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Deep tissue incision enhances spinal dorsal horn neuron activity during static isometric muscle contraction in rats

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Abstract

Translational correlates to pain with activities after deep tissue injury have not been studied to a great extent. We hypothesized that deep tissue incision causes greater activation of nociception-transmitting neurons evoked by muscle contraction. *In vivo* neuronal activity was recorded in 203 dorsal horn neurons (DHNs) from 97 rats after sham, skin-only, or skin + deep muscle incision. We evaluated DHN responses to static, isometric muscle contractions induced by direct electrical stimulation of the muscle. The effect of pancuronium on DHN response to contractions was also examined. Approximately 50% of DHNs with receptive fields in the hindpaw was excited during muscle contraction. One-second, 0.5- and 1.0-gram muscle contractions produced greater DHN activity after skin + deep muscle incision [median (interquartile range): 32 (5 – 39); $p = 0.021$ and 36 (26 – 46) impulses; $p = 0.006$, respectively] than after sham [6 (0 – 21) and 15 (8 – 32) impulses, respectively]. Neuromuscular blockade with pancuronium inhibited the muscle contractions and DHN activation during electrical stimulation, demonstrating contraction-induced activation. The greater response of spinal DHNs to static muscle contraction after skin + deep muscle incision may model and inform mechanisms of dynamic pain after surgery.

Perspective: Completing various activities are important milestones for recovery and hospital discharge after surgery. Skin + deep muscle incision caused greater activation of nociception-transmitting dorsal horn neurons evoked by muscle contraction compared to skin-only incision. This result suggests an important contribution of deep muscle injury to activity-evoked hyperalgesia after surgery.

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Keywords

Deep tissue incision; postoperative pain; static isometric muscle contraction; spinal dorsal horn neuron activity; *in vivo* extracellular recordings

Introduction

Severe acute postoperative pain often complicates the recovery process after surgery,^{15, 27} resulting in prolonged hospitalization associated with respiratory and cardiovascular complications.^{5, 8, 15, 33}

To better understand the mechanisms for pain caused by surgery, we previously have generated a rat model of postoperative nociception.^{3, 4} We demonstrated that incision in skin + deep muscle caused more spontaneous activity in nociceptors and dorsal horn neurons (DHNs), compared to skin incision alone,^{3, 12, 34, 35} suggesting that deep tissue injury is one of the generators of ongoing nociception. Cutaneous responses are most often studied; however, pain associated with deep tissue injury is of increasing significance for evaluating clinical, pathologic pain conditions and improving its treatment.

Despite its clinical significance, translational correlates to pain with activities have not been studied to a great extent. Pain with activities is associated in part with muscle contraction and muscle function.^{1, 8, 10} Previous work on DHNs and primary afferents suggest that evoked response to von Frey filaments after the incision is largely a cutaneous response,^{34, 35} and this may have little relation to evoked response to muscle contractions that occur during activities. The present study used muscle contraction elicited by electrical stimulation as a stimulus to simulate contractions during activities. We examined the effect of static, isometric muscle contraction on DHN activity. The DHN responses to muscle contraction were examined in rats that underwent sham incision, skin incision or skin + fascia and muscle incision of the hindpaw. We hypothesized that deep muscle incision causes greater activation of nociception-transmitting neurons evoked by muscle contraction.

Material and Methods

General

All experiments were reviewed and approved by the University of Iowa Animal Care and Use Committee (Approval number: 1109205), Iowa City, Iowa, USA and conformed to the NIH Guide for the Care and Use of Laboratory Animals. Adult male Sprague-Dawley rats (250 – 300 g, 8–10 weeks old, Harlan, Indianapolis, IN, USA) were used in this study. Rats were housed in groups of 2 in clear plastic cages (40 × 60 × 30 cm) on fresh bedding with free access to food and water. The environment was controlled with a 12-hour light-dark cycle and a room temperature of 22.0 ± 2.0 °C. The physical conditions of the animals were carefully monitored every weekday for clinical signs of illness during the experiments. The animals did not show any signs of stress (except nociception-related behavior) or illness throughout the experiment. Altogether, 97 rats were used in this study.

Surgical incisions

Anesthesia was induced by placing the animal in a sealed plastic box with 3 – 5% isoflurane mixed with air. Then anesthesia was maintained at 1.5 – 2% isoflurane delivered via a nose cone during the following surgical procedures. The duration of surgery was approximately 10 – 15 minutes.

The left hindpaw was prepared in a sterile manner with 10% povidone-iodine. For rats subjected to skin-only incision, a 1-cm longitudinal incision was made beginning 0.5 cm from the proximal edge of the heel, using a specially designed handle and blade device.³⁵ During the incision, the blade handle was applied against the plantar surface indenting the skin to make the skin incision. The incised skin was then opposed with three subcutaneous mattress sutures with 6–0 nylon on a P-1 needle (Ethicon, Somerville, NJ, USA) while visualized under a binocular dissecting microscope to limit deep tissue damage.

