Official Title: Evaluation of Motor Unit Abnormalities after Experimentally Induced Sensitization Using Capsaicin: A Randomized, Double-Blinded, Placebo-Controlled Study

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Abstract

Central sensitization is a condition that represents a cascade of neurological adaptations, resulting in an amplification of nociceptive responses from noxious and non-noxious stimuli. However, whether this abnormality translates into motor output and more specifically, ventral horn abnormalities, needs to be further explored. Twenty healthy participants aged 20-70 will be randomly allocated to topical capsaicin or a placebo topical cream which will be applied onto their left upper back to induce a transient state of sensitization. Visual analogue scale (VAS) ratings of pain intensity and brush allodynia score (BAS) will be used to determine the presence of pain and secondary allodynia. Surface electromyography (sEMG) and intramuscular electromyography(iEMG) will be used to record motor unit activity from the upper trapezius and infraspinatus muscles before and twenty minutes after application of capsaicin/placebo. Motor unit recruitment and variability will be analyzed in the sEMG and iEMG, respectively. An independent t-test will be performed on the data. This preclinical evidence may provide some explanation for the influence of central sensitization on changes in movement patterns that occur in patients who have pain encouraging of further clinical investigation.

Keywords: Central Sensitization; Surface Electromyography; Intramuscular Electromyography; Motor Unit; Recruitment

1. Introduction

Chronic pain is characterized by pain lasting longer than three months [1-3]. It is a common condition affecting 1 in 5 people [4] and is rising due to the aging population and increased prevalence of comorbid conditions such as diabetes [5,6] and obesity [7]. Chronic pain produces

a significant socioeconomical burden with the annual estimated cost in Canada exceeding 6 billion dollars [4,8-11]. As such, the assessment and diagnostic efficacy of chronic pain is of vital importance to improving its management throughout society.

Central sensitization describes a state of neuronal hyper-excitability in the central nervous system that may occur due to malfunction of spinal and supraspinal pain facilitatory and inhibitory circuits resulting in amplification of somatosensorial responses [12]. Beyond somatosensorial changes, alteration in motor function can also be present with pain, and may be a reflex of neuromuscular function impairment [13,14]. A normal afferent input and normal central processing circuitry is essential to deliver normal efferent output. However, the influence of the changes that occur within the dorsal horn on the ventral horn remain largely ill defined [15,13].

Motor unit assessment is crucial in evaluating diseases and abnormalities within the ventral horn [16]. Activity of the ventral horn, where anterior horn cells reside, is very important for motor unit activation [16]. Surface EMG (sEMG) [17] and intramuscular EMG (iEMG) [18] can be used to assess the neural drive to muscles, by recording motor units to understand the effects of central sensitization on motor control and the ventral horn. Based on Henneman's size principle, motor units should be recruited in the same order, with smaller units being recruited first [19]. This principle presents an opportunity to investigate if central sensitization creates abnormalities on the motor unit level.

Previously, central sensitization has been induced in healthy subjects to examine its neurophysiological effects via capsaicin [20-22]. Capsaicin, a chilli pepper extract, can be used to effectively induce experimental transient states of central sensitization. [23-25,21,22]. The presence of expanded sensorial responses and the involvement of the spinal nociceptive system

post capsaicin have been largely tested by means of quantitative sensory testing methods and electromyography (EMG) [25,21,22,24].

Despite the usefulness of experimental capsaicin to better understand the sensorial abnormalities, its impact on motor function and motor unit recruitment are lesser studied. Evidence suggests that nociceptive input by peripheral capsaicin exerts a centrally-mediated inhibitory effect on motor function [26]. A decrease in root mean squared (RMS) amplitude during exercise at the time of peak sensitization was measured by needle EMG [26]. However, the effect that capsaicin-induced sensitization has on individual motor units or on their recruitment patterns has not been previously examined.

The purpose of this study is to determine whether topical capsaicin-induced sensitization has any influence on ventral horn activity. We hypothesize that capsaicin induces a change in individual motor unit activity, as well as the recruitment pattern of many motor units, and may affect motor unit activity at different segmental levels from the level of capsaicin application.

2.0 Methods

2.1 Study Participants

Ethical approval was obtained from the Ethics Board of the Toronto Rehabilitation Institute, University of Toronto prior to commencing the study. This was in accordance with the World Health Organization statement of ethical principles for medical research involving human subjects [27].

