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Movement evoked pain and mechanical hyperalgesia after intramuscular injection of nerve growth factor: A model of sustained elbow pain

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Running title: Functional effects of sustained elbow pain

DISCLOSURES

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ABSTRACT

Objective

Chronic lateral epicondylalgia (LE) presents as lateral elbow pain provoked by upper limb tasks. An experimental model of elbow pain provoked by movement/muscle contraction and maintained over several days is required to understand sustained elbow pain. This study investigated the time course and pain location induced by nerve growth factor (NGF) injection into a wrist extensor muscle, and whether movement and muscle contraction/stretch provoked pain.

Methods

On Day 0 twenty-six pain free volunteers were injected with NGF (N=13) or isotonic saline (randomized) into the extensor carpi radialis brevis (ECRB) muscle of the dominant arm. On Day 2 pain was induced in all participants by hypertonic saline injection into ECRB. A Likert scale and patient-rated tennis elbow evaluation (PRTEE) was used to assess pain and functional limitation (Days 0-10). Pain intensity during contraction/stretch of ECRB, and pressure pain thresholds (PPTs) were recorded before and after injections on Days 0 and 2, and Days 4 and 10.

Results

Compared with isotonic saline, NGF evoked: i) greater Likert pain ratings from 12 hours post-injection until Day 6, ii) greater PRTEE scores on Days 2 and 4, iii) greater pain during ECRB contraction/stretch on Day 2, and iv) lower PPTs on Day 4.

Conclusions

This paper presents a novel experimental human pain model suitable to study the sustained effects of lateral elbow pain on sensorimotor function and to probe the mechanisms underlying persistent musculoskeletal pain.

Key words: Nerve growth factor (NGF), elbow pain, muscle contraction, muscle stretch, hyperalgesia.

INTRODUCTION

Patients with lateral epicondylalgia (LE) present with lateral elbow pain provoked by gripping and other manual tasks. Chronic LE involves sensorimotor changes, including bilateral mechanical hyperalgesia and reduced pain free grip strength (1), and strength deficits of wrist, elbow, and shoulder muscles (2,3). Whether the sensorimotor deficits found in chronic LE are a cause or effect of sustained pain and hyperalgesia remains unclear.

Experimental models of pain have been used to investigate mechanisms that underlie sensorimotor changes during acute muscle pain, such as delayed muscle activation (4). Although these studies provide insight, interpretation is limited by the transience of the induced pain. This could explain inconsistencies between the effects of acute experimental pain and impairments of musculoskeletal pain conditions; e.g. pain provocation by muscle contraction/stretch (5), deep-tissue hyperalgesia (6). Models of sustained pain and hyperalgesia that mimic typical behavior of chronic musculoskeletal pain conditions are needed to study the transition from acute to sustained musculoskeletal pain (7).

The combined effect of delayed onset muscle soreness (DOMS) induced by eccentric exercise of the wrist extensor muscles and intramuscular injection of hypertonic saline has been used to study sustained elbow pain. That method induced mechanical hyperalgesia for two days, and reduced grip and wrist extension force at 24 hours following exercise (8). However, damage to contractile elements by eccentric exercise (9) can directly influence function, which precludes investigation of the independent effects of pain/nociceptive stimulation. An alternative is nerve growth factor (NGF), an endogenous neuromodulator vital for nerve development and reconstruction (10). Intramuscular injection of NGF induces mechanical hyperalgesia for up to 14 days and mild pain during muscle contraction that lasts up to 3 days after injection into the tibialis anterior (11,12), masseter (13) and supraspinatus muscles (14). NGF injection provides a viable method to study sustained hyperalgesia, but the pain response to muscle contraction remains unclear. A recent study found electrically-stimulated muscle contraction evoked pain that was no

worse whether the muscle fascia was injected with NGF or isotonic saline (15). However, that study does not preclude provocation of NGF-induced muscle pain by muscle contraction, as electrical stimulation was limited to twitches, which do not replicate function, and hyperalgesia of fascia might not respond similarly to muscle hyperalgesia during contraction. Investigation of pain and hyperalgesia after NGF injection into elbow muscle and the relationship to muscle contraction and function is required to determine whether NGF injection could be a suitable model to study a potential cause-effect relationship between pain and sensorimotor changes in sustained elbow pain.

This study investigated, in healthy subjects: 1) the time course of pain and hyperalgesia induced by injection of NGF into a wrist extensor muscle, and 2) whether movement and muscle contraction provoke pain in the NGF-induced hyperalgesic muscle.

METHODS

Participants

Twenty-six healthy volunteers (age 25.8 ± 5.4 years (mean \pm SD); 7 females) participated in this study. Participants were excluded if they had a recent history of pain that affected the upper limb and/or neck, a history of neurological, musculoskeletal or mental illness, were currently using analgesics and/or anti-inflammatory medications, or if they were participating in more than two sessions of muscle training exercises per week that involved the upper limbs. All participants were given a written and verbal explanation of the study and written informed consent was obtained prior to inclusion. The study was approved by the local ethics committee (N-201200640) and conformed to the Declaration of Helsinki. Data collection was conducted at Aalborg University, Denmark.

Study design

A randomized, double blind, placebo-controlled study design was used to study the nature and time course of pain induced by NGF injection. Participants attended four experimental sessions over 11 days (Figure 1). On Day 0, participants were randomized into one of two groups: NGF

group (n = 13; 5 females) or control group (n = 13; 2 females). Participants were blinded to group allocation for the duration of the study. On Day 0 participants received an injection of NGF (NGF group) or isotonic saline (control group) into the extensor carpi radialis brevis (ECRB) muscle of the dominant upper limb. On Day 2 hypertonic saline-induced pain was evoked in the ECRB muscle of the dominant limb in all participants to investigate whether NGF injection sensitized the muscle to chemical irritation. The behavior of pain induced by NGF to a range of stimuli was studied to identify whether it reacted in a manner consistent with clinical pain. To address this issue, assessments of the muscle pain and functional limitation, movement-evoked pain, response to muscle contraction and stretch, and pressure pain sensitivity were performed before and after injections on Days 0 (NGF/ISO) and Day 2 (hypertonic saline), and on Days 4 and 10. Participants completed a daily diary of their elbow pain from Day 0 to Day 10.

