Quantitative Response of Healthy Muscle Following the Induction of Capsaicin: An Exploratory Randomized Controlled Trial

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Abstract

Background: Myofascial pain syndrome (MPS) is a prevalent chronic pain disorder primarily characterized by myofascial trigger points (MTrP). There is limited knowledge on the pathophysiology and mechanisms underlying MTrP and its development. Research has previously demonstrated the identification of MTrPs using ultrasound and vibration sonoelastography, although there is some contradictory evidence regarding if MTrPs present as hyper or hypoechoic regions. Electromyography (EMG) investigations of MTrP have demonstrated that MTrP are usually located proximal to innervation zones where the peak surface EMG signals are obtained from. Central sensitization has been proposed as the primary mechanism underlying MTrP development. Central sensitization is associated with hyperexcitability of neuronal responses to normal or noxious stimuli. There is a need for a study that measures ultrasound image textural changes and motor unit activity responses in the muscle following sensitization. The purpose of this study is to determine whether sensitizing healthy muscle using capsaicin induces a regional change in image texture variables within the specific and surrounding muscles, as well as the motor unit frequency and amplitude changes that accompany them.This is an exploratory trial that aims to provide preliminary evidence on whether central sensitization is a direct cause of taut band and MTrP development. Methods: The study conforms to the Consolidated Standards of Reporting Trials recommendations. Ethical approval will be sought from the University Health Network (UHN) Research Ethics Board. This proposed study is a single centered, factorial, randomized placebo-controlled trial with two independent variables, depth of capsaicin application and dose of capsaicin, for a total of four treatment arms and two control treatment groups. Discussion: This will be the first study that assesses the B-mode ultrasound image texture of induced sensitized muscles, and will provide more evidence on muscle motor unit activity and regional changes of central sensitization. Findings from this study may support one of few hypotheses proposed delineating the involvement of central sensitization in the development of trigger points.

Background

Myofascial pain syndrome (MPS) is a prevalent chronic pain disorder primarily characterized by myofascial trigger points (MTrP). MTrP are stiff taut bands of muscle distinguished by hypersensitivity

on palpation and local twitch responses on snapping palpation.[1] A current clinical challenge in chronic pain care, applicable to myofascial pain, is early detection and effective treatment targeting the underlying pathophysiology.

There is limited knowledge on the pathophysiology and mechanisms underlying MTrP and its development. Ultrasound studies have been able to characterize trigger points[32,45,46], however there are some contradictory findings on whether these areas are hyperechoic or hypoechoic[45,46]. More recently, texture feature analysis have been shown to differentiate between healthy and MPS as well as active and latent trigger points[32]. Trigger points are typically located within the region of the muscle belly, where there is a high distribution of motor endplates.[2,3] Seventy-one percent of acupoint locations in the body, which are regions associated with motor points, motor endplates, or major motor nerve pathways overlap with common MTrP.[4]

Electromyography (EMG) investigations of MTrP have demonstrated that MTrP are usually located proximal to innervation zones where the peak surface EMG signals are obtained from.[3] Motor endplates at MTrP present with spontaneous endplate activity (SEA) associated with excessive acetylcholine release.[5,6] In latent and active trigger points, SEA is characterized by continuous low amplitude action potentials. Active MTrP, which are more painful clinically, elicit intermittent spikes in addition to SEA. This continuous activity is thought to overexert and hyper contract the muscle resulting in the presentation of a taut band.[7] Studies assessing the contractility of MTrP have found increased muscle fatigability in active and latent MTrP relative to unaffected muscles. Furthermore, it has also been demonstrated that there is increased fatigability and EMG activity in active MTrP relative to latent ones.[8] This data suggests that perturbations in the efferent system involved in muscle control is a vital component of the MTrP pathophysiology.

Central sensitization has been proposed as the primary mechanism underlying MTrP development. Central sensitization is associated with hyperexcitability of neuronal responses to normal or noxious stimuli.[9] Furthermore, this concept offers an explanation of the afferent, efferent and abnormal signal modulation at the spinal cord level.[9,10] Patients with myofascial pain present with hyperalgesia and decreased pain threshold, i.e., signs of central sensitization, particularly at the

region of MTrP.[5,11] Experimental evidence has demonstrated that afferent nociceptive fibers at the region of MTrP are sensitized. Neurons in the dorsal horn segment corresponding to the location of MTrP are also sensitized, manifesting clinically as hyperalgesia within the dermatome affected by MTrP.[12] This is evidenced by Kim et al's[12] findings whereby they reported transcutaneous electric stimulation increased MTrP pressure pain thresholds when applied at a remote region within the same dermatome. Srbley et al.[1] further presented evidence of a sensitization arc that maintains the MTrP contracture. They experimentally induced sensitization at a remote region in the dermatome wherein a latent MTrP is located using topical capsaicin, and subsequently found a decrease in pressure pain thresholds at the MTrP.