The skin + deep muscle incision was made similarly to that described previously.^{34, 35} At the same plantar site as in the skin incision, a 1-cm longitudinal incision was made through the skin, underlying fascia and the plantar flexor digitorum brevis muscle with a No. 11 surgical blade. Blunt curved forceps were then inserted through the incision into the muscle to further divide and retract the muscle. The muscle origin and insertion remained intact. This method was similar to that described previously,⁴ except that the muscle was not elevated at its dorsal surface. The wound was then closed with three subcutaneous mattress sutures as described for the skin incision. A sham surgical procedure included isoflurane anesthesia and sterile preparation of the hindpaw, but no incision was made.

Behavioral studies

A total of 44 rats, randomly divided into three groups, underwent behavioral study prior to electrophysiological studies. One day after sham, skin incision, or skin + deep muscle incision, the rats underwent abbreviated testing for guarding behavior.^{4, 34} Unrestrained rats were placed individually on a small plastic mesh floor (grid 8 × 8 mm) covered with a plastic chamber (21 × 27 × 15 cm). Using an angled magnifying mirror, the position of the rat hindpaw was closely observed during a 1-minute period repeated for every 5 minutes for 30 minutes. Based on the hindpaw position during the majority of the 1-minute observation period, a score of 0, 1 or 2 was given. Zero was given for full weight bearing position of the incised area of the hindpaw which was blanched or distorted by the mesh; 1 was given if the incised area of the hindpaw just touched the mesh without blanching or distortion; 2 was given if the incised area of the hindpaw was completely off the mesh. We abbreviated the scoring to 6 scores during the 30-minute session, and the 6 scores were added together (0 – 12) for each paw. Then the cumulative pain score was determined by subtracting the sum of the score of non-incised hindpaw from that of incised hindpaw. The maximum possible score was 12. The person performing the behavioral experiments was blinded to the type of incision.

Electrophysiological studies

General—The surgical preparation of the animal for electrophysiological recording was performed according to the previous methods.^{34, 37} Briefly, one day after sham, skin

incision, or skin + deep muscle incision, anesthesia was induced with 3 – 5% isoflurane in air through a sealed box and then maintained with 2% isoflurane in air via a nose cone. The common carotid artery was cannulated to monitor the blood pressure. Then a tracheotomy was performed and the rat trachea was connected to a ventilator (Harvard Apparatus, Inc., South Natick, MA, USA) via the tracheal cannula. The animal was artificially ventilated with oxygen and anesthesia was maintained with 2% isoflurane. The rat was then positioned in a stereotactic frame. The head and vertebral column of the rat were stabilized with ear bars and vertebral clamps, respectively. Limited laminectomies were performed to expose the dorsal spinal cord at the lumbar enlargement between 13th thoracic and 3rd lumbar vertebra. The underlying dura was removed, and the spinal cord was covered with mineral oil. The rat's paw was placed plantar side up and elevated from the base of the recording platform by 1 cm. The ankle and dorsum of the paw were embedded in clay. The three middle digits were glued together with cyanoacrylate and attached to a P11T strain gauge amplifier (Grass Instruments, Quincy, MA, USA) with a 6-0 nylon suture (Fig. 1).

After these surgical procedures, anesthesia was decreased to 1.2% isoflurane during the subsequent recording period. For DHN recording, small holes were made with fine forceps on the pia mater of the left side lumbar enlargement between the L4 and L6 segments. A tungsten parylene-coated electrode (1 – 1.5 m Ω impedance, Microprobe Inc., Clarksburg, MA, USA) was then driven into the dorsal spinal cord through the pia mater hole. Neuronal activity was amplified (Grass Instruments, Quincy, MA, USA), displayed on an oscilloscope and discriminated on the basis of amplitude and waveform (BAK Electronics, Germantown, MD, USA). All data were recorded and stored in a computer with a data acquisition system 1401-plus and Spike2 software (Cambridge Electronic Design, Cambridge, UK).

During the DHN recording, the mean arterial blood pressure was at least 90 mmHg. The body temperature was maintained at a range of 35 – 37 °C with a servo-controlled electric heating lamp and an underbody heating pad. The person performing the electrophysiology was not blinded to the type of incision.

Recording protocol—According to the previous method,^{34, 36} the recording electrode was lowered to the surface of the spinal cord onto the pia mater hole, and the depth of the electrode was set as 0 μ m. Then the electrode was advanced into the spinal cord slowly at 10 μ m/step via a micropositioner (David Kopf Instruments, Tujunga, CA, USA) until a neuron with ongoing, spontaneous activity was encountered or depth of approximately 1000 μ m was reached. If a neuron with ongoing activity was detected, the mechanical receptive field of the neuron was determined by applying mechanical stimulation (tapping and a 228-mN von Frey filament) to the plantar hindpaw. A neuron was accepted for further study only if its mechanical receptive field included at least part of the incision site. If no neurons met the criteria, and the 1000- μ m depth was reached, the electrode was then driven backward to the dorsal surface of the spinal cord at a rate of 10 μ m/step via the micropositioner while touching and tapping were applied to the plantar hindpaw. Repeated mechanical stimulation was used to search for DHNs that received input from the plantar hindpaw and did not necessarily have ongoing activity. Again, these neurons without ongoing activity were accepted for further study if the mechanical receptive field included at least part of the

incision site. The depth of the neuron recorded was based on the electrode position optimized for the action potential amplitude.

