Contribution of Behavior, Psychosocial, and Physiological Domains in the Progression of Temporomandibular Disorders

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Abstract:

Title: Contribution of Behavior, Psychosocial, and Physiological Domains in the Progression of Temporomandibular Disorders (TMD)

Objective: The primary objective of this pilot study was to evaluate if there was a relationship between autonomic nervous system (ANS) activity during sleep, as quantified by a time domain measure of heart rate variability (SDNN), and masticatory muscle activity, as measured by the percent of the time a given muscle was active (duty factor). A secondary objective of this investigation was to analyze if anxiety (GAD-7), depression (PHQ-9), and physical symptoms (PHQ-15) scores, were correlated with ANS and masticatory muscle activity during sleep.

Materials and Methods: Adults that were ≥18 years of age were recruited as subjects if they were in good oral and overall health. Informed consent was obtained. Subjects completed GAD-7, PHQ-9, PHQ-15 questionnaires, and performed biting tasks to calibrate masseter and temporalis muscle electrical activities per Newton of biting force. Subjects were trained to use portable recording equipment for use in their own homes. Night-time electromyography (EMG) and electrocardiography (ECG) recordings were collected over three nights for ≥ 6 hours per night. Masseter and temporalis nocturnal duty factors were calculated for each subject. Subjects were empirically categorized into A or B Groups, with relatively high (≥1.5%) or low duty factors (<1.5%), respectively. Nocturnal ultradian cycling of ANS activity was determined by quantifying ECG derived heart rate variability measures of low (sympathetic) and high (parasympathetic) frequency spectral powers. A polynomial regression was fit to data in order to identify peaks and valleys of ANS ultradian cycling. Over 20-minute epochs at two peaks and two valleys of ultradian cycling, time domain standard deviations of inter-beat intervals (SDNN) were quantified. Linear regressions were created for each subject to compare masticatory muscle

duty factors (%) and heart rate variability (SDNN). Independent samples T-tests were used to assess differences between Groups A and B regression of slopes and y-intercepts from muscle duty factor versus normalized SDNN regressions. Independent samples T-test were also used to compare slopes of regressions between subjects with normal and raised anxiety, or depression, or physical symptoms scores. A p-value of ≤ 0.05 was considered significant.

Results: Data were collected from 16 subjects, six males and ten females. Complete EMG and ECG recordings were missing from two subjects, thus eight were assigned to Group A and six to Group B. Three subjects had mild and two had moderate anxiety scores. Two subjects had mild depression scores, five subjects had low and three subjects had medium physical symptoms scores. Group B subjects had significantly higher muscle duty factor versus normalized SDNN regression slopes for both the masseter ($p=0.01$) and temporalis ($p=0.01$) muscles compared to Group A. Group B subjects had significantly lower regression y-intercepts compared to Group A for both muscles ($p<0.02$). Subjects with increased anxiety had significantly lower duty factor versus normalized SDNN regression slopes for the masseter muscle ($p=0.03$). No significant differences were found for muscle duty factor versus normalized SDNN regressions between subjects with increased depression, or increased physical symptoms, and subjects with normal scores.

Conclusions: Group A subjects with higher night-time muscle duty factors had lower regression slopes and higher y-intercepts compared to Group B subjects. A lower muscle duty factor versus normalized SDNN regression slope correlated with maintaining either high sympathetic or low parasympathetic activity at peaks and valleys of nocturnal ANS ultradian cycling. Modulating autonomic nervous system tone may be a potential therapeutic target in the treatment of

increased nocturnal muscle activity, thereby ameliorating muscle activity as a contributing factor to TMD-related pain.

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1. Background and Significance:

Temporomandibular disorders (TMD) are a group of orofacial (face, head, or neck) conditions involving the temporomandibular joint (TMJ), the masticatory muscles, and the contiguous tissue components (1). Between 5-12% of the population is afflicted by these disorders, a majority of whom are young females (2). Historically, orthodontics has been blamed for causing TMD, but has also been touted as being protective against TMD (3). Although there is a lack of evidence to support the etiological and corrective roles of orthodontics (4, 5), due to the high prevalence of TMD, orthodontists are likely to encounter patients suffering from the disease. TMD is costly to both individuals and society because current therapies are not evidence-based and fail to prevent or cure symptoms predictably (6).