Investigations assessing neuronal responsivity to noxious stimuli have demonstrated increased sensitization at the region of primary nociception and the development of a secondary nociceptive field.[13-15] This has been confirmed using EMG and quantitative sensory testing methods to assess the activity of sensory nociceptive, mechanoheat, and chemoreceptive nerve fibers following the injection or application of capsaicin.[13-15]. Many other factors are likely involved in the chronic pain patient including disordered ascending and descending signals within the central nervous system, termed nocioplastic pain by the IASP.[18,19,49] Dideriksen et al.[16] and Falla et al.[17] demonstrated that motor unit potentials reduce in amplitude following the injection of nociceptive hypertonic saline into the upper trapezius muscle. This is in line with the EMG characteristics of MTrPs cited earlier.[7,8] However, other studies, such as the study directed by Birch et al. (2000), present contradictory results, indicating that no significant differences in motor unit discharge rates were found. Studies assessing the EMG activity of trigger points failed to locate the aberrant activity that characterizes MTrP using surface EMG but were able to demonstrate the presence of SEA, intermittent spikes in active MTrP, as well as activity associated with local twitch responses using intramuscular EMG recordings due to the method's specificity.[5]

In regards to MTrP structure, Shankhar et al. (2012) was able to identify taut bands of MTrPs using 3D and 2D ultrasound[47]. They discovered that the muscle fascicles surrounding the taut band presented a different appearance than the adjacent healthy muscles. The study identified MTrPs as

hyperechoic regions, as in another study confirmed by Lewis et al.(1999)[46]. In contrast to these studies, Sikdar et al. (2008) noted that MTrPs appear as hypoechoic elliptical reigions within the ultrasound image[48]. Although there is some evidence on the changing structure of myofascial trigger points, to the best of our knowledge, there has been no evidence of how central sensitization affects muscle structure through image analysis. There is a need for a study that measures specific B-mode ultrasound textural changes within sensitized areas, as well as motor unit activity responses in the muscle following sensitization.

The purpose of this study is to determine whether sensitizing healthy muscle using capsaicin, a chilli pepper extract, induces a regional change in ultrasound texture features of the targeted muscle, or of the muscles in that are in close proximity. This regional change will be accompanied by electromyography (EMG) recordings to confirm the presence of abnormality. Central sensitization will be induced using topical capsaicin and injectable capsaicin at three different concentrations. This is an exploratory trial that aims to provide preliminary evidence on whether central sensitization is a direct cause of taut band and MTrP development.

Our specific research questions are followings:

a. Does sensitization cause a significant regional change in the texture features of the targeted muscle or surrounding muscles? Our hypothesis is that that there will be a structural change within the sensitized or adjacent muscles.

b. Does capsaicin induced sensitization elicit a change in motor unit action potential(MUAP) amplitude by approximately 20 to 25% within the region of experimentally induced central sensitization or in the region of texture feature changes? Our hypothesis for this portion of the study is that sensitization modifies the anterior horn cell activity which will be measured by the amplitude of the potentials and the change in texture features.

c. Does capsaicin induced sensitization elicit continuous electrical activity as observed in MTrPs? This tests the hypothesis that sensitization creates the presence of continuous low amplitude action potentials.

d. Does capsaicin induced sensitization influence the rate of recruitment of motor units in the muscle

within the region induced? Does this occur within the regions identified by the changes in texture features? The recruitment of motor units is normally expected to follow the Henneman size principle. [20-23] Our hypothesis for this portion of the study is that sensitization will cause an aberration in recruitment. If this is demonstrated, then there is modification of the normal processing at both dorsal and ventral horns of the spinal cord.

e. Is there a location dependent EMG response to capsaicin induced sensitization? In other words, do the texture features or EMG responses from muscles that lie anatomically distant from the location of the site of intramuscular capsaicin injection have changes from their baseline calculations? This would test the hypothesis that type III and IV afferents have an influence on the anterior horn cells that lie distant to the ones that are supplying muscle fibers in the vicinity of the stimulated sensory afferents.

f. Is there a dose dependent EMG response of motor units to capsaicin induced sensitization? Here, the hypothesis of causality will be assessed in a preliminary manner. If there is a relationship then further experiments that more thoroughly assess causality will be needed.

Methods And Design

Study Design:

The study conforms to the Consolidated Standards of Reporting Trials recommendations. Ethical approval is approved by the University Health Network (UHN) Research Ethics Board. This study is registered under the National Institutes of Health ClinicalTrials.gov.

This proposed study is a single centered, factorial, randomized placebo-controlled trial with two independent variables, depth of capsaicin application and dose of capsaicin, for a total of nine treatment arms. The first between groups variable will be topical capsaicin application and injectable capsaicin injection. Within each partition there will be three treatments: control, 50 micrograms, 100 micrograms. One-hundred micrograms reported to be an effective high dose of capsaicin.[13] The control group will receive a topical skin lotion which is inert and has no sensitization effect. An equal number of participants will be allocated to each of the six treatment groups using an electronic randomization generator. Block randomization will be used to ensure equal allotment into each group. An alternate member of the research team will conduct the randomization schedule a priori.

Participant allocation will be concealed by placing their assignment in an envelope and delivered to participants by the same member of the research team uninvolved in the measurement or randomization protocols. Participants and investigators will be blinded to the delivered dose; however, the type of capsaicin delivery cannot be blinded from either participants or investigators. The member of the research team conducting the randomization schedule and concealing allocation will have knowledge of and keep track of the doses contained in the containers and vials of the topical and injectable capsaicin, respectively. They will deliver the appropriate dose to the team member implementing the experimental protocol to ensure there is blinding with respect to dose. This individual will not be involved in participant recruitment.