Twenty-three healthy participants, age between 20-70 years old, with no direct trauma to cervicothoracic region within the past 30 days, no past medical history of inflammatory disorders as rheumatoid arthritis, no neurodegenerative disorders such as Parkinson's disease nor motor

neurone diseases as amyotrophic lateral sclerosis, or other neuromuscular disorder will be recruited for this study. Also, we will include subjects that have a normal body mass index (18.5 – 24.9) [28] and have a pain visual analogue scale (VAS) below 3 indicating low pain severity [29]. Since prevalence of neck pain in the general population is high, mild pain or aches are not necessarily related to an abnormality of the underlying muscle [30]. Participants have to be able to communicate in English. Participants will be excluded if they had persistent pain for more than 3 months. This will be determined by the physician on our research team (DK).

2.2 Dataset Collection

An initial screening will be performed to assess eligibility in the study. Each participant will be seated upright with their hands comfortably on their lap and asked to relax their neck and shoulder muscles. The physician member of the research team will then assess the patients' pain intensity by visual analogue scale (VAS). VAS ranges from 0 to 100 mm which 0 mm reflecting no pain at all and 100 mm representing the worst imaginable pain [29]. Following this, brush allodynia, a clinical technique used to identify pain due to a stimulus that does not normally provoke pain, will be performed to confirm presence of central sensitization [12]. To map out borders of secondary allodynia, subjects will be instructed to recognize a distinct alteration in the sensation perception such as increased burning, intense pricking, or an unpleasant sensation, and that location will be marked [31]. Brush allodynia score (BAS) will be calculated as the distance between the farthest points marked on the superior and inferior axis multiplied by the distance between the farthest points marked on the medial and lateral axis as previously described by Cavallone et. al. [31]. The VAS and the presence of central sensitization by means of BAS will be assessed at baseline (pre) before the induction of sensitization and twenty minutes after (post).

Upon successful screening of inclusion and exclusion criteria, participants will have their left side area of skin (overlying the upper trapezius and infraspinatus muscles) cleansed with alcohol preparation pads and water. The skin will be abraded with '3M Red Dot' abrasive strips before application of the surface electromyogram (Trigno Galileo sensors, *Delsys Inc.*). Electrodes will be placed in 4 areas: the muscle belly of the upper trapezius, and the infraspinatus, as well as reference electrodes on C7 and the acromion. These electrodes are 4 channel EMG sensors and have their signals filtered from 20 – 450 Hz. The sEMG recordings are wirelessly transmitted to the Trigno base station, which relays and compiles the data to Neuromap (*Delsys Inc.*) for signal analysis. A monopolar needle electrode will be inserted into the upper fibers of trapezius muscle and its reference will be placed at the mid-clavicle point. Using this setup intramuscular recordings of single motor units will be performed using an Excaliber, *Natus Medical* clinical electrodiagnostic machine.

2.2.1 Electromyograms recordings before application of intervention

Participants will then be instructed to perform horizontal shoulder abductions from 0° to 90° and then from 90° to 0° for 1 minute. The study subject will be verbally cued to move their arm every 2 seconds. sEMGs were recorded during this time. Upon completion of this task, a monopolar intramuscular needle electrode will be placed directly into the upper trapezius muscle. Participants will then be instructed to gently contract their trapezius muscle by shrugging their shoulder, enough to recruit only the first motor unit in that region. Visual feedback of the signal will be given to the subject to ensure that only the first motor unit was activated for the movement. iEMG recordings will be recorded for 30 seconds at a sampling frequency of 6 kHz.

2.2.2 Application of intervention

Participants will receive either a dose of 2.5 ml (75µg/ml) capsaicin cream (treatment, Zostrix brand) or skin lotion (placebo) which is inert and causes no sensitization effects. Participants will also blinded to the delivered treatment, using concealed containers for the creams. The location of application will be a 10 cm by 10 cm square on trapezius muscle which extends from T3 to T8 on the left side that all recordings were conducted.

After collection of the baseline sEMG and iEMG recordings, a trained medical professional will apply the capsaicin / placebo cream directly to the region of skin in a standardized 10 cm x 10 cm square at the spinal levels T3-T8, to sensitize the nociceptive afferents within that region. A twenty-minute waiting period will be used to enable the sensitizing effects of capsaicin to take effect.

2.2.3 Electromyograms recordings after application of intervention

To confirm the presence of central sensitization, brush allodynia will be used to detect mechanical allodynia outside the region of the primary nociception – region of topical placement – which is the region of secondary allodynia [25]. Upon confirmation of central sensitization, in participants with application of topical capsaicin, participants will be entered into the experimental arm of the study.

2.3 Study Procedures for EMG Analyses

This section outlines the procedures for the EMG analysis. For the sEMG data, the pre-recording motor units will be matched with an algorithm implemented in MATLAB (Mathworks, version 2018a), described in section 2.3.1, in order to determine the aberration of recruitment pattern after treatment.

