A Pilot Study of Temporomandibular Disorder (TMD) Pain and Nocturnal Autonomic Nervous System (ANS) Associated Masticatory Muscle Activity

Brandon Zegarowski, DDS

A thesis submitted in partial fulfillment for the
Degree of Master of Science in Orthodontics

Oregon Health & Science University
Portland, OR
December 2023

Committee members:
J. Nickel
Y. Wu
L. Iwasaki

Table of Contents

List of Figures

List of Tables

1. Abstract:

Title: A Pilot Study of Temporomandibular Disorders (TMD) Pain and Nocturnal Autonomic Nervous System (ANS) Associated Masticatory Muscle Activity

Objective: Pain associated with TMD causes detrimental effects on daily function and quality of life of affected individuals. This pilot study tested for differences in autonomic nervous system and masticatory muscle activities in subjects with and without TMD-associated pain (±Pain).

Materials and Methods: Subjects were 18 years or older. Subjects' levels of characteristic pain intensity (CPI) were assessed using validated instruments of the Diagnostic Criteria of Temporomandibular Disorders (DC/TMD). Subjects participated in two laboratory calibration sessions to quantify masseter and temporalis muscle activities per N of bite-force. Subjects were trained to use a portable electromyography (EMG) and electrocardiography (ECG) recorder, recording data for 3 nights, with each recording being greater than 6 hours. Characterization of nocturnal activity of the autonomic nervous system was accomplished using commercial software to quantify heart rate variability (HRV). Data of night-time ultradian cycling of sympathetic/parasympathetic tone for each night recording was fitted with a higher order polynomial. Peaks and valleys of ANS tone were identified based on the polynomial, and time (pNN50, parasympathetic activity) and frequency (sympathetic/parasympathetic, Low Frequency/High Frequency) domain measures of HRV were determined at inflection points. For these inflection points, muscle activities were quantified for activities ranging from >1 to <5 N of load on the mandible and represented as a % of total recording time. Analysis of variance (ANOVA) and post-hoc tests were used to determine if there were differences ($p < 0.05$) in muscle duty factors between ±Pain groups. Three-dimensional (3D) regression analysis tested for

correlations between independent variables of ANS tone and muscle duty factor, and the dependent variable of CPI.

Results: Twenty-seven subjects completed the study protocols. Subjects with pain (+P) had significantly higher average muscle duty factors ($p < 0.02$) compared to subjects without pain (-P) subjects. There was a positive correlation ($R^2 = 0.75$) between muscle duty factors, sympathetic nervous system activity, and CPI scores. The combination of independent variables of the ratio of sympathetic/parasympathetic activity (LF/HF), and parasympathetic tone (pNN50), showed a significant correlation ($R^2 = 0.67$) with pain scores.

Conclusions:

1. There were significant differences in muscle duty factors at low-levels of jaw-loading between subjects with and without chronic pain.
2. There was a positive correlation between 6-month chronic pain intensity (CPI) scores versus masticatory muscle duty factors at low levels of jaw-loading and peak-to-valley ratios of sympathetic/parasympathetic activities (LF/HF)
3. There was a correlation between higher CPI scores versus lower parasympathetic activity (pNN50) and higher sympathovagal (LF/HF) activity.

2. Introduction:

Temporomandibular disorders (TMD) affect 5-12% of the human population with a higher prevalence in females than males and younger rather than older individuals.[1] Pain stemming from TMD can cause negative impacts on daily function of individuals and a lower quality of life. TMD are best diagnosed using physical measures of pain, dysfunction, and temporomandibular joint (TMJ) tissue changes identified via imaging, which are also used in conjunction with psychosocial assessment.[2] The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD)[3] have been the most commonly utilized classification system based off an Axis I physical assessment and Axis II psychosocial status and pain-related disability assessment. An update to increase target sensitivity and specificity of Axis I was introduced as Diagnostic Criteria (DC)/TMD in 2014 through creation of a new protocol[4] that could be used in any clinical setting, including a screener for pain-related TMD and diagnostic criteria for differentiating the most common pain-related TMD and intra-articular disorders.[4] The protocol also standardized language used for clinicians and started to combine both mechanism and etiological basis for DC/TMD. The Orofacial Pain: Prospective Evaluation and Risk Assessment study[2] furthered the development of TMD diagnosis by classifying individuals with pain into subtypes with a focus on etiology on top of anatomic pain location.[2] Research regarding TMD is important for diagnosis and to establish quantifiable subject differences in TMD prevalence. Current evidence does not support orthodontics as a treatment modality for TMD therefore it is important to continue developing diagnostic methods that can lead to individualized evidence-based therapies.

TMJ Regulation and Jaw Behavior:

The temporomandibular joint includes the bony components of the mandibular condyle and the glenoid fossa, and the articular disc which is composed of fibrocartilage.[5] The motor innervation of masticatory muscles and the sensory innervation of the TMJ is derived from the mandibular branch of the trigeminal nerve. Sensory innervation of accessory muscles includes both trigeminal and cervical nerves. Neural control of motor components of the TMJ involves the recruitment of multiple motor units that has been postulated to lead to high force exertion by the masticatory muscles, such as the masseter and temporalis, and subsequently high loading of the TMJ.[5] Voluntary movement of the jaw during opening and closing is controlled by the motor cortex while cyclic movement during mastication involves an area of the brainstem called the central pattern generator. The central pattern generator receives input from teeth, muscle spindles, and the TMJ capsule and facilitates adaptive responses to changes in dental occlusion over time and immediate changes in food textures to help mitigate excessive forces or load vectors from causing physiologic harm. Reflex movement is controlled via muscle spindles and brainstem interneurons which help contribute to control of muscle contraction, velocity, and muscle length.[5] In the presence of pain, these internal controls are disrupted and can lead to functional dysregulation of both simple and complex jaw movements – as found in some TMD.

Sympathetic and parasympathetic responses in relation to fear or psychological stress may promote dysregulated jaw movements which can further amplify TMD via abnormal loading of the TMJ complex and subsequent neural inflammation.[5] Rodent models have shown abnormal loading of the TMJ can result in inflammatory changes by upregulation of biochemical drivers associated with degradation like vascular endothelial growth factor and hypoxia inducible factor 1α, as well as matrix metalloproteinases which mediate nociceptor activity in the TMJ

tissues.[6] In addition, self-reported presence of depression and physical symptoms are associated with waking-state oral parafunctional behaviors.[7] As a result, TMD have a complex multifactorial etiology that involves both motor and sensory components with consequent hyperalgesia affecting the masticatory muscles of TMD subjects. The Generalized Hypervigilance Hypothesis emerged in 1996 which was based on concepts of occlusal interferences leading to hypersensitization and amplification of nociceptive sensory information (Appendix 1).[8] Of the many past approaches towards TMD treatment, occlusal changes via occlusal adjustment, equilibration, or orthodontic treatment were previously employed as ways to reduce occlusal interferences, and thus, combat TMD. However, to date there is insufficient evidence to support the use of occlusal treatments for the treatment of TMD.[9] Hence, randomized clinical trials which are directed toward identifying treatments that would improve in quality of life, reduce pain, restore function, and enhance the physical, social, and psychological well-being of individuals are needed.[5] To date, such clinical trials have resulted in recommendations towards multidisciplinary care of TMD as it generally has a multifactorial etiology that is best treated with a collaboration of providers in medicine, dentistry, and physical therapy.

Mechanobehavior of the TMJ complex:

Disc displacement causing TMD is an intracapsular biomechanical disorder involving the condyle-disc-fossa/eminence complex, with a malposition of the disc in relation to the condyle causing a lock or click during jaw opening or closing or both.[10] Degenerative joint disease causing TMD is characterized by the deterioration of articular tissue with osseous changes in the condyle and/or articular eminence. The articular disc is especially prone to degenerative change due to its avascularity and high oxygen and glucose gradients due to high rate of nutrient consumption and cell density.[10] When the joint is strained mechanically, hypoxia and limited

glucose concentrations affect the synthesis of extracellular matrices which subsequently limits regenerative properties of the disc. This mechanical strain can be measured by the concentration of work input to articulating tissues known as the TMJ energy density.[11] A study of 68 adult subjects in three groups, those with TMJ disc displacement and pain (+DD+P), those with disc displacement without pain (+DD-P), and controls without disc displacement or pain (-DD-P), showed that +DD+P subjects had significantly higher TMJ energy densities compared to both other groups during symmetrical jaw closing.[11] Additionally, in a study of healthy subjects (17 females, 17 males with -DD-P), energy densities during asymmetric (laterotrusive) jaw closure were 3.6-times and significantly larger than during symmetric jaw closure and during asymmetric jaw closure the contralateral TMJ energy densities were 2-times and significantly larger in healthy females compared to healthy males.[12] Thus, with respect higher energy densities increasing the liability for fatigue failure of TMJ cartilage tissues, the type of jaw-use behavior may be a factor as well as sex, because females have significantly smaller TMJ disc cartilage volume compared to males.[12]

Mechanobehavioral scores (MBS) combine jaw biomechanics and behavior by calculating the product of TMJ energy density and the Duty Factor (DF, %) of the jaw muscles.[11] Muscle DF represent jaw muscle activity as a percentage of time over a total recording time. Duty Factors are calculated as a percentage of electromyographic (EMG) recordings of masseter and temporalis muscle activities in the home environment over total recording time. Previous studies have shown Interclass Correlation Coefficients of 0.74 for masseter and 0.42 for temporalis in reliability tests of EMG activity recording.[13] Differences in masticatory muscle usage were reported between subjects with and without TMJ disc displacement for forces on the mandible of $\leq$ 20N.[14] The study used DC/TMD protocols in conjunction with imaging data and

EMG output versus bite-force calibrations of masseter and temporalis muscle activities of the subjects.[14] The results demonstrated that for subjects with disc displacement and pain, at loading forces of ≤20N, temporalis muscle DFs were significantly higher than masseter DFs in these subjects as well as in subjects without disc displacement and pain. A commonly believed hypothesis that myofascial TMD can be explained by sleep bruxism was investigated in a laboratory-based polysomnographic investigation performed by Raphael et al. comparing subjects with myofascial TMD with controls. It was found that most participants, both those with myofascial TMD and controls, did not exhibit sleep bruxism and thereby rejecting this hypothesis.[15]

Females with disc displacement and no pain also used their temporalis and masseter muscles at lower levels for a greater percentage of time compared to females with disc displacement and pain and both males and females without disc displacement and pain.[12] These findings indicate that earlier mechanical fatigue of the TMJ articulating surfaces and cartilage failure could be due to the variables of frequency of loading and the magnitude of mechanical stress on the joint.[14] Iwasaki et al. further investigated the difference in muscle forces used during static biting in subjects with or without pain and disc displacement and found that mean temporalis duty factors at 20N were higher than masseter duty factors in +DD+P subjects, while masseter DF at 5-9% of the 20N biting tasks was significantly higher in +DD-P women than +DD-P men and women, and men in both other diagnostic groups. Night-time DFs at 5-9% in +DD-P women were also significantly higher than in -DD-P men and women. Thus, these authors concluded that part of the reason for higher muscle forces and EMG amplitudes for the same biting task between groups could be attributable to physical differences in masseter and temporalis muscle orientations.[16]

Heart Rate Variability and Assessment of Autonomic Nervous System Tone:

Temporalis and masseter muscle activities in adult subjects have been shown to be modulated by the ANS via sympathetic and parasympathetic nervous system activity.[17] Noradrenaline is a vital excitatory neuromodulator released from sympathetic nerve fibers that assists with arousal-related stimulation of motor neuron excitability. Studies have shown that during rapid eye movement (REM) sleep excitatory effects of noradrenaline on motor output are lost without the presence of endogenous glutamatergic drive.[17] The presence of an endogenous glutamatergic drive is necessary for noradrenaline to trigger muscle activity at the level of the motor neurons, giving an insight to part of the etiology of certain sleep motor disorders.

Heart rate variability reflects the regulation of autonomic balance, blood pressure, vascular tone, and potentially facial muscles, with short term variability generated by the parasympathetic and sympathetic activities of the ANS.[18] Analysis of heart rate variability is based on the heart's sinus rhythm and recorded via electrocardiography (ECG) data where the electrical signals of the heart are traced versus time.[19] The two critical frequency domain parameters are low frequency (LF) power from 0.04-0.15 Hz, and high frequency (HF) power from 0.15-0.4 Hz which reflect the modulation of sympathetic and parasympathetic tone, respectively.[19,20] A ratio of low frequency to high frequency indicates the balance between sympathetic and parasympathetic tone and the power ratios during sleep mark the recurrent periods known as ultradian cycling.[21] To contrast the ratio of LF/HF, also known as sympathovagal tone, the measure of pNN50 is used as a time measure of parasympathetic activity. This measure determined the percentage (%) of parasympathetic power intervals that exceeded 50 milliseconds (ms).

