attachment by which the vertebra was moved. Little of the multifidus muscle was detached from the L_6 vertebra using this method because it's aponeurotic tendon inserts onto the process's caudal edge (Bogduk 1980). The sciatic nerve on the right side was cut close to its emergence from the iliac fossa to reduce afferent input from the hindlimb. The lumbar spine was mechanically secured at the L_4 spinous process and the iliac crests using a Kopf spinal unit (David Kopf Instruments, Tujunga, CA). Paraspinal tissues were bathed in warm mineral oil (37°C) to prevent tissue desiccation. The dura mater was incised and the L_6 dorsal root was cut close to the spinal cord. Thin dorsal root filaments were teased until single unit activity with a receptive field in the paraspinal musculature could be identified. The L_6 spinal nerve innervates the fascicles of the multifidus and longissimus muscles attaching to the L_6 vertebra (Bogduk 1976).

Activity from a putative muscle spindle in the paraspinal muscles was first identified by gently compressing the tissues or by manually moving the forceps attached to the L_6 vertebra ventralward to evoke a high frequency discharge. Only afferents whose discharge was highest in response to probing the trunk muscles compared with the gluteal, hip or leg regions were used. Prior to SCh injection, we isolated and confirmed that the afferent's receptive field was in a lumbar paraspinal muscle by removing the sacrocaudalis dorsalis lateralis (lumbococcygeus) muscle which lies between the lumbar multifidus and longissimus muscle. First and second sacral nerves innervate the lumbococcygeus muscle so that none of the afferent recordings were lost by its removal. Calibrated von Frey hairs were applied to determine the most sensitive area for mechanically activating the afferent. Action potentials were passed through a high impedance probe (HIP511, Grass, West Warwick, RI), amplified (P511K, Grass) and monitored using an audiomonitor (Grass) and digitally stored. Data were recorded with a CED 1401 interface (Cambridge Electronic Design, UK) and off-line analysis was performed with Spike 2 (Cambridge Electronic Design, Cambridge, UK) data acquisition software.

Mechanical Loading of the L_6 vertebra

Controlled movement of the L_6 vertebra was applied to stretch the paraspinal muscles using an electronic feedback control system (Lever System Model 310; Aurora Scientific, Aurora, Ontario Canada) as in previous studies (Pickar 1999;Ge et al. 2005;Cao et al. 2009a;Ge et al. 2011). One end of the forceps (1x2 teeth) was clamped to the L_6 spinous process and the other was attached to the Lever System. Thus L_6 vertebral movement was obligatorily coupled to and determined from lever arm movement. Prior to ramp and hold testing, the

lumbar spine was initially positioned in a neutral position with the spine producing no external force against the Lever System. Movements consisted of ramp (5mm s^{-1}) and hold (1.5s) translations of the L₆ vertebra applied every 4 seconds in a dorsal-ventral direction. Hold magnitudes were either 1.5mm (n=124), 1.6mm (n=20), or 1.7mm (n=50).

Afferent Identification and Classification

Muscle spindle afferents were confirmed and characterized based upon a) their ability to respond to muscle vibration, b) silencing during direct muscle stimulation, and c) their discharge pattern to ramp and hold vertebral movements before and after an intra-arterial (i.a.) SCh injection ($100\text{--}400\mu\text{g}\cdot\text{kg}^{-1}$). Small amplitude vibrations (50-90Hz) were applied to the most sensitive area of their receptive field. Direct muscle electrical stimulation ($0.12\text{--}6\text{mA}$, $10\text{--}100\mu\text{s}$) was applied with a constant current stimulus isolation unit (Grass Instrument, PSIU6, West Warwick, RI) and a square wave stimulator (Grass Instrument, S88) using two needle electrodes inserted into either side of the most sensitive portion of the afferent's receptive field.

