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Spinal Manipulative Therapy Reduces Peripheral Neuropathic Pain in the Rat

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Abstract

Objective.—Spinal manipulative therapy, including low velocity variable amplitude spinal manipulation (LVVA-SM), relieves chronic low back pain, especially in patients with neuropathic radiating leg pain following peripheral nervous system insult. Understanding the underlying analgesic mechanisms requires animal models. The objective of the current study was to develop an animal model for the analgesic actions of LVVA-SM in the setting of peripheral neuropathic pain.

Methods.—Adult male Sprague Dawley rat sciatic nerve tibial and common peroneal branches were transected, sparing the sural branch (spared nerve injury, SNI). 15–18 days later, rats were randomly assigned to 1 of 3 groups (n = 9 each group): LVVA-SM at 0.15 Hertz or 0.16 Hertz or a sham control procedure. LVVA-SM (20° flexion at the L5 vertebra with an innovative motorized treatment table) was administered in anesthetized rats for 10 minutes. Control rats only underwent anesthesia and positioning on the treatment table. 10, 25, and 40 minutes later. The plantar skin of the hindpaw ipsilateral to SNI was tested for mechanical sensitivity (paw withdrawal threshold to a logarithmic series of Semmes-Weinstein monofilaments) and cold sensitivity (duration of paw lifting, shaking, and/or licking to topical acetone application).

Results.—SNI produced behavioral signs of mechanical and cold allodynia. LVVA-SM reduced mechanical, but not cold, hypersensitivity as compared to Control (0.15 Hertz: $P = 0.04$ at 10 minutes; 0.16 Hertz: $P < 0.001$ at 10 minutes, $P = 0.04$ at 25 minutes).

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Conclusion.—The analgesic effect of LVVA-SM in chronic low back pain patients with neuropathic leg pain can be reverse-translated to a rat model.

Keywords

Allodynia; Chiropractic; flexion distraction; low back-related leg pain; low velocity variable amplitude spinal manipulation; manual therapy; nociception; pain; peripheral neuropathy; spared nerve injury

Introduction

Neuropathic pain that radiates to the legs following peripheral nervous system insult is a common component of chronic low back pain and significantly increases its debilitating burdens [1]. Pharmacological agents are the mainstay of treatment, but neuropathic pain often is refractory to them [2]. Opiates are particularly problematic as they contribute to the current epidemic of drug abuse, addiction, and overdose [3]. The American College of Physicians recently recommended that clinicians should initially select non-pharmacologic approaches for their acute, subacute, and chronic low back patients [4]. Complementary and integrative health mind and body interventions are cost-effective and safe non-pharmacologic approaches to the management of low back pain with (or without) a neuropathic component [5].

One such intervention is spinal manipulative therapy (SMT). Non-thrust low velocity variable amplitude spinal manipulation (LVVA-SM) is a frequently utilized [6] SMT procedure for the management of low back pain [7,8]. It is also referred to as distraction manipulation, flexion distraction, or Cox distraction [9–11]. One month of LVVA-SM produced greater analgesia in chronic low back patients who had radiating leg pain as compared to those without leg pain [10]. A better understanding of the underlying analgesic mechanisms requires new animal models of LVVA-SM.

To this end, we developed an innovative custom-made, motorized table to simulate LVVA-SM in the anesthetized rat [12]. Our procedure involves the cyclical lowering and raising of the caudal section of the table while providing vertebral column traction and applying constant manual pressure in the rostral direction to the spinous process of the lumbar (L5) vertebra (Fig. 1). This simulates clinical LVVA-SM, in which the patient lies prone on a motorized or manual treatment table that is divided into stationary rostral and moveable caudal sections [7,8,10,11]. Passive flexion motion is administered multiple times to the lumbar spine in a controlled cyclical manner with both ankles secured. Continuous mild pressure is applied at the same time with the clinician's hand in the rostral direction to the contact point of a lumbar vertebra spinous process.

We previously reported [12] that LVVA-SM (20° flexion at L5 vertebra for a clinically relevant duration of 10 minutes [13]) reduced nociceptive behavior during the rat hindpaw formalin test, an experimental model of inflammatory pain. The objective of the current study was to develop an animal model for the analgesic actions of LVVA-SM in the setting of peripheral neuropathic pain. We chose the spared nerve injury (SNI) model of peripheral neuropathic pain, which involves transection of 2 of the 3 primary sciatic nerve branches,

because it is well characterized, reproducible, and associated with robust rat hindpaw mechanical and cold hypersensitivities that persist for months [14,15]. All of these features are similar to the clinical presentation of neuropathic pain symptoms [2].