Recently reported data have demonstrated a correlation with masticatory muscle activity and ANS tone during night-time ultradian cycling of sympathetic and parasympathetic spectral powers.[22] A 2019 study on night-time autonomic nervous system ultradian cycling and masticatory muscle activity showed that normalized jaw muscle DFs were linearly related positively with sympathetic spectral power and negatively with parasympathetic spectral power.[22] Night-time parasympathetic spectral power differences between ultradian cycles was also significantly different between subjects at either low or high risk for chronic systemic inflammation. Throughout the duration of sleep, low risk subjects had increased, and high-risk subjects had decreased parasympathetic tone respectively. This suggests increased susceptibility to systemic inflammation in higher risk groups.

3. Purpose:

The proposed research is important as it tests for differences between activities in the temporalis and masseter muscles and ANS tone, via HRV, during the night in subjects with and without TMD-associated pain. This project will test the following working hypothesis of whether or not:

1. There were significant differences in muscle duty factors between subjects with and without chronic pain.
2. There was a correlation between the independent variables of:
    i. Sympathetic/parasympathetic activity ratio (low frequency/high frequency – LF/HF)
    ii. Masticatory muscle duty factors

    and the dependent variable of:

iii. 6-month chronic pain intensity (CPI) scores

3. There was a correlation between the independent variables of:

   i. Sympathetic/parasympathetic activity ratio (low frequency/high frequency – LF/HF)

   ii. Parasympathetic activity (pNN50)

   and the dependent variable of:

   iii. 6-month chronic pain intensity (CPI) scores

4. Materials and Methods:

Subjects:

Male and female subjects that were 18 years old or older were recruited. Subjects were enrolled in the study in accordance with University at Buffalo School of Dental Medicine (UBSDM) and Oregon Health and Science University (OHSU) Institutional Review Board oversight (Appendix 2). Subjects were recruited at UBSDM (Timepoint 2, T2) as part of a longitudinal study using the inclusion criteria of individuals who had participated in a parent study (Timepoint 1, T1) with previous TMJ images ≥4 years old and exclusion criteria of individuals who were unable to complete the experimental protocols, pregnancy, musculoskeletal or rheumatological disease, TMJ degenerative disease based on cone-beam computed tomography imaging, multiple missing teeth, large dental restorations, or fixed orthodontic appliances. A summary of the protocols and subjects' activities is found in Table 1.

Table 1. Summary of Subjects' Study Activities in Buffalo, NY

Activity [Estimated Time]	Purpose	Application to project

Clinical Visit [2.5 hours]	- Collect: • Informed consent • Maxillary & mandibular dental impressions (1 of each arch) - Conduct DC/TMD exam & complete questionnaires	- Collected material will permit • Protection of subject's rights • Custom devices (oral clutch with reference system, target frames) to be constructed • Application of DC/TMD to determine classification, change/no change over time
Imaging Visit #1 [1 hour]	Attain bilateral cone beam computed tomography (CBCT) with reference system	- Application of imaging aspects of DC/TMD for group classification - Characterize 3D craniomandibular anatomy for dynamic stereometry
Imaging Visit #2 [1.5 hour]	Attain magnetic resonance (MR) images of TMJs	
Lab Visit #1 [2 hours]	- Record jaw-tracking with reference system & during jaw movements - EMG calibration tasks - Train subject for in-field recordings	- Link jaw-tracking with images via reference system (dynamic stereometry) to provide variables to calculate energy density - For in-field data and analyses, subject will • Perform calibration biting tasks during laboratory EMG recording • Learn how to use equipment & supplies
In-field recordings	- Record in-field masseter & temporalis EMG, ECG, and body position for 3 day & 3 nights - Collect 8 day-time saliva samples and 3 day-time pain intensity scores for 2 days	- From 3 awake- & 3 night-time sessions: Quantify behavior in terms of duty factor of jaw loading via the masseter & temporalis muscles; ANS tone - From 2 day-time sessions: Quantify salivary cortisol, pain scores
Lab Visit #2 [1 hour]	- Return equipment & data	- Assess completed in-field recordings

Subjects' levels of characteristic pain intensity (CPI) were assessed using validated instruments (DC/TMD). As in previous studies, a calibrated examiner determined pain (+P) or no pain (-P) grouping based on presence of absence of myalgia criteria.[23] The CPI had 3 questions about pain intensity: current, worst, and average in the past 6 months with ratings of 0 = no pain to 10 = worst possible pain.[23] The total CPI scores were the mean of 3 ratings x 10 ranged from 0 to 100 with 0 defined as no pain (-P) and >0 defined as pain (+P).[24]

Data Acquisition - Protocol for Ambulatory recording of Electromyography (EMG) and Electrocardiography (ECG):

To assess ANS tone and DFs, the subjects used portable equipment to record heart-beat and masseter and temporalis muscle activities at home for 3 days and 3 nights (Figure 2).

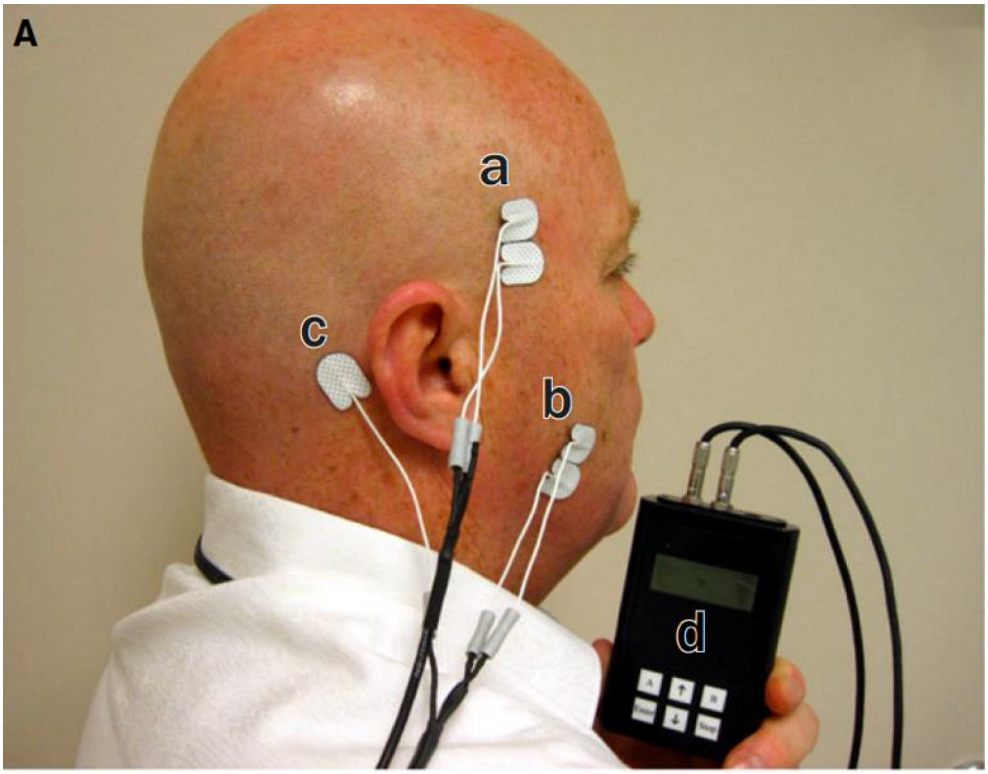


Figure 1: Example of portable EMG recorder showing electrodes set-up for self-recording, with attachments to the (a) temporalis, (b) masseter, and (c) mastoid process.

In this study, subjects were trained to use portable recorders that permit bilateral in-field EMG recording and simultaneous ECG recording during Lab Visit #1. Training of subjects to perform self-recordings of EMG and ECG in their natural environments with the portable equipment was completed by study personnel (Drs. Jeff Nickel and Laura Iwasaki). At Lab Visit #2 the 6 recordings were obtained and checked for completeness and recorders were returned (Figure 3).

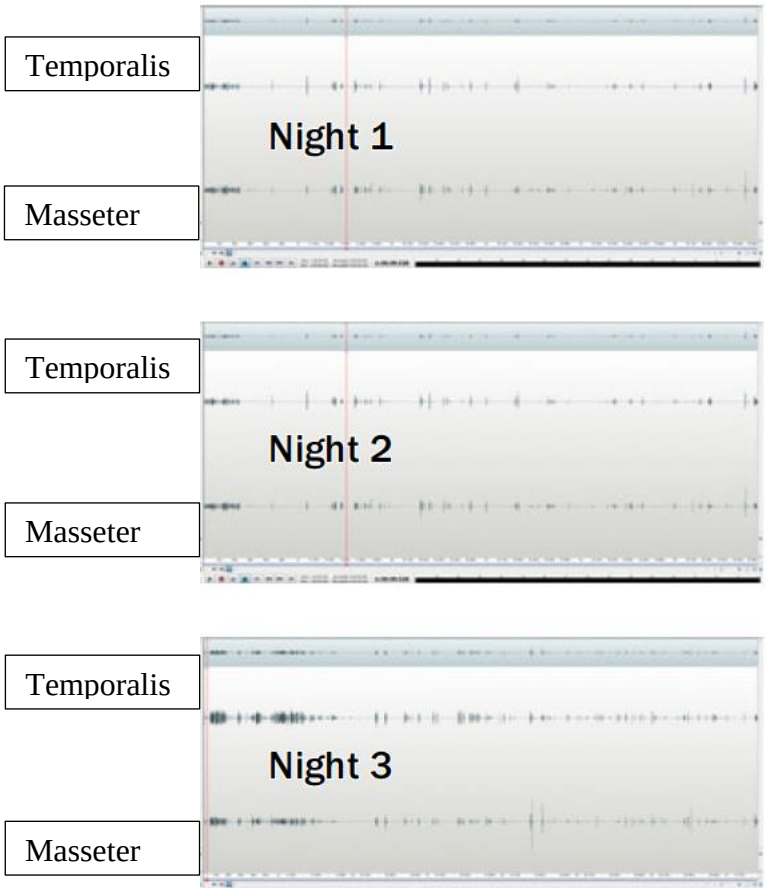


Figure 2: Example of 3 night EMG recordings from one subject

Data Acquisition - Protocol for Laboratory Estimation of mV thresholds per 20 N bite-force:

Subjects participated in two laboratory visits to perform calibration exercises that comprised static and dynamic unilateral biting on the right and left first molars/bicuspids while muscle activities and bite forces were recorded via EMG equipment and a calibrated force transducer device, respectively. Subjects were asked to produce light to medium biting forces, as defined by the subject, without the aid of visual feedback. A series of 5 static bites and then a series of approximately 5 dynamic bites were performed at 4 frequencies (0.5, 1.0, 1.5, 2.0 Hz), aided by a digital metronome, on one side and then repeated on the other side at each visit. The resulting EMG and bite-force data were plotted for each subject visit and linear regression

analyses defined the slope of right and left masseter muscle activities per bite-force (mV/N). The slopes from two visits per subject were averaged.

EMG Data Processing:

Raw EMG signals were processed through commercial software (Dark Audacity, Catalase Systems ltd, Ireland; WavePad, NCH Software, Canberra, Australia) to reduce noise and formatted for utilization in custom built programs. As described in previous published work, the ambulatory masseter and temporalis EMG recordings were processed using customized software (MatLab®, MATHWORKS, Natick, MA), to detect, delimit, and calculate root-mean-square values for EMG segments (EMGRMS, mV) defined by 128 ms contiguous rectangular sliding Hamming windows.[14,16] Average slope, mV/N, (Appendix Table 2) for each subject's masseter and temporalis muscle from laboratory calibration exercises were used to quantify magnitudes of muscle activities in the sleep-state EMG recordings made in each subject's natural environments.[16] Lower first molars 36 and 46, as identified by the FDI numbering system, were evaluated for both right and left masseter activities, with the mV data averaged for muscle output associated with a 20 N bite-force. By this method, it was possible to calculate masseter and temporalis muscle duty factors for mandibular load and duration thresholds over epochs defined by ANS ultradian cycling, where Duty Factor = Cumulative Time of Muscle Activity/Total Recording Length .

ANS Data Processing:

EMG recordings were catalogued as Waveform Audio Files (WAV) identified by the title "Channel 6" and analyzed with Heart Rate Variability Analysis software (MindWare

Technologies, Ohio, USA). Channels 1, 2, 3, and 4 contained masticatory muscle data solely from the right temporalis, right masseter, left temporalis, and left masseter respectively. Channel 6 EMG data were analyzed in segments written at 300-second intervals. Data from all the written segments of EMG data were exported to a spreadsheet (Excel, Microsoft, Redmond, WA), where analysis was conducted using a custom program (MatLab®, MATHWORKS, Natick, MA) in which researchers input the HRV analysis data, coefficients for right and left temporalis and masseters for each subject and combined Channels 1-2 and 3-4 WAV files. Low (sympathetic drive, 0.04-0.15 Hz) and high (parasympathetic drive, 0.15-0.4 Hz) frequency spectral powers were calculated to produce a sympathetic/parasympathetic spectral power ratio as a measure of ANS tone, and pNN50 as a time domain measure of parasympathetic activity (% of intervals that exceeded 50ms), for subjects' night time recordings.