In order to classify spindle afferents based upon intrafusal fiber innervation and compare them with previous studies of jaw (masseter, temporalis, pterygoid), gastrocnemius, and longissimus muscle spindles by Taylor and colleagues (Taylor et al. 1992a; 1992b; Durbaba et al. 2006) we used a similar approach and similar metrics to those they described. Thus, we report values for initial frequency (IF) and dynamic difference (DD) and their changes following succinylcholine injection (ΔIF and ΔDD). In addition, values for DD following SCh injection (SChDD) are presented as previously reported for the longissimus muscle (Durbaba et al. 2006). Afferent activity was typically recorded during a control period consisting of 5 or 6 ramp and hold cycles, followed by a rapid injection of SCh (100 or $200\mu\text{g}\cdot\text{kg}^{-1}$ in saline) over less than a cycle, followed by ramp and hold cycles for the next 4-5 minutes similar to that described by others (Rack and Westbury 1966; Taylor et al. 1992a; 1992b; Durbaba et al. 2006). No measures were taken to increase blood flow to the trunk muscles. Consequently, an additional dose of SCh was occasionally needed (24 of 194 afferents; 12%) to elicit a spindle response. No more than a total of $400\mu\text{g}\cdot\text{kg}^{-1}$ of SCh was administered in a given preparation. Ramp and hold responses were averaged over three cycles prior to and during 60-90s following the SCh injection. A 60-90s time interval yields a reasonable steady state of representative responses (Taylor et al. 1992a). Cycle histograms of spindle afferent activity using 50ms and 100ms bin widths were constructed. IF was calculated as the mean frequency during the 0.5s preceding the ramp. Peak frequency (PF) was the maximum frequency occurring toward the end of the dynamic stretch which eliminated any influence of the initial burst. Dynamic difference ($\text{DD} = \text{PF} - \text{IF}$) was derived from these measurements. Statistical tests used included Kruskal-Wallis one-way ANOVA ($P > 0.05$) to compare responses between the three hold magnitudes and the established method of separating muscle spindle subpopulations derived from Hald (1952) using logarithmic transformation of distribution counts, parabolic fitting, and linear re-transformation of the counts (Taylor et al. 1992b; Taylor et al. 1992a; Schafer 1994).

In addition to intrafusal fiber classification, qualitative criteria described by Scott (1990) to identify an afferent as terminating in primary or secondary endings were used to further characterize all 194 afferents. To be considered as terminating in primary endings, an afferent met at least two of the three following criteria: (i) the presence of an initial burst at the onset of a $5\text{mm}\cdot\text{s}^{-1}$ ramp stretch; (ii) a clear discontinuity in the response at the end of the stretch (iii) the absence of discharge during the ramp release. Afferents with secondary endings showed (i) a slow continuous increase in instantaneous frequency during ramp-up; (ii) slow adaptation at the end of ramp-up; (iii) continued firing at the beginning of ramp down. Intermediate endings show mixed characteristics.

RESULTS

Spindle afferents were isolated from the longissimus (n=159) or the multifidus (n=35) muscles. Biomechanically both muscles are considered local muscles attaching directly to the lumbar vertebra (Bergmark 1989). Ninety-five percent (185/194) of spindle afferents had a resting discharge with the lumbar spine lying in a neutral position prior to SCh injection. In the 9 afferents without a resting discharge, direct stimulation was tested following SCh injection which provided a resting discharge. Direct muscle stimulation decreased the response of all afferents, a characteristic of muscle spindles.

Primary vs Secondary Classification

Based upon qualitative criteria described by Scott (1990) 96% (186/194) of spindle afferents were classified as having primary endings while 2% (4/194) were classified as having secondary endings. Representative examples of primary and secondary endings are shown in Figure 1. The remaining 2% were classified as intermediate afferents showing characteristics common to both primary and secondary endings. Of the 155 afferents tested with low frequency vibration (50-90Hz) all but two were able to follow frequencies above 50Hz. Being driven by frequencies above 40Hz has been suggested to distinguish between primary and secondary afferent endings (Matthews and Watson 1981).

Succinylcholine increases dynamic responsiveness by inducing b_1 fiber contracture. Figure 2 shows two representative examples of responses from lumbar muscle spindle afferents during ramp and hold movements of the L_6 vertebra before and after SCh injection. During the control period in Fig. 2a, the afferent had an IF of 24 impulses s^{-1} . Its discharge fell silent during the release from hold. Following a single SCh injection ($100\mu g \cdot kg^{-1}$) IF increased substantially to a maximum of 140 impulses s^{-1} accompanied by an increase dynamic sensitivity ($\Delta DD = 121$ impulses s^{-1}) over the 60-90s period following injection. Figure 2b shows an afferent with a resting IF of 27 impulses s^{-1} and a distinct dynamic response during the ramp vertebral movement prior to SCh injection similar to that shown in Fig. 2a. Following SCh, IF increased much less than the afferent shown in Fig. 2a (maximum of 52 impulses s^{-1}) and was accompanied by a decrease in dynamic response ($\Delta DD = -24$ impulses s^{-1}). It is these distinctive dynamic and bias response characteristics following SCh injection that form the basis for intrafusal fiber (b_1c , b_2c , b_1b_2c , or c) classification.