Participants

Recruitment

Participants will be recruited from UHN and the University of Toronto clinics, employees, volunteers, visitors, students, and external participants. UHN is a tertiary healthcare centre with eight hospitals located in Toronto, Canada and the University of Toronto is an academic institution with a main campus located in close proximity to the main UHN hospitals in Toronto. Advertisements, flyers, and in person recruitment will be employed to collect the necessary sample size for the study. The recruitment procedures will run in line with the approved guidelines from the UHN research ethics boards. Participants will not be coerced into participating and will be informed that they have the right to withdraw at any time during the experimental procedures. They will also be informed they have the right to withdraw their data prior to publication. Participants will be briefed and consented on recruitment prior to commencing the study procedures. Participant treatment allocation will be recorded on a separate document until all of the data collection is complete and the data is analyzed.

Eligibility Criteria

Female or male participants who meet the following inclusion criteria will be included in the study: (1) participants are healthy with no past medical history (2) a visual analogue score below 3 indicating low pain severity, however, ideally who complain of no pain (3) right or left handed, (4) normal body mass index, (5) have sufficient command of the English language to provide informed consent and to

understand the study protocols, (6) participants agree to sign a consent to volunteer for the research.

Exclusion Criteria

Participants who meet one or more of the following criteria will be excluded: (1) history of pain, (2) physical examination detection of myofascial trigger points, (3) participants present with a history of pain related disturbances such as poor sleep, cognitive disturbances, psychiatric disorders, (4) history of general medical disorder that may affect the outcome of the study such as diabetes mellitus, (5) and a history of cervical radiculopathies or (6) history of inflammatory arthropathy.

Experimental Protocol

This experimental protocol will be carried out at the Kumbhare Lab, Toronto Rehabilitation Institute to ensure any medical emergencies can be promptly cared for. Following the completion of the preliminary intake forms and participant debriefing, informed consent will be obtained. Each participant will be seated upright with their hands comfortably on their lap in a chair that has a high supportive back. They will be asked to relax their neck and shoulder muscles. A member of the research team will then apply the inclusion and exclusion criteria. The presence of a MTrP will be assessed by palpation of the upper trapezius muscle since this is the current method utilized in clinical practice. If there is a MTrP, then they will be asked to withdraw from the study since they do not satisfy the inclusion and exclusion criteria.

Participants will sit on a chair with one arm extended downward, and with the other arm on an armrest of the chair. The extended arm will be fixed by a wristcuff and a chain down to the floor, with a loadcell between the floor and the end of chain. The loadcell will measure the exerted force. The anatomical location for ultrasound probe and electrode placement will be identified for each participant. The ultrasound probe will be placed on 3 muscles: the trapezius, the supraspinatus, and the infraspinatus. Two ultrasonic pictures will be taken of each. Care will be taken to image the region of the muscle that was not mechanically impacted by the insertion of the needle or injection of the substance. The area will then be cleaned with alcohol and water. The Delsys Galelio surface sensor, and the intramuscular needle electrode will be placed directly on the trapezius' identified area.

Participants will be asked to gently contract their trapezius muscle. They will be instructed to perform a gradually increasing contraction in isometric condition, in a controlled manner with a monitor showing the exerted force as well as the target force. They will hold this contraction at 30% of their maximal voluntary contraction, and then perform a gradually decreasing contraction to rest. This will be performed four times for each type of study intervention and before as well as after each intervention, namely topical control cream, topical capsaicin at 50ug and 100 ug, injection of the superior aspect of the superior fascia followed by intramuscular injection with these two concentrations of capsaicin. The placement of these latter will be verified by ultrasound guidance. The intramuscular needle will then be removed and participants will be bandaged and cared for appropriately by the expert physician performing the experiment.Following this, ultrasound pictures will be taken again from the same muscles mentioned previously. Participants will be re-examined to determine if there were any adverse effects from the experimental procedures. If any occurred, then these will be carefully managed by the medical members of the research team and recorded. Participants will be asked to remain at the lab for an additional 30 minutes to ensure they are well prior to leaving (Figure 2).

Central sensitization and measurement techniques

Inducing central sensitization

Central sensitization will be induced using capsaicin according to the established technique.[13,24,25] It induces increased pain severity at high doses relative to heat and elicits a dose dependent pain responses, allowing for more easily quantifiable measurements.[26] Capsaicin delivery induces both primary and secondary nociception suggesting that central mechanisms are involved in the nociceptive response, also termed as central sensitization.[13,14] Previous evidence has confirmed that capsaicin can sensitize nociceptive and mechanoheat receptors outside of the region of primary hyperalgesia, with speculation that it can also sensitize chemonociceptive receptors.[13] Therefore, capsaicin can effectively be used to induce central sensitization.

Capsaicin will be applied directly to the region of the innervation zone at the muscle belly to sensitize the neurons within the region of taut band development. Topical and intramuscular capsaicin will be

used. The capsaicin formula will be compounded by a registered pharmacist. Topical capsaicin will be delivered in a cream and injectable capsaicin will be intermixed with saline prior to injection. The control group in the topical capsaicin arm will be treated with the cream base used during the experiment without added capsaicin and the injection arm will be injected with saline. A trained medical professional on the research team will apply the topical capsaicin or topical placebo treatments. The region of topical application will measure 5cm squared in the dermatome zone location to cover an area of approximately 25cm2. A trained physician in physical medicine and rehabilitation will deliver injectable capsaicin using a 27-gauge needle at the location of the superior fascia of the upper trapezius muscle with ultrasound guidance. The fascial layer was chosen as it is the region with the lowest pressure pain threshold relative to skin and muscle, suggesting a high density of nociceptors within the layer, and it possesses connections to the spinal cord.[27] Changes in muscle fascia properties and nociception at muscle fascia, compared to nociception at muscle or subcutaneous tissue, has also been implicated in the development of pain.[28,29] Furthermore, intramuscular capsaicin will also be injected using ultrasound guidance to avoid the superior or inferior fascia.