NGF-induced pain and hyperalgesia

A single bolus of NGF (5 µg, 0.2 ml; recombinant human NGF, prepared by the pharmacy at Aalborg University Hospital), or isotonic saline (0.2 ml 0.9%) was injected into the ECRB muscle of the dominant upper limb on Day 0. The injection site was 1 cm lateral to a point 5 cm distal to the lateral epicondyle along a line from the lateral epicondyle to the midline of the wrist. Palpation during contraction (radial deviation and extension of the wrist) and ultrasound imaging of the anatomical boundaries of the muscle confirmed that this site related to ECRB. Separate examiners prepared and administered the injection, and performed the assessments to ensure blinding of the assessor and participant.

Questionnaires on pain intensity and functional limitation

A modified 7-point Likert scale that relates the pain intensity to specific activities (9,11) was used to assess muscle pain intensity at the beginning of each session: 0 = 'a complete absence of pain/soreness'; 1 = 'a light pain/soreness in the muscle felt only when touched/a vague ache'; 2 = 'a

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3 moderate pain/soreness felt only when touched/a slight persistent ache'; 3 = 'a light muscle
4 pain/soreness when lifting objects or carrying objects'; 4 = 'a light muscle pain/soreness, stiffness
5 or weakness when moving the wrist or elbow without gripping an object'; 5 = 'a moderate muscle
6 pain/soreness, stiffness or weakness when moving the wrist or elbow'; 6 = 'a severe muscle
7 pain/soreness, stiffness or weakness that limits my ability to move'. The patient-rated tennis elbow
8 evaluation (PRTEE) was used to measure pain and functional limitation (16) at the beginning of
9 each session. It has excellent test-retest reliability ($r=0.93$) and good correlation with other
10 functional scales such as the Disability of Arm and Shoulder (DASH) questionnaire ($r=0.87$) in the
11 tennis elbow population (16). The task-related questions are scored on an 11-point Likert scale,
12 with calculation of separate subscales for pain and function (Function A: activities specific to the
13 upper limb; Function B: general activities), and a total score ranging from 0 (no pain and no
14 functional limitation) to 100 (worst imaginable pain with a very significant functional limitation).
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32 *Location of NGF-induced pain*

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34 Participants drew the distribution of their pain induced by the injection of NGF or isotonic
35 saline on an anatomical drawing of the upper limb at the beginning of each session. These drawings
36 were digitized (Matlab 7.14) and the size of the painful area represented as a percentage of the total
37 surface area of the anterior and posterior surfaces of the upper limb as represented by the drawing.
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45 *Pain diary*

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47 Participants completed a pain diary at approximately midday and in the evening on Days 0-4
48 and only in the evening on Days 5-10. The diary consisted of the 7-point modified Likert scale, an
49 anatomical drawing of the upper limb upon which the pain area was drawn, and four questions
50 where participants rated their pain on an 11-point numerical rating scale (NRS): i) when the arm
51 was at rest; ii) when doing a task with repeated arm movements; iii) when pain was at its least; and
52 iv) when pain was at its worst.
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Contraction- and stretch-evoked pain

The influence of contraction and stretch of the ECRB muscle on pain intensity was examined for both upper limbs. Participants performed the muscle contraction tasks (i.e. wrist extension and gripping; order randomized) with the upper limb supported on a platform in 90° shoulder flexion, elbow extension and forearm pronation. Participants were instructed to maintain this upper limb position during each contraction. A force sensor (MC3A 250, AMTI, USA) was mounted above the hand being tested to record the force exerted during the wrist extension contractions. Gripping force was measured with a custom-made grip dynamometer (grip width = 64 mm), consisting of a strain gauge (CCT Transducers, Italy) interposed between two padded bars. Three maximal voluntary contractions (MVC) with strong verbal encouragement were performed for each task. Force was gradually increased to a maximum within each 5 s trial. Each trial was separated by 1 min to limit possible effects of fatigue. Immediately after each contraction the participants indicated whether pain intensity increased, decreased or was unchanged during the contraction, and verbally rated the pain intensity on an 11-point NRS anchored with 'no pain' at 0 and 'maximum pain imaginable' at 10. The maximum force achieved during the three MVC trials was used for the submaximal trials. Three submaximal contractions were performed before and after the injections. The MVC recorded on Day 0 (i.e. before NGF/ISO injection) was used to calculate the 10% MVC force target required for submaximal trials performed on Days 0, 2 and 4. A target force of 10% MVC was chosen as it was comparable to the amount of force required for many everyday tasks, and pilot tests (n=3) indicated that it allowed participants to perform three submaximal contractions without onset of forearm muscle fatigue. In the submaximal tasks participants gradually increased force from zero to the 10% MVC target (displayed on a computer screen) over 5 s, maintained the target force for 10 s, and then reduced force to zero over 5 s. Participants were instructed to match the 10% MVC target as closely as possible. Participants rested for 30 s between submaximal contractions. Immediately after each contraction the participants

indicated whether there was an increase, decrease or no change in pain intensity during the contraction, and verbally rated the pain intensity on the 11-point NRS.

For the stretching task, the upper limb was supported on a platform in 90° shoulder flexion, elbow extension, and the forearm in neutral rotation. The wrist was passively moved into flexion or ulnar deviation in separate trials (order randomized), held for 5 s, and then returned to the starting position (17). One trial of each stretch (i.e. flexion, ulnar deviation) was performed at each experimental session. Immediately after each stretch, participants indicated whether there was an increase, decrease or no change in pain during the stretch, and verbally rated the pain intensity on the 11-point NRS.

Pressure pain sensitivity

Pressure pain thresholds (PPT) were measured bilaterally with an electronic algometer (Algometer Type II, Somedic AB, Sollentuna, Sweden) applied to the ECRB muscle (injection site), low back (3 cm lateral to the spinous process of the 4th lumbar vertebra), and over the tibialis anterior muscle belly. Pressure applied via the algometer probe (1 cm²) was increased at a rate of 30 kPa/s, and the participant was instructed to press a button when the pressure sensation changed to one of pain, at which point the application of pressure ceased. Three measurements were recorded at each site and the mean value used for analysis. The PPT data were expressed as a percentage of the PPT measures recorded at the baseline session (Day 0 pre-injection).