Historically, dentists have considered night-time oral behaviors, such as bruxing and clenching, as etiological to the development of chronic pain associated with TMD (7), and self-reported data support this concept (8, 9). However, sleep data from both sleep laboratories and natural environments has shown that bruxing and clenching at high levels of muscle activations are rare in both subjects with and without TMD (7). Raphael et al. in 2012 found similar rates of sleep bruxism between patients with and without myofascial TMD when observed for two nights using laboratory polysomnography. They also found similar levels of sub-threshold sleep bruxism between the two groups, suggesting that patients with TMD do not have a greater incidence of sleep bruxism (10). Iwasaki et al. in 2015 found that bruxing and clenching rarely occurred in patients with TMD symptoms (11). The low magnitudes and durations of masticatory muscle use suggest that other factors may be more important in the development of TMD than night-time bruxing and clenching (7).

Mechanobehavior and the TMJ:

The mandibular condyle and articular eminence contain cartilages, which are sensitive to their mechanical environments (12). In 1986, Copray et al. demonstrated in rats that the mandibular condylar cartilage was not a primary growth center. It was, instead, a secondary growth site that was affected by magnitudes and frequencies of loads on the cartilage. The mechanics of how the mandibular cartilage was loaded affects the mitotic rates of extracellular matrix synthesis by secondary cartilage cells (13-15). The mechanical environment of the TMJ evolves with age and changes with various behaviors (7). The cells of the secondary cartilage of the condyle respond to loading conditions and determine the morphology of the TMJ and may contribute to the development of degenerative joint disease in the second and third decades of life (7). The osseous changes that occur in this age group occur over a decade earlier than in other human joints (16).

Dynamic stress concentrations within the TMJ depend on individual joint-specific congruity and movements during jaw functions (7). The TMJ disc matrix is susceptible to early mechanical fatigue (17, 18), which is compounded by the unique physiological susceptibility of the TMJ fibrocartilage cells (7). Alterations to the mechanical environment of the TMJ could be indicative of degenerative joint changes (19, 20). A better understanding of the mechanical environment and its effects on the degeneration of the TMJ may improve treatments aimed at preventing or reversing degenerative joint disease (7).

Autonomic Nervous System and Heart Rate Variability:

The autonomic nervous system (ANS) affects both afferent (sensory) and efferent (motor) pathways. The afferent pathway transmits sensory information from the periphery to the central nervous system (CNS) (21). The efferent pathway is responsible for modulating circadian cycles, sleep stages, and hearty rhythmicity (22). During nonrapid eye movement (NREM) sleep,

the cardiovascular system is stable and parasympathetic (cholinergic) cardiac modulation is stronger. During rapid eye movement (REM) sleep, the cardiovascular system is unstable and greatly influenced by surges in sympathetic (noradrenergic) activity (23-26).

Heart rate variability is the physiological phenomenon of variation in heart beats (27). It is a measure of neurocardiac function and is generated by heart-brain interactions and dynamic non-linear ANS processes (28). Heart rate variability reflects regulation of autonomic balance, blood pressure, gas exchange, gut, heart, and vascular tone, and possibly facial muscles (28). Short term heart rate variability is generated by the relationship between the sympathetic and parasympathetic branches of the ANS.

Heart rate variability analysis, based on the heart's sinus rhythm, is done using electrocardiography (ECG) data collected at 250 Hz (27) and where the electrical signals from the heart are traced versus time. The largest deflections in the heartbeat tracing represent the ventricular depolarization and muscle contraction, which are defined by a recognizable pattern called the QRS complex (Figure 1), and where the time between the peak R-waves are identified (RR) as the inter-beat intervals (Figure 1) (27). These data can be separated into components that operate within different frequency ranges (29, 28). The two critical frequency domain parameters obtained from spectral analysis are low frequency (LF) power (0.04-0.15 Hz) which represents sympathetic influences, and high frequency (HF) power (0.15-0.4 Hz) which reflects the modulation of vagal (parasympathetic) tone (30, 27). LF/HF ratio indicates balance between sympathetic and vagal tones (27). The standard deviation of the inter-beat interval of normal sinus beats (SDNN) is a time-domain index that quantifies the amount of heart rate variability over a monitoring period (31). Both the sympathetic and parasympathetic nervous systems contribute to the SDNN, and the SDNN is highly correlated with ultra-low frequency, very low

frequency, and low frequency band power, as well as with total power (31). Sympathetic versus parasympathetic spectral power ratios during sleep mark the recurrent periods known as ultradian cycling (32).