To confirm the presence of central sensitization, brush allodynia will be used to detect mechanical hyperalgesia outside the region of primary nociception—region of topical placement or injection—which is the region of secondary hyperalgesia.[13-15] The size of the region of secondary hyperalgesia will be measured to characterize the extent of central sensitization. This will be accomplished using a tape measure and the perpendicular dimensions of the region of secondary hyperalgesia will be recorded in square centimeters.

Ultrasound analysis

The Sonosite Ultrasound machine located at the Toronto Rehabilitation Institute clinic will be used. The ultrasound probe will be placed onto the trapezius before and after the sensitization is induced and an ultrasonic image will be captured. Texture feature analysis will be performed using MATLAB and the Signal Processing Toolbox. Analyses will include first order parameters such as mean and

standard deviation of the pixel level values, as well as second order parameters, which provide details on the spatial distribution of pixel values. These include co-occurance and run-length matrices, local binary pattern and blob analysis[Quant text ref].

Measurement of motor unit activity using EMG

The Delsys Trigno surface EMG system will be used in this experiment. The system offers the benefit of having a 4-pin mini-grid that can extract individual motor unit data, without causing participants discomfort. The analysis will also be complemented by the use of intramuscular EMG, since it offers high location specificity when measuring EMG activity, while only a local region is analzed. The intramuscular EMG data will be analyzed by a Cadwell Sierra Wave device.

The anatomical location for electrode placement, along the C7-acromion line, will be identified for each participant. Baseline EMG measurements, as well as the ultrasonic images will be taken from the upper trapezius muscle to obtain the baseline motor unit recruitment curve. The trapezius muscle was chosen for study since it is a common site for MTrPs, given the high load cervical muscles carry as well as the general increase sedentariness that perpetuates poor posture and cervical muscle strain, leading to trigger point presentation. Furthermore, the trapezius muscle has been used frequently in previously published work, which analyze motor unit parameters, power spectrum and amplitude measurements.[31,32,33]

sEMG Analysis

After the identification for electrode placement and the captured baseline ultrasonic images, the area will be cleaned with alcohol and water. The Delsys Galelio sensor will be placed directly on the identified area. The EMG sensor (bandwidth of 20-450Hz) consists of 4 metal contacts for detecting the signal at the skin surface. Both the right and left sides of the trapezius will be measured.

The Delsys Trigno system, which includes sensors, the Trigno base station, and the EMGworks software will be used to decompose the acquired EMG signals into individual motor units. The following parameters will be analyzed: the firing times and frequency of individual motor units, motor unit action potential amplitude and shapes[36-38], the root mean square value (RMS) of each channel and of the entire signal[16], the coefficient of variation for force steadiness[39], and the centroid of

the EMG signal in the cranial-caudal and the medial-lateral directions[16]. This will be repeated for each contraction.

Intramuscular EMG analysis

A 27g monopolar electromyography needle will be placed into the midbelly of the upper fibers of the trapezius muscle using ultrasound guidance. The intramuscular EMG signals will be measured using Cadwell Sierra Wave. Since this system does not offer motor unit decomposition, we will use the intramuscular EMG analysis to provide us with more details of the gross EMG measurements such as the overall RMS value, the coefficient of variation for force steadiness, and the centroid measurements.

Determination of Central Sensitization:

Before measuring outcomes, approximately 20 minutes after capsaicin application we will analyze the area of secondary hyperalgesia for each study subject, then determine whether central sensitization has in fact been induced. The presence of central sensitization is confirmed by expansion of the receptive field and area of secondary hyperalgesia beyond the 25 cm2 initial application area. If it has, the study participant will undergo the rest of the experimental methodology. If it has not, the capsaicin will be applied once more and the region of secondary hyperalgesia will be remeasured in 20 minutes later. The above described process for confirming the presence of central sensitization will take place again. If the study participant has not had sensitization at this stage they will be withdrawn from the study.

Primary Outcomes

The primary outcome measures:

1. Textural regional changes

2. Recruitment and Amplitude regional changes

These will be recorded for each arm of the study, please see figure 1.

Secondary outcomes:

1. A record of adverse events

2. Safety evaluation The investigating physician monitors the patient clinically for signs of distress.

The methods and timing for assessing, recording, and analyzing safety parameters. Any signs of distress noted by investigating physician will be assessed clinically and appropriately managed. The information will be recorded on the patient's data form. The study team will discuss all incidents and any potential causal link to the study interventions. Patients will be provided with a contact number for the principal 18

investigator in order to report any changes that occur during or after the study. Our publication will include a list of any adverse events or intercurrent illness encountered. Any patient who experiences an adverse event will be triaged to their family physician or local emergency department as necessary. They will be contacted after to obtain information on the course of their treatment and the outcome of the event.