Custom programs (MatLab®, MATHWORKS, Natick, MA) were developed which used ECG frequency domain data to plot a 10^{th} order polynomial (Figure 4). To assess ultradian amplitude effects of ANS activity, investigator-driven selection of 2 peaks and 2 valleys were used per night recording to define ±10-minute epochs about each peak and valley. Then, for each 20-minute epoch, parasympathetic activity (pNN50, %) and sympathovagal tone (LF/HF) were calculated by measuring the ANS ultradian amplitude of the peaks-valleys, with larger amplitudes indicating a larger response, and smaller amplitudes indicating a diminished response for the respective sympathetic/parasympathetic metric . Additionally, for each 20-minute epoch, temporalis and masseter muscle duty factors were calculated for thresholds of magnitude of load (1-<2 N, 2-<5 N, and 5-10 N) and duration thresholds of (0.5-1 s, 1-<2 s, 2-<5 s). HRV and EMG data were exported to spreadsheets (Excel, Microsoft, Redmond, WA) for statistical analysis.

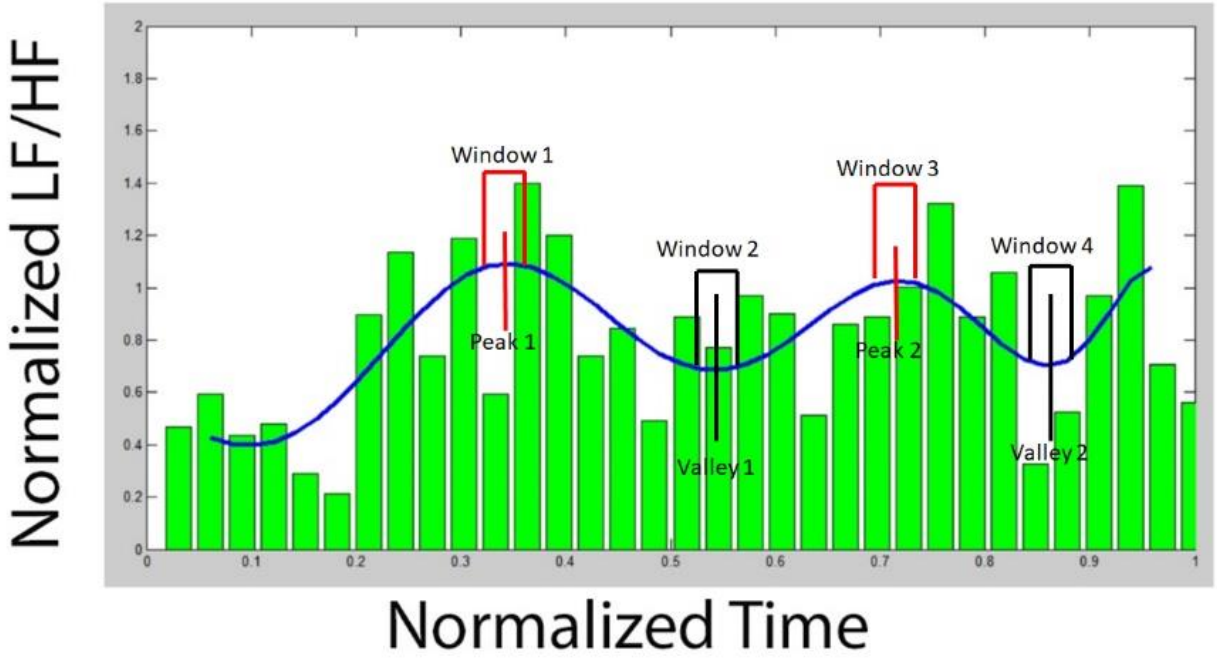


Figure 3: Peak and valley assessment of night-time ulradian cycling, to identify 20 minute epochs (Windows) that were +/- 10 minutes about each peak and valley. , Parasympathetic (pNN50) activity, sympathovagal (LF/HF) tone, and masticatory muscle duty factors were then measured for each epoch.

Data and Statistical Analyses:

Subjects' CPI levels were assessed using validated instruments (DC/TMD, Appendix 3) at Timepoints 1 and 2 and quantified as -P if the subject exhibited a score of 0, with positive scores being quantified as +P.

Analysis of Variance (ANOVA) was used to determine if there were differences in ANS activity and muscle duty factors between +/- pain groups. 3D regression analysis tested for correlations between the peak-valley ratios of independent variables of ANS tone (pNN50, LF/HF), overall averaged muscle duty factors, and the dependent variable of CPI.

5. Results:

A total of n = 27 subjects completed study protocols. Of the subjects, 16 were female and 11 were male with an average age ± standard deviation (SD) of 38.35 ± 12.0 years at T1 and 42.2 ± 12.0 years at T2. Subjects #016 and #025 did not complete the study and were omitted from the results. Subjects #003, #008, and #021 completed 2 of the 3 nights of the recording protocol and were included in the results. A total of 78 recordings were completed across all subjects with an average length of 7.8 hours (Appendix 3).

Subjects' CPI levels were [Describe any changes in +P/-P status amongst subjects from T1 to T2]. What numbers of subjects were in +P, -P groups and sub-groups by sex? What were ranges and averages +/- SDs of CPI scores for subjects overall, males, females at T2 – should describe these here and refer to Appendix 4]

Table 2: Subject Characteristic Pain Intensity (CPI) results from T1-T2: M = Male, F = Female, P = Pain

Subject #	Sex	T1 Age (years)	T1 CPI	T2 Age (years)	T2 CPI
001	M	46.8	-P	54.2	-P
002	F	33.4	+P	38.7	+P
003	F	61.6	-P	65.0	-P
004	F	23.7	-P	32.8	-P
005	F	49.8	-P	59.3	-P
006	F	44.3	-P	53.0	+P
007	F	26.7	+P	32.0	+P
008	F	32.5	+P	36.9	+P
009	F	45.8	+P	50.0	+P
010	F	28.4	-P	35.5	-P
011	M	33.1	+P	39.2	+P
012	M	29.0	-P	36.1	-P
013	M	39.0	-P	46.9	-P
014	F	26.6	+P	31.2	+P
015	M	63.4	-P	67.4	-P
017	F	23.4	-P	31.5	-P
018	M	25.3	-P	32.4	-P
019	M	26.3	-P	34.0	-P

020	F	36.4	+P	41.8	+P
021	M	59.9	-P	69.8	-P
022	F	52.0	+P	58.9	+P
023	F	23.6	+P	33.5	+P
024	F	43.8	-P	55.7	+P
026	M	43.3	-P	51.7	+P
027	M	26.1	+P	41.9	+P
028	F	28.6	+P	35.9	+P
029	M	42.6	+P	48.4	+P

Subjects' masseter and temporalis duty factors were averaged for both -P and +P groups to represent a normalized masticatory muscle duty factor. Subjects with chronic pain on average had higher masticatory muscle duty factors (Figure 4, Appendix Table 4).

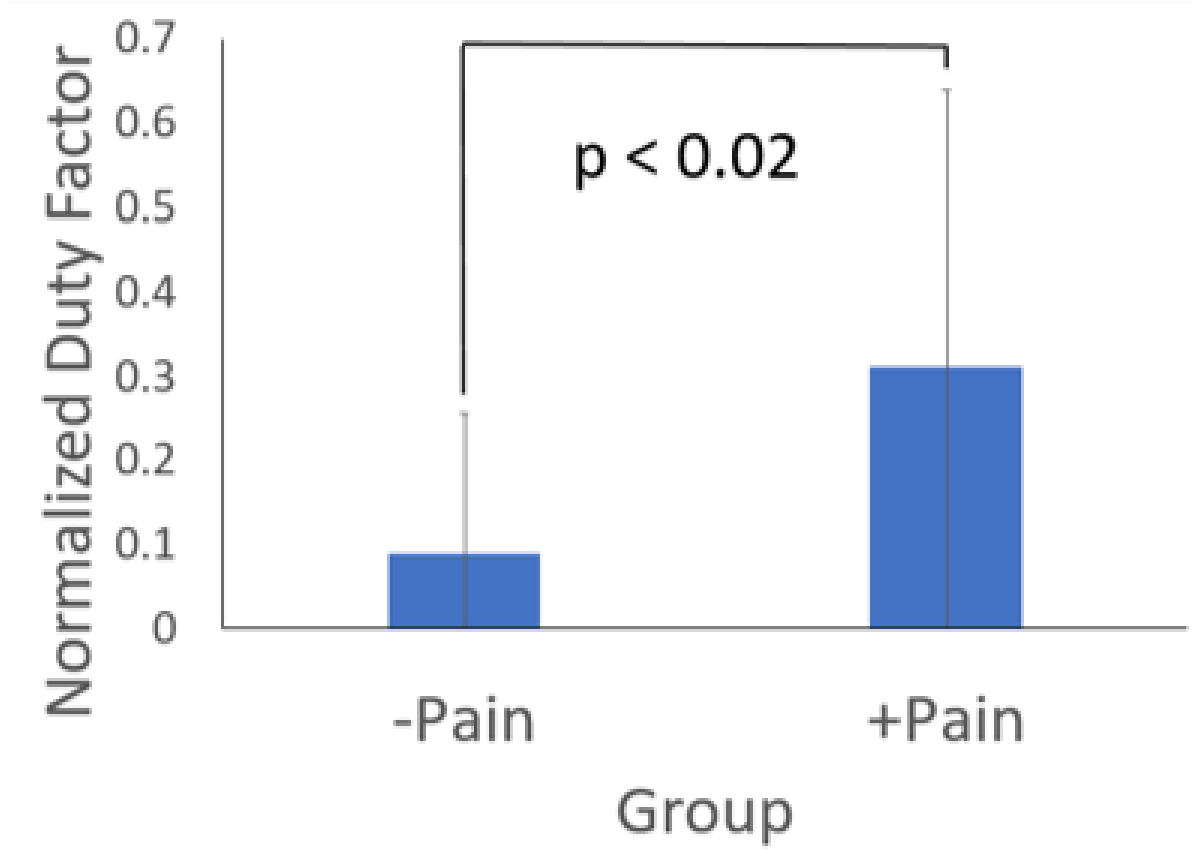


Figure 4: Comparison of normalized averaged temporalis and masseter duty factors to subjects in – Pain and + Pain groups

Regression analysis (Figure 5) of the dependent variable of normalized CPI scores (Appendices 4 and 5) versus independent variables of normalized average muscle duty factor and normalized peak-to-valley ratio of LF/HF values showed R^2=0.75. Subjects with higher CPI scores tended to have higher average muscle DFs and a ratio of sympathetic/parasympathetic (LF/HF) activities.