For the iEMG data, the pre- and post-recording motor units will also be matched using an algorithm implemented in MATLAB (Mathworks, version 2018a), described in 2.3.2. The variability of that motor unit shape will be compared before and after capsaicin and between groups.

2.3.1   Surface EMG

Recorded sEMGs will be analyzed in *Neuromap* [32]*,* which provides an average template, average amplitude, and order of recruitment of each motor unit detected and isolated from the raw signal using *Delsys'* motor unit decomposition algorithm [33]. Each motor unit template will then be analyzed based on their "overall match" in shape and amplitude.

Shape Analysis

The motor unit signal that will be obtained from M electrode contacts at T consecutive time samples will be assembled into an $M \times T$ matrix. This matrix will then be compressed into a single vector to create a motor unit "signature" [34]. This will be done by taking the recordings from all electrode contacts at a single time step and concatenating it to the recordings from all contacts at the next time point. This concatenation will be repeated to the time T to create the signature **(Figure 1)**. This process will be performed for the pre- and post-recording motor units for each participant.

The shape of each detected pre-recording motor unit will be compared to the shape of each post-recording motor unit via the cross-correlation function, *xcorr*, in *Matlab* (Mathworks,2018a). This function returns a normalized value between $0 - 1$ between each pre- and post-recording motor unit. Cross-correlation values between pre- and post-recording motor unit pairs that are

below a preset threshold of 0.8 [35] will be set to 0 as the pair is unlikely to be from the same motor unit.

Amplitude Analysis

The motor unit average amplitudes obtained from the *Neuromap* software will be used for calculating an amplitude ratio between pre- and post-recording motor units. This amplitude ratio will be calculated for each pre/post motor unit pair to determine similarities in the amplitude. Similar to the shape analysis, amplitude ratios that are below a preset threshold of 0.8 or above 1.2 are set to 0. This ratio will be normalized to be within a scale from 0 – 1 to allow for comparison between shape and amplitude using the following equation (**Eq.1**) when the amplitude ratio is above one.

$$Normalized\ Amp.Ratio\ =\ 1 - Amp.Ratio \qquad (1)$$

The numbers from the shape analysis and amplitude analysis, which are not set to 0, will be averaged together to express an "overall match" between pre- and post- motor units from each participant. As mentioned, based on Henneman's size principle, motor units should be recruited in the same order with smaller units being recruited first and subsequent motor units are recruited as more force is needed [19]. To observe whether this principle is upheld or violated, the reorganization of the recruitment will be identified by how much earlier or later the similar motor units were recruited. In order to quantify the recruitment, the difference in the order of recruitment between the pre and post recording will be calculated. Recruitment order of motor units will be

determined through a custom automated search algorithm (Algorithm 1), which will determine the best match between pre- and post-recording motor units.

Algorithm 1: Automated Motor Unit Matching method

Input: Matrix of averaged values from shape and amplitude analysis

Output: Matched pre-post motor units

1:    **loop** through each pre-motor unit (row) and each post-motor unit (col)

2:    **if** value at current location is the highest in that row and column, **and** the number
      is not equal to zero

3:      **Record** match between the pre- and post- motor unit, and set the row and col

          to 0 (i.e. remove the pre- and post- motor unit from further pairing)

4:    Return matched pre-post motor unit pairs

The average recruitment order difference for each motor unit will be obtained for each participant. Following this, for each participant, the recruitment difference for all of their motor units will be averaged, to obtain one vector of average recruitment changes per person. This final matrix containing numbers on a continuous scale, will be used for statistical testing. See Appendix A for a detailed explanation of the matrix set up.

2.3.2   Intramuscular EMG analysis using template matching

Recorded iEMGs will be thresholded using the median absolute deviation estimate approach (**Eq.2**) to find the peak location of motor unit action potentials (MUAPs) [36].

$$Threshold = 10 \times \frac{Median(|X|)}{0.6745} \quad (2)$$

Each detected peak, along with 12.5 ms before and after will be stored and used for analyses. These detected MUAPs will then be clustered to determine the number of motor units present in the recording. In particular, the first detected peak and its surrounding environment (i.e. the MUAP) forms the first motor unit template. Subsequent detected MUAPs will be compared to the first template using cross correlation. If the cross correlation is more than 0.85, it will be determined as the same MUAP and added to that motor unit cluster and the template will be updated. However, if the cross-correlation is less than 0.85, it will be classified as a different motor unit and a new template cluster will be created for comparison.