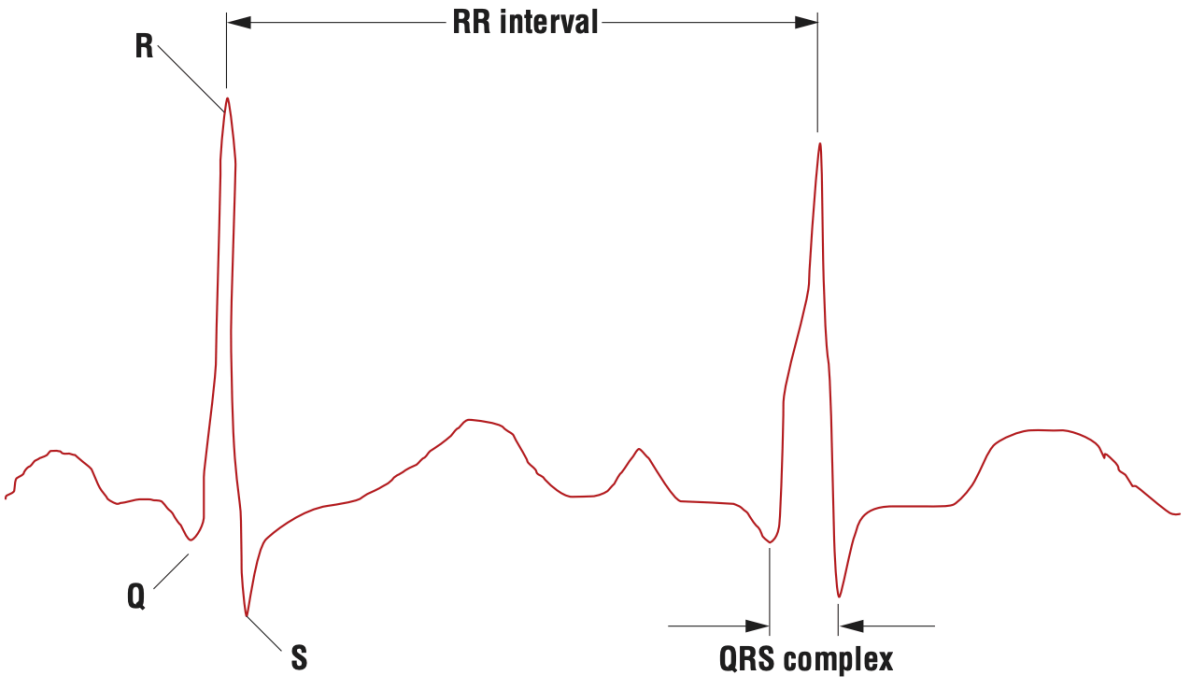

Figure 1 (33): An example of the graphic representation of the electrical current flowing through electrocardiogram (ECG) leads containing information about the heart's rhythm. QRS complex represents the ventricular depolarization that occurs during each heartbeat. The RR interval indicates the time between heartbeats (33). ECG information is used to perform heart rate analysis.

Heart rate variability analysis allows for an accurate and detailed determination of the functional regulatory characteristics of the ANS (34), and it is a good indicator for the non-invasive assessment of ANS activity in response to psychophysiological stress (35).

Psychological states may have an impact on sympathovagal balance in the absence of any palpable changes in heart rate or respiration rate (36).

Jaw Behavior and ANS Tone:

Excitatory noradrenergic neurotransmission in the brainstem is closely coupled to changes in muscle activity across the sleep-wake cycle (37). Secretion of noradrenaline is tightly correlated to changes in motor activity (38). One of the principal roles of noradrenaline is to enhance the gain of motoneuron output in response to excitatory transmitter input (39-41). Noradrenaline can potentiate the excitatory actions of glutamate on motoneurons and amplifies endogenous glutamatergic transmission (37, 42).

The masseter, along with the temporalis muscle, is innervated by the trigeminal nerve. The masseter and temporalis muscles are two of the four muscles of mastication. This trigeminal nerve receives both glutamatergic (43-45) and noradrenergic (46, 47) stimulation. It has been previously shown that the masseter muscle's motor behavior during waking is partially recapitulated during active REM sleep (48).