Figure 3 shows the raw distributions of IF and DD during the control period (open bars) and following SCh injection (filled bars) in cats grouped according to "hold" magnitudes (1.5 to 1.7mm) used during movement of the L_6 vertebra. Resting discharge during the control period was similar across the three hold magnitudes (Fig. 3; left column, open bars) with control IFs having similar distributions. The majority of afferents had control IFs below 30 impulses s^{-1} . In all 3 groups following SCh injection, IF increased substantially to values less than 125 impulses s^{-1} with mean IFs (SD) of 62.0 (16.2), 63.7 (21.9), and 70.6 (24.5) impulses s^{-1} for the 1.5, 1.6, and 1.7mm displacement holds, respectively. Control values for DD extended up to 130 impulses s^{-1} and increased to 264 impulses s^{-1} following SCh injection. Mean control DD was 58.3 (23.1), 57.1 (22.3), and 68.7 (26.7) impulses s^{-1} and 60.1 (48.7), 58.7 (43), and 82.2(60) impulses s^{-1} following SCh for the 1.5, 1.6, and 1.7mm displacement groups, respectively. Distribution patterns were not substantially different using 100ms bin widths compared to the 50ms bin widths, therefore only 50ms bin width data are shown in Fig. 3 and subsequent figures.

To enable comparisons of raw data with previous studies (Taylor et al. 1992a; 1992b; Durbaba et al. 2006), we report values for ΔIF , ΔDD , and SChDD (Fig.4). Mean ΔIF (SD) was 36.3 (19.2), 39.7 (22.8), and 45.0(22.2) impulses s^{-1} for the 1.5, 1.6, and 1.7mm,

respectively. As seen in Figure 4 (middle column), ΔDD was positive for 73 of the 194 afferents (37.6%) and negative for the remaining 121 (62.4%). The impact of this high percentage of lumbar spindle afferents with a negative ΔDD is reflected in the low mean values for ΔDD of 1.7 (38.4), 1.6 (32.7) and 13.5 (56.8) impulses s^{-1} for 1.5, 1.6, and 1.7mm displacements respectively. Mean SChDD was 60.1 (48.7), 58.66 (43.0), 82.2 (60.0) for the 1.5, 1.6, 1.7mm displacements respectively. In previous muscle spindle classification studies, the frequency distributions for SChDD and ΔIF in the longissimus muscle (Durbaba et al. 2006) and ΔDD and ΔIF in the leg and jaw muscles (Taylor et al. 1992b; Taylor et al. 1992a) were subsequently used as a basis for identifying intrafusal fiber subpopulations. Therefore, to specifically determine and compare our findings with previous findings in the back (Durbaba et al. 2006) we used the distributions for SChDD and ΔIF . The 1.5, 1.6, 1.7mm hold magnitude groups were combined for both SChDD and ΔIF because there were no differences between groups using the Kruskal-Wallis one-way ANOVA ($P > 0.05$).

To determine threshold afferent discharge rates for dividing intrafusal fibers subpopulations, the distributions of SChDD and IF were logarithmically transformed according to a previously established method (Taylor et al. 1992b; Taylor et al. 1992a; Schafer 1994) derived from Hald (1952) as shown in Figure 5. The completely transformed distributions (Figs. 5b and 5e) each could be fit with two parabolic distributions representing two populations. After taking the anti log of the ordinate, the cross-over point or threshold frequency between the two subpopulations for $\ln(SChDD)$ occurred at $\ln(3.87)$ impulses s^{-1} (Fig. 5c) corresponding to a discharge rate of 47.9 impulses s^{-1} . Similarly, the cross-over point between the distributions for $\ln(\Delta IF)$ occurred at 2.50 impulses s^{-1} (Fig. 5f) corresponding to a threshold frequency of 12.2 impulses s^{-1} . SChDD was then plotted against the corresponding values of ΔIF (Fig. 6). Horizontal and vertical lines were drawn at the respective threshold frequencies for SChDD and ΔIF determined from Figs. 5c and 5f. Two lumbar spindle afferents (1%) could be classified as terminating exclusively on b_1 fibers (quadrant 1: high dynamic response, low static response), 46.4% as terminating on b_1b_2c fibers (quadrant 2: high dynamic and static responses), 46.9% as terminating on b_2c fibers (quadrant 3: low dynamic response and high static response) and 5.7% as terminating on c fibers. The b_1b_2 afferent population had a mean (SD) ΔIF and SChDD of 48.7 (20.1) and 105.5(51.9) impulses s^{-1} respectively; while the b_2c afferents had a mean ΔIF and SChDD of 33.2(16.1) and 30.7(10.3) impulses s^{-1} .