3. Sample size calculation.

Using GPower V3.0.10 (Dusseldorf, Germany), considering a medium effect size (Hedge's G) of 0.5 and a moderate correlation coefficients (0.5) among repeated measures, sample size calculation determined that a minimum of 70 participants would be required to detect differences via repeated measures ANOVA (two measurement as before and after among 7 groups) with 80% power and an alpha of 0.05. We aim to recruit 84 participants to account for potential attrition (~20%) of participants given the study duration and frequency of follow-up visits.

We plan to recruit equal number of men and women. To date, there are no reported sex differences in induction of Central Sensitization using Capsaicin among males and females with neurological impairments.

4. Statistical analysis plan

Baseline participants' characteristics including demographics will be analyzed using appropriate descriptive statistics. Mean and standard deviation will be calculated for continuous variables. Categorical variables will be presented as numbers and percentages. To test the change in ultrasound images from baseline to experimental conditions, the texture features will be computed in MATLAB and principal component analysis will be computed to reduce the variables. A repeated measures MANOVA will be applied for each intervention group. To test the change in EMG within and between

groups a repeated measurement ANOVA will be applied considering each intervention group as factor. The dependent variable would be considered as pain intensity, duration, and area of pain in separate analysis. Data distributions would be first checked with the Shapiro-Wilk to conform the normal distribution of the data. Additionally, we will test for the homogeneity of variance using Mauchly's test as well as correcting the degrees of freedom if necessary [40]. If the assumption of normality not met, for all skewed data an appropriate transformation will be used. We will construct univariate and multivariable linear regression models of differences in EMG amplitude (differences between after and before) as a function of intervention groups when adjusted for important covariates. Due to small sample size in each group, there is not possible to adjust for several covariates. Therefore, in a univariate model we will find the most important ones. For initial selection of relevant variables, all variables with a p-value≤0.2 in the univariate analysis will be selected and retained in the multivariable analysis.

All Statistical analyses will be conducted with SAS for Windows (version 9.3; SAS Institute, Inc, Cary, NC). Using a two-sided test a P-value ≤ 0.05 will be considered as statistically significant.

Discussion

This will be the first study that assesses the effect of capsaicin induced central sensitization on ultrasound texture features and muscle motor unit activity. Findings from this study may support one of few hypotheses proposed delineating the involvement of central sensitization in the development of trigger points. A prominent hypothesis in the literature is the integrated hypothesis. The integrated hypothesis suggests that unaccustomed eccentric activity or submaximal to maximal concentric muscle exertion leads to muscle fiber damage, segmental hypercontraction within the muscle fibers, and ischemia.[6] The resultant damage and ischemia at the muscle instigates an inflammatory biomarker cascade that potentiates the activity of motor neurons, subsequently increasing the release of acetylcholine and inducing muscle contraction, and sensitizes sensory neurons.[6,41] The sensitization of peripheral sensory and motor neurons is thought to contribute to the sensitization of dorsal horn neurons in the associated spinal segment, which likely influences efferent neuronal activity at the muscle. From this hypothesis, it would be expected to observe uncoordinated motor

unit activity and activation given that muscle fiber damage has occurred and motor units may be differentially potentiated or sensitized. The findings from studies inducing acute nociception in the muscle support this hypothesis.[16,42] Hypertonic saline injections have been shown to reorganize muscle activity within the muscle.[42] Previous findings also suggest that motor unit discharge frequency changes variably among motor units, although predominantly decreasing, as a consequence of external nociception.[16] This study should elucidate whether a nociceptive stimulus that causes central sensitization induces uncoordinated motor unit activation and differential discharge patterns among the units. The integrated hypothesis presupposes that the cause of myofascial trigger points is exogenous and induces endogenous changes in muscle. The Cinderella and the neurogenic hypothesis suggest otherwise.

The Cinderella hypothesis suggests sustained low threshold motor unit activation metabolically overloads oxidative muscle fibers, leading to muscle fiber damage or 'ragged fiber' presentation and metabolic changes such as ischemia, hypoxia, and insufficient ATP.[7,41] This may result in disturbed calcium homeostasis and the development of the muscle contracture known as the trigger point. Low threshold motor unit activity was demonstrated in a number of articles assessing the effect of continued muscle contraction and psychologically demanding tasks—muscle overexertion and persistent psychological stress are theorized triggers for chronic pain and trigger point development. [43,44] The sustained contracture in the muscle maintains the hypoxic and acidic environment, leading to peripheral and central sensitization of neurons in the associated spinal segment.[7] This suggests an endogenous and exogenous cause for the trigger points. Under the premise of the Cinderella hypothesis, it would be expected to observe sustained low threshold motor unit activity with central sensitization.

The neurogenic hypothesis is distinct from the integrated and Cinderella hypotheses as it poses trigger point development is a result of endogenous central sensitization.[1] Central sensitization would result from persistent nociceptive input from other peripheral mechanic or systemic pathologies leading to neurogenic inflammation, such as trauma or endocrine disease. Subsequently, inflammatory and algogenic substances would be released from peripheral nociceptors onto tissue

and sensitizing the region. Sensitization of motor and sensory neurons innervating somatic muscle may lead to the phenomenon of trigger points. Ultimately, the neurogenic hypothesis stipulates the trigger point is a secondary outcome central sensitization, which is the primary pathology. Continuous low threshold motor unit activity would also be an anticipated observation as neurons will be increasingly sensitized. It would also be expected to observe increased low threshold motor unit activity in remote muscles that present with signs of central sensitization, e.g. hyperalgesia, however this is outside the scope of this article.