After a neuron with the receptive field that included the incision site was identified using either search stimulus (ongoing activity or mechanically evoked activity from the hindpaw), the action potential amplitude was optimized. A baseline measurement for ongoing activity was recorded for 3 minutes. Ongoing activity of the neuron during this period was averaged. A neuron with a mean activity rate greater than 0.1 impulses/second was considered to be spontaneously active.

Following the measurement of spontaneous activity, the cutaneous response properties to brush, touch, pinch, and monofilaments were determined as described previously.^{34, 36} Low threshold neurons were not recorded. As shown by others, neurons were all classified as WDR neurons since all responded to low threshold brush and/or touch and pinch.²⁴ We recognize the limitations of this cutaneous classification given that touch was used as a search stimulus for locating DHNs. Also, some rats had undergone hindpaw incision which affects cutaneous response properties. Finally, needle electrodes were inserted into the hindpaw in all experiments (see below). This may further limit the utility of the cutaneous classifications.

Static, 5-second isometric contractions (Part 1): *In vivo* extracellular DHN recording was performed after the behavioral tests in 44 rats. Contraction of plantar flexor digitorum brevis muscle was elicited by the electrical stimulation delivered through a pair of 30-gauge insulated stainless steel needles that were inserted into the muscle medial to the incision, approximately 0.5 cm apart. The stimulation frequency was 50 Hz; pulse duration was 50 µseconds. Voltage was varied to generate a static isometric contraction to the targeted peak tension. The tension generated by electrical stimulation was monitored and recorded with the data acquisition system. The tension index was defined as the area under the tension curve during muscle contraction.

The baseline tension was set at 1.0 gram. A static isometric contraction was produced using a 5-second stimulus targeted to generate a peak tension of 1.0 and 3.0 grams above baseline. Criteria for a DHN response to muscle contraction were the same as those used for responses to mechanical stimuli:¹² If baseline spontaneous activity was present, the DHN was considered as responsive when action potential rate during muscle contraction was increased at least two standard deviations (SD) greater than the baseline activity; If spontaneous activity was not present, the DHN was considered responsive when at least one action potential was elicited during the contraction. The response to either the 1.0-gram or 3.0-gram tension was required for the DHN to be considered responsive to contraction.

In some neurons, we were able to detect activity elicited during stimulation and contraction, but upon review, could not completely discriminate the neuronal activity from the stimulus artifacts. These neurons were designated as responding to contraction but not quantifiable.

1-second isometric contractions (Part 2): In part 2, we examined neural response to the brief and less forceful contractions in separate groups of rats. The contractions were 1-

second duration generating targeted peak tension of 0.5, 1.0, and 1.5 grams from a resting tension of 1.0 gram. In part 1, we recognized that higher voltages might be required to generate the same tension in rats that had undergone skin + deep muscle incision. Therefore, in part 2, we recorded voltages required to elicit these brief, isometric contractions. The same search criteria were used to select DHNs as part 1, and all neurons had the receptive field that included the incision site.

First, we examined the neural response to 1-second isometric contractions in 37 rats (18 rats in the sham group and 19 in the skin + deep muscle incision group). We then examined the effect of pancuronium on DHN response to isometric contractions in another 16 rats (8 rats in each group) as described below (Part 3). Data obtained from all these 53 rats were incorporated and analyzed to evaluate DHN response to 1-second isometric contractions.

Effect of pancuronium (Part 3).: Only the neurons activated by muscle contraction were selected for testing with pancuronium. Sixteen neurons, 8 from the sham-operated group and 8 from the skin + deep muscle incision group, were tested with both 5-second, 1.0- and 3.0-gram isometric contractions and 1-second, 0.5-, 1.0-, and 1.5-gram isometric contractions. Then pancuronium (0.5 mg/kg, Hospira, Lake Forest, IL, USA) was administered via a right femoral vein catheter (polyethylene 50 tubing). Five minutes later, the same voltages were used to elicit muscle contractions, and the corresponding tension and neuronal activity were again recorded.

Statistical analysis

For continuous data, the Shapiro-Wilk test was used to determine whether the normality assumption is satisfied. The parametric tests were used when the normality assumption was satisfied. The two-way ANOVA with repeated measures on one factor followed by Šidák's multiple comparisons test was used to evaluate the tension and tension index generated during 5-second contractions. The unpaired t-test was used to compare the depth in the spinal cord between the DHNs with spontaneous activity *vs.* those without spontaneous activity. The depth of the neurons between the DHNs responding *vs.* not responding to contractions was also compared using the unpaired t-test.

The nonparametric tests were applied when the normality assumption was not satisfied. The Kruskal-Wallis test followed by Dunn's multiple comparisons test was used to compare cumulative pain scores and rates of spontaneous activity among the three incision groups. The Chi-square test with the Bonferroni correction for multiple tests was used to compare the percentage of neurons with and without spontaneous activity, and neurons responding and non-responding to muscle contraction among groups. The generalized linear mixed model was used to test main effects and interactions for DHN impulses during 1-second and 5-second contractions, tension and voltage during 1-second contractions, and tension generated each second during 5-second contractions; significant main effects or interactions were further tested with the Kruskal-Wallis test or Friedman test with Šidák correction for multiple comparisons followed by Dunn's multiple comparisons test. DHN impulses and tension generated before and after administration of pancuronium were also tested with the generalized linear mixed model; significant main effects or interactions were further tested

The Friedman test with Šidák correction followed by Dunn's multiple comparisons test or the Wilcoxon matched-pairs signed rank test with Šidák correction.