Discussion

Limitations in using conduction velocities to classify muscle spindle afferents has led to the use of new approaches for characterizing them, including their intrafusal fiber terminations. The use of succinylcholine accompanied by muscle stretch has provided quantitative estimates for the number of contributions of bag_1 and bag_2 terminations in muscles of the limb, jaw, and low back (Dutia 1980; Taylor et al. 1992a; 1992b; Durbaba et al. 2006). These muscles had been stretched by isolating and directly pulling on their tendons. The purpose of this study was to determine whether leaving lumbar muscles attached to their vertebra and then stretching them using controlled ramp and hold movements of an individual lumbar vertebra could be used to classify intrafusal fiber terminations despite not knowing the actual changes in muscle length. The ability to use vertebral movement of the intact spine in lieu of using direct tendon stretch would permit physiological investigation of the majority of paraspinal muscles whose tendons are either too difficult or impossible to isolate. Despite not being able to directly measure changes in paraspinal muscle length in the current preparation, this could be estimated based on geometric considerations using the Pythagorean theorem and anatomical attachment sites of the longissimus and multifidus (Bogduk 1980; Cao et al. 2009b; Ge et al. 2011). With the longissimus and multifidus muscle lying oblique to the spine's long axis and containing multiple-layered fiber bundles oriented

in a complex fashion (Bogduk 1980), the magnitude of muscle lengthening is not the same as the magnitude of vertebral translation. Nevertheless, since L_6 actuation in both the sagittal (dorsal-ventralward) and longitudinal (caudal-cranialward) planes has been shown to evoke similar muscle spindle discharge it has been estimated that for every 0.20mm change in L_6 actuation longissimus and multifidus muscles would lengthen by 0.19mm and 0.16mm respectively (Cao et al. 2009b;Ge et al. 2011).

Our control values for IF and DD in lumbar muscles inclusive of multifidus and longissimus were similar to those previously reported for the longissimus muscle (Durbaba et al. 2006) where the majority of their 119 afferents had IFs less than 25 impulses s^{-1} and DDs less than 90 impulses s^{-1} in response to ramp pulls of the tendon. The majority of the 194 afferents in the present study had IFs of 30 impulses s^{-1} and DDs 80 impulses s^{-1} in response to ramp movements of the vertebra (Fig. 3). These control DDs for the lumbar muscles are greater than those reported for jaw (Taylor et al. 1992a) and limb (Taylor et al. 1992b) muscles.

The higher control DD values in back muscles, despite using deep pentobarbital anesthesia to depress central motor outflow as evidenced by the elimination of both cardiovascular responses to noxious pinch and all withdrawal, pinna and corneal reflexes has previously been attributed to persistent tonic γ_d activity (Durbaba et al. 2006), similar to the pentobarbital-resistant γ activity found in intercostals muscles (Sears 1964). However, unlike in the intercostal study, where the ventral roots were cut, there has been no direct evidence showing that γ_d activity persists in the lumbar paraspinal muscles under pentobarbital anesthesia. Indirect evidence from lumbar paraspinal muscles (Ge et al. 2007) suggests that anesthesia resistant γ_d activity does not persist under deep pentobarbital. This raises the possibility that control DD is actually higher in back muscles compared to the jaw or limb muscles. Other differences between trunk and limb muscles with regards to their anatomy and physiology have been described by a number of investigators (Richmond and Abrahams 1979;Price and Dutia 1987;1989;Taylor et al. 1992a;Durbaba et al. 2006;Cao et al. 2009a;2009b). For example, peak dynamic sensitivity for spindles in the biverter cervicis muscle following SCh administration was nearly half that reported for soleus spindles (Price and Dutia 1987). Explanations for higher control DD values in the trunk as opposed to the limb may include but are not limited to different patterns of muscle innervation, and/or inherit diversity among spindle encoder sites, signal integration and summation (Carr et al. 1996;Mileusnic et al. 2006) within back musculature perhaps due to unique postural maintenance and respiratory demands.