Saline-induced muscle pain and related measures

A single bolus of hypertonic saline (0.5 ml, 5.8%) was injected into the muscle belly of ECRB (same location as NGF/ISO injection) on Day 2. The pain intensity was recorded continuously on a 10-cm electronic visual analogue scale (VAS; sampling frequency of 1 Hz), where 0 cm indicated ‘no pain’ and 10 cm ‘maximum pain imaginable’. Participants performed gripping and wrist extension tasks (see above) immediately after the injection. Participants were

1 instructed to begin rating the saline-induced pain intensity immediately after the injection and to
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3 update their pain rating after each repetition of the gripping and wrist extension tasks until the pain
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5 ceased. The maximum VAS scores reported by each participant during each task (i.e. gripping and
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7 wrist extension) were used for further analysis. After the saline-induced pain had ceased,
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9 participants drew their pain distribution on the standardized drawing of the upper limb.
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16 *Statistical analysis*

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18 Statistical analysis was performed using Statistica 9 (Statsoft, Tulsa, OK, USA). According
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20 to a Kolmogorov-Smirnov test for normality the majority of PPT data, pain area data, and VAS
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22 scores during saline-induced pain were normally distributed. The contraction- and stretch-evoked
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24 pain and questionnaire data (e.g. Likert scale, PRTEE, pain at rest, worst pain) were not normally
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26 distributed and were therefore analyzed with non-parametric tests. Data are reported as mean and
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28 95% confidence intervals or median and interquartile range when appropriate. Significance was set
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30 at $P < 0.05$ for all analyses.
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34 *Comparison of the effects of injection of NGF and ISO:* To determine whether NGF
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36 injection induced muscle hyperalgesia, PPTs were compared between *sessions* (Day 0 post-
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38 injection vs. Day 2 pre-injection vs. Day 4 vs. Day 10), and between *groups* (NGF vs. ISO) with a
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40 mixed-model repeated measure analysis of variance (RM-ANOVA). To determine the time course
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42 of area of pain, these data were compared between *sessions* (Day 0 post-injection and 15
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44 subsequent assessments) and a between-subject factor of *group* (NGF vs. ISO) with a mixed-model
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46 RM-ANOVA. A Bonferroni post-hoc test was used for the PPT and area of pain data. To determine
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48 whether pain induced by NGF was provoked by muscle contraction and stretch, the non-normally
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50 distributed NRS data during these tasks were analyzed in several ways. First, a Kruskal-Wallis test
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52 on ranks was used to test for differences between *groups/side* (Group: NGF, ISO; Side: ipsilateral,
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54 contralateral) at each *session*. This was followed by a Mann Whitney U test to probe the specific
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56 differences when significant. Second, a Friedman test was used to test for differences between
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sessions within each *group* (NGF, ISO) and *side* (ipsilateral, contralateral). This was followed by a Wilcoxon matched pairs test when significant to investigate differences between individual sessions. Bonferroni corrections were used to adjust p-values for multiple comparisons.

Effects of hypertonic saline: To determine whether NGF injection sensitized the muscle to chemical irritation PPTs were compared between *sides* (Ipsilateral vs. contralateral), *sessions* (Day 2 pre-injection vs. Day 2 post-injection) and a between-subject factor of *group* (NGF vs. ISO) with a mixed-model RM-ANOVA. The VAS scores during saline-induced pain were analyzed with a two-way RM-ANOVA with a between-subject factor of *group* (NGF vs. ISO), and the *task-sequence* (the task that was performed first: gripping vs. extension). A Bonferroni post-hoc test was used for the PPT and VAS scores data. An independent t-test (two tails) was used to compare the pain area data.

RESULTS

Self-reported NGF-induced pain intensity

The 7-point Likert scale scores were higher in the NGF group than the ISO group from the evening of Day 0 until Day 6 ($P < 0.003$, Figure 2A). For the NGF group, peak pain was experienced on the morning of Day 2 ($P = 0.001$) and then gradually returned to zero by Day 10 ($P = 0.068$). No participants in either group reported elbow pain at rest ($P = 1.00$). When participants reported the worst pain they experienced in the preceding 12 hours (Days 0-4) or 24 hours (Days 5-10), the NRS scores were greater in the NGF group than the ISO group from the evening of Day 0 until Day 5 ($P < 0.003$, Figure 2B). Those in the NGF group reported greater pain with repeated arm movements than the ISO group, reflected by higher NRS scores recorded in the pain diary, between Day 0 and Day 4 ($P < 0.003$, Figure 2C).

The total PRTEE and component scores (Pain, upper limb activities, general activities) for participants injected with NGF were greater than those in the ISO group when measured on both Day 2 ($P < 0.001$) and Day 4 ($P < 0.001$, Figure 3).

Participants injected with NGF reported a larger area of pain than those injected with isotonic saline (RM-ANOVA interaction: group x session: $F_{15} = 6.29$, $P < 0.001$) from the evening of Day 0 until the evening of Day 4 (post-hoc: $P < 0.05$, Table 1, Figure 4).

Contraction-evoked pain after NGF vs. ISO

On Day 2 (before the hypertonic saline injection) participants reported greater pain provocation during maximal wrist extension contraction (i.e. higher NRS scores) for the limb injected with NGF than the limb injected in the ISO group and the contralateral limbs in either group (NGF, ISO) ($P < 0.017$, Figure 5). There were no differences in pain intensity evoked by contraction at 10% MVC ($P > 0.15$). No participants reported pain (NRS = 0) following muscle contraction of the contralateral limb (i.e. non-injected limb).

Stretch-evoked pain after NGF vs. ISO

When the ECRB muscle was stretched by passively moving the wrist into flexion there was greater provocation of pain (i.e. higher NRS scores) for the injected limb of the NGF group than the injected side of the ISO group, and the contralateral limb in either group (NGF, ISO) on Day 2 ($P < 0.001$, Figure 5). Stretch into ulnar deviation had negligible effect on pain (Figure 5). The stretch of the ECRB muscle in the limb contralateral to the injection did not produce pain for participants in either group (NRS = 0).