All data are presented as mean $\pm$ SD or median with interquartile range. Values of $P < 0.05$ were considered significant. All tests were performed using SPSS Statistics (version 25, IBM, Armonk, NY, USA) and GraphPad Prism software (version 7.0a, GraphPad Software, Inc., San Diego, CA, USA).

Results

General—A total of 97 rats were used in this study, and no serious adverse events in each experimental group.

Behavioral study—In 44 rats, the median abbreviated cumulative pain score with interquartile range was 0 (–1 to 1), 3 (1 to 5), and 8 (6 to 9) in the sham ($n = 18$), skin incision ($n = 11$), and skin + deep muscle incision ($n = 15$) groups, respectively (Fig. 2A). The cumulative pain score of the skin + deep muscle incision group was greater than that of the sham ($p < 0.0001$) and skin incision groups ($p = 0.0282$).

Electrophysiological studies: Static, 5-second isometric contractions (Part 1)

General: One hundred and fifteen DHNs in 44 rats had a mechanical receptive field in the plantar hindpaw, part of which included the incision or the corresponding site in the sham, unincised hindpaw. Forty-two neurons from 18 rats in the sham group, 37 neurons from 11 rats in the skin incision group, and 36 neurons from 15 rats in the skin + deep muscle incision groups were recorded. As noted, all were WDR neurons. Forty-nine responded to brush and all 115 were activated by touch and pinch.

Spontaneous activity: The percentage of neurons with spontaneous activity was 16.7% (7/42), 18.9% (7/37), and 36.1% (13/36) in the sham, skin incision, and skin + deep muscle incision groups, respectively (Fig. 2B). There was no difference in the percentage of neurons with spontaneous activity among the groups ($p = 0.0949$). The median rate (and interquartile range) of spontaneous activity was 6.1 (3.6 – 10.0), 1.0 (0.7 – 5.7) and 9.0 (2.9 – 30.2) impulses/second in the sham, skin incision, and skin + deep muscle incision groups, respectively (Fig. 2C). There was a significant difference between the skin incision vs. the skin + deep muscle incision groups ($p = 0.0464$).

Contraction-responsive DHNs: The percentage of DHNs activated by muscle contraction was 38.1% (16/42), 48.6% (18/37), and 58.3% (21/36) in the sham, skin incision, and skin + deep muscle incision groups, respectively (Fig. 2D); these proportions were not different ($p = 0.2022$). Among these DHNs, responses were evident but not quantifiable during the 1.0-gram contraction in 6 neurons from the sham group, 5 neurons from the skin incision group and 4 neurons in skin + deep muscle group. During the 3.0-gram contraction, 6 neurons in the sham group, 5 neurons from the skin incision group and 7 neurons from the skin + deep muscle group responded, but the neuronal activity was not quantifiable.

An example of responses during 5-second contractions is shown in Fig 3A, and data for all quantifiable DHN are summarized Fig. 3B and C. The DHN responses and tension

generated during 1.0- and 3.0-gram contractions were significantly greater than baseline in all three groups (Supplementary Fig. S1). DHNs in the skin + deep muscle incision group showed increased responses during 1.0-gram, 5-second contractions compared to the sham and skin incision groups (Fig. 3B and Supplementary Fig. S1C); the neuronal responses of the skin + deep muscle incision group to 1.0-gram contractions were greater compared to sham at 2 ($p = 0.0027$), 3 ($p = 0.0020$), and 4 ($p = 0.0014$) seconds, and at 2 ($p = 0.0251$), 3 ($p = 0.0180$), and 4 ($p = 0.0354$) seconds compared to skin incision. There was no significant difference in contraction-induced neuronal activity between the sham and skin incision groups. No difference among groups was found in the neuronal responses during 3.0-gram contraction. The contractions elicited the same tension in all 3 groups (Fig. 3C and Supplementary Fig 1).

Total activity of the DHNs in the skin + deep muscle incision group was greater compared with the sham group during 1.0-gram contraction (Fig. 4A). There were no significant differences in total activity between sham vs. skin incision during both 1.0- and 3.0-gram contraction. Analyses of DHN activity, after subtracting the baseline activity, showed similar results with the data analyzed without subtracting baseline activity (Fig. 4B). There were no significant differences between groups in tension generated (Fig. 4C) and tension index (Fig. 4D) during both 1.0- and 3.0-gram, 5-second contractions.

Characteristics of DHNs: The recording depths of neurons with and without spontaneous activity were compared within each group (Fig. 5A-C). The depth of spontaneously active neurons [(SA (+))] was greater than the depth of those without spontaneous activity [SA (-)] for all 3 groups (Fig 5A-C). The neurons responding to muscle contraction were deeper compared to those not responding to muscle contraction in the sham and skin incision groups (Fig. 5D and E). There was no difference in depth between those responding and not responding to contractions in the skin + deep muscle incision group (Fig. 5F).