Iwasaki et al. have demonstrated that ANS tone, as measured by the ratio of LF/HF spectral powers, is significantly correlated with low-level jaw muscle activity during sleep. Higher masticatory muscle activity durations at low magnitude, as measured by electromyography (EMG) were positively correlated with higher LF/HF spectral power ratios. The former were measured via jaw muscle duty factors (DF), which is the duration of muscle activity above a certain threshold during a period of recording (duration of muscle activity/duration of recording period x100, %). (49). In 2019, Iwasaki et al. found in a pilot study that night-time parasympathetic spectral power differences were significantly different between low and high-risk subjects from four nights worth of EMG/ECG recordings. The seven subjects

were categorized as low and high risk for systemic inflammation (49). Those with relatively increased sympathetic or decreased parasympathetic spectral powers, defined as high ANS tone, had longer durations of low-level masseter and temporalis muscle activity (49). Highest average duty factors were associated with 1-2 N of bite-force and showed cumulative masseter and temporalis activities of 9.2 and 8.8 seconds/20-minute epoch, respectively (49). Recently, animal studies have shown that low-level masseter and temporalis EMG cycling occurs alongside ANS ultradian cycling, further suggesting that masticatory muscle activities reflect ANS function (37).

ANS dysregulation or high sympathetic tone, marked by the increase in sympathetic spectral powers combined with reduced parasympathetic spectral powers, leads to the production of inflammatory cytokines from glial cells which increase the excitability of peripheral afferents in the trigeminal ganglia and secondary interneurons in the trigeminal subnucleus caudalis (51-54). The increase in sympathetic tone is associated with increased excitability of masticatory motoneurons through dopamine and noradrenergic enhanced responses of motoneurons to glutamate (37, 55).

Anxiety and Depression and ANS Tone:

Psychosocial state is associated with the onset and development of chronic pain (56, 57). The development of chronic TMD-related pain is associated with somatization, anxiety, and depression (58, 59). Abnormal ANS tone and Hypothalamic-Pituitary Axis (HPA) axis dysregulation promote chronic inflammation and pain (60-63). ANS dysregulation produces both central and peripheral neuroplastic changes via astroglia cells, particularly in the trigeminal subnucleus caudalis and the peripheral trigeminal ganglia satellite glial cells, affecting non-nociceptive and nociceptive afferent processing (64-69). The activation of microglia by insults or stress results in profound morphological and secreted molecular profile changes that could

influence neuronal plasticity and behavior (64). Chronic pain or stress has an effect on microglia which influence anxiety and depression, which in turn could affect ANS tone.

In order to assess how chronic anxiety and pain could alter ANS tone and how it relates to TMD, it is necessary to first obtain proper diagnosis of patients with TMD. The Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD) were first published in 1992 (70). The criteria were split up into Axis I diagnoses, or physical assessments, and Axis II diagnoses, or assessments of psychosocial status and pain-related disability (70). Axis I diagnoses were validated in 2010 (71) and Axis II diagnoses were validated in 2002 (72). As a result of the validation studies, Axis I was modified to improve reliability but the original Axis II assessments, which were found to be reliable, remained the same. The criteria were revised and renamed the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) in 2014 (73). This classification system was based on the biopsychosocial model of pain, incorporating both physical and psychosocial components in a diagnosis (Figure 2) (73). Axis II is comprised of several different domains, including surveys to quantify anxiety, depression, and physical symptoms of pain (Figure 3) (74). The presence of significant psychosocial distress should be considered as a particularly important comorbid condition contributing to TMD onset as well as being associated with chronic TMD pain (75, 76).

Clinical and Research Applications of Selected DC/TMD Axis I and Axis II Tests

	Axis I: Physical diagnosis		Axis II: Psychosocial status	
	Pain diagnoses	**Joint diagnoses**	**Distress and pain disability**	
Application	**Clinical or research**		**Clinical**	**Clinical or research**
Screening test	TMD pain screener	DC/TMD for disc displacements, degenerative joint disease, and sublaxation	PHQ-4 and GCPS	PHQ-9, GAD-7, PHQ-15, and GCPS
Confirmatory test	DC/TMD for myalgia, arthralgia, and headache attributed to TMD	Imaging: MRI for disc displacements, CT for degenerative joint disease, and panoramic radiographs, MRI, or CT for sublaxation	Consultation with mental health provider	Structured psychiatric or behavioral medicine interview

Patient Health Questionnaire-4 (PHQ-4), Graded Chronic Pain Scale (GCPS), Patient Health Questionnaire-9 (PHQ-9), Generalized Anxiety Disorder-7 (GAD-7), Patient Health Questionnaire-15 (PHQ-15).