Methods

Animals

Thirty-four male Sprague Dawley rats (268–366 grams at the start of experimentation; Envigo, Indianapolis, IN, USA) were singly-housed on Tek-Fresh bedding (Envigo) with environmental enrichment (PVCT tube, chew toys) and maintained on a 12 hour light-dark cycle (lights on 7:00AM – 7:00PM). Access to 19% Rodent Diet (7433, Kent Feeds, Muscatine, IA) and tap water was provided *ad libitum*. Rats were acclimatized to their housing environment 1 week prior to experimentation. All methods were approved by the Palmer College of Chiropractic Institutional Animal Care and Use Committee.

Habituation

Habituation to the behavior testing laboratory began at the same time of each morning (7:30 – 8:00) after transportation from the housing room. After 15 minutes in the home cage, each rat was transferred into a transparent plexiglass chamber on a raised metal mesh grid for 30 minutes [14,15]. Habituation was performed once daily 4 times before and once again at 15–18 days after SNI.

Mechanical sensitivity

Eight Semmes-Weinstein monofilaments of logarithmic stiffness (Stoelting Co., Wood Dale, IL) were used as described previously [14,15]. The 50% withdrawal threshold (grams) was determined using the up-down method of Dixon, as modified by Chaplan. While all paws rested on the grid, a monofilament was gently applied perpendicularly to the lateral plantar surface of the left hindpaw with enough force to produce slight bending. A positive response was a rapid withdrawal of the hindpaw within 2 seconds. The next larger or smaller monofilament was used if a positive or negative response occurred, respectively. Rats that exhibited mechanical thresholds greater than 5 grams at baseline testing 15–18 days after SNI were excluded from the study [14,15].

Cold sensitivity

As described previously [14,15], an approximately 10 microliter drop of room temperature acetone (Pharmco-AAPER, Brookfield, CT) was applied to the lateral plantar surface of the left hindpaw from a modified 2 millimeter diameter tip of PE-90 tubing attached to a 1 milliliter syringe. The duration (seconds) spent lifting, shaking, and/or licking the hindpaw during the first 30 seconds after acetone application was recorded. Three trials were performed with an inter-trial interval of 3 minutes. The cold response durations of the 3 trials were averaged. Rats that exhibited cold response durations less than 1 second at baseline testing 15–18 days after SNI were excluded from the study [14,15].

Spared nerve injury

All rats were anesthetized in an induction chamber with isoflurane (2.5%, Butler Schein Animal Health, Dublin, OH) in oxygen (1 Liter/minute) for 3 minutes. Anesthesia with isoflurane (1.5–2%) in oxygen (1 Liter/minute) continued through a nose cone. Ophthalmic ointment (Altaire Pharmaceutical Inc., Aquebogue, NY) was placed over the eyes. The antibiotic Baytril (10 milligrams/kilogram, Bayer Healthcare LLC, Shawnee Mission, KS) was injected subcutaneously. The lateral left hindlimbs were shaved between the hip and the knee then wiped with povidone-iodine (Well @ Walgreens, Deerfield, IL) and isopropyl alcohol (Well @ Walgreens) 3 times. Each rat was placed prone over a draped homeothermic blanket (Harvard Apparatus Inc., Holliston, MA). Using aseptic technique, an incision was made in the skin over the left femur. The fascia between the biceps femoris and gluteus superficialis muscles was cut. The muscles were retracted to expose the sciatic nerve. As described previously [14,15], the tibial and common peroneal nerves branches were ligated with 6–0 silk (Covidien, Mansfield, MA) and then transected 1 millimeter distal and proximal to the ligation. Care was taken to avoid damage to the sural nerve branch. The muscles and fascia were closed with 5–0 coated VICRYL* sutures (Ethicon, Somerville, NJ). The skin was closed with 11 millimeter Michel wound clips (Fine Science Tools, Foster City, CA). Bacitracin Zinc Ointment (Well @ Walgreens) was applied to the skin wound. Postoperatively, rats were placed in clean cages with fresh bedding and on heating pads for recovery and then into the housing room. Twice daily physical examinations and once daily weighing were performed during the first week post-surgery. Seven days post-operatively, the wound clips were removed and the lower back was shaved under isoflurane anesthesia. Afterwards, once daily physical examinations and weighing were done.