In the sham group, 5 of 7 SA (+) neurons (71%) and 12 of 30 SA (-) neurons (40%) responded to contractions ($p = 0.0467$). In the skin incision group, 6 of 7 SA (+) neurons (86%) and 15 of 27 SA (-) neurons (56%) responded to contractions ($p = 0.1434$). In the skin + deep muscle incision group, 9 of 13 SA (+) neurons (69%) and 12 of 23 SA (-) neurons (52%) responded to contractions ($p = 0.3187$). Except for the sham group, there was no relationship between SA (+) neurons and contraction-responsive neurons in either incision group. Altogether, neurons with spontaneous activity were deeper in the dorsal horn than those without spontaneous activity. Neurons responding to contraction tended to be deeper in the dorsal horn than those that did not respond.

Electrophysiological studies: 1-second isometric contractions (Part 2)

General: Eighty-eight DHNs were recorded in 53 rats (26 rats from the sham group and 27 rats from the skin + deep muscle incision group). Among these, 40 DHNs (18 neurons from 16 rats in the sham group and 22 from 18 rats in the skin + deep muscle incision group) responded to contraction elicited by brief electrical stimulation and could be quantified. Forty-eight neurons did not respond or could not be quantified. We did not continue recording from DHNs that were not quantifiable.

Contraction-responsive DHNs: Examples for 0.5-, 1.0-, or 1.5-gram increases are shown in Fig. 6A. The contractions produced a tension-dependent activation of DHNs in both groups (Fig. 6B). While contractions with higher tension levels generated greater DHN responses compared to baseline in both the incision and the sham groups, the median DHN responses of the skin + deep muscle incision group were greater than the sham group at 0.5- and 1.0-gram contractions (Fig. 6B). Analyses of DHN activity, after subtracting the baseline activity, showed similar results (Supplementary Fig. S2). The mean tension values generated in the sham vs. skin + deep muscle incision groups were not significantly different (Fig. 6C). When we compared voltages used to generate the targeted increase in tension to 0.5, 1.0, and 1.5 grams, greater voltages were required to elicit the same tension in incised muscle compared with the sham group (Fig. 6D).

Effect of pancuronium on muscle contractions and DHN responses (Part 3)

Effect of pancuronium on DHN responses to 5-second isometric contractions: An example of a contraction-responsive neuron, from a rat that had undergone skin + muscle incision, before and after pancuronium is shown in Fig. 7A. Before administration of pancuronium, the DHN activity and tension generated during 3.0-gram contractions were significantly greater than the baseline values in both the skin + deep muscle incision and the sham groups (Fig. 7B and C and Supplementary Fig. S3A and B). On the other hand, after administration of pancuronium, neuronal activity and tension generated during contractions were not different from the baseline values. In both the skin + muscle incision and the sham groups, the tension values generated during both 1.0- and 3.0-gram contractions were less after administration of pancuronium compared to values obtained before administration of pancuronium (Fig. 7B). The DHN responses during 1.0- and 3.0-gram contractions before administration of pancuronium were significantly greater compared with those after administration of pancuronium (Fig. 7C and Supplementary Fig. S3A and C).

Effect of pancuronium on DHN responses to 1-second isometric contractions: An example of DHN response to 1-second contractions before and after administration of pancuronium is shown in Fig. 8A. Before administration of pancuronium, the DHN activity and tension generated during 1.0- and 1.5-gram contractions were significantly greater than the baseline values in both the skin + deep muscle incision and sham groups (Fig. 8B and C and Supplementary Fig. S4A and B). On the other hand, after administration of pancuronium, neuronal activity and tension generated during contractions were not different from the baseline values. In both the skin + deep muscle incision and the sham groups, the tension levels generated during 0.5-, 1.0- and 1.5-gram contractions were significantly less after administration of pancuronium, compared with values obtained before pancuronium (Fig. 8B). In the skin + muscle incision group, the DHN responses during 0.5-, 1.0- and 1.5-gram contractions before administration of pancuronium were significantly greater compared with those after administration of pancuronium (Fig. 8C and Supplementary Fig. S3A and C). In the sham group, DHN activity before administration of pancuronium was significantly greater compared with the value after pancuronium only during 1.5-gram contractions.

Discussion

The present study, for the first time, examined the activity of nociception-transmitting DHNs during muscle contraction induced by electrical stimulation of muscle after incision. Approximately 50% of neurons with somatic receptive fields in the hindpaw were excited during isometric muscle contraction. Increasing tension of the contraction produced greater responses of the DHNs. Neuromuscular blockade with pancuronium blocked the muscle contraction and reduced the DHN activity during electrical stimulation, demonstrating contraction-induced activation. Those neurons responding to contraction tended to be located deeper in the dorsal horn than those not responding to contraction. Finally, contraction of the muscle produced greater DHN activity after skin + deep muscle incision than after sham or skin-only incision.