Pressure pain sensitivity after NGF vs. ISO

The RM-ANOVA of the PPTs recorded at the ipsilateral elbow showed an interaction between group and session ($F_3 = 3.19$, $P = 0.029$; Figure 6A). PPTs were lower in the NGF group at Day 2 than Day 0 post-injection (post-hoc: $P = 0.005$) and Day 10 (post-hoc: $P < 0.001$), and lower on Day 4 than Day 0 post-injection (post-hoc: $P = 0.027$) and Day 10 (post-hoc: $P < 0.001$). There were no such differences between sessions for the ISO group. PPT was lower for the NGF group

than the ISO group on Day 4 (post-hoc: $P = 0.03$) but not at any other session (post-hoc: $P > 0.05$). For the contralateral elbow there was a main effect of session ($F_3 = 12.36$, $P < 0.001$). PPT on Day 10 was greater (regardless of group) than all other sessions (post-hoc: $P < 0.05$). As expected, PPT was not significantly affected at the low back (Figure 6B) or tibialis anterior (Figure 6C).

Effect of hypertonic saline on induced pain behavior

There was no difference between groups (NGF: 6.6 ± 2.9 arbitrary units; ISO: 5.1 ± 3.3) with respect to the area of pain following the hypertonic saline injection (Figure 4). During pain induced by hypertonic saline, the peak VAS scores recorded when participants performed the submaximal contraction tasks (i.e. gripping and wrist extension) were greater for participants in the NGF group (7.3 ± 0.8 cm) than the ISO group (6.2 ± 0.6 cm; RM-ANOVA main group effect: $F_1 = 5.01$, $P = 0.036$). Hypertonic saline injection at the elbow did not change the PPTs for either group at the elbow, low back or tibialis anterior muscle (Table 2).

DISCUSSION

This study is the first to demonstrate that intramuscular injection of NGF in the ECRB muscle induces lateral elbow pain and leads to reduced function lasting for several days. A unique feature of this model is the provocation of pain with movement of the upper limb and by contraction and stretch of the injected muscle. These features indicate that intramuscular injection of NGF induces pain that responds in a manner typical of sustained clinical pain, and is therefore a suitable model to study the effect of sustained lateral elbow pain on motor control of the upper limb.

Self-reported pain and functional effects of intramuscular NGF injection

Participants who received an injection of NGF reported lateral elbow pain that peaked 48 hours after injection and lasted for an average of 6 days. Although sustained pain/soreness following NGF injection has been reported, there are discrepancies between the present and

previous results. A single NGF injection given into the tibialis anterior muscle induced pain that peaked after 24 hours with a lower intensity (Likert scale: 2) and lasted for 7 days (11), whereas pain after three separate injections on consecutive days peaked 24 hours after the third injection (Likert scale: 3) and lasted for a further 14 days (12). Injection of NGF into ECRB induced sustained muscle pain that was more intense than after injection into tibialis anterior (i.e. higher scores on the Likert scale) but had a similar duration, which implies duration might be independent of initial pain intensity.

Data from the PRTEE, which evaluates pain and functional limitation, concurs with findings from the Likert scale. Participants injected with NGF reported greater pain and reduced function on the PRTEE (total score and sub-scales) than those in the ISO group at Day 2 and Day 4. Furthermore, the Day 2 PRTEE scores of individuals injected with NGF (18 ± 7) were similar to that reported by patients with mild chronic LE (24 ± 6 (mean \pm SD); 1), which provides evidence that injection of NGF into the ECRB muscle induces pain and functional limitation comparable to mild chronic LE.

The area of pain was greatest 48 hours after the NGF injection and was primarily located around the injection site. Pain spread into the proximal half of the forearm in 12/13 participants, similar to delayed onset muscle soreness (DOMS) at the elbow (6). An increase in the area of pain has also been reported following injection of NGF into the tibialis anterior muscle (12). Increased pain area is thought to be explained by expansion of the receptive fields of nociceptive neurons with prolonged noxious input (18).

Contraction-evoked pain

Maximal wrist extension of the arm injected with NGF evoked lateral elbow pain of $\sim 2/10$ from a resting intensity of zero. Similar pain intensity has been reported during contraction of leg (11) and shoulder (14,19) muscles that were injected with NGF. Provocation of pain with movement and muscle contraction is a feature of the NGF model of sustained pain that is not

consistently associated with other common models of deep tissue pain (e.g. hypertonic saline; 5). However, in the present study, lateral elbow pain was only provoked by maximal wrist extension and not the 10% contraction intensity. Previous studies have reported pain (~2-3/10) during submaximal contractions of shoulder (14,19) and lower limb muscles (11) injected with NGF. It is unclear whether differences in contraction-evoked pain intensity between studies are due to differences in contraction intensity or the dynamic/static nature of the tasks.

Stretch-evoked pain

This is the first study to demonstrate provocation of pain by stretch of a muscle injected with NGF. This is best explained as a result of mechanical sensitization (i.e. also demonstrated by reduced PPT) of the muscle following NGF injection. Surprisingly, only the wrist flexion stretch, and not ulnar deviation stretch, was provocative. A greater range of motion is available for wrist flexion (~90°) compared to ulnar deviation (~35°) (17), which may result in a greater change in muscle length and thus greater pain provocation.

Pressure pain sensitivity

Pressure pain threshold at the elbow injection site was less in the NGF group than the ISO group at Day 4. Similarly, intramuscular injection of NGF into tibialis anterior (11,12), trapezius (19) and masseter (13,20) muscles induced mechanical hyperalgesia at the injection site that lasted for approximately one week.

Effects of superimposed injection of hypertonic saline

Intramuscular injection of hypertonic saline into ECRB elicited more intense pain during the contraction tasks in the NGF group than the ISO group, but there was no difference in the size of the painful area between the two groups. These findings concur with an earlier study that found men (but not women) reported more intense pain in the leg that was injected with NGF than the

contralateral leg injected with isotonic saline, but with no difference in the area of pain between the two legs (11).

PPTs at the elbow were not affected by injection of hypertonic saline in either group. Injection of hypertonic saline alone (i.e. no prior injection of NGF) into ECRB (8) did not affect PPT at the injection site, which suggests that injection of hypertonic saline into ECRB does not affect PPT at the elbow, whether pre-sensitized with NGF or not.