Limitations And Conclusions

Overall, the findings from this study should present preliminary evidence to inform central sensitization's effects on motor unit activity. Results may provide plausibility for the aforementioned hypotheses. A limitation of this study is the degree of invasiveness needed to increase the specificity of the EMG measurements, however, the trade-off between invasiveness and specificity is necessary to ensure we capture a sufficient effect size with a feasible sample size. Additionally, the experimental protocol will be carried out on healthy participants with induced central sensitization to determine the effects of this phenomenon on muscle activity. Although these results are not directly generalizable to participants with myofascial pain, this study's results should provide insight as to whether central sensitization does perturb muscle activity and structure, and inform further investigations into the pathophysiology of myofascial pain. Another limitation of this study in light of the integrated and Cinderella hypotheses is that sensitization will be induced prior to the assessment of EMG activity at motor neurons. However, it is important to understand whether central sensitization is a direct cause of aberrant motor unit activity, as a fraction of the population develops myofascial trigger points following precipitating events such as injury, stress, or poor posture. The presence of central sensitization at a specific severity or 'tipping point' may predispose some individuals to developing trigger points—or induce trigger points with increased severity—and the mechanisms described by the integrated or Cinderella hypotheses may lead to the manifestation of the trigger point. These hypotheses need not be mutually exclusive, but rather may be complementary or present as alternate etiologies to the development of the trigger point. It is possible the anatomical

changes resulting from each hypothesis leads to a different manifestation of trigger points that appear clinically equal. Further studies assessing central sensitization markers and EMG activity of muscle innervated by spinal segments remote from the region of primary sensitization are needed to elucidate the overarching pathophysiology of trigger point presentation.

Declarations

Acknowledgements: N/A

Ethics approval and consent to participate:

The study conforms to the Consolidated Standards of Reporting Trials recommendations. Informed consent will be obtained from all subjects participating in this study. Ethical approval was approved by the University Health Network (UHN) Research Ethics Board(Approval Number: 18-6163). The approval form is included in the supplementary files of this submission. This study is registered under the National Institutes of Health ClinicalTrials.gov. Our study is approved. It has been approved by the University Health Network Research Ethics Board. The submission ID is CAPCR-ID: 18-6163

Consent for publication:

Not applicable.

Availability of data and material:

The datasets used and/or analyzed during the study will be available from the corresponding author upon reasonable request.

Data Monitoring Committee

A DMC is not applicable for this study since it is not a multi-location study.

Trial Status:

Protocol version and date: Version 2, May 1,2019

Recruitment: Starting June 2019- November 2019

The Delsys Trigno EMG machine has been ordered. The pharmacy has approved the intended capsaicin formula that will be used in the experiment. Participants will be recruited from June 2019-November 2019

Competing interests:

The authors declare that they have no competing interests.

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Authours' Contribution:

All authors (VE, KM, DK) contributed to the study design; drafted, reviewed and finalized the study

protocol; critically revised the manuscript and approved the final manuscript.

References

1. Srbely JZ. New trends in the treatment and management of myofascial pain syndrome. Curr Pain

Headache Rep 2010;14(5):346-52.

2. Masuda T, Sadoyama T. Distribution of innervation zones in the human biceps brachii. J

Electromyogr Kinesiol 1991;1(2):107-15.

3. Barbero M, Cescon C, Tettamanti A et al. Myofascial trigger points and innervation zone locations in

upper trapezius muscles. BMC Musculoskeletal Disord 2013;14(1):179.

4. Melzack R, Stillwell DM, Fox EJ. Trigger points and acupuncture points for pain: correlations and

implications. Pain 1977 Feb 1;3(1):3-23..

5. Hong CZ, Simons DG. Pathophysiologic and electrophysiologic mechanisms of myofascial trigger

points. Arch Phys Med Rehabil 1998;79(7):863-72.

6. Gerwin RD, Dommerholt J, Shah JP. An expansion of Simons' integrated hypothesis of trigger point

formation. Current pain and headache reports. 2004 Dec 1;8(6):468-75.

7. Bron C, Dommerholt JD. Etiology of myofascial trigger points. Curr Pain Headache Rep

2012;16(5):439-44.

8. Yu SH, Kim HJ. Electrophysiological characteristics according to activity level of myofascial trigger

points. J Phys Ther Sci. 2015;27(9):2841-3.

9. Woolf CJ. Central sensitization: implications for the diagnosis and treatment of pain. Pain

2011;152(3):S2-15.

10. Graven-Nielsen T, Arendt-Nielsen L. Assessment of mechanisms in localized and widespread

musculoskeletal pain. Nat Rev Rheumatol 2010;6(10):599.

11. Fernández-de-las-Peñas C, Cuadrado ML, Arendt-Nielsen L et al. Myofascial trigger points and sensitization: an updated pain model for tension-type headache. Cephalalgia 2007;27(5):383-93.

12. Kim Y, Kim J, Shim JK et al. The hypoalgesic effect of remote tactile sensory modulation on the mechanical sensitivity of trigger points: A randomized controlled study. NeuroRehabilitation 2014;35(3):607-14.

13. LaMotte RH, Lundberg LE, Torebjörk HE. Pain, hyperalgesia and activity in nociceptive C units in humans after intradermal injection of capsaicin. J Physiol 1992;448(1):749-64.