DHN responses to muscle contraction

As in previous studies, incision of deep muscle tissue produced greater guarding scores and either an increase in the proportion of DHNs with spontaneous activity or a greater average rate of spontaneous activity compared to either sham or skin incision. Electrical stimulation of the flexor digitorum brevis muscle, which produced similar tension during contractions in all groups, caused greater DHN activity in the muscle incision group. This greater response in the muscle incision group during 5-second isometric contractions was evident at the relatively weaker tension level, 1.0-gram, but not at 3.0-gram. We then further examined the effect of incision on the DHN activity during brief, 1-second contractions with tension levels 0.5 – 1.5 gram; these abrupt episodes of contractions may be similar to some activities that generate a very abrupt fast response, such as coughing. Again, our results showed greater DHN response in the muscle incision group at the relatively weaker tension levels, 0.5- and 1.0-gram, compared with the sham group.

Skin incision was not sufficient to enhance the DHN response to contraction compared with sham (no incision). In the previous histologic studies, incision of skin, fascia, and muscle produced edema and muscle injury that resolved by 7 days later.³⁴ When we attempted to injure only skin, a histologic exam revealed fascial edema and inflammation that, in the present study, did not affect responses to contraction.

Neuromuscular blockade with pancuronium has been used by others to block the contraction produced by electrical stimulation of the ventral root or sciatic nerve. In our preparation, pancuronium blocked the contraction induced by the narrow pulse width and high-frequency stimulus which likely activated large diameter, efferent motor axons. Large diameter, Type I or Type II afferents could also have been excited.²⁹ Considering that muscle contractions were completely abolished after administration of pancuronium, it is quite unlikely that direct depolarization of the muscle fibers contributed in any significant degree to contractions induced by electrical stimuli used in the present study. Blocking the contraction, in the presence of electrical stimulation, significantly reduced the neuronal response to electrical stimulation, suggesting that direct stimulation of primary afferents was not the major contributor to the increased DHN response during contractions.

Incision of deep muscle tissue, in this study, did not significantly increase the proportion of DHNs responding to contraction. This suggests we did not convert DHNs from contraction-unresponsive to contraction-responsive DHNs by incision. Rather, those that are responsive to contraction had increased their response magnitude after incision.

In this study and in previous studies, DHNs with spontaneous activity after incision were located deeper in the dorsal horn compared to those without spontaneous activity.³⁴ These findings suggest that muscle tissue afferents projecting to the deeper laminae of the spinal cord may have the greater contribution to the spontaneous and contraction-evoked neural activity, compared to the cutaneous and muscle afferents projecting to the superficial laminae.^{2, 9, 23, 34} In the present study, contraction-responsive neurons also tended to be located deeper in the dorsal horn compared with the neurons unresponsive to muscle contraction, but it was not consistent among groups: the depth of DHNs responding to contraction was deeper compared to those not responding to muscle contraction in both the sham and skin incision groups, but not in the skin + deep muscle incision group.

Postoperative pain during activities

After surgery, patients typically rate their pain at rest, without provocations, and pain with activities using various scales.^{8, 10, 11, 15} Pain with activities is usually greater than pain at rest. In patients undergoing major surgery, common activities such as turning in bed, getting in and out of a chair, or walking generate minimal to no pain before surgery, but are notably painful after surgery. Therefore, pain with activities after surgery is hyperalgesia.

Previously, we have frequently used heat or mechanical stimuli to study sensitization after incision. Uniquely, in the present study, we used muscle contraction as the stimulus to begin to understand pain with muscle contraction after injury. Our intent for these experiments was to better understand pain with activities after surgery by using static, isometric contraction to simulate activity before and after surgery. Both brief 1-second and sustained 5-second contractions were used.

Static isometric contraction and pain

Static isometric contraction of hindlimb skeletal muscle in cats has been used to study the pressor reflex response.^{19, 20} The superficial dorsal horn of the spinal cord is the major site of the first synapse of this reflex. Interactions of the reflex pressor response and pain are likely.²² Static isometric contractions of uninjured muscle in human subjects are generally not painful. If isometric contractions are sustained, subjective fatigue and subsequent pain are perceived.^{7, 30}

There are few experimental human models of pain with muscle contraction after injury. Delayed onset muscle soreness (DOMS) is a painful human muscle injury induced by repeated concentric and eccentric exercise.^{7, 30} After DOMS, subjects underwent isometric muscle contraction of the DOMS quadriceps muscles and the contralateral, uninjured quadriceps muscle. Isometric contraction of DOMS quadriceps muscle produced pain whereas the contralateral non-sore muscle was not painful during contractions. This isometric contraction of injured muscle also produced widespread activation in the primary somatosensory and motor (S1, M1) cortices on functional magnetic resonance imaging.³⁸

Although the mechanism of injury in DOMS is different from injury induced by surgery, these findings demonstrated that isometric contraction of injured muscle induced pain in human subjects.

Metabonociceptors, which are thought to contribute to sensing fatigue and nociceptive muscle stimuli, are activated by high concentrations of metabolites such as low pH, increased lactic acid, reactive oxygen species and adenosine triphosphate.^{14, 18, 21, 25} These metabolites are produced in the muscles during ischemic contractions.^{21, 25} We have previously shown that an ischemic-like condition is present in muscle tissue after incisions and this ischemic-like condition may contribute to the ongoing activity of nociceptors in incised muscle.^{13,16,17,31,32} Furthermore, if these ischemic-like conditions were increased by muscle contractions, then these metabolites could contribute to nociception and the enhanced responses in DHNs after incision.