Low velocity variable amplitude spinal manipulation and sham control

The rats were anesthetized in an induction chamber with isoflurane (3%) in oxygen (2 liters/minute) for 2 minutes. As described previously [12], they were placed prone on a water circulating heating pad on top of the stationary rostral section of our custom-made treatment table (Fig. 1). Anesthesia with isoflurane (2%) in oxygen (1 liter/minute) was continued through a nose cone. The head was stabilized with a tooth bar positioned within the nose cone caudal to the incisors. The L5 vertebra was positioned over the fulcrum point of the table. Rats were then randomly assigned to 1 of 3 groups: LVVA-SM at 0.15 Hertz, LVVA-SM at 0.16 Hertz, or a sham control procedure (n = 9 each group). For the LVVA-SM groups, a piece of Velcro® brand tape was placed around the base of the tail. A 3–0 silk suture (Surgical Specialties Corp., Reading, PA) was tied over the tape and draped over a pulley behind the tail. A 100 gram weight was hung from the other end of the suture. At 5 minutes after anesthesia induction, isoflurane was further reduced to 1.5%. LVVA-SM was then administered for 10 minutes. The caudal section of the table was cyclically lowered to 20° flexion and then back to the neutral position with a computer-controlled motor at 0.15 Hertz (88 total cycles) or 0.16 Hertz (94 total cycles). Mild manual pressure was constantly applied for the 10 minutes to the L5 spinous process in the rostral direction by an experimenter's index and middle fingers as done clinically during LVVA-SM [7,8,10,11]. Isoflurane was again further reduced to 1% and 0% during the ninth and tenth minutes, respectively. Control rats only underwent the anesthesia and positioning methods on the treatment table. The rats were returned to their cages on heating pads following LVVA-SM

or the control procedure. All rats were ambulatory within 5 minutes. They were placed in the plexiglass chamber at this time for an additional 5 minutes before behavioral testing.

Timeline

Habituation to the behavior testing laboratory preceded testing of mechanical and cold sensitivity before and after SNI. 15–18 days after SNI, rats were tested for baseline mechanical and cold sensitivity. As determined using *a priori* exclusion criteria described above, 7 of 34 rats were removed from the study because at post-SNI baseline testing they exhibited mechanical thresholds greater than 5 grams and/or cold response durations less than 1 second [14,15]. The remaining 27 rats were randomly assigned to be treated with either 0.15 Hertz LVVA-SM, 0.16 Hertz LVVA-SM, or Control ($n = 9$ each group). Testing of mechanical sensitivity and then cold sensitivity was repeated 10, 25, and 40 minutes later. These time-points were based on our previous studies using the formalin test [12] and from studies of rat knee joint [16] and mouse ankle joint [17] mobilization. The individual performing the assessments was blind to group assignment and not involved in the LVVA-SM or the control procedure. Rats were euthanized after the last behavior test with Fatal-Plus® (0.88 milliliters/kilogram, intraperitoneal, Vortech Pharmaceuticals Ltd., Dearborn, MI) followed by thoracotomy [12].

Statistical methods

SAS/STAT (release 9.4; SAS Institute, Inc., Cary, NC) was used for data analyses. The mechanical and cold sensitivity responses were skewed and therefore were log-transformed for analyses. One-way analysis of variance was used to compare these responses among groups pre-SNI. Linear mixed-effect models of these responses were analyzed over the 4 time-points post-SNI (baseline and 10, 25, and 40 minutes after intervention) with an unstructured covariance and terms for group, time, and group X time interactions. Least-squares means, mean differences, and 95% confidence intervals from the models were transformed back to the original scales for reporting. Between-group comparisons were made between each of the 2 LVVA-SM groups and the control group at each time-point. A $P < 0.05$ was considered statistically significant.

Results

Effect of LVVA-SM on mechanical hypersensitivity

Mean mechanical thresholds assessed before SNI did not differ between groups (0.15 Hertz LVVA-SM [13.1; 95% confidence interval (CI) 11.4 to 15.1], 0.16 Hertz LVVA-SM [14.4; 95% CI 12.6 to 16.6], Control [15.0; 95% CI 13.1 to 17.2]; $F_{2,24} = 2.16$, $P = 0.36$). SNI decreased baseline mean mechanical thresholds to a similar extent across groups (Fig. 2). The control procedure did not change mean mechanical thresholds when assessed at the 10, 25, and 40 minute time points. 0.15 Hertz LVVA-SM ($P = 0.04$) and 0.16 Hertz LVVA-SM ($P < 0.001$) significantly increased mean mechanical thresholds at 10 minutes compared to Control. 0.16 Hertz LVVA-SM significantly increased mean mechanical thresholds compared to Control at 25 minutes ($P = 0.04$).

No effect of LVVA-SM on cold sensitivity

Cold response durations before (0 seconds for all groups) and after SNI did not differ between groups (Fig. 3). LVVA-SM did not change mean cold response duration at any time-point after SNI ($F_{6,24} = 0.65$, $P = 0.69$).

Discussion

Despite the analgesic effectiveness of non-thrust LVVA-SM, particularly in chronic low back patients with neuropathic leg pain [10], no one has reverse-translated these findings to animal models. The present study addresses this gap using a reductionist approach and shows that LVVA-SM reduced rat hindpaw mechanical hypersensitivity following traumatic peripheral nerve injury. This result extends our previous report [12] indicating that LVVA-SM reduced rat hindpaw nociceptive behavior in an experimental model of inflammatory pain.