This process will be repeated until all of the detected MUAPs in the pre- and post-recording are classified. The MUAP cluster with the highest number of MUAPs, for the pre-recording, will be compared with all MUAP clusters found in the post-recording for each participant. The MUAP cluster in the post that has the highest cross-correlation value will be considered "matched." Participants that do not have matched MUAPs with cross-correlation values above or equal to 0.95 will not be used for statistical analysis.

2.4 Statistical Methods

2.4.1 Surface EMG

An independent samples t-test will be performed on the difference in recruitment position using treatment as the factor for the trapezius muscle. In particular, the statistical test will compare

post-pre capsaicin recruitment differences against post-pre placebo recruitment differences. The average difference in recruitment order will be used to avoid bias of having multiple data points per participant that correspond to different motor units.

2.4.2 Intramuscular EMG

From the intramuscular EMG algorithm mentioned in section 2.3.2, the resultant matrices will be the variances for the pre-recording motor unit trains. The statistical model used will assess the difference between the pre- and post- recording motor units for each capsaicin and placebo as well as a between group comparison (Post-Pre capsaicin motor unit variance vs. Post-Pre placebo motor unit variance). These analyses would provide insight into our hypothesis that sensitization induced by capsaicin effects upon the motor unit characteristics. Either an independent samples t-test or a Kruskal-Wallis H test will be performed, depending on how many participants' pre and post motor units match up.

3   Sample Size Calculation

The minimum sample size required per group to detect whether a significant difference exists between two means for one dependent variable was calculated with a confidence level of 95%, a 80% statistical power level and 5% error. A mean detectable difference of 5 for the dependent variable, mean motor unit recruitment change, and an estimated standard deviation of 3 was used. The estimated sample size was 7 people per group; however, considering a 20% drop out rate, the suggested sample size was 9 people per group. This analysis was completed using PASS Software, version 15.

4 Conclusions

This may provide some insights for the neurophysiological influence of central sensitization on changes in efferent responses and movement patterns that occur in patients with persistent pain. However, the clinical implication of this experimental findings on the movement properties needs to be further investigated.

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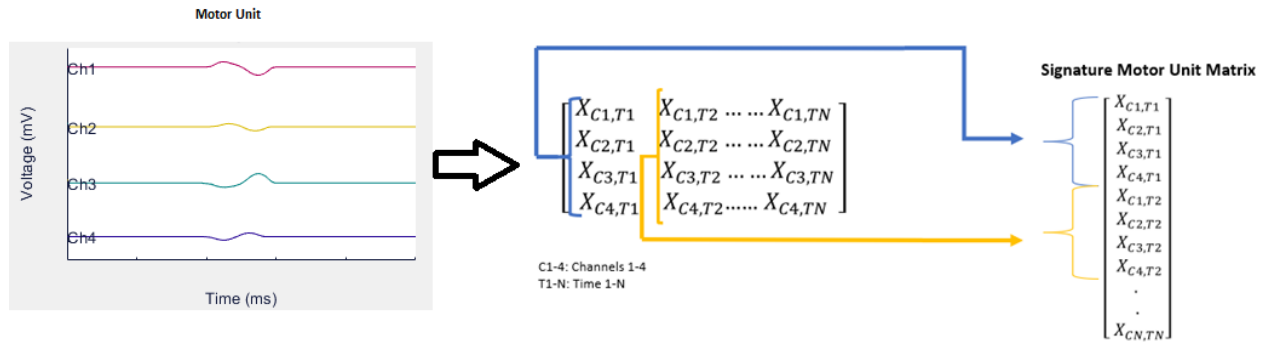
Figure 1: Process of creating the motor unit signature from a set of recordings. Each column of the matrix corresponds to the recordings from all electrode contacts at a single time step up to the time *T*. The columns are then concatenated to create a single vector corresponding to motor unit signature.

APPENDIX A

MUPRE	MUPOST	Difference Array
1	9	8
2	8	6
4	13	9
5	16	11
6	15	9
8	19	11
14	18	4
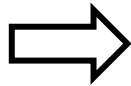
Average Difference per person (for T test USE):

$$= Average(Differences)_{per\ person}$$

$$= \frac{(8) + (6) + (9) + (11) + (9) + (11) + (4)}{7}$$

= 8.286

Supplementary Figure 1: Pre/Post Difference

In order to compare the reorganization in recruitment, the difference in order of recruitment was calculated and put into an array. This difference array was calculated for each person, and an average difference in recruitment per person was analysed using a t-test. The reason for having an average per person was to avoid any bias by having multiple data points per person that correspond to different motor units. Assumptions of normality and homogeneity of variances were verified from the Shapiro- Wilk test (p=0.187) and Levenes Statistic(p=0.081), therefore a t-test can be used.