Increases in dynamic sensitivity (ΔDD) and bias (ΔIF) following SCh injection have been used to demonstrate relative contributions from b_1 and b_2 intrafusal fiber contraction respectively to the discharge of individual spindle afferents (Gladden 1976). In large populations of muscle spindle afferents, ΔIF and ΔDD distributions following SCh injection and their logarithmic transformation were used to quantify the percentage of spindle afferents that terminate on b_1 and/or b_2 intrafusal fibers in limb and jaw muscles (Taylor et al. 1992a;1992b). However, using direct tendon pull to stretch the longissimus muscle, Durbaba et al. (2006) found that neither ΔIF or ΔDD provided assistance in distinguishing between b_1 and b_2 fiber contributions leading them to search for alternative approaches for delineating intrafusal fiber subpopulations. It should be noted that ΔIF responses should be independent of the method used to stretch the muscles because IF represents resting values prior to the stretch. For the dynamic response, Durbaba et al.(2006) used DD values following SCh injection (SchDD) and their logarithmic transformation [$\ln(\text{SchDD})$] to quantify the percentage of spindle afferents terminating on b_1 and/or b_2 intrafusal fibers. Using this metric instead of ΔDD was motivated by the assumption that tonic γ_d activity likely created a ceiling effect on the dynamic response to succinylcholine. For the

longissimus, Durbaba et al.(2006) determined the best ln (SChDD) dividing point between intrafusal fiber populations to be 3.9 corresponding to a value of 50 impulses s^{-1} . Our larger data set suggested nearly the same value of 3.87 corresponding to a value of 47.9 impulses s^{-1} . We also report a similar proportion of muscle spindle afferents terminating on b_2c intrafusal fibers (46.9%) in lumbar muscles compared to the 51.4% reported by Durbaba et. al. (2006) in the longissimus muscle. Additionally, our b_2c fiber percentage is closer to the 43% that occur for spindle afferents in the gastrocnemius muscle using a threshold frequency value derived from ΔDD (Taylor et al. 1992b). We report a distribution of 46.4% b_1b_2 fibers in the back muscles compared to the 41.9% reported in the longissimus muscle by Durbaba et al.(2006). Our b_1 fiber percentage (1%) and c fiber percentage(5.7%) confirm the 1.3% and 5.4% distribution reported using direct stretch of the longissimus muscle tendon on a smaller number of back afferents (Durbaba et al. 2006). Of the intrafusal fiber classification studies to date, jaw muscles appear to have the greatest b_1 fiber innervation (7%) in comparison to either the back or hindlimb (Taylor et al. 1992a;1992b;Durbaba et al. 2006). The functional significance of intrafusal fiber variation remains to be determined and continues to present a formidable challenge in this field of study.

A characteristic of the ΔDD distribution which attracted our attention was the presence of negative values (Fig. 4, middle column). This has been reported previously but not addressed in any detail. Negative ΔDD reflects a dynamic response that is diminished in the presence of succinylcholine. Sixty-two percent of our population had negative ΔDD s. Using tendon pull to create ramp stretches, 42% of Durbaba et al.'s (2006) population also had negative ΔDD s. These rather large percentages in the lumbar muscles are 3-4 times greater than the approximate 16% found in the gastrocnemius muscle (Taylor et al. 1992b) and somewhat closer to the approximate 33% found in jaw muscles (Taylor et al. 1992a). What could potentially account for negative ΔDD s? One possibility is neuromuscular blockade at the b_1 fusimotor terminals described by Carr et al. (1996). In the presence of tonic γ_d activity, succinylcholine in doses similar to those we and Durbaba et al. (2006) used, produces smaller dynamic responses compared to control. While we have previously argued against the presence of tonic γ_d activity in the pentobarbital-anesthetized cat preparation (see above), direct experimental evidence for this conclusion is lacking. A second possibility arises from a study of muscle spindles in the cervical spine. Dynamic responses lower in magnitude after succinylcholine compared to before were attributed to a depolarizing block at the fusimotor neuromuscular junction (Price and Dutia 1987). It was not clear whether this occurred predominately at b_1 terminals. If any of the negative ΔDD s are a consequence of a succinylcholine-induced blockade at b_1 fusimotor terminals, some of the b_2c spindle afferents may be misclassified. The mechanisms underlying the high proportion of muscle spindle afferents with dynamic responses that decrease following succinylcholine injection and the consequences for classifying muscle spindle afferents according to the intrafusal fibers on which they originate requires further investigation.