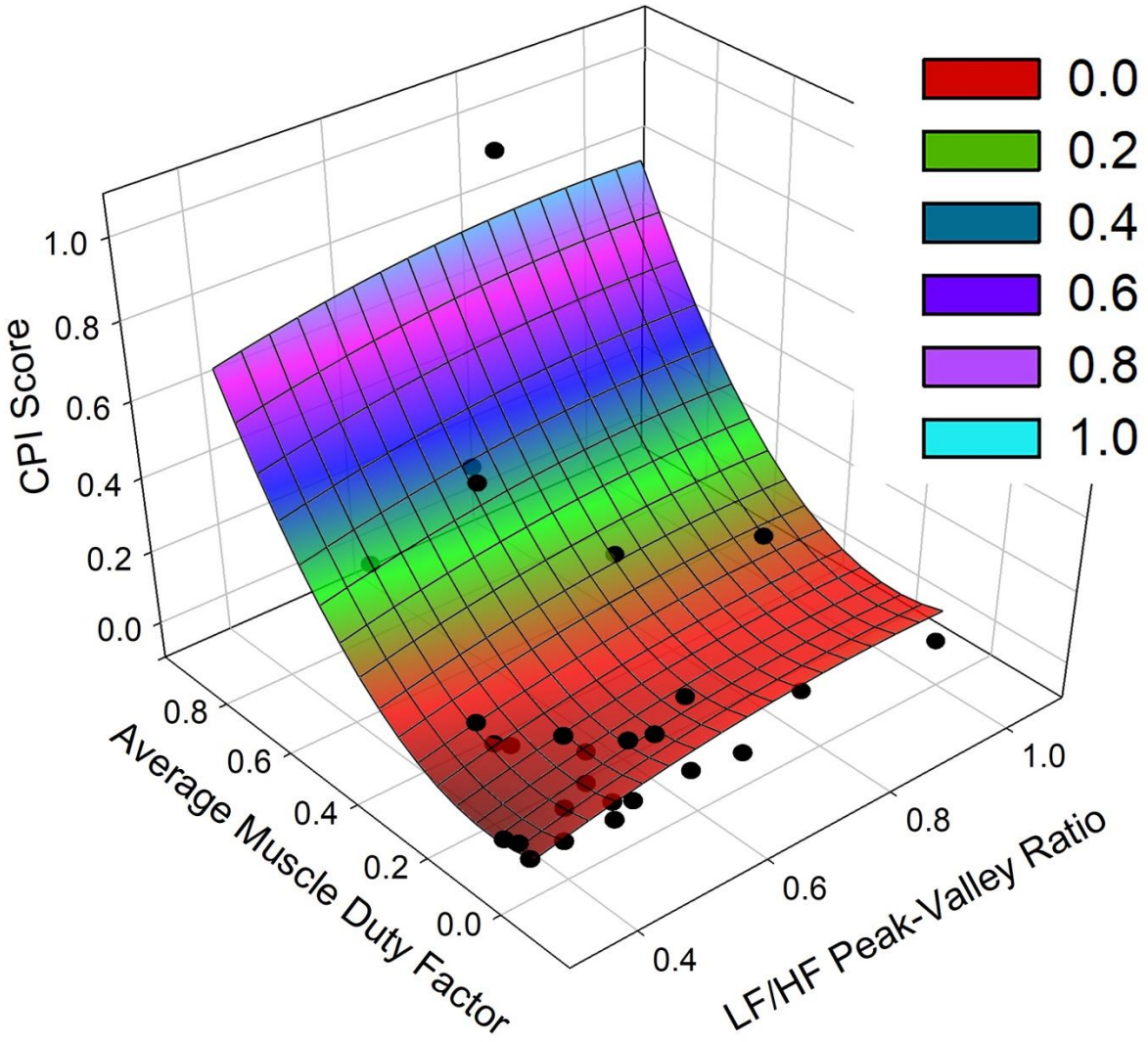


Figure 5: 3D regression showing non-linear effects of normalized 6-month CPI scores versus masticatory muscle duty factors and peak-to-valley ratio of sympathetic/parasympathetic activities (Low Frequency/High Frequency, LF/HF).

Regression analysis (Figure 6, R^2= 0.67) was made of the normalized dependent variable of CPI scores versus independent variables of normalized ultradian cycling peak-valley ratios of parasympathetic activity (pNN50, %) and normalized low frequency (sympathetic) over high frequency (parasympathetic) ratios.

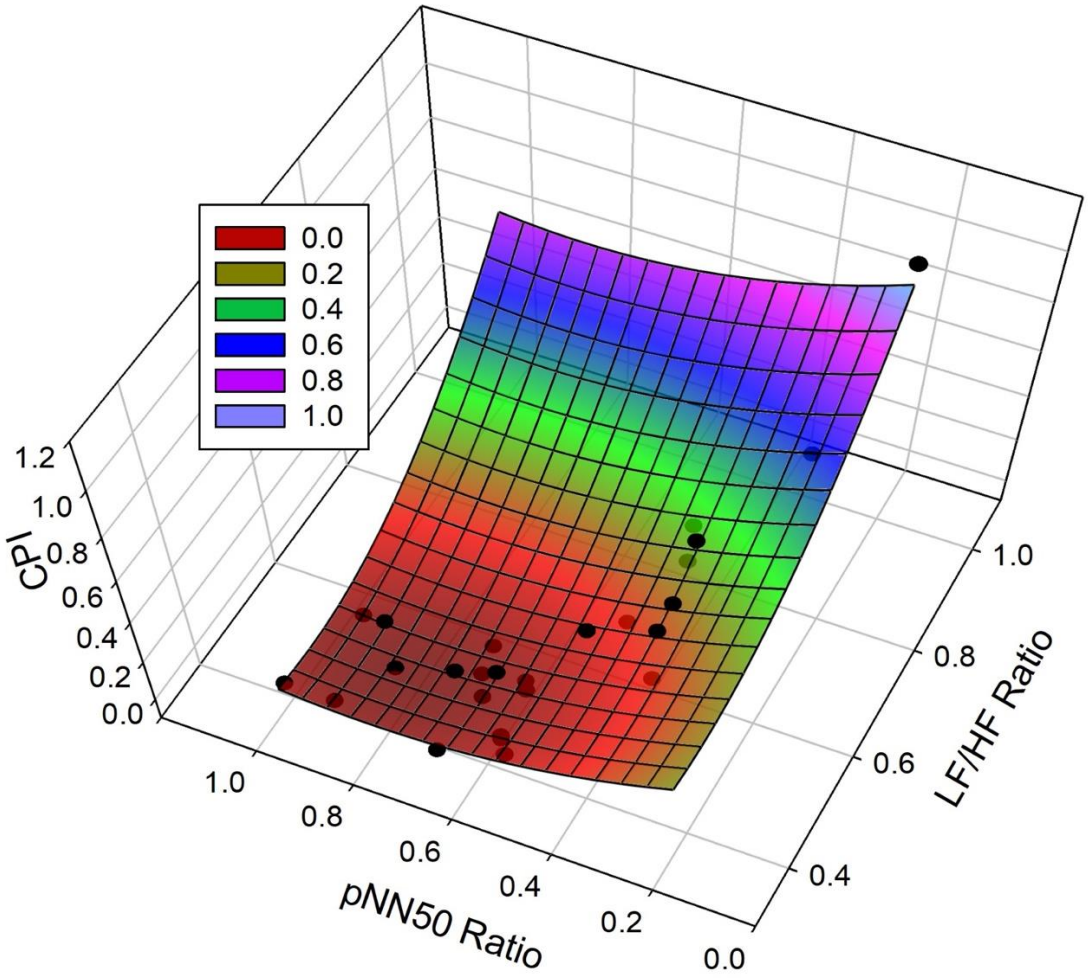


Figure 6: 3D regression showing non-linear effects of normalized 6-month CPI versus normalized parasympathetic activity (pNN50) and sympathetic/parasympathetic activity (Low Frequency/High Frequency).

The lowest normalized CPI values were associated with relatively higher normalized peak-to-valley ratios of pNN50 values and relatively lower normalized peak-to-valley ratios of LF/HF.. Subjects with the highest pain intensity scores had the highest LF/HF ratios (sympathetic

compared to parasympathetic activity) and lowest pNN50 values (parasympathetic activity) during sleep.

6. Discussion

Pain has a subjective component which is influenced by biological, psychological, and social factors.[25] HRV as an method of measuring ANS activity has been widely used in pain studies to provide measures of activity in both the sympathetic and parasympathetic branches.[25] In subjects with pain, HRV studies have shown an association between the ANS and the subjective experience of pain, with higher parasympathetic activity being associated with better self-regulation and higher pain inhibition capacities. Patients with chronic pain show a reduction in HRV primarily due to changes in the efferent sympathetic and parasympathetic cardiac activity, with a shift toward increased sympathetic nervous system activity and the related release of catecholamines. Parasympathetic activity on the other hand has been shown to be an inhibitor of pain transmission in the subnucleus caudalis via cholinergic receptors.[26] During sleep, the fluctuations in parasympathetic and sympathetic tone demonstrate ultradian cycling which have peaks and valleys over the course of sleep duration.[22] When investigating ultradian cycling during sleep, understanding the role of the suprachiasmatic nucleus (SCN) as a chronobiological controller of circadian rhythms as well as the ultradian dynamics used to measure physiological or pathological aberrations is vital. Peripheral oscillators in the central nervous system (CNS) work along with the SCN, ranging from almost fully autonomous to completely dependent on the SCN, and function all together as part of the multioscillator system.[27] When evaluating the potential effect of pathologies on ultradian cycling, three variables must be considered: phase, period length, and amplitude. Observations in recent studies have demonstrated a correlation

between the weakening of the circadian system with resulting misalignment, reduction, or even loss of amplitudes.[27]

When assessing subjects in the current study for pain in relation to ANS tone during sleep, we used ultradian dynamics in terms of amplitude to correlate pNN50 and LF/HF as a way of assessing parasympathetic activity and sympathetic compared to parasympathetic tone respectively. When the peak-to-valley ratio of pNN50 is high, this means there is a larger ultradian amplitude of parasympathetic tone and higher HRV. In the current study, this was associated with lower CPI scores. Conversely, when the peak-to-valley ratio of LF/HF is high, there is either increased sympathetic or decreased parasympathetic activity or both. In the current study, this was associated with higher CPI scores when peak-to-valley ratios of pNN50 were lower or if the average masticatory muscle DF was higher.

Studies have investigated the use of the beta-blocker medication propranolol for the treatment of TMD, however there have not been statistically significant differences in pain reduction compared to placebo .[28] Based on current results, which implicates both the PNS and SNS in the regulation of pain, using dual alpha/beta blocker medications should be further investigated with the idea that sympathetic tone in the subnucleus caudalis would be reduced, in theory reducing pain in subjects by reducing ultradian amplitude of sympathetic signals. By blocking both alpha and beta receptors, it is likely we could decrease sympathetic and increase parasympathetic activities, potentially leading to a reduction in pain.

In patients with or without chronic pain HRV is also related to endogenous pain modulation (EPM) which depends on the excitation-inhibition of neurovisceral networks. Historically, pain in relation to TMD was also postulated to be related to high load of the TMJ complex causing nociceptive signaling directly leading to a painful response (Appendix 1),

hence treatment revolved over attempting to reduce high biting forces.[8] However, current data suggests TMD pain could be associated with lower loads on the mandible, in the range of ≤5N, and muscle activities of < 5s. These findings are similar to reports from polysomnography based[15] and ambulatory recording methodologies[29]. Hence there may be other factors and indirect pathways leading to pain signaling in the CNS. Pre-clinical and clinical data show exaggerated nociception occurs with heightened neuroinflammatory signaling in the spinal cord.[30] Microglial production of cytokines Interleukin-1β and Tumor Necrosis Factor-α promotes the development of allodynia in patients with peripheral nerve injury (PNI), developing mechanical pain hypersensitivity.[31] As a result, pharmacological intervention targeting microglial activation prevented neuropathic pain associated with PNI.[31] Repeated social defeat (RSD) protocols in animal models of psychosocial stress involve activation of microglia to increase neuroinflammatory signaling, which augments pain and anxiety-like behaviors. Studies that have used RSD in mice have shown microglial activation selectively within the nociceptive pathway of the dorsal horn of the lumbar cord.[32] The findings of these studies indicate that RSD induces an inflammatory gene profile in the lumbar spinal cord with increased proinflammatory cytokine expression, chemokine ligand/receptor interactions, adhesion molecules and immunoregulatory markers.[32] Within the same study, microglial depletion with a colony stimulating factor 1 receptor (CSF1R) antagonist prevented the development of mechanical allodynia in RSD mice.[32] This demonstrates that psychosocial stress-related glial inflammation enhances pain transmission, as non-nociceptive information gets into pain related pathways, stimulating secondary interneurons to transmit pain-related signals. This is especially important when identifying etiological factors behind TMD pain as further studies examining modulation

of both ANS and microglial activity in TMD patients would be valuable in identifying potential therapies.

7. Limitations

This study has several limitations that should be considered for future investigations. The study had subjects self-applying instruments to measure ECG and EMG. As a result, variability in positioning the electrodes between nights and between subjects could have contributed to data inconsistencies. A further limitation was also not looking as sex as a biological variable and recruiting equal sample size sub-groups of +P and -P in males and females.

An additional limitation was assessing subjects for sleeping disorders or quality of sleep prior to gathering data. Obstructive sleep apnea or subjects having irregular diets or alcoholic drinks could affect the consistency of data as sleep could be disturbed by depressive or stimulating substances. This would in turn affect both the ECG and EMG recordings. Ideally, controlled sleep environments with assessment of sleep biometrics could provide more consistent data as subjects could be limited to blue-light exposure or detrimental habits that can affect sleep quality. By assessing lifestyle of subjects prior to gathering data, future studies could investigate the potential effects of substance use or diet with pain. In addition, future studies should evaluate duty factors with subjects with defined anxiety, depression, and/or physical symptoms as it could help specify the type of treatment that could regulate sympathetic or parasympathetic tone.

Comparison of awake-state recordings with night-time recordings is also vital as previous studies indicated muscle duty factors are higher than at night and reflect psychosocial state.[7] Because CPI is evaluated during daytime, it is important data to consider when comparing the relationship of ANS activity, muscle duty factors, and pain.