Figure 2: Components of the current Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) Axis I and Axis II tests (73). The DC/TMD is used for the diagnosis of TMD, with Axis I diagnoses containing physical assessments of the joint and pain, and Axis II diagnoses containing assessments of psychosocial status and pain-related disability. For research purposes, Axis II screening tests can be used to classify subjects with questionnaires. Generalized Anxiety Disorders-7 (GAD-7) is a 7-item questionnaire validated to screen for anxiety. Patient Health Questionnaire 9 (PHQ-9) is a 9-item questionnaire validated to screen for depression. Patient Health Questionnaire 15 (PHQ-15) is a 15-item questionnaire validated to screen for somatization (physical symptoms).

Recommended Axis II Assessment Protocol

Domain	Instrument	No. of items	Screening	Comprehensive
Pain intensity	Graded Chronic Pain Scale (GCPS)	3	✓	✓
Pain locations	Pain drawing	1	✓	✓
Physical function	Graded Chronic Pain Scale (GCPS)	4	✓	✓
Limitation	Jaw Functional Limitation Scale-short form (JFLS)	8	✓	
	Jaw Functional Limitation Scale-long form (JFLS)	20		✓
Distress	Patient Health Questionnaire-4 (PHQ-4)	4**	✓	
Depression	Patient Health Questionnaire-9* (PHQ-9)	9**		✓
Anxiety	Generalized Anxiety Disorder-7 (GAD-7)	7**		✓
Physical symptoms	Patient Health Questionnaire-15* (PHQ-15)	15		✓
Parafunction	Oral Behaviors Checklist (OBC)	21	✓	✓

*The RDC/TMD depression and non-specific physical symptoms instruments could be substituted for the PHQ-9 and PHQ-15, respectively, if continuity with legacy data is important.
**Each of the PHQ-4, PHQ-9, and GAD-7 include one additional item beyond the number listed above; the additional item is a global reflective question regarding functional interference due to any of the endorsed symptoms on that instrument.

Figure 3: List of the domains included in the Axis II of the Research Diagnostic Criteria for Temporomandibular Disorders (RDC/TMD), now called the Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) (74). The current Diagnostic Criteria for Temporomandibular Disorders (DC/TMD) are used for the diagnosis of TMD and contains both Axis I diagnoses and Axis II diagnoses. Axis I diagnoses assess physical assessments of the joint and pain, and Axis II diagnoses are assessments of psychosocial status and pain-related disability. This chart outlines all of the components of the Axis II diagnoses. For research purposes, Axis II screening tests can be used to classify subjects with questionnaires. Generalized Anxiety Disorders-7 (GAD-7) is a 7-item questionnaire validated to screen for

anxiety. Patient Health Questionnaire 9 (PHQ-9) is a 9-item questionnaire validated to screen for depression. Patient Health Questionnaire 15 (PHQ-15) is a 15-item questionnaire validated to screen for physical symptoms.

Trigeminal motoneurons are under the control of the neurotransmitter glutamate. Glutamate is responsible for regulating muscle tone throughout various behavioral states, such as waking state, NREM sleep, and REM sleep (77). REM sleep can be broken down into two components: tonic and phasic REM. Tonic REM is a parasympathetically driven state, whereas phasic REM is sympathetically driven. In 2008, Burgess et al. demonstrated that basal masseter muscle tone is tightly coupled to changes in behavioral state (77), and that glutamate was contributing to the pattern of muscle tone during wakefulness, NREM sleep, and phasic REM sleep, but not during tonic REM sleep. Gonzalez et al. in 2018 demonstrated, using the DC/TMD to assign psychosocial scores to 67 subjects, 31 with elevated psychosocial scores compared to 36 with normal scores, that those with elevated psychosocial scores showed significantly higher jaw muscle duty factors at low magnitudes of jaw loading. (78). This may be due to ANS dysregulation, which increases motoneuron excitability which could lead to physical symptoms (54). In 2014, using the experience sampling method, Glaros et al. showed that participants with more frequent and severe headaches reported significantly higher levels of depression and general somatization, and significantly more participants who experienced headaches reported myofascial pain (59).