In summary these results confirm the findings of a single smaller intrafusal fiber classification study within back musculature (Durbaba et al. 2006). More importantly, this study indicates that stretch of the back muscles as defined by the magnitude of controlled vertebral movement along a single axis can be used in lieu of direct muscle tendon stretch to investigate trunk proprioception. This novel approach of using vertebral movement in lieu of direct tendon stretch will greatly facilitate intrafusal fiber classification and physiological characterization of proprioceptors in the majority of trunk muscles in which muscle tendon isolation in situ is either very difficult if not impossible. Greater understanding of trunk-related proprioceptive feedback could provide key insights into proprioceptive deficits associated with low back pain while at the same time perhaps lead to more effective clinical interventions for both acute and chronic low back pain.

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Reference List

1. Bakker DA, Richmond FJR. Muscle spindle complexes in muscles around upper cervical vertebrae in the cat. *J Neurophysiol.* 1982; 48:62–74. [PubMed: 6214618]
2. Bergmark A. Stability of the lumbar spine. A study in mechanical engineering. *Acta Orthop Scand Suppl.* 1989; 230:1–54. [PubMed: 2658468]
3. Bogduk N. The lumbosacral dorsal rami in the cat. *J Anatomy.* 1976; 122:653–662.
4. Bogduk N. The dorsal lumbar muscles of the cat. *Anatomy Anz*, Jena. 1980; 148:55–67.
5. Boyd, IA. The action of the three types of intrafusal fibre in isolated cat muscle spindles on the dynamic and length sensitivities of primary and secondary sensory endings. In: Taylor, A.; Prochazka, A., editors. *Muscle receptors and movement.* Oxford University Press; New York: 1981. p. 17-32.
6. Boyd, IA. Intrafusal muscle fibres in the cat and their motor control. In: Barnes, WJP.; Gladden, MH., editors. *Feedback and motor control in invertebrates and vertebrates.* Croom Helm; London: 1985. p. 123-144.
7. Brumagne S, Cordo P, Lysens R, Verschueren S, Swinnen S. The role of paraspinal muscle spindles in lumbosacral position sense in individuals with and without low back pain. *Spine.* 2000; 25:989–994. [PubMed: 10767813]
8. Brumagne S, Janssens L, Knapen S, Claeys K, Suuden-Johanson E. Persons with recurrent low back pain exhibit a rigid postural control strategy. *Eur Spine J.* 2008; 17:1177–1184. [PubMed: 18594876]
9. Cao DY, Khalsa PS, Pickar JG. Dynamic responsiveness of lumbar paraspinal muscle spindles during vertebral movement in the cat. *Exp Brain Res.* 2009a; 197:369–377. [PubMed: 19590861]
10. Cao DY, Pickar JG, Ge W, Ianuzzi A, Khalsa PS. Position sensitivity of feline paraspinal muscle spindles to vertebral movement in the lumbar spine. *J Neurophysiol.* 2009b; 101:1722–1729. [PubMed: 19164108]
11. Carr RW, Morgan DL, Proske U. Impulse Initiation in the mammalian muscle spindle during combined fusimotor stimulation and succinylcholine infusion. *J Neurophysiol.* 1996; 75:1703–1713. [PubMed: 8727407]
12. Claeys K, Brumagne S, Dankaerts W, Kiers H, Janssens L. Decreased variability in postural control strategies in young people with non-specific low back pain is associated with altered proprioceptive reweighting. *Eur J Appl Physiol.* 2011; 111:115–123. [PubMed: 20824281]
13. Durbaba R, Taylor A, Ellaway PH, Rawlinson S. Classification of longissimus lumborum muscle spindle afferents in the anaesthetized cat. *J Physiol.* 2006; 571:489–498. [PubMed: 16410280]
14. Dutia MB. Activation of cat muscle spindle primary, secondary and intermediate sensory endings by suxamethonium. *J Physiol.* 1980; 304:315–330. [PubMed: 6449590]
15. Ge W, Cao DY, Long C, Pickar JG. Plane of vertebral movement eliciting muscle lengthening history in the low back influences the decrease in muscle spindle responsiveness of the cat. *J Appl Physiol.* 2011; 111:1735–1743. [PubMed: 21960662]
16. Ge W, Cao DY, Pickar JG. Resting discharge of paraspinal muscle spindles is stable under deep anesthesia using sodium pentobarbital. *Society for Neuroscience.* 2007; 27:14
17. Ge W, Long CR, Pickar JG. Vertebral position alters paraspinal muscle spindle responsiveness in the feline spine: effect of positioning duration. *J Physiol.* 2005; 569:655–665. [PubMed: 16210357]
18. Gladden M. Structural features relative to the function of intrafusal muscle fibres in the cat. *Progress in Brain Research.* 1976; 44:51–59. [PubMed: 137428]
19. Hald, A. *Statistical Theory with Engineering Applications.* John Wiley; New York: 1952.