8. Conclusion

The aim of this study was to test if there were correlations between the independent variables of ANS activities and jaw muscle duty factors, and the dependent variable of 6-month CPI scores. The results of this study showed that:

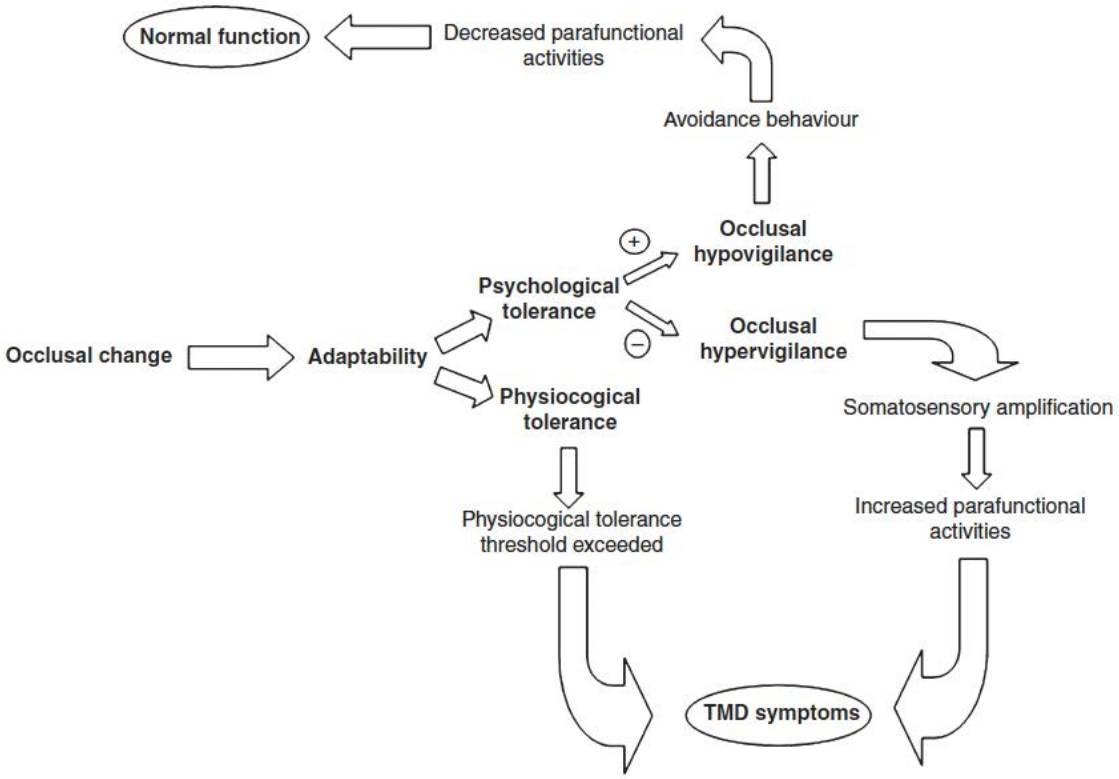
4. There were significant differences in muscle duty factors at low-levels of jaw-loading between subjects with and without chronic pain.
5. There was a positive correlation between 6-month chronic pain intensity (CPI) scores versus masticatory muscle duty factors at low levels of jaw-loading and peak-to-valley ratios of sympathetic/parasympathetic activities (LF/HF)
6. There was a correlation between higher CPI scores versus lower parasympathetic activity (pNN50) and higher LF/HF.

This study demonstrated that low-level masticatory muscle activity, when combined with increased sympathetic nervous system tone, may affect the intensity of pain experienced by individuals with TMD.

9. Appendices

Appendix 1:[8]

(+) = increases
(-) = decreases


Normal function
Decreased parafunctional activities
Avoidance behaviour
Occlusal hypovigilance
Psychological tolerance
Occlusal change
Adaptability
Occlusal hypervigilance
Physiocogical tolerance
Somatosensory amplification
Physiocogical tolerance threshold exceeded
Increased parafunctional activities
TMD symptoms

Appendix 2 – IRB approval memo

OREGON HEALTH & SCIENCE UNIVERSITY

IRB MEMO

Research Integrity Office
3181 SW Sam Jackson Park Road - L106RI
Portland, OR 97239-3098
(503)494-7887 irb@ohsu.edu

APPROVAL OF SUBMISSION

March 28, 2023

Dear Investigator:

On 3-28-2023, the IRB reviewed the following submission:

IRB ID:	STUDY00018800	MOD or CR ID:	MOD00048255
Type of Review:	Modification / Update		
Title of Study:	Contribution of Mechanobehavioral, Psychosocial, and Physiological Domains in the Progression of Temporomandibular Disorders		
Title of modification	Revised protocol to increase study sample accrual to 35		
Principal Investigator:	Laura Iwasaki		
Funding:	Name: American Association of Orthodontists Foundation, PPQ #: 1014638		
IND, IDE, or HDE:	None		
Documents Reviewed:	• Protocol • Protocol_Prog TMD_no EN_032723_tracked.docx		

The IRB granted final approval on 3/28/2023. The study is approved until 3/1/2024.

Review Category: Expedited-Minor Modification

Appendix 3: Bite Force (N) vs. Muscle Activity (µV) regression slopes

Biting Tooth: 36 = left mandibular first molar, 46 = right mandibular first molar
Muscle: R = Right, L = Left, M = Masseter, T = Temporalis
Slope: Bite-force vs. Muscle Activity Regression average slope (mV/N) of muscle activity for unilateral biting on right and left first molars
Intercept: Bite-force vs. Muscle Activity Regression average intercept
mV output at 20N: Averaged masticatory muscle output (mV) per 20N of bite force on right and left first molars

Subject #	Tooth	Muscle	slope	intercept	mV output at 20N	
001	36	RM	0.18E-03	1.50E-03		
001	46	RM	0.05E-03	5.70E-03	5.88E-03	RM
001	36	RT	0.36E-03	55.00E-03		
001	46	RT	0.31E-03	58.00E-03	63.20E-3	RT
001	36	LM	0.09E-03	18.10E-03		
001	46	LM	0.07E-03	6.40E-03	13.80E-03	LM
001	36	LT	0.45E-03	-3.20E-03		
001	46	LT	0.12E-03	11.20E-03	9.70E-03	LT
002	36	RM	0.35E-03	6.33E-03		
002	46	RM	0.08E-03	10.30E-03	12.70E-03	RM
002	36	RT	0.36E-03	56.80E-03		
002	46	RT	0.08E-03	62.40E-03	64.00E-03	RT
002	36	LM	0.64E-03	2.57E-03		
002	46	LM	0.12E-03	3.65E-03	10.70E-03	LM
002	36	LT	0.63E-03	7.00E-03		
002	46	LT	0.07E-03	14.20E-03	17.70E-03	LT
003	36	RM	0.39E-03	1.01E-03		
003	46	RM	0.29E-03	2.17E-03	8.33E-03	RM
003	36	RT	0.51E-03	57.00E-03		

003	46	RT	0.72E-03	57.60E-03	69.70E-03	RT
003	36	LM	0.87E-03	9.96E-03		
003	46	LM	0.70E-03	6.19E-03	23.80E-03	LM
003	36	LT	1.30E-03	22.20E-03		
003	46	LT	1.15E-03	1.62E-03	36.40E-03	LT
004	36	RM	0.42E-03	12.80E-03		
004	46	RM	0.38E-03	14.20E-03	21.50E-03	RM
004	36	RT	0.24E-03	59.20E-03		
004	46	RT	0.64E-03	56.10E-03	66.40E-03	RT
004	36	LM	1.29E-03	14.20E-03		
004	46	LM	0.66E-03	16.70E-03	35.00E-03	LM
004	36	LT	1.01E-03	12.40E-03		
004	46	LT	0.60E-03	1.12E-03	22.90E-03	LT
005	36	RM	0.96E-03	7.41E-03		
005	46	RM	1.30E-03	6.22E-03	9.07E-03	RM
005	36	RT	0.23E-03	65.60E-03		
005	46	RT	0.72E-03	66.40E-03	66.90E-03	RT
005	36	LM	0.85E-03	21.30E-03		
005	46	LM	0.22E-03	19.10E-03	23.20E-03	LM
005	36	LT	0.30E-03	12.40E-03		
005	46	LT	0.25E-03	8.18E-03	15.80E-03	LT
006	36	RM	0.19E-03	5.72E-03		
006	46	RM	0.20E-03	6.74E-03	10.20E-03	RM
006	36	RT	0.60E-03	59.90E-03		
006	46	RT	0.52E-03	64.30E-03	73.20E-03	RT

006	36	LM	0.44E-03	7.52E-03		
006	46	LM	0.19E-03	9.19E-03	14.70E-03	LM
006	36	LT	0.82E-03	-1.83E-03		
006	46	LT	0.52E-03	3.00E-03	14.00E-03	LT
007	36	RM	0.86E-03	9.27E-03		
007	46	RM	0.57E-03	7.26E-03	9.69E-03	RM
007	36	RT	0.11E-03	63.50E-03		
007	46	RT	0.95E-03	62.90E-03	6.53E-03	RT
007	36	LM	0.17E-03	19.00E-03		
007	46	LM	0.17E-03	18.60E-03	22.20E-03	LM
007	36	LT	0.48E-03	22.70E-03		
007	46	LT	0.29E-03	7.66E-03	22.90E-03	LT
008	36	RM	0.53E-03	5.18E-03		
008	46	RM	0.70E-03	3.81E-03	5.73E-03	RM
008	36	RT	0.49E-03	61.00E-03		
008	46	RT	0.18E-03	56.90E-03	61.30E-03	RT
008	36	LM	0.17E-03	4.04E-03		
008	46	LM	0.13E-03	1.90E-03	5.93E-03	LM
008	36	LT	0.18E-03	0.75E-03		
008	46	LT	0.52E-03	4.75E-03	5.10E-03	LT
009	36	RM	0.26E-03	9.50E-03		
009	46	RM	0.46E-03	9.10E-03	16.50E-03	RM
009	36	RT	0.19E-03	63.00E-03		
009	46	RT	0.13E-03	66.00E-03	67.80E-03	RT
009	36	LM	0.53E-03	14.30E-03		

009	46	LM	1.53E-03	10.20E-03	32.80E-03	LM
009	36	LT	7.78E-03	1.07E-03		
009	46	LT	1.50E-03	2.50E-03	94.60E-03	LT
010	36	RM	0.29E-03	7.40E-03		
010	46	RM	0.19E-03	5.85E-03	11.40E-03	RM
010	36	RT	0.32E-03	61.00E-03		
010	46	RT	0.58E-03	55.60E-03	67.30E-03	RT
010	36	LM	0.96E-03	18.50E-03		
010	46	LM	0.49E-03	5.10E-03	26.30E-03	LM
010	36	LT	2.22E-03	3.10E-03		
010	46	LT	1.25E-03	0.63E-03	36.60E-03	LT
011	36	RM	0.22E-03	14.30E-03		
011	46	RM	0.15E-03	15.70E-03	15.40E-03	RM
011	36	RT	0.18E-03	59.80E-03		
011	46	RT	0.17E-03	61.00E-03	60.70E-03	RT
011	36	LM	0.60E-03	7.90E-03		
011	46	LM	0.51E-03	9.49E-03	9.81E-03	LM
011	36	LT	0.96E-03	13.20E-03		
011	46	LT	0.55E-03	11.00E-03	13.60E-03	LT
012	36	RM	0.86E-03	20.50E-05		
012	46	RM	0.65E-03	15.40E-03	9.22E-03	RM
012	36	RT	0.19E-03	90.10E-03		
012	46	RT	0.23E-03	99.00E-03	98.80E-03	RT
012	36	LM	0.16E-03	13.30E-03		
012	46	LM	0.18E-03	20.60E-03	20.30E-03	LM

012	36	LT	0.48E-03	47.00E-03		
012	46	LT	0.35E-03	17.60E-03	40.60E-03	LT
013	36	RM	0.36E-03	6.10E-03		
013	46	RM	0.10E-03	4.70E-03	6.79E-03	RM
013	36	RT	0.29E-03	61.00E-03		
013	46	RT	0.11E-03	58.00E-03	60.90E-03	RT
013	36	LM	0.19E-03	15.50E-03		
013	46	LM	0.14E-03	8.04E-03	15.10E-03	LM
013	36	LT	0.12E-03	31.00E-03		
013	46	LT	0.15E-03	12.60E-03	24.50E-03	LT
014	36	RM	0.66E-03	4.19E-03		
014	46	RM	0.90E-03	5.64E-03	6.47E-03	RM
014	36	RT	0.57E-03	59.30E-03		
014	46	RT	0.36E-03	55.00E-03	61.30E-03	RT
014	36	LM	0.32E-03	13.40E-03		
014	46	LM	0.36E-03	14.00E-03	20.50E-03	LM
014	36	LT	0.55E-03	5.75E-03		
014	46	LT	0.51E-03	8.35E-03	17.70E-03	LT
015	36	RM	0.09E-03	9.59E-03		
015	46	RM	0.11E-03	12.50E-03	13.10E-03	RM
015	36	RT	0.03E-03	60.60E-03		
015	46	RT	0.02E-03	59.30E-03	60.40E-03	RT
015	36	LM	0.15E-03	11.00E-03		
015	46	LM	0.12E-03	8.30E-03	12.30E-03	LM
015	36	LT	0.21E-03	22.30E-03		