We found that LVVA-SM did not change cold hypersensitivity. This is consistent with a clinical report indicating that a single cervical high velocity low amplitude thrust spinal manipulation did not provide relief from remote cold pain in persons with chronic lateral epicondylalgia [18]. However as in our rat study, this SMT procedure did relieve mechanical pain. We speculate that SMT exerts greater analgesic effects on mechanical allodynia as compared to cold allodynia. Perhaps SMT fails to interrupt the mechanisms that drive cold hypersensitivity, such as loss of cold-sensitive A δ afferent suppression of C-fiber input to spinal cord dorsal horn lamina I [15]?

Stimulation frequency determines which pain relieving central nervous system mechanisms are activated by transcutaneous electrical stimulation (TENS). For example, 4 Hertz, but not 100 Hertz, TENS at the inflamed knee joint increased serotonin concentration in the spinal cord dorsal horn [19]. However, the frequency, duration, and intensity of SMT have not been optimized to maximize efficacy for the treatment of neuropathic pain. For future studies, we will modify our equipment to systematically investigate the analgesic effects of more variable frequencies as well as duration and intensity of LVVA-SM. We found that a single treatment with LVVA-SM yielded only a short duration analgesic effect. Although the immediate analgesic effects of LVVA-SM have not been studied in humans, 1 month of repeated LVVA-SM sessions produced analgesia in chronic low back pain patients that lasted up to a year [20]. Our proof-of principle study sets the stage for future studies to evaluate the temporal effects of repeated LVVA-SM in rodent models of neuropathic pain. Such studies will inform clinical practice.

By what mechanism(s) could LVVA-SM reduce mechanical hypersensitivity? LVVA-SM likely stimulates low-threshold mechanoreceptors (LTMRs) in skin and joints (muscles, tendons, ligaments, and bones) [9,21,22]. Activation of LTMRs could induce spinal and supraspinal descending inhibition [23,24]. Indeed, the latter could explain our finding that LVVA-SM yielded an analgesic effect at a remote site. Indeed, LVVA-SM? attenuated the ability of knee or ankle joint mobilization to reduce hindpaw mechanical hypersensitivity [16,17]. Similarly, pharmacological blockade of spinal cord serotonin receptors (5-HT_{1/2}, 5-HT_{2A}, 5-HT₃) attenuated the ability of 4 Hertz TENS to reduce hindpaw thermal

hypersensitivity [25]. Descending inhibition hypotheses can now be explored with our new model of the analgesic actions of LVVA-SM in the setting of peripheral neuropathic pain, for example with pharmacological blockade of spinal monoamine (5-HT_{1/2}, 5-HT_{1A}, α_2 -adrenergic) or cannabinoid (CB1) receptors.

Low back pain is exceedingly complex. Neuropathic pain that radiates to the legs can result from peripheral nervous system insult [2]. Our findings indicate that LVVA-SM may be particularly effective at exerting an analgesic effect in the setting of neuropathic pain. Importantly, this potentially translatable data could improve the rationale of clinicians for using LVVA-SM especially since non-pharmacologic SMT treatments are recommended for initially treating low back pain patients [4].

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Conflicts of Interest and Source of Funding

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Figure 1. The LVVA-SM technique.

An anesthetized rat lies prone on our custom-made, motorized table. The caudal section of the table is cyclically lowered and raised between the neutral position (A) and 20° flexion (B) by a computer-controlled motor. Vertebral column traction is provided using an incisor bar in an anesthesia nose cone (a) and a 100 gram weight (c). Constant manual pressure is applied to the L5 spinous process by an experimenter's fingers (b).

Figure 2. Mechanical thresholds after SNI surgery and after either LVVA-SM or a sham control procedure.

Data are presented as means and 95% confidence intervals (CI) from the linear mixed-effect model. Baseline post-SNI mean mechanical thresholds (B) were not different among the groups. The mean mechanical threshold of the 0.16 Hertz LVVA-SM group ($n = 9$) was higher than that of the control group ($n = 9$) at 10 (** $P < 0.001$) and 25 (* $P = 0.04$) minutes (min) after completion of LVVA-SM or the control procedure. The mean mechanical threshold of the 0.15 Hertz LVVA-SM group ($n = 9$) was higher than that of the control group at 10 minutes (^ $P = 0.04$).

Figure 3. Cold response durations after SNI surgery and after either LVVA-SM or Sham LVVA-SM.

Data are presented as means and 95% confidence intervals (CI) from the linear mixed-effect model. The mean cold response durations were not different at any time between the control ($n = 9$), 0.15 Hertz LVVA-SM ($n = 9$), and 0.16 Hertz LVVA-SM ($n = 9$) groups.