14. Srbely JZ, Dickey JP, Bent LR, Lee D, Lowerison M. Capsaicin-induced central sensitization evokes segmental increases in trigger point sensitivity in humans. J Pain 2010;11(7):636-43.

15. Torebjörk HE, Lundberg LE, LaMotte RH. Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. J Physiol 1992;448(1):765-80.

16. Dideriksen JL, Holobar A, Falla D. Preferential distribution of nociceptive input to motoneurons with muscle units in the cranial portion of the upper trapezius muscle. J Neurophysiol 2016;116(2):611-8.

17. Falla D, Farina D. Motor units in cranial and caudal regions of the upper trapezius muscle have different discharge rates during brief static contractions. Acta physiologica 2008;192(4):551-8.

18. Lee U, Kim M, Lee K et al. Functional Brain Network Mechanism of Hypersensitivity in Chronic Pain. Scientific Rep 2018;8(1):243.

19. Schmidt-Wilcke T, Clauw DJ. Fibromyalgia: from pathophysiology to therapy. Nature Rev Rheumatol 2011;7(9):518.

20. Henneman E, Somjen G, Carpenter DO. Functional significance of cell size in spinal motoneurons. J Neurophysiol 1965;28(3):560-80.

21. Henneman E, Mendell LM. Functional organization of motoneuron pool and its inputs. Handbook of Physiology. The Nervous System. Motor Control. 1981;1:423-507.

22. Ertas M, Stålberg E, Falck B. Can the size principle be detected in conventional EMG recordings?. Muscle & Nerve 1995;18(4):435-9.

23. Vilensky JA, Gilman S. Renaming the" Henneman Size Principle". Science 1998;280(5372):2027-.

24. Szolcsanyi J. A pharmacological approach to elucidation of the role of different nerve fibres and receptor endings in mediation of pain. J Physiologie 1977;73(3):251-9.

25. Carpenter SE, Lynn B. Vascular and sensory responses of human skin to mild injury after topical treatment with capsaicin. Br J Pharmacol 1981;73(3):755-8.

26. Simone DA, Baumann TK, LaMotte RH. Dose-dependent pain and mechanical hyperalgesia in humans after intradermal injection of capsaicin. Pain 1989;38(1):99-107.

27. Ishimaru K, Kawakita K, Sakita M. Analgesic effects induced by TENS and electroacupuncture with different types of stimulating electrodes on deep tissues in human subjects. Pain 1995;63(2):181-7.

28. Langevin HM, Fox JR, Koptiuch C et al. Reduced thoracolumbar fascia shear strain in human chronic low back pain. BMC Musculoskel Dis 2011;12(1):203.

29. Schilder A, Hoheisel U, Magerl W et al. Sensory findings after stimulation of the thoracolumbar fascia with hypertonic saline suggest its contribution to low back pain. Pain 2014;155(2):222-31.

30. Barbero M, Gatti R, Conte LL et al. Reliability of surface EMG matrix in locating the innervation zone of upper trapezius muscle. J Electromyogr Kinesiol 2011;21(5):827-33.

31. Kumbhare D, Shaw S, Grosman-Rimon L, Noseworthy MD. J Ultrasound Med 2017; 36(12):2559-68.

32. Kumbhare DA, Ahmed S, Behr MG, Noseworthy MD. Crit Rev Biomed Eng 2018;46(1).

33. Farina D, Madeleine P, Graven-Nielsen T et al. Standardising surface electromyogram recordings for assessment of activity and fatigue in the human upper trapezius muscle. Eur J App Physio 2002;86(6):469-78.

34. Holobar A, Zazula D. Correlation-based decomposition of surface electromyograms at low contraction forces. Med & Bio Eng and Comput 2004;42(4):487-95.

35. Holobar A, Zazula D. Multichannel blind source separation using convolution kernel compensation. IEEE Transactions on Signal Processing 2007;55(9):4487-96.

36. Holobar A, Farina D, Gazzoni M et al. Estimating motor unit discharge patterns from high-density surface electromyogram. Clin Neurophysio 2009;120(3):551-62.

37. Holobar A, Minetto MA, Botter A et al. Experimental analysis of accuracy in the identification of motor unit spike trains from high-density surface EMG. IEEE Transactions on Neural Systems and

Rehabilitation Engineering 2010;18(3):221-9.

38. Holobar A, Glaser V, Gallego JA et al. Non-invasive characterization of motor unit behaviour in pathological tremor. J Neur Eng 2012;9(5):056011.

39. Bandholm, T., Rasmussen, L., Aagaard, P., Diederichsen, L., & Jensen, B. R. (2008). Effects of experimental muscle pain on shoulder-abduction force steadiness and muscle activity in healthy subjects. European Journal of Applied Physiology, 102(6), 643-650.

40. Huynh H, Feldt LS. Estimation of the Box correction for degrees of freedom from sample data in randomized block and split-plot designs. J Edu Stat 1976;1(1):69-82.

41. Shah JP, Gilliams EA. Uncovering the biochemical milieu of myofascial trigger points using in vivo microdialysis: an application of muscle pain concepts to myofascial pain syndrome. J Bodyw Mov Ther 2008;12(4):371-84..

42. Falla D, Farina D, Graven-Nielsen T. Experimental muscle pain results in reorganization of coordination among trapezius muscle subdivisions during repetitive shoulder flexion. Exp Brain Res 2007;178(3):385-93.