015	46	LT	0.20E-03	15.20E-03	22.80E-03	LT
016	36	RM	0.17E-03	6.56E-03		
016	46	RM	0.09E-03	7.48E-03	8.12E-03	RM
016	36	RT	0.01E-03	59.80E-03		
016	46	RT	0.07E-03	58.80E-03	60.10E-03	RT
016	36	LM	0.06E-03	10.00E-03		
016	46	LM	0.16E-03	9.50E-03	12.00E-03	LM
016	36	LT	0.07E-03	13.30E-03		
016	46	LT	0.13E-03	8.45E-03	12.90E-03	LT
017	36	RM	0.08E-03	1.89E-03		
017	46	RM	0.23E-03	6.15E-03	7.09E-03	RM
017	36	RT	0.18E-03	55.60E-03		
017	46	RT	0.29E-03	64.00E-03	64.40E-03	RT
017	36	LM	0.24E-03	0.67E-03		
017	46	LM	0.18E-03	7.67E-03	8.33E-03	LM
017	36	LT	0.62E-03	-2.81E-03		
017	46	LT	0.72E-03	0.49E-03	12.20E-03	LT
018	36	RM	0.20E-03	16.60E-03		
018	46	RM	0.11E-03	13.50E-03	18.10E-03	RM
018	36	RT	0.16E-03	61.50E-03		
018	46	RT	0.25E-03	56.30E-03	63.00E-03	RT
018	36	LM	0.24E-03	14.10E-03		
018	46	LM	0.12E-03	9.43E-03	15.40E-03	LM
018	36	LT	0.32E-03	16.50E-03		
018	46	LT	0.18E-03	17.50E-03	22.10E-03	LT

019	36	RM	0.04E-03	12.50E-03		
019	46	RM	0.02E-03	16.30E-03	15.00E-03	RM
019	36	RT	0.02E-03	62.10E-03		
019	46	RT	0.03E-03	61.60E-03	62.40E-03	RT
019	36	LM	0.07E-03	10.30E-03		
019	46	LM	0.02E-03	7.51E-03	9.86E-03	LM
019	36	LT	0.21E-03	4.32E-03		
019	46	LT	0.06E-03	5.21E-03	7.39E-03	LT
020	36	RM	0.20E-03	6.54E-03		
020	46	RM	0.08E-03	10.40E-03	11.30E-03	RM
020	36	RT	0.15E-03	58.70E-03		
020	46	RT	0.18E-03	69.90E-03	67.60E-03	RT
020	36	LM	0.29E-03	14.60E-03		
020	46	LM	0.16E-03	14.50E-03	19.00E-03	LM
020	36	LT	0.17E-03	8.17E-03		
020	46	LT	0.04E-03	8.58E-03	10.40E-03	LT
021	36	RM	0.18E-03	18.70E-03		
021	46	RM	0.25E-03	17.80E-03	22.60E-03	RM
021	36	RT	0.09E-03	61.80E-03		
021	46	RT	0.07E-03	62.70E-03	63.90E-03	RT
021	36	LM	0.37E-03	10.10E-03		
021	46	LM	0.26E-03	11.30E-03	17.00E-03	LM
021	36	LT	0.61E-03	2.93E-03		
021	46	LT	0.51E-03	8.04E-03	16.70E-03	LT
022	36	RM	0.74E-03	-7.46E-03		

022	46	RM	0.13E-03	2.15E-03	6.85E-03	RM
022	36	RT	0.22E-03	69.20E-03		
022	46	RT	0.52E-03	50.10E-03	67.00E-03	RT
022	36	LM	0.28E-03	21.30E-03		
022	46	LM	0.35E-03	12.20E-03	23.10E-03	LM
022	36	LT	0.50E-03	34.80E-03		
022	46	LT	0.65E-03	8.18E-03	33.00E-03	LT
023	36	RM	0.48E-03	64.50E-03		
023	46	RM	0.29E-03	83.50E-03	81.70E-03	RM
023	36	RT	0.12E-03	6.91E-03		
023	46	RT	0.87E-03	17.30E-03	21.60E-03	RT
023	36	LM	0.64E-03	11.10E-03		
023	46	LM	0.45E-03	10.10E-03	21.50E-03	LM
023	36	LT	0.60E-03	8.60E-03		
023	46	LT	0.26E-03	7.79E-03	16.80E-03	LT
024	36	RM	0.07E-03	60.00E-03		
024	46	RM	0.34E-03	59.10E-03	63.70E-03	RM
024	36	RT	0.52E-03	10.50E-03		
024	46	RT	1.02E-03	12.30E-03	26.80E-03	RT
024	36	LM	0.14E-03	6.86E-03		
024	46	LM	0.51E-03	3.76E-03	11.80E-03	LM
024	36	LT	0.73E-03	18.80E-03		
024	46	LT	0.81E-03	17.40E-03	33.40E-03	LT
025	36	RM	0.33E-03	75.50E-03		
025	46	RM	0.38E-03	74.00E-03	81.80E-03	RM

025	36	RT	0.68E-03	5.98E-03		
025	46	RT	1.19E-03	21.80E-03	32.60E-03	RT
025	36	LM	0.61E-03	23.80E-03		
025	46	LM	0.56E-03	19.90E-03	33.60E-03	LM
025	36	LT	2.10E-03	7.00E-03		
025	46	LT	1.07E-03	1.92E-03	29.20E-03	LT
026	36	RM	0.20E-03	61.90E-03		
026	46	RM	0.17E-03	63.60E-03	66.40E-03	RM
026	36	RT	0.49E-03	25.00E-03		
026	46	RT	0.87E-03	20.70E-03	36.40E-03	RT
026	36	LM	0.21E-03	8.84E-03		
026	46	LM	0.22E-03	12.00E-03	14.80E-03	LM
026	36	LT	1.17E-03	4.17E-03		
026	46	LT	1.27E-03	-2.92E-03	25.00E-03	LT
027	36	RM	0.95E-03	50.20E-03		
027	46	RM	0.48E-03	63.70E-03	71.30E-03	RM
027	36	RT	0.67E-03	11.90E-03		
027	46	RT	0.79E-03	19.90E-03	30.50E-03	RT
027	36	LM	0.28E-03	6.66E-03		
027	46	LM	0.28E-03	11.90E-03	14.80E-03	LM
027	36	LT	2.15E-03	12.50E-03		
027	46	LT	1.41E-03	12.50E-03	4.81E-03	LT
028	36	RM	0.52E-03	61.70E-03		
028	46	RM	0.36E-03	65.10E-03	72.20E-03	RM
028	36	RT	0.41E-03	11.50E-03		

028	46	RT	0.10E-03	14.60E-03	18.10E-03	RT
028	36	LM	0.37E-03	52.30E-03		
028	46	LM	0.91E-03	4.99E-03	33.30E-03	LM
028	36	LT	1.101E-03	14.90E-03		
028	46	LT	0.45E-03	4.42E-03	25.20E-03	LT
029	36	RM	0.20E-03	64.00E-03		
029	46	RM	0.62E-03	64.20E-03	64.80E-03	RM
029	36	RT	0.71E-03	10.60E-03		
029	46	RT	0.15E-03	11.60E-03	13.30E-03	RT
029	36	LM	0.29E-03	4.53E-03		
029	46	LM	0.26E-03	3.04E-03	6.63E-03	LM
029	36	LT	0.24E-03	16.40E-03		
029	46	LT	0.47E-03	0.79E-03	15.70E-03	LT

Appendix 4: Timepoint 2 (T2) Pain Scores and Night Recording Durations

T2 CPI: Characteristic Pain Intensity score assessed at Timepoint 2
Night 1/2/3 hrs: Duration of recording in hours
Average: Average recording duration over 3 nights
N/A = non-applicable

Subject	Sex	T2 CPI	Night1 hrs	Night2 hrs	Night3 hrs	Average hrs
001	Male	0	6.5	6.5	6.8	6.6
002	Female	11	7.1	6.2	6.1	6.5
003	Female	0	N/A	5.1	7.9	6.0
004	Female	0	7.3	8.1	7.5	7.6
005	Female	0	9.0	10.3	8.3	9.2
006	Female	8	6.5	9.9	7.9	8.1
007	Female	9	8.7	9.5	8.6	8.9
008	Female	17	6.4	N/A	8.1	7.2
009	Female	12	6.7	7.3	6.7	6.9
010	Female	0	8.7	7.5	8.3	8.1
011	Male	16	7.3	6.6	7.5	7.2
012	Male	0	7.9	7.8	9.1	8.3
013	Male	0	8.9	12.0	6.9	9.3
014	Female	9	9.1	8.4	7.2	8.3
015	Male	0	7.9	7.5	7.0	7.5
016	Male	N/A	N/A	N/A	N/A	N/A
017	Female	0	9.7	9.7	10.3	9.9
018	Male	0	8.9	7.7	7.9	8.2
019	Male	0	9.0	7.8	7.5	8.1

020	Female	24	9.6	6.2	10.4	8.7
021	Male	0	7.1	N/A	8.1	7.6
022	Female	7	6.3	4.8	5.2	5.4
023	Female	5	5.6	6.4	9.6	7.2
024	Female	46	8.8	8.3	9.3	8.8
025	Male	N/A	8.0	8.3	7.5	7.9
026	Male	2	6.8	7.3	7.9	7.3
027	Male	10	6.3	7.9	8.4	7.5
028	Female	0	7.2	9.2	9.2	8.6
029	Male	5	8.2	8.0	6.7	7.6

Appendix 5: Timepoint 2 (T2) Pain Scores and Muscle Duty Factors

T2 CPI: Characteristic Pain Intensity score assessed at Timepoint 2
Av. Duty Factor: Averages of masticatory muscle duty factors (%) over 3 nights for 20 minute epochs about ultradian peaks and valleys of ANS tone

Subject#	Sex	T2 CPI	Muscle	Av. Duty Factor (%)
001	Male	0	Masseter	10.0E-4
001	Male	0	Temporalis	3.1E-04
002	Female	11	Masseter	9.4E-03
002	Female	11	Temporalis	1.8E-03
003	Female	0	Masseter	6.7E-04
003	Female	0	Temporalis	0.0E+00
004	Female	0	Masseter	0.0E+00
004	Female	0	Temporalis	0.0E+00
005	Female	0	Masseter	0.0E+00
005	Female	0	Temporalis	1.7E-04
006	Female	8	Masseter	0.0E+00
006	Female	8	Temporalis	0.0E+00
007	Female	9	Masseter	1.0E-03
007	Female	9	Temporalis	3.1E-04
008	Female	17	Masseter	9.4E-03
008	Female	17	Temporalis	1.8E-03
009	Female	12	Masseter	1.2E-04
009	Female	12	Temporalis	0.0E+00
010	Female	0	Masseter	1.1E-04
010	Female	0	Temporalis	1.9E-04

011	Male	16	Masseter	4.7E-04
011	Male	16	Temporalis	2.1E-04
012	Male	0	Masseter	5.2E-03
012	Male	0	Temporalis	0.0E+00
013	Male	0	Masseter	8.3E-05
013	Male	0	Temporalis	0.0E+00
014	Female	9	Masseter	0.0E+00
014	Female	9	Temporalis	0.0E+00
015	Male	0	Masseter	3.8E-04
015	Male	0	Temporalis	5.6E-04
017	Female	0	Masseter	2.5E-04
017	Female	0	Temporalis	0.0E+00
018	Male	0	Masseter	3.8E-05
018	Male	0	Temporalis	9.4E-05
019	Male	0	Masseter	2.8E-04
019	Male	0	Temporalis	7.3E-04
020	Female	24	Masseter	3.7E-03
020	Female	24	Temporalis	7.7E-04
021	Male	0	Masseter	2.7E-04
021	Male	0	Temporalis	9.8E-04
022	Female	7	Masseter	1.6E-03
022	Female	7	Temporalis	1.5E-03
023	Female	5	Masseter	8.8E-04
023	Female	5	Temporalis	2.5E-03

024	Female	46	Masseter	8.9E-04
024	Female	46	Temporalis	8.1E-04
026	Male	2	Masseter	5.7E-03
026	Male	2	Temporalis	2.8E-03
027	Male	10	Masseter	5.7E-03
027	Male	10	Temporalis	1.4E-03
028	Female	0	Masseter	6.9E-04
028	Female	0	Temporalis	1.5E-03
029	Male	5	Masseter	6.2E-03
029	Male	5	Temporalis	5.4E-03