Sensitization of peripheral and central mechanisms following NGF injection

Intramuscular injection of NGF sensitizes high threshold mechanosensitive afferent fibers (i.e. muscle nociceptors) (21). Under normal conditions these muscle afferents do not respond to weak, everyday stimuli (e.g. muscle contraction, stretch) and require tissue-threatening stimulation to be activated (22). In the current study, contraction, stretch and direct pressure stimulation of the ECRB muscle after NGF injection evoked pain, which indicates involvement of peripheral sensitization. Evidence of sensitized central mechanisms such as sensitization of dorsal horn neurons (23), distinct areas of referred pain (11), and spreading hyperalgesia (12) have been found following NGF injection. The extensive spreading of pain including referred pain suggests that sensitization of central mechanisms cannot be excluded.

In the current study, an injection of hypertonic saline into pre-sensitized muscle did not induce further mechanical hyperalgesia at the elbow or referred pain, but did elicit more intense pain compared to the isotonic saline group. Hypertonic saline activates dorsal horn neurons, induces hyperalgesia one day after injection (23), and produces distinct areas of referred pain (7), but it does not alter the mechanical thresholds of muscle afferents (24), which suggests that hypertonic saline may sensitize central, rather than peripheral, mechanisms. Further, the strong nociceptive barrage caused by hypertonic saline may excite the pool of dorsal horn neurons to the same extent independent of a potential sensitization of the central neurons (i.e. a ceiling effect). Thus it is

unclear to which degree facilitated central mechanisms was involved within the short period of NGF-induced pain.

NGF as a model of sustained elbow pain

It is critical experimental models of sustained pain reflect typical features of musculoskeletal conditions, including prolonged pain (rather than a brief, transient event) and provocation of pain with contraction, stretch and function. Data from the present study and previous reports for other muscles provide evidence that intramuscular injection of NGF more effectively replicates these features of musculoskeletal pain conditions than injection of hypertonic saline or DOMS for several reasons. First, NGF injection induced pain that was evoked during movement for approximately one week after a single injection (current study; 11) and two weeks after multiple injections (12). In contrast, pain from hypertonic saline injection lasted for up to 10 minutes and DOMS-related pain was sustained for 2-3 days after exercise (8). Second, pain that is induced by injection of NGF was evoked by contraction and stretch of ECRB. This contrasts the potential for pain to decrease during contraction/stretch of a muscle injected with hypertonic saline (5). Third, injection of NGF in the current study induced lateral elbow pain during movement of the upper limb that was more intense than exercise-induced DOMS of the wrist extensor muscles (6) and more similar in intensity to that reported by people with mild LE (1).

Conclusions

This study shows that a single intramuscular injection of NGF induces sustained elbow pain that is provoked by contraction, stretch and functional use of the muscle. As such, this experimental pain model may be suitable to study the sustained effects of lateral elbow pain on sensorimotor function and to probe the mechanisms underlying persistent musculoskeletal pain.

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None

CONFLICT OF INTEREST/DISCLOSURE SUMMARY

The authors declare that there are no conflicts of interest related to this study.

For Review Only

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LEGENDS FOR TABLES**Table 1:** Size of the painful area

NGF – nerve growth factor group

ISO – isotonic saline group

Mean (95% confidence interval)

* – Significantly enlarged compared with the ISO group, Bonferroni: $P < 0.05$ # – Significantly enlarged compared with Day 0 am, Bonferroni: $P < 0.05$ **Table 2:** Pressure pain thresholds for the elbow, low back and tibialis anterior

NGF – nerve growth factor group

ISO – isotonic saline group

Mean (95% confidence interval) pressure pain thresholds normalized to values recorded pre-injection on Day 0 (i.e. 0–100 %)

Pre – Pre hypertonic saline injection at Day 2

Post – Post hypertonic saline injection at Day 2

LEGENDS FOR FIGURES

Figure 1: Timeline of experiment. Participants attended four experimental sessions (Days 0, 2, 4, and 10), and completed a daily diary of their elbow pain (Day 0 to Day 10) at approximately midday and in the evening of Days 0-4 and only in the evening on Days 5-10.

AM – morning; PM – evening; PRTEE – patient rated tennis elbow evaluation; PPT – pressure pain threshold; NGF – nerve growth factor; ISO – isotonic saline.

Figure 2: Median scores (75th percentile) from the 10 day pain diary for the NGF (nerve growth factor, open bars) and ISO (isotonic saline; median score was always 0, data for the 75th percentile is shown on the right side of the NGF data for each time point) groups including (A) the 7-point Likert scale (0 = ‘a complete absence of pain/soreness’; 6 = ‘a severe muscle pain/soreness, stiffness or weakness that limits my ability to move’), (B) the numerical rating scale of worst pain intensity (0-10), and (C) the numerical rating scale of the pain experienced with repeated arm movements (0-10).

* – Significant increase compared with the ISO group, Mann Whitney and Bonferroni: $P < 0.003$.

– Significant increase compared with Day 0 am, Wilcoxon and Bonferroni: $P < 0.003$.

Figure 3: Median (75th percentile) total score for the patient rated tennis elbow evaluation (PRTEE) questionnaire for the NGF (nerve growth factor, open bars) and ISO (isotonic saline, solid bars) groups at Day 2 and 4. The total score is further represented by the three subscales Pain, Function A (activities specific to the upper limb), and Function B (general activities).

* – Significant increase compared with the ISO group, Mann Whitney and Bonferroni: $P < 0.017$.