43. Mclean L, Urquhart N. The influence of psychological stressors on myoelectrical signal activity in the shoulder region during a data entry task. Work Stress 2002;16(2):138-53.

44. Westad C. Motor control of the upper trapezius. 2005

45. Sikdar S, Shah JP, Gebreab T, Yen RH, Gilliams E, Danoff J, Gerber LH. Novel applications of ultrasound technology to visualize and characterize myofascial trigger points and surrounding soft tissue. Arch Phys Med Rehabil. 2009;90:1829-38

46. Lewis J, Tehan P. A blinded pilot study investigating the use of diagnostic ultrasound for detecting active myofascial trigger points. Pain. 1999;79:39-44.

47. Shankar H, Reddy S. Two- and three-dimensional ultrasound imaging to facilitate detection and targeting of taut bands in myofascial pain syndrome. Pain Med. 2012;13:971-5.

48. Sikdar S, Shah JP, Gilliams E, Gebreab T, Gerber LH. Assessment of myofascial trigger points (MTrPs): a new application of ultrasound imaging and vibration sonoelastography. Conf Proc IEEE Eng Med Biol Soc. 2008;5585-8.

49. International Association for the Study of Pain. (2018). IASP terminology. Retrieved from

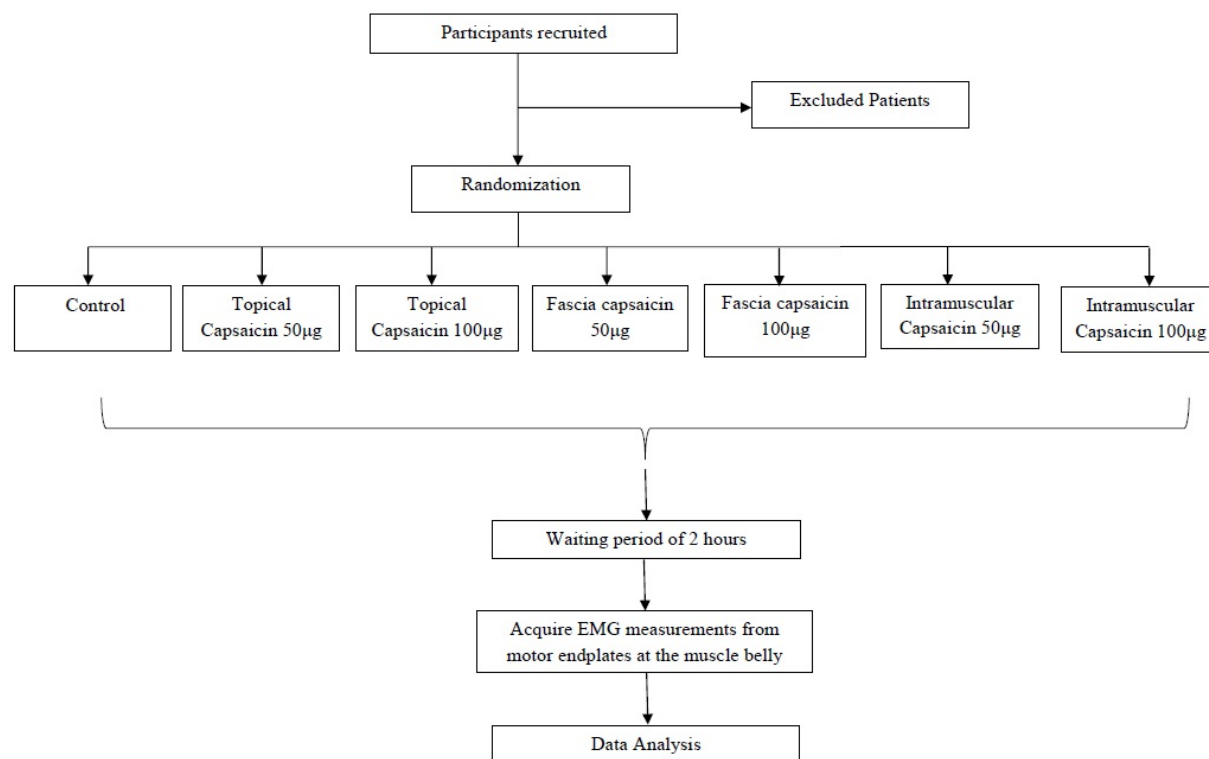
https://www.iasp-pain.org/Education/Content.aspx?ItemNumber=1698

Figures

Figure 1

Flow chart of methods

ACTIVITY	TIME															
	0:00-0:04	0:05-0:09	0:10-0:14	0:15-0:19	0:20-0:24	0:25-0:29	0:30-0:34	0:35-0:39	0:40-0:44	0:45-0:49	0:50-0:54	0:55-1:00	1:05-1:09	1:10-1:14	1:15-1:19	1:20-1:24
Participants consented and briefed	█															
Participants trained to perform 90^0 elbow abductions		█	█													
Surface EMG set-up				█												
Intramuscular EMG set-up					█											
Period until intramuscular needle application EMG spikes remit						█										
Topical or injectable capsaicin applied							█									
Wait period								█	█							
Primary and secondary regions of hyperalgesia confirmed using BA										█						
EMG recording period											█					
Participants perform three 90^0 elbow abductions												█	█			
EMG recording period														█	█	█

Figure 2

Proposed experimental protocol timeline