Appendix 6: Subjects' ANS Activity at Ultradian Cycle Peaks and Valleys

S# = subject #
M= male
F= female
T2= Timepoint 2 Characteristic Pain Intensity
N(1-3)= Night 1 through 3
ANS: A= LF/HF and B= pNN50
P1-P3= ultradian cycle peaks 1 through 3
V1-V3= ultradian valleys 1 through 3
P/tP = average valley
V/tV = average peak
P/V= average peak valley ratios for 1 through 3
N/A = not-applicable

S#	Sex	T2	N	ANS	P1	P2	P3	V1	V2	V3	P/tP	V/tV	P/V
001	M	0	1	A	2.6	1.5	4.3	0.1	0.1	0.2	2.8	0.1	22.0
001	M	0	2	A	2.1	3.1	3.2	1.6	0.7	1.7	2.8	1.3	2.1
001	M	0	3	A	7.2	3.0	2.7	1.7	1.8	1.3	4.3	1.6	2.7
001	M	0	1	B	68.1	35.6	39.7	34.0	15.4	23.0	47.8	24.1	2.0
001	M	0	2	B	30.1	52.1	37.2	0.7	29.5	27.2	39.8	19.1	2.1
001	M	0	3	B	16.4	29.3	33.5	10.0	17.8	25.2	26.4	17.7	1.5
002	F	11	1	A	0.7	1.9	1.3	0.1	0.8	1.1	1.3	0.7	1.9
002	F	11	2	A	0.8	2.3	2.3	0.3	1.0	1.1	1.8	0.8	2.3
002	F	11	3	A	0.5	1.3	2.4	0.4	1.0	1.0	1.4	0.8	1.8
002	F	11	1	B	40.5	19.2	15.4	9.8	14.9	7.3	25.0	10.6	2.4
002	F	11	2	B	18.6	12.5	7.1	16.9	7.4	3.6	12.7	9.3	1.4
002	F	11	3	B	57.0	67.4	21.7	53.4	22.3	9.1	48.7	28.3	1.7
003	F	0	1	A	10.6	8.9	11.9	6.0	1.4	7.6	10.5	5.0	2.1
003	F	0	2	A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
003	F	0	3	A	16.9	15.3	12.5	5.6	13.2	2.7	14.9	7.2	2.1

003	F	0	1	B	20.4	5.9	6.7	0.1	0.1	0.1	11.0	0.1	109.7
003	F	0	2	B	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
003	F	0	3	B	3.2	4.7	4.7	2.2	2.2	2.7	4.2	2.4	1.8
004	F	0	1	A	2.5	2.3	1.7	0.1	1.9	1.2	2.2	1.1	2.0
004	F	0	2	A	2.1	2.5	2.5	0.5	1.4	1.7	2.4	1.2	1.9
004	F	0	3	A	1.3	2.3	2.7	0.1	1.8	1.3	2.1	1.0	2.0
004	F	0	1	B	41.8	20.8	38.4	8.7	12.1	22.1	33.7	14.3	2.3
004	F	0	2	B	21.9	16.4	20.3	0.1	8.7	17.0	19.5	8.6	2.3
004	F	0	3	B	35.1	19.0	28.9	13.1	2.4	14.6	27.7	10.0	2.8
005	F	0	1	A	5.1	2.1	2.6	1.9	1.4	1.1	3.3	1.5	2.3
005	F	0	2	A	2.9	2.5	2.0	2.1	2.2	0.8	2.4	1.7	1.4
005	F	0	3	A	4.8	3.4	6.6	0.7	2.7	1.8	4.9	1.8	2.8
005	F	0	1	B	18.6	10.3	10.6	0.1	0.1	31.1	13.2	10.4	1.3
005	F	0	2	B	5.9	28.5	36.2	0.1	1.8	9.2	23.5	3.7	6.4
005	F	0	3	B	1.9	5.3	4.8	1.0	0.4	0.5	4.0	0.6	6.7
006	F	8	1	A	2.4	6.3	4.4	0.9	1.5	1.9	4.4	1.4	3.1
006	F	8	2	A	3.5	2.7	4.3	1.8	2.6	1.6	3.5	2.0	1.7
006	F	8	3	A	3.7	3.8	3.3	2.0	1.4	1.6	3.6	1.7	2.1
006	F	8	1	B	7.0	2.8	1.5	1.3	0.7	0.7	3.8	0.9	4.4
006	F	8	2	B	2.5	2.5	5.1	0.0	0.3	2.0	3.4	0.8	4.3
006	F	8	3	B	17.9	9.4	12.2	11.2	5.5	3.2	13.1	6.6	2.0
007	F	9	1	A	2.4	2.0	2.3	1.6	1.4	1.1	2.2	1.4	1.6
007	F	9	2	A	3.7	2.0	2.6	1.4	0.9	1.1	2.8	1.2	2.4
007	F	9	3	A	2.7	1.3	2.3	1.2	1.1	1.2	2.1	1.2	1.8
007	F	9	1	B	45.4	56.2	52.9	27.0	51.7	42.1	51.5	40.3	1.3

007	F	9	2	B	54.8	52.6	50.3	16.4	35.8	45.6	52.6	32.6	1.6
007	F	9	3	B	45.6	54.6	57.2	31.3	44.8	50.1	52.5	42.1	1.2
008	F	17	1	A	0.9	0.8	1.3	0.2	0.8	0.5	1.0	0.5	2.0
008	F	17	2	A	2.2	2.1	1.1	0.1	0.9	0.6	1.8	0.5	3.5
008	F	17	3	A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
008	F	17	1	B	21.6	5.5	31.7	0.1	3.9	13.4	19.6	5.8	3.4
008	F	17	2	B	24.6	7.3	29.2	0.0	5.6	23.4	20.4	9.7	2.1
008	F	17	3	B	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
009	F	12	1	A	1.5	1.3	1.7	0.8	0.8	0.8	1.5	0.8	1.8
009	F	12	2	A	1.9	2.0	1.0	0.4	0.3	0.4	1.6	0.4	4.5
009	F	12	3	A	0.9	1.3	2.4	0.4	0.2	0.1	1.5	0.2	6.7
009	F	12	1	B	20.6	14.9	32.2	3.7	15.7	17.9	22.6	12.4	1.8
009	F	12	2	B	19.3	25.2	46.2	14.8	28.5	39.9	30.2	27.7	1.1
009	F	12	3	B	43.7	25.3	35.6	20.9	24.3	39.3	34.9	28.2	1.2
010	F	0	1	A	2.6	4.4	2.9	0.5	1.6	0.8	3.3	0.9	3.5
010	F	0	2	A	5.3	3.1	3.1	1.9	1.6	2.0	3.8	1.9	2.1
010	F	0	3	A	6.3	2.9	2.6	2.3	2.5	0.8	3.9	1.9	2.1
010	F	0	1	B	12.8	19.7	33.4	2.7	6.5	5.8	22.0	5.0	4.4
010	F	0	2	B	28.7	32.5	29.1	17.3	21.5	29.1	30.1	22.6	1.3
010	F	0	3	B	40.6	33.9	37.5	34.3	25.6	22.5	37.3	27.5	1.4
011	M	16	1	A	3.4	3.5	1.9	2.8	2.4	2.1	2.9	2.4	1.2
011	M	16	2	A	2.6	3.8	2.7	1.3	2.3	1.5	3.0	1.7	1.8
011	M	16	3	A	3.7	2.5	2.4	1.6	2.0	1.5	2.8	1.7	1.7
011	M	16	1	B	5.6	14.4	37.2	0.2	2.3	7.1	19.1	3.2	6.0
011	M	16	2	B	3.6	8.1	25.1	0.1	0.1	4.8	12.2	1.7	7.4

011	M	16	3	B	3.2	9.1	34.4	0.1	0.1	7.0	15.5	2.4	6.4
012	M	0	1	A	8.3	4.8	5.7	1.4	3.0	3.2	6.3	2.6	2.5
012	M	0	2	A	3.9	4.0	5.4	1.9	3.3	4.2	4.4	3.1	1.4
012	M	0	3	A	6.6	4.1	8.3	4.0	1.6	3.2	6.3	3.0	2.1
012	M	0	1	B	8.7	9.7	16.4	1.6	6.0	6.7	11.6	4.7	2.4
012	M	0	2	B	3.1	5.7	9.2	0.1	4.8	5.8	6.0	3.6	1.7
012	M	0	3	B	1.9	15.8	9.4	0.8	0.6	5.4	9.0	2.2	4.0
013	M	0	1	A	4.7	3.2	2.6	0.3	1.1	0.8	3.5	0.7	4.8
013	M	0	2	A	4.5	2.8	3.5	3.0	1.6	2.2	3.6	2.3	1.6
013	M	0	3	A	5.9	4.3	3.4	0.5	3.6	1.9	4.6	2.0	2.3
013	M	0	1	B	33.3	25.6	15.4	11.1	14.6	13.3	24.8	13.0	1.9
013	M	0	2	B	17.8	22.2	16.6	5.6	0.3	6.4	18.8	4.1	4.6
013	M	0	3	B	7.1	3.6	5.4	1.2	3.1	4.5	5.4	2.9	1.8
014	F	9	1	A	1.0	1.7	1.9	0.5	0.4	0.6	1.5	0.5	3.1
014	F	9	2	A	1.1	0.8	0.9	0.6	0.7	0.9	0.9	0.7	1.3
014	F	9	3	A	1.0	1.7	1.5	0.4	1.0	0.7	1.4	0.7	1.9
014	F	9	1	B	60.7	51.7	44.8	23.8	31.9	29.7	52.4	28.5	1.8
014	F	9	2	B	19.5	19.6	19.6	32.6	25.9	35.2	19.6	31.3	0.6
014	F	9	3	B	59.6	34.6	44.5	31.1	32.8	31.6	46.2	31.8	1.5
015	M	0	1	A	5.7	4.1	4.8	0.7	2.3	0.2	4.9	1.0	4.7
015	M	0	2	A	1.6	2.8	2.2	0.1	1.4	0.7	2.2	0.7	3.0
015	M	0	3	A	0.6	2.3	2.0	0.4	0.2	1.2	1.6	0.6	2.7
015	M	0	1	B	25.8	22.2	13.2	16.3	9.0	2.0	20.4	9.1	2.2
015	M	0	2	B	19.9	18.5	26.6	17.2	10.9	10.3	21.7	12.8	1.7
015	M	0	3	B	29.7	35.1	6.0	28.1	26.5	5.5	23.6	20.0	1.2

017	F	0	1	A	0.9	1.6	1.5	0.4	1.1	1.4	1.3	1.0	1.3
017	F	0	2	A	1.4	1.9	1.3	0.5	1.0	1.3	1.5	0.9	1.7
017	F	0	3	A	0.8	1.4	1.2	0.2	0.9	0.9	1.1	0.7	1.6
017	F	0	1	B	73.6	51.3	47.7	38.9	39.1	46.0	57.5	41.3	1.4
017	F	0	2	B	67.9	46.2	55.8	16.4	46.6	56.6	56.6	39.9	1.4
017	F	0	3	B	67.4	61.5	53.0	25.2	44.4	39.1	60.6	36.2	1.7
018	M	0	1	A	0.8	1.4	1.3	0.5	0.6	1.3	1.2	0.8	1.4
018	M	0	2	A	3.8	2.7	2.6	1.1	2.5	2.3	3.0	2.0	1.5
018	M	0	3	A	1.9	2.1	1.5	0.3	1.6	0.8	1.8	0.9	2.1
018	M	0	1	B	9.2	20.9	16.3	0.1	6.8	6.7	15.5	4.6	3.4
018	M	0	2	B	0.7	2.4	4.6	0.3	0.3	1.5	2.6	0.7	3.8
018	M	0	3	B	0.7	4.7	5.9	0.1	0.1	1.4	3.7	0.5	7.0
019	M	0	1	A	1.2	1.4	1.4	0.2	0.8	1.0	1.3	0.7	2.0
019	M	0	2	A	1.1	1.3	1.9	0.9	0.6	1.0	1.5	0.9	1.7
019	M	0	3	A	2.0	1.4	1.2	0.5	0.8	1.1	1.5	0.8	1.9
019	M	0	1	B	48.0	34.2	29.7	23.0	26.0	22.0	37.3	23.6	1.6
019	M	0	2	B	49.8	47.0	46.4	14.6	39.6	44.5	47.7	32.9	1.4
019	M	0	3	B	51.9	32.6	45.0	31.3	24.0	31.5	43.2	28.9	1.5
020	F	24	1	A	0.9	2.1	1.2	0.5	0.4	0.4	1.4	0.4	3.3
020	F	24	2	A	0.9	1.0	0.7	0.4	0.7	0.2	0.9	0.5	1.9
020	F	24	3	A	0.1	1.3	1.1	1.3	1.6	2.2	0.8	1.7	0.5
020	F	24	1	B	49.3	30.6	58.6	20.6	29.9	26.7	46.2	25.7	1.8
020	F	24	2	B	12.7	5.9	21.1	7.2	0.1	2.7	13.2	3.3	4.0
020	F	24	3	B	11.0	2.4	8.7	0.1	0.1	1.4	7.4	0.5	13.6
021	M	0	1	A	1.2	1.3	0.6	0.6	0.3	0.4	1.0	0.4	2.4