Figure 4: Pain chart drawings of painful areas immediately after the NGF/ISO (nerve growth factor/isotonic saline) injection (Day 0 post-injection), the evening of Day 0 (Day 0 pm), before the hypertonic saline injection on Day 2 (Day 2 pre-injection), pain evoked by hypertonic saline

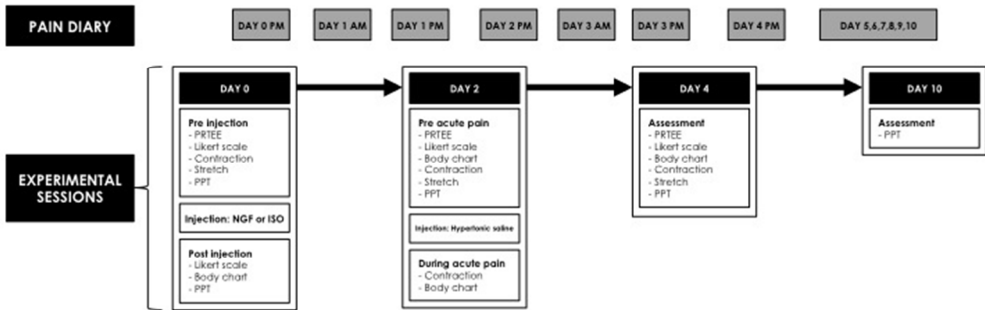
injection (Day 2 hypertonic saline injection), and the evenings of Day 4, 6, 8 and 10. The number of participants in each group who reported pain is indicated for each time-point. The crosses indicate the injection site.

Figure 5: Median (75th percentiles) pain intensity scores on a numerical pain scale (0-10) in the NGF (nerve growth factor, open bars) and ISO (isotonic saline, solid bars) groups at Day 2 and 4 during maximal (A) and submaximal wrist extension (B), maximal (C) and submaximal gripping (D), and when ECRB was stretched by passively moving the wrist into maximal flexion (E), and ulnar deviation (F).

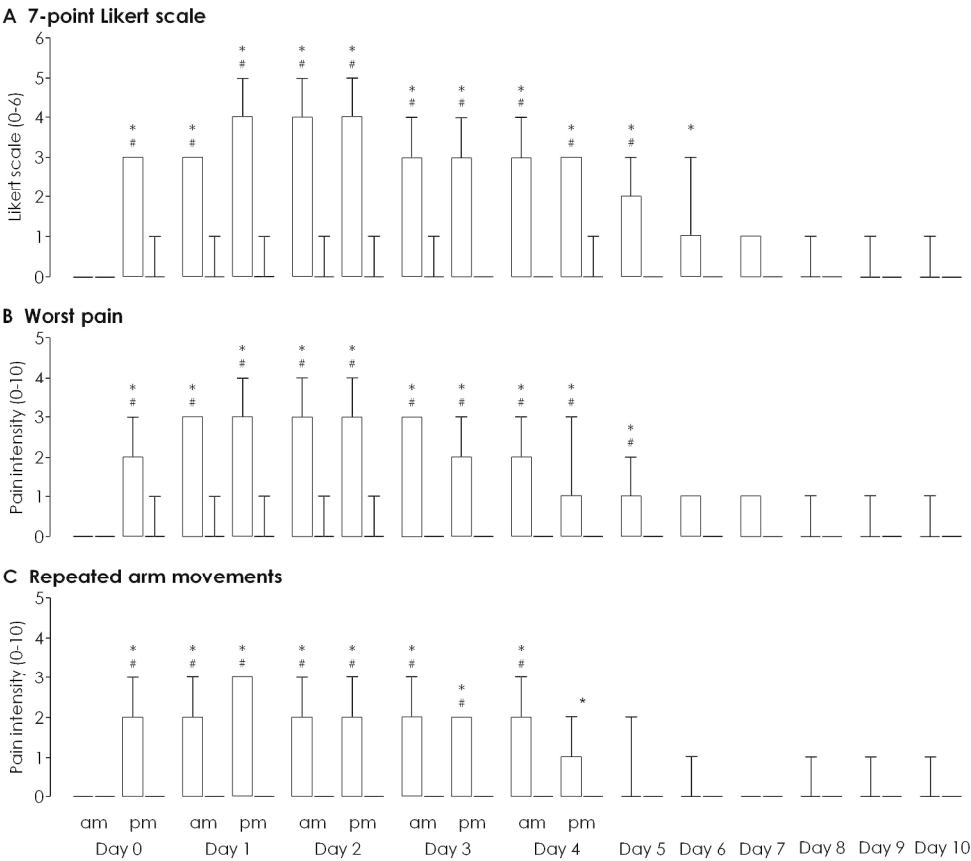
* – Significant increase compared with the ISO group, Mann Whitney and Bonferroni: $P < 0.017$.

Figure 6: Mean (95% confidence interval) of pressure pain threshold from the NGF (nerve growth factor, open bars) and ISO (isotonic saline, solid bars) groups normalized to values recorded pre-injection on Day 0 (i.e. baseline) for the elbow (A), low back (B) and tibialis anterior muscle (C).

* – Significant increase compared with the ISO group, Bonferroni: $P < 0.05$.



Timeline of experiment. Participants attended four experimental sessions (Days 0, 2, 4, and 10), and completed a daily diary of their elbow pain (Day 0 to Day 10) at approximately midday and in the evening of Days 0-4 and only in the evening on Days 5-10.
AM – morning; PM – evening; PRTEE – patient rated tennis elbow evaluation; PPT – pressure pain threshold; NGF – nerve growth factor; ISO – isotonic saline.
254x96mm (72 x 72 DPI)

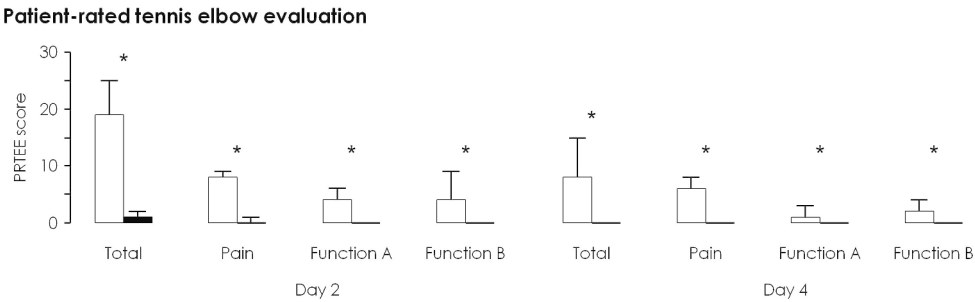


Median scores (75th percentile) from the 10 day pain diary for the NGF (nerve growth factor, open bars) and ISO (isotonic saline; median score was always 0, data for the 75th percentile is shown on the right side of the NGF data for each time point) groups including (A) the 7-point Likert scale (0 = 'a complete absence of pain/soreness'; 6 = 'a severe muscle pain/soreness, stiffness or weakness that limits my ability to move'), (B) the numerical rating scale of worst pain intensity (0-10), and (C) the numerical rating scale of the pain experienced with repeated arm movements (0-10).