Limitations

First, in some recordings, DHNs were excited by contraction but could not be quantified due to artifacts and other DHN activity during contractions. Second, because higher voltages were required in incised muscle to generate the same tension compared to unincised muscle, we used pancuronium to demonstrate that direct activation of afferent fibers by electrical stimulation was limited. Greater voltage requirement in the muscle incision group could be explained at least in part by the availability of fewer muscle fibers for force production due to the incisional injury and by structural damage to the fascia. Higher voltages likely recruited more motor units to produce the intended tension. These structural changes and also edema and reflexive contributions, in addition to pain, may contribute to the limited muscle function observed in patients after surgery. Finally, we do not know if these DHN responses are related to postoperative pain, postoperative fatigue or a combination of both.

Conclusion

Completing various activities is important milestones for recovery and hospital discharge after surgery. Activity-evoked hyperalgesia after surgery may be a result of injury to several tissues like skin, fascia, muscle, tendon and serous membranes. Pain with activities is, in part, an example of a static, isometric muscle contraction.^{6, 28} Modeling and understanding mechanisms for dynamic pain after incisional injury will further our understanding of postoperative pain mechanisms.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Highlights

- Rat dorsal horn neuron response to muscle contraction after incision was examined.
- Muscle contraction excited ~50% of neurons with receptive fields in the hindpaw.
- Contraction-evoked activity was higher after skin + muscle incision than skin-only.
- Pancuronium inhibited contraction by electrical stimulation and neuronal activity.
- This suggests a contribution of muscle injury to activity-evoked pain after surgery.

Figure 1. Schematic for *in vivo* extracellular dorsal horn neuron (DHN) recording and the flexor digitorum brevis muscle contraction preparation. (A) The DHNs were recorded at the L4–6 segment of the spinal cord. (B) Static isometric contraction of the flexor digitorum brevis muscle was elicited by electrical stimulation using a pair of 30-gauge insulated stainless steel needles inserted into the muscle. (C) The tension in grams was measured by a strain gauge amplifier attached to the middle 3 digits of the hindpaw by a 6–0 nylon suture.

Figure 2.

Summary of behavior and dorsal horn neuron (DHN) responses in rats after incision. (A) Guarding behavior of rats that underwent DHN recording 1 day after sham, skin incision, or skin + deep muscle incision. Guarding pain behavior was measured during a 30-minute period. The results are presented as median with interquartile range. $\dagger p < 0.05$; $\dagger\dagger\dagger\dagger p < 0.0001$ by Kruskal-Wallis test followed by Dunn's multiple comparisons test. (B) Percentage of neurons with spontaneous activity in the sham, skin incision, and skin + deep muscle incision groups. Numbers in parentheses represent the number of DHNs which had spontaneous activity and the total number of neurons tested. The proportions of spontaneously active neurons were compared using the chi-square test. (C) The rate of spontaneous activity. The results are presented as median with interquartile range. $\dagger p = 0.0401$ by Kruskal-Wallis test followed by Dunn's multiple comparisons test. (D) Percentage of neurons activated by 5-second isometric muscle contraction in the sham, skin incision, and skin + deep muscle incision groups. Numbers in parentheses represent the

number of DHNs activated and the total number of neurons tested. The proportions of contraction-responsive neurons were compared using the chi-square test.

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Figure 3.

Median dorsal horn neuronal impulses per second before, during, and after 5-second muscle contractions in rats that underwent sham, skin incision, and skin + deep muscle incision. (A) Sample recording showing a spontaneously active DHN that was activated by muscle contraction. DHN responses to 1.0-gram and 3.0-gram increases in tension by 5-second contractions are shown. Activity is shown before, during, and after 5-second muscle contractions. The upper panel is tension generated, the middle panel is the digitized oscilloscope trace of the action potentials, and the lower panel is the peristimulus histogram of the action potentials (bin width = 1 second). The inset shows several representative single action potentials. (B and C) Summary of the contraction-responsive DHN's activity (B) and the tension generated (C) during 1.0-gram and 3.0-gram increases in tension. Data are presented as median with interquartile range. Main effects and interactions tested with the generalized linear mixed model are shown in the graphs. †† $p < 0.01$ vs. sham; & $p < 0.05$

vs. skin by Kruskal-Wallis test with Šidák correction for multiple comparisons followed by Dunn's multiple comparisons test. The detailed results of *post hoc* analyses are given in Supplementary Figure S1. Imp = impulses.

Figure 4.

Summary of dorsal horn neuron (DHN) response to 5-second contractions. (A) Total neuronal impulses during 5-second muscle contractions using targeted increases in tension to 1.0- and 3.0 gram. (B) Total neuronal impulses during 5-second muscle contractions, after subtracting the baseline activity. In spontaneously active neurons whose baseline activity was greater than 0.1 impulses/second before 1-gram contraction, background activity was subtracted from total neuronal impulses recorded during each subsequent experimental period. Data are expressed as median with interquartile range in (A) and (B). In (A) and (B), significant main effects and interaction detected with the generalized linear mixed model are shown in the graphs. A significant main effect of the group detected by generalized linear mixed model was further tested by comparing groups at each target tension the by Kruskal-Wallis test with Šidák correction for multiple comparisons followed by Dunn's multiple comparisons test: †† $p < 0.01$ vs. sham. A significant effect of target tension was further tested with the Friedman test in each incision group with Šidák correction for multiple comparisons followed by Dunn's multiple comparisons test: * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$ vs. baseline before 1.0-gram contraction. (C) Summary of the average tension generated during contractions. (D) Mean tension index during 1.0- and 3.0-

gram contraction. Data are expressed as mean $\pm$ SD in (C) and (D). In (C) and (D), **** indicates $p < 0.0001$ vs. baseline by two-way ANOVA with repeated measures in one factor followed by Šidák's multiple comparisons test. Imp = impulses.