021	M	0	2	A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
021	M	0	3	A	1.6	1.2	1.0	0.9	0.7	0.4	1.3	0.7	1.8
021	M	0	1	B	12.1	46.6	28.3	0.1	0.1	15.6	29.0	5.3	5.5
021	M	0	2	B	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A	N/A
021	M	0	3	B	6.9	9.9	8.6	3.0	2.9	7.0	8.5	4.3	2.0
022	F	7	1	A	5.4	2.3	2.7	0.1	1.8	0.7	3.5	0.9	4.0
022	F	7	2	A	5.4	4.2	10.0	4.2	0.1	0.1	6.5	1.5	4.5
022	F	7	3	A	1.5	1.3	0.4	1.2	0.0	0.1	1.1	0.5	2.3
022	F	7	1	B	18.2	48.1	64.7	0.1	15.6	0.1	43.7	5.3	8.3
022	F	7	2	B	15.9	9.8	8.0	7.0	7.9	0.1	11.3	5.0	2.2
022	F	7	3	B	11.0	96.0	72.9	0.1	0.1	53.6	59.9	17.9	3.3
023	F	5	1	A	2.4	1.8	2.3	0.9	0.4	1.8	2.1	1.0	2.1
023	F	5	2	A	1.1	1.4	1.8	0.6	0.8	1.2	1.4	0.9	1.6
023	F	5	3	A	1.5	1.6	1.3	0.4	1.1	0.9	1.4	0.8	1.8
023	F	5	1	B	1.8	3.3	2.9	0.2	0.8	1.5	2.6	0.8	3.2
023	F	5	2	B	14.0	7.1	7.3	0.1	0.1	3.5	9.5	1.2	7.7
023	F	5	3	B	7.7	30.3	51.7	0.1	0.1	21.1	29.9	7.1	4.2
024	F	46	1	A	3.9	2.4	3.8	0.3	2.0	1.1	3.4	1.1	3.0
024	F	46	2	A	1.4	1.8	4.0	0.4	1.0	2.0	2.4	1.1	2.2
024	F	46	3	A	3.1	5.2	4.1	0.3	2.1	1.4	4.1	1.3	3.2
024	F	46	1	B	9.5	8.5	7.3	1.1	5.9	3.9	8.4	3.6	2.3
024	F	46	2	B	10.5	7.3	2.7	3.7	3.0	1.7	6.8	2.8	2.4
024	F	46	3	B	3.2	4.8	4.1	1.7	0.8	1.6	4.1	1.4	3.0
026	M	2	1	A	3.4	3.4	2.4	0.9	0.1	1.2	3.1	0.7	4.2
026	M	2	2	A	3.0	4.1	3.4	2.8	2.3	2.3	3.5	2.5	1.4

026	M	2	3	A	4.6	2.8	3.7	4.5	0.6	0.5	3.7	1.8	2.0
026	M	2	1	B	6.8	6.1	5.0	4.4	2.0	0.9	6.0	2.4	2.5
026	M	2	2	B	2.7	1.7	1.6	0.1	0.4	0.5	2.0	0.3	6.1
026	M	2	3	B	1.4	2.5	6.0	0.3	0.1	1.1	3.3	0.5	6.4
027	M	10	1	A	2.2	3.7	2.0	0.1	0.5	1.3	2.6	0.6	4.3
027	M	10	2	A	1.8	3.2	4.2	0.3	2.5	1.4	3.1	1.4	2.2
027	M	10	3	A	4.6	4.2	1.5	1.3	1.1	1.3	3.4	1.3	2.7
027	M	10	1	B	44.6	59.2	50.1	43.3	45.3	45.0	51.3	44.5	1.2
027	M	10	2	B	60.1	62.2	57.2	57.2	50.0	40.4	59.9	49.2	1.2
027	M	10	3	B	48.4	57.0	53.1	12.7	43.7	45.1	52.8	33.8	1.6
028	F	0	1	A	6.3	11.5	16.3	3.8	5.6	10.5	11.4	6.6	1.7
028	F	0	2	A	3.3	3.2	3.9	1.4	2.2	3.1	3.5	2.2	1.5
028	F	0	3	A	10.8	3.1	9.1	3.4	1.3	1.8	7.6	2.1	3.6
028	F	0	1	B	2.7	3.4	11.1	0.3	1.7	0.3	5.7	0.8	7.5
028	F	0	2	B	11.8	3.7	5.2	5.6	2.9	3.7	6.9	4.1	1.7
028	F	0	3	B	40.5	30.7	10.9	9.7	14.6	2.4	27.4	8.9	3.1
029	M	5	1	A	4.6	1.8	2.8	0.8	1.2	1.0	3.1	1.0	3.1
029	M	5	2	A	16.7	9.5	6.8	9.7	5.2	1.8	11.0	5.6	2.0
029	M	5	3	A	19.3	6.7	2.5	2.2	1.4	2.1	9.5	1.9	5.0
029	M	5	1	B	9.2	4.7	4.6	3.5	4.4	4.6	6.1	4.1	1.5
029	M	5	2	B	14.6	6.3	4.2	4.7	4.1	3.0	8.4	3.9	2.1
029	M	5	3	B	7.8	4.7	3.9	3.2	3.3	2.5	5.5	3.0	1.8

10. References

1. NIDCR. Prevalence of TMJD and its Signs and Symptoms. Available from: https://www.nidcr.nih.gov/research/data-statistics/facial-pain/prevalence [Last Accessed; February 8, 2022].
2. Bair E, Gaynor S, Slade GD, et al. Identification of clusters of individuals relevant to temporomandibular disorders and other chronic pain conditions. Pain 2016;157(6):1266-1278, doi:10.1097/j.pain.0000000000000518
3. Dworkin LLSF. Research Diagnostic criteria for temporomandibular disorders: Review criteria, examinations and specifications, critique. J Craniomandib Disord 1992;6(301-355
4. Schiffman E, Ohrbach R, Truelove E, et al. Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) for Clinical and Research Applications: recommendations of the International RDC/TMD Consortium Network* and Orofacial Pain Special Interest Groupdagger. J Oral Facial Pain Headache 2014;28(1):6-27, doi:10.11607/jop.1151
5. The National Academies of Sciences E, and Medicine. Temporomandibular Disorders: Priorities for Research and Care. In: Temporomandibular Disorders: Priorities for Research and Care. (Yost O, Liverman CT, English R, et al. eds.) Washington (DC); 2020.
6. Kartha S, Zhou T, Granquist EJ, et al. Development of a Rat Model of Mechanically Induced Tunable Pain and Associated Temporomandibular Joint Responses. J Oral Maxillofac Surg 2016;74(1):54 e1-10, doi:10.1016/j.joms.2015.09.005

7. Khawaja SN, Nickel JC, Iwasaki LR, et al. Association between waking-state oral parafunctional behaviours and bio-psychosocial characteristics. Journal of Oral Rehabilitation 2015;42(9):651-656, doi:10.1111/joor.12302

8. Michelotti A, Iodice G. The role of orthodontics in temporomandibular disorders. Journal of Oral Rehabilitation 2010;37(6):411-429, doi:10.1111/j.1365-2842.2010.02087.x

9. Luther F, Layton S, McDonald F. Orthodontics for treating temporomandibular joint (TMJ) disorders. Cochrane Database Syst Rev 2010;7):CD006541, doi:10.1002/14651858.CD006541.pub2

10. Schiffman EL, Ahmad M, Hollender L, et al. Longitudinal Stability of Common TMJ Structural Disorders. J Dent Res 2017;96(3):270-276, doi:10.1177/0022034516679396

11. Gallo LM, Iwasaki LR, Gonzalez YM, et al. Diagnostic group differences in temporomandibular joint energy densities. Orthod Craniofac Res 2015;18 Suppl 1(0 1):164-9, doi:10.1111/ocr.12074

12. Gallo LM, Fankhauser N, Gonzalez YM, et al. Jaw closing movement and sex differences in temporomandibular joint energy densities. J Oral Rehabil 2018;45(2):97-103, doi:10.1111/joor.12588

13. Gonzalez Y, Iwasaki LR, Mccall Jr WD, et al. Reliability of electromyographic activity vs. bite-force from human masticatory muscles. European Journal of Oral Sciences 2011;119(3):219-224, doi:10.1111/j.1600-0722.2011.00823.x

14. Iwasaki LR, Gonzalez YM, Liu H, et al. A pilot study of ambulatory masticatory muscle activities in temporomandibular joint disorders diagnostic groups. Orthod Craniofac Res 2015;18 Suppl 1(0 1):146-55, doi:10.1111/ocr.12085

15. Raphael KG, Sirois DA, Janal MN, et al. Sleep bruxism and myofascial temporomandibular disorders: a laboratory-based polysomnographic investigation. J Am Dent Assoc 2012;143(11):1223-31, doi:10.14219/jada.archive.2012.0068

16. Iwasaki LR, Liu H, Gonzalez YM, et al. Modeling of muscle forces in humans with and without temporomandibular joint disorders. Orthodontics & Craniofacial Research 2015;18(170-179, doi:10.1111/ocr.12075

17. Schwarz PB, Mir S, Peever JH. Noradrenergic modulation of masseter muscle activity during natural rapid eye movement sleep requires glutamatergic signalling at the trigeminal motor nucleus. J Physiol 2014;592(16):3597-609, doi:10.1113/jphysiol.2014.272633

18. Shaffer F, Ginsberg JP. An Overview of Heart Rate Variability Metrics and Norms. Front Public Health 2017;5(258, doi:10.3389/fpubh.2017.00258

19. Li K, Rudiger H, Ziemssen T. Spectral Analysis of Heart Rate Variability: Time Window Matters. Front Neurol 2019;10(545, doi:10.3389/fneur.2019.00545

20. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology. Eur Heart J 1996;17(3):354-81

21. Gronfier C, Simon C, Piquard F, et al. Neuroendocrine processes underlying ultradian sleep regulation in man. J Clin Endocrinol Metab 1999;84(8):2686-90, doi:10.1210/jcem.84.8.5893

22. Iwasaki LR, Gallo LM, Markova M, et al. Night-time autonomic nervous system ultradian cycling and masticatory muscle activity. Orthod Craniofac Res 2019;22 Suppl 1(107-112, doi:10.1111/ocr.12267

23. Nickel JC, Gonzalez YM, Wu Y, et al. Chronic Pain-Related Jaw Muscle Motor Load and Sensory Processing. J Dent Res 2022;101(10):1165-1171, doi:10.1177/00220345221099885

24. Von Korff M, Ormel J, Keefe FJ, et al. Grading the severity of chronic pain. Pain 1992;50(2):133-149, doi:10.1016/0304-3959(92)90154-4

25. Forte G, Troisi G, Pazzaglia M, et al. Heart Rate Variability and Pain: A Systematic Review. Brain Sci 2022;12(2), doi:10.3390/brainsci12020153

26. Chichorro JG, Porreca F, Sessle B. Mechanisms of craniofacial pain. Cephalalgia 2017;37(7):613-626, doi:10.1177/0333102417704187

27. Hardeland R. Melatonin and the pathologies of weakened or dysregulated circadian oscillators. J Pineal Res 2017;62(1), doi:10.1111/jpi.12377

28. Tchivileva IE, Hadgraft H, Lim PF, et al. Efficacy and safety of propranolol for treatment of temporomandibular disorder pain: a randomized, placebo-controlled clinical trial. Pain 2020;161(8):1755-1767, doi:10.1097/j.pain.0000000000001882

29. Gonzalez YM, Nickel JC, Scott JM, et al. Psychosocial Scores and Jaw Muscle Activity in Women. J Oral Facial Pain Headache 2018;32(4):381-388, doi:10.11607/ofph.2133

30. Maier SF, Watkins LR. Immune-to-central nervous system communication and its role in modulating pain and cognition: Implications for cancer and cancer treatment. Brain Behav Immun 2003;17 Suppl 1(S125-31, doi:10.1016/s0889-1591(02)00079-x

31. Raghavendra V, Tanga F, DeLeo JA. Inhibition of microglial activation attenuates the development but not existing hypersensitivity in a rat model of neuropathy. J Pharmacol Exp Ther 2003;306(2):624-30, doi:10.1124/jpet.103.052407

32. Sawicki CM, Kim JK, Weber MD, et al. Microglia Promote Increased Pain Behavior through Enhanced Inflammation in the Spinal Cord during Repeated Social Defeat Stress. J Neurosci 2019;39(7):1139-1149, doi:10.1523/JNEUROSCI.2785-18.2018