* – Significant increase compared with the ISO group, Mann Whitney and Bonferroni: $P < 0.003$.

– Significant increase compared with Day 0 am, Wilcoxon and Bonferroni: $P < 0.003$.

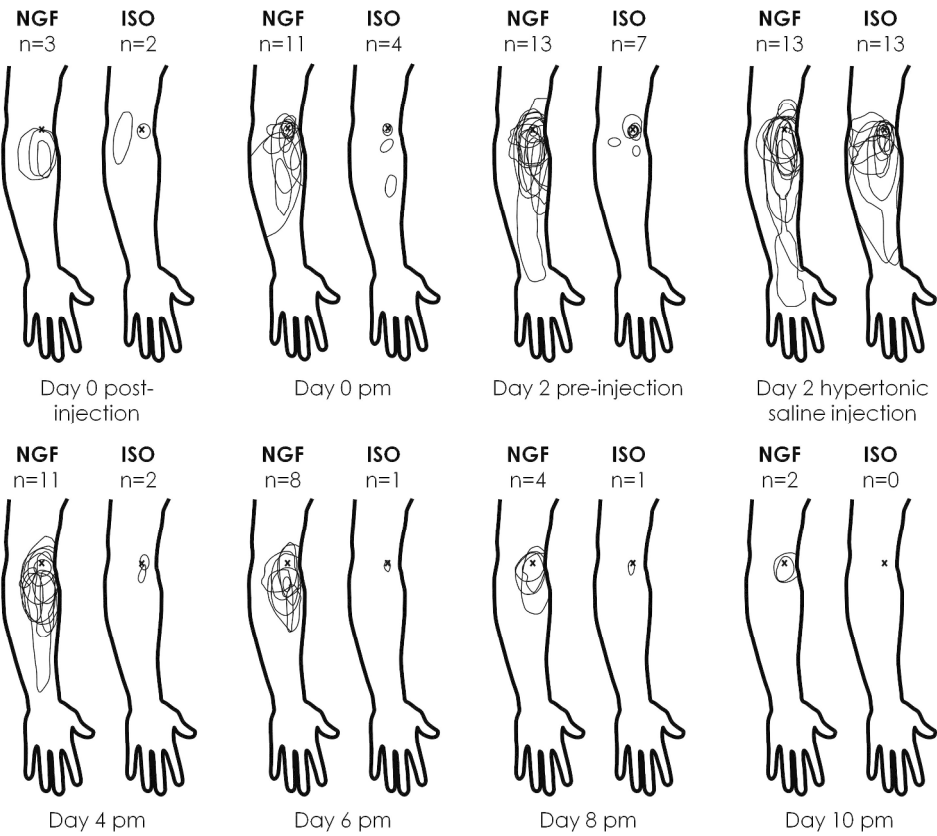
503x438mm (155 x 155 DPI)



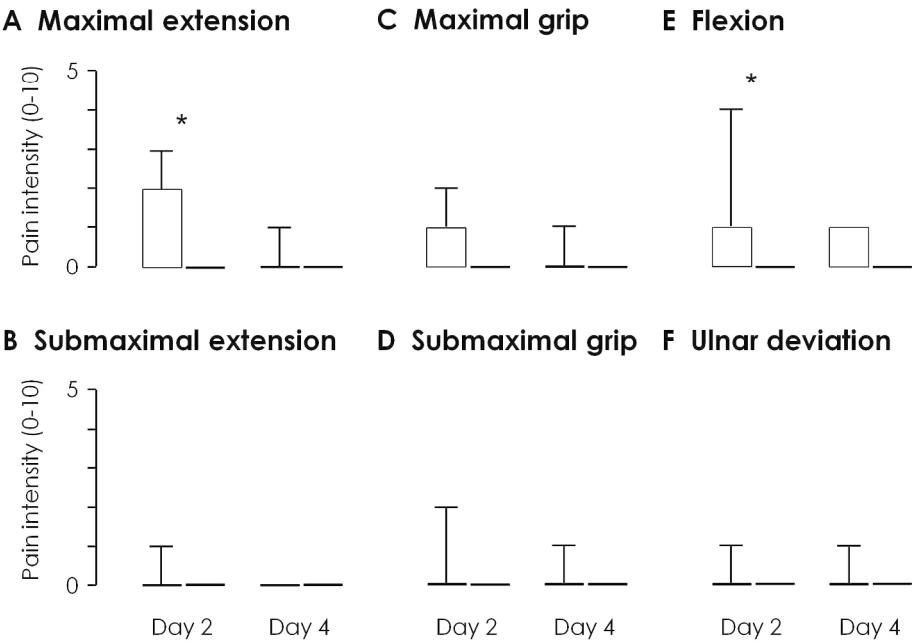
Median (75th percentile) total score for the patient rated tennis elbow evaluation (PRTEE) questionnaire for the NGF (nerve growth factor, open bars) and ISO (isotonic saline, solid bars) groups at Day 2 and 4. The total score is further represented by the three subscales Pain, Function A (activities specific to the upper limb), and Function B (general activities).

* – Significant increase compared with the ISO group, Mann Whitney and Bonferroni: $P < 0.017$.