20. Hodges P. Altered trunk muscle recruitment in people with low back pain with upper limb movement at different speeds. *Arch Phys Med Rehabil.* 1999; 80:1005–1012. [PubMed: 10489000]
21. Hulliger M. The mammalian muscles spindle and its central control. *Rev Physiol Biochem Pharmacol.* 1984; 101:1–110.
22. Johanson E, Brumagne S, Janssens L, Pijnenburg M, Claeys K, Paasuke M. The effect of acute back muscle fatigue on postural control strategy in people with and without recurrent low back pain. *Eur Spine J.* 2011; 20:2152–2159. [PubMed: 21533851]
23. Matthews PB, Watson JD. Action of vibration on the response of cat muscle spindle Ia afferents to low frequency sinusoidal stretching. *J Physiol.* 1981; 317:365–381. [PubMed: 6458696]
24. Mileusnic MP, Brown IE, Lan N, Loeb GE. Mathematical models of proprioceptors. I. Control and transduction in the muscle spindle. *J Neurophysiol.* 2006; 96:1772–1788. [PubMed: 16672301]
25. Panjabi MM. The stabilizing system of the spine. Part I. Function, dysfunction, adaptation and enhancement. *J Spinal Disorders.* 1992; 5:383–389.
26. Pickar JG. An in vivo preparation for investigating neural responses to controlled loading of a lumbar vertebra in the anesthetized cat. *J Neurosci Methods.* 1999; 89:87–96. [PubMed: 10491938]
27. Pickar JG, Sung PS, Kang YM, Ge W. Response of lumbar paraspinal muscles spindles is greater to spinal manipulative loading compared with slower loading under length control. *Spine J.* 2007; 7:583–595. [PubMed: 17905321]
28. Price RF, Dutia MB. Properties of cat neck muscle spindles and their excitation by succinylcholine. *Exp Brain Res.* 1987; 68:619–630. [PubMed: 2961588]
29. Price RF, Dutia MB. Physiological properties of tandem muscle spindles in neck and hind-limb muscles. *Prog Brain Res.* 1989; 80:47–56. [PubMed: 2699377]
30. Rack PMH, Westbury DR. The effects of suxamethonium and acetylcholine on the behavior of cat muscle spindles during dynamic stretching and during fusimotor stimulation. *J Physiol.* 1966; 186:698–713. [PubMed: 4226417]
31. Reeves NP, Cholewicki J, Lee AS, Mysliwiec LW. The effects of stochastic resonance stimulation on spine proprioception and postural control in chronic low back pain patients. *Spine.* 2009a; 34:316–321. [PubMed: 19214090]
32. Reeves NP, Cholewicki J, Narendra KS. Effects of reflex delays on postural control during unstable seated balance. *J Biomech.* 2009b; 42:164–170. [PubMed: 19121523]
33. Richmond FJ, Abrahams VC. Physiological properties of muscle spindles in dorsal neck muscles of the cat. *J Neurophysiol.* 1979; 42:604–617. [PubMed: 154558]
34. Schafer SS. Regularity in the generation of discharge patterns by primary and secondary muscle spindle afferents, as recorded under a ramp and hold stretch. *Exp Brain Res.* 1994; 102:198–209. [PubMed: 7705500]
35. Scott JJ. Classification of muscle spindle afferents in the peroneus brevis muscle of the cat. *Brain Res.* 1990; 509:62–70. [PubMed: 2306639]
36. Sears TA. Efferent discharges in alpha and fusimotor fibres of intercostal nerves of the cat. *J Physiol.* 1964; 174:295–315. [PubMed: 14244124]
37. Taylor A, Durbaba R, Rodgers JF. The classification of afferents from muscle spindles of the jaw-closing muscles of the cat. *J Physiol.* 1992a; 456:609–628. [PubMed: 1293289]
38. Taylor A, Ellaway PH, Durbaba R. Why are there three types of intrafusal muscle fibers? *Prog Brain Res.* 1999; 123:121–131. [PubMed: 10635709]
39. Taylor A, Rodgers JF, Fowle AJ, Durbaba R. The effect of succinylcholine on cat gastrocnemius muscle spindle afferents of different types. *J Physiol.* 1992b; 456:629–644. [PubMed: 1293290]

Fig. 1.