Figure 5.

Summary for recording depth of dorsal horn neurons in sham, skin incision, and skin + deep muscle incision groups. Each circle represents the recording depth of one neuron. In the upper panels (A, B, and C), open and filled circles represent neurons without spontaneous activity [SA (-)] and neurons with spontaneous activity [SA (+)], respectively. In the lower panels (D, E, and F), open and filled circles represent neurons unresponsive to muscle contraction [Contract (-)] and neurons responsive to muscle contraction [Contract (+)], respectively. Results are expressed as mean $\pm$ SD. P values are calculated by unpaired t-test. Note: Each circle is randomly arranged on the horizontal axis and does not reflect the medial or lateral position of the neuron in the spinal cord.

Figure 6.

Dorsal horn neuron (DHN) responses to 1-second contractions using targeted increases in tension to 0.5-, 1.0-, and 1.5-gram. (A) Example recording of DHN activated by 1-second, 0.5-, 1.0-, and 1.5-gram contractions. The tension generated, digitalized oscilloscope trace of the action potentials, and the peristimulus activity histogram (bin width = 1 second) of the DHN are shown. (B) Summary of the DHN activity during 0.5-, 1.0-, and 1.5-gram muscle contractions in the sham and skin + deep muscle incision groups. (C) Summary of the average tension generated during contractions. (D) Summary of the voltage used. Data are presented as median with interquartile range in (B) to (D). Significant main effects detected by generalized linear mixed model are shown. There was no significant interaction effect. † $p < 0.05$; †† $p < 0.01$; ††† $p < 0.001$; †††† $p < 0.0001$ vs. sham by *post hoc* Kruskal-Wallis test with Šidák correction for multiple comparisons followed by Dunn's multiple comparisons test. ** $p < 0.01$; **** $p < 0.0001$ vs. baseline by *post hoc* Friedman test in each group with Šidák correction for multiple comparisons followed by Dunn's multiple comparisons test. imp = impulses.

Figure 7.

Dorsal horn neuron (DHN) responses to 5-second muscle stimulation before and after pancuronium. (A) Example recording of DHN response to 5-second, 1.0-gram and 3.0-gram muscle contractions before and after pancuronium (0.5 mg/kg) in a rat with skin + deep muscle incision. Tension (upper panel), digitized oscilloscope trace of the action potentials (middle panel) and activity histogram (lower panel; bin width = 1 second) are shown. The inset shows several representative single action potentials. The line under the tension tracing indicates the 5-second period of stimulation. (B) Summary of tension generated during 5-second muscle contractions before and after pancuronium in the sham and the skin + deep muscle incision groups. (C) Summary of DHN activity during 5-second muscle contractions before and after administration of pancuronium in the sham the skin + deep muscle incision groups. Data are presented as median with interquartile range. Main effects and interactions tested with the generalized linear mixed model are shown in the boxes on the right (B and

C). *** $p < 0.001$ vs. Baseline by Friedman test in each group with Šidák correction for multiple comparisons followed by Dunn's multiple comparisons test. ¶ $p < 0.05$ vs. values at the comparable tension level after pancuronium administration by Wilcoxon matched-pairs signed rank test with Šidák correction for multiple comparisons. The detailed results of *post hoc* analyses for a significant three-way interaction (C) are given in Supplementary Figure S3. imp = impulses.

Figure 8.

Dorsal horn neuron (DHN) responses to 1-second muscle stimulation before and after pancuronium. (A) Example recording of DHN response to 1-second, 0.5-, 1.0- and 1.5-gram contractions before and after pancuronium (0.5 mg/kg) in a rat with skin + deep muscle incision. The upper panel is tension generated, the middle panel shows digitized oscilloscope trace of the action potentials, and the lower panel shows the peristimulus histogram (bin width = 1 second). (B) The median increase in tension generated during muscle stimulation before and after pancuronium. (C) Median DHN activity during muscle stimulation in the sham and the skin + deep muscle incision groups before and after pancuronium. Data are presented as median with interquartile range. Main effects and interactions tested with the generalized linear mixed model are shown in the boxes on the right (B and C). * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$ vs. Baseline by Friedman test in each group with Šidák correction for multiple comparisons followed by Dunn's multiple comparisons test. ¶ $p < 0.05$ vs. Baseline by Friedman test in each group with Šidák correction for multiple comparisons followed by Dunn's multiple comparisons test.

0.05 vs. values at the comparable tension level after pancuronium administration by Wilcoxon matched-pairs signed rank test with Šidák correction for multiple comparisons. The detailed results of *post hoc* analyses for a significant three-way interaction are given in Supplementary Figure S4. imp = impulses.