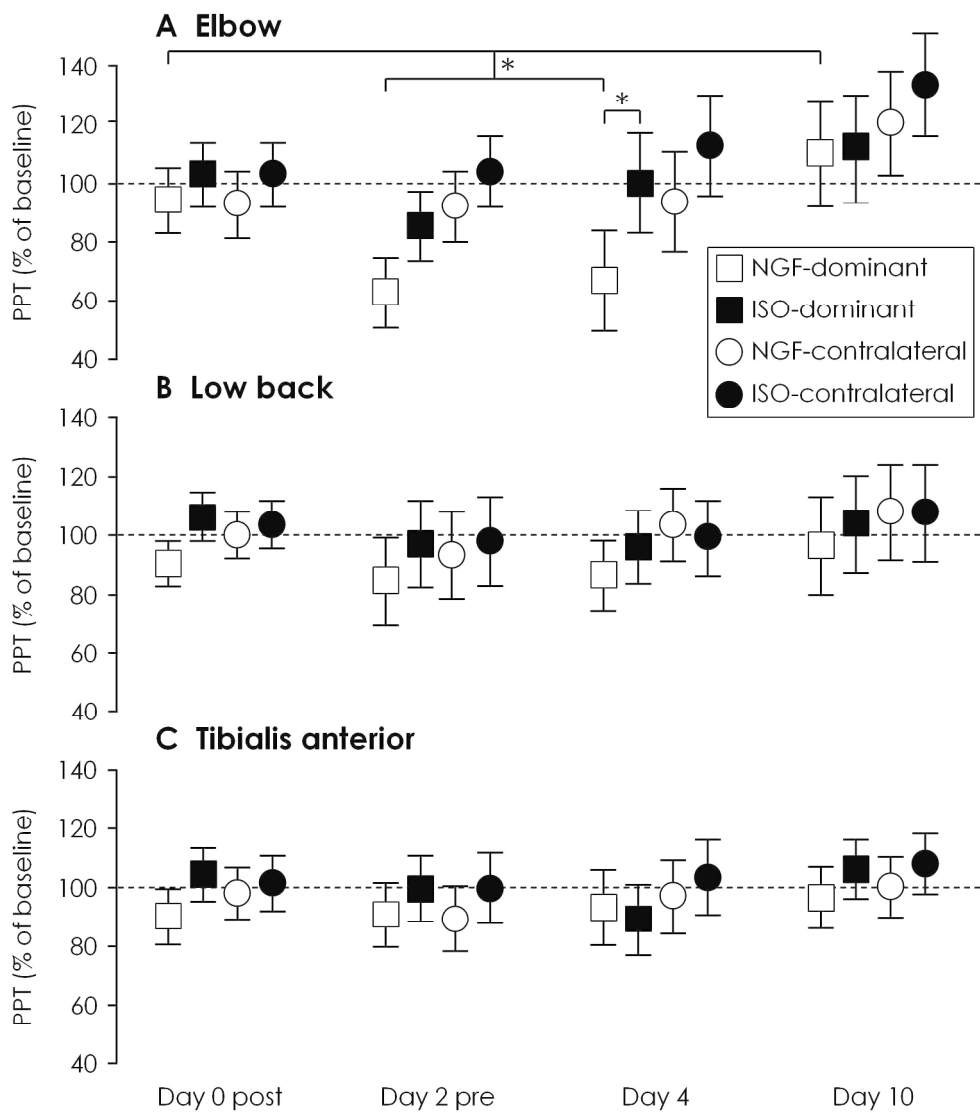
349x114mm (223 x 223 DPI)



Pain chart drawings of painful areas immediately after the NGF/ISO (nerve growth factor/isotonic saline) injection (Day 0 post-injection), the evening of Day 0 (Day 0 pm), before the hypertonic saline injection on Day 2 (Day 2 pre-injection), pain evoked by hypertonic saline injection (Day 2 hypertonic saline injection), and the evenings of Day 4, 6, 8 and 10. The number of participants in each group who reported pain is indicated for each time-point. The crosses indicate the injection site.
269x230mm (289 x 289 DPI)



Median (75th percentiles) pain intensity scores on a numerical pain scale (0-10) in the NGF (nerve growth factor, open bars) and ISO (isotonic saline, solid bars) groups at Day 2 and 4 during maximal (A) and submaximal wrist extension (B), maximal (C) and submaximal gripping (D), and when ECRB was stretched by passively moving the wrist into maximal flexion (E), and ulnar deviation (F).
* – Significant increase compared with the ISO group, Mann Whitney and Bonferroni: $P < 0.017$.
254x169mm (300 x 300 DPI)



Mean (95% confidence interval) of pressure pain threshold from the NGF (nerve growth factor, open bars) and ISO (isotonic saline, solid bars) groups normalized to values recorded pre-injection on Day 0 (i.e. baseline) for the elbow (A), low back (B) and tibialis anterior muscle (C).
* – Significant increase compared with the ISO group, Bonferroni: $P < 0.05$.
230x260mm (300 x 300 DPI)

	Day 0		Day 1		Day 2		Day 3		Day 4		Day 5	Day 6	Day 7	Day 8	Day 9	Day 10
	am	pm	am	pm	am	pm	am	pm	am	pm						
NGF	0.0	3.4*	4.6*,#	5.0*,#	5.5*,#	5.3*,#	4.9*,#	4.5*,#	3.6*,#	4.2*,#	2.7	2.2	1.8	1.2	0.6	0.3
	(0.0)	(2.9)	(1.8)	(1.1)	(1.8)	(1.4)	(1.5)	(1.6)	(1.4)	(1.8)	(1.2)	(1.1)	(1.1)	(1.1)	(0.6)	(0.4)
ISO	0.0	0.2	0.2	0.2	0.3	0.4	0.1	0.1	0.1	0.1	0.1	0.0	0.0	0.0	0.0	0.0
	(0.0)	(0.1)	(0.1)	(0.1)	(0.2)	(0.3)	(0.1)	(0.2)	(0.1)	(0.1)	(0.1)	(0.0)	(0.0)	(0.0)	(0.0)	(0.0)

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	Elbow		Low back		Tibialis anterior	
	Day 2 pre	Day 2 post	Day 2 pre	Day 2 post	Day 2 pre	Day 2 post
NGF-dominant	62.6 (13.3)	64.6 (16.6)	85.0 (9.7)	89.4 (14.4)	90.8 (10.7)	88.1 (12.3)
NGF-contralateral	92.2 (5.8)	93.1 (9.1)	93.4 (13.1)	100.1 (13.9)	89.4 (9.9)	91.8 (11.8)
ISO-dominant	85.5 (12.8)	78.3 (13.4)	97.2 (14.2)	95.9 (8.4)	99.0 (12.3)	99.1 (11.7)
ISO-contralateral	104.0 (12.7)	101.6 (12.3)	98.3 (17.9)	103.8 (22.1)	99.5 (11.5)	106.3 (8.6)

For Review Only