Examples of the qualitative criteria used to classify afferent endings as primary (top panel) or secondary (middle panel), during ramp and hold movement of the L₆ vertebra (bottom panel). Primary endings exhibited at least two of the following three characteristics: A initial burst at ramp-up onset; B discontinuity in response at end of ramp-up; and C absence of response during ramp release. Secondary endings exhibited all of the following characteristics: D a slow continuous frequency increase during ramp-up; E slow adaption at end of ramp-up; and F continued firing during ramp down

Fig. 2.

Examples of two types of responses from muscle spindle afferents to a single injection (shown at arrow) of succinylcholine (Sch; $100\mu\text{g} \cdot \text{kg}^{-1}$) during repetitive ramp and hold movements of the L₆ vertebra. Hold displacements were 1.7mm in (2a) and 1.5 mm in (2b). Note the initial frequency (IF) is similar for both afferents prior to Sch injection. Note the large bias and dynamic difference (DD) characteristic of spindle afferents with b₁b₂c intrafusal terminations (2a) and the small bias and no increase in DD characteristic of b₂c intrafusal terminations (2b) following Sch injection

Fig. 3.

Frequency histograms showing the distributions of initial frequency (IF, left column) and dynamic difference (DD, right column) for 194 lumbar muscle spindle afferents in response to ramp and hold movements of the L₆ vertebra prior to and following succinylcholine (SCh) injection. Three hold magnitudes were used: 3a 1.5mm (n=124); 3b 1.6mm (n=20); and 3c 1.7mm (n=50). Open bars represent control values prior to SCh injection and filled bars represent responses 60-90s following SCh injection. Control and post-SCh distributions of IF and DD were similar across magnitudes and were used to make comparisons with distributions of raw data from previous studies (see Discussion)

Fig. 4.

Frequency histograms showing the distributions of the change in initial frequency (ΔIF , left column), change in dynamic difference (ΔDD , middle column), and dynamic difference following succinylcholine injection (SChDD, right column) for 194 lumbar muscle spindle afferents during ramp and hold movements of the L_6 vertebra following succinylcholine injection. Three hold magnitudes were used: 1.5mm ($n=124$); 1.6mm ($n=20$); 1.7mm ($n=50$). Distributions of ΔIF , ΔDD and SChDD were similar across hold magnitudes

Fig. 5.

Steps in the process of dividing the frequency distributions of SChDD (left column) and ΔIF (right column) into two subpopulations (see text describing basis for approach). For SChDD, distributions were logarithmically transformed (5a, 5b) and fitted with a parabolic curve (5b) first for abscissa values from 4.3 to 5.6 (○). Points for the second parabola included values to the left of abscissa value 4.3 (●). In addition, where original data (+) used for the second parabola overlapped with the first parabola, points for the second parabola (Δ) were obtained by subtracting values along the first parabola from the vertically-aligned original data (+). In 5c ordinate values have been transformed back into linear values and plotted separately to derive the subpopulation threshold values (dashed line). The process was repeated for ΔIF in the right column. The first parabola was fitted for abscissa values from 2.8 to 4.6 and the second parabola included abscissa values to the left of 2.8. Subpopulation threshold values (shown in 5c and 5f) using controlled vertebral movement were similar to those values previously obtained using direct tendon pull methods (see Discussion)

Fig. 6.

Subpopulations of muscle spindle afferents based upon threshold values. Changes in initial frequency (ΔIF) versus dynamic response to succinylcholine (SChDD) have been plotted for all 194 spindle afferents. The position of the horizontal line divides SChDD values at 48 impulses s^{-1} as indicated by Fig. 5c and the vertical line divides ΔIF values at 12 impulses s^{-1} as indicated by Fig. 5f. Subpopulations of spindle afferents are designated by quadrants 1-4 as follows: b_1c (quadrant 1), b_1b_2c (quadrant 2), b_2c (quadrant 3) and c intrafusal fiber (quadrant 4) terminations. The number of afferents in each subpopulation is shown in parentheses. The majority of spindle afferents terminated on b_1b_2c or b_2c fibers. The overall number of afferents in each of these two subpopulations was similar.