Heart Rate Variability and Masticatory Muscle Activities:

Rhythmic masticatory muscle activities and sleep bruxism events are related to the activation of the central and autonomic nervous systems (79). Transient cortical arousals and

increased sympathetic activity contribute to increased cortical excitatory input to the central pattern generator through the cortico-bulbar projection (79). Increases in sympathetic activity have been shown to occur prior to sleep bruxism episodes which are followed by an increase in cortical alpha and theta wave electroencephalographic activities along with an increase in heart rate and arterial blood pressure (9, 80). Data from Zhong et al. in 2019 suggest that heart rate increases associated with rhythmic masticatory muscle activations may be intrinsic to the cortical arousal response and autonomic activation (79).

Animal and human studies of ANS dysregulation demonstrated that night-time masticatory muscle use may also reflect parasympathetic and sympathetic activities (37, 81). The research of Cady et al. in 2014 showed that sympathetic hyperactivity in rats produced inflammation in glial cells and hyper-excitability of primary and secondary nociceptive neurons in the trigeminal ganglia and the spinal tract subnucleus caudalis (82). This ANS dysregulation could be the cause of the development of chronic myalgia and mechanical allodynia of TMD, rather than bruxing and clenching (49).

Measuring ANS tone through ECG, and how it relates to night time masticatory muscle use may help to explain why bruxing and clenching at high levels of muscle activations are rare in patients both with and without TMD. This may also help to explain how muscle use is modulated by the ANS and help address gaps that currently exist in knowledge, diagnosis, and treatment of TMD.

2. Purpose:

There were two primary objectives of this study. The first objective was to test whether or not there was a correlation between the nocturnal ANS activity, as measured by the standard deviation of ECG inter-beat intervals (SDNN), and masticatory muscle activity, as measured by duty factors. The second primary objective was to test for diagnostic group differences in nocturnal masticatory muscle and ANS activity, as measured by muscle duty factor versus normalized SDNN regression slopes and y intercepts, where the diagnostic groups were categorized based on high and low masticatory muscle duty factors. A secondary objective of this investigation was to analyze if anxiety, physical symptoms, and depression were correlated with masticatory muscle duty factor versus normalized SDNN regression slopes. The underlying scientific rationale of the approaches used was based on the need to reduce heterogeneity of TMD diagnoses by elucidating the relationship between nocturnal ANS ultradian cycling, masticatory muscle activity, and psychosocial state.

3. Materials and Methods:

Subjects:

Subjects were recruited and gave informed consent to participate at the University at Buffalo School of Dental Medicine (UBSDM) in Buffalo, NY according to University Institutional Review Board (IRB) ethical standards and the Helsinki Declaration of 1975, revised in 1983. The Oregon Health & Science Institutional Review Board (IRB) was designated the lead IRB and approved the study protocols (Appendix A). Subject were recruited for inclusion in the study if they were ≥18 years of age. Inclusion criteria required subjects to be in good general and oral health as determined by reviewing medical history and performing an intra-oral exam. Exclusion criteria included signs of gross tissue inflammation or dental caries, missing dental restorations or missing teeth anterior to the second permanent molars, and inability to sleep while using portable equipment for recording ECG and EMG signals.

Subject Protocols:

DC/TMD Questionnaires:

DC/TMD Axis II data were collected at UBSDM during the initial visit. Specifically, subjects were asked to complete the Generalized Anxiety Disorders-7 (GAD-7), Patient Health Questionaire-9 and 15 (PHQ-9, PHQ-15) forms, for the assessment of anxiety, depression, and physical symptoms, respectively (Appendix B).

Laboratory EMG Recordings During Biting:

Subjects participated in two laboratory visits. During the first laboratory visit, a previously described protocol (Iwasaki et al., 2015, 2017) (11, 83) was used to calibrate masseter and temporalis muscle electrical activities per Newton of biting force. In brief, bilateral surface electrodes (Ambu Neuroline 720, Columbia, MD) were positioned on centers of the bellies

(centroids) the anterior temporalis and superficial masseter muscles, and a single ground electrode was attached to the mastoid process on the right side of the subject. The locations of centroids for electrode placement over masseter and temporalis muscles was determined by palpation during jaw clenching. As previously described (85), a calibrated bite-force transducer was sequentially placed between the right and left first molars, and between central incisors. For each of the 3 biting locations, each subject was asked to produce 5 static bites, each static bite lasting about 3 seconds in duration, with forces ranging from light to moderate as determined by the subject. No visual or auditory feedback was provided to the subject. Following static biting, each subject was asked to produce dynamic biting at 4 frequencies of 0.5, 1, 1.5, 2 Hz. These frequencies corresponded to a range of 30 beats per minute (BPM) to 120 BPM. During the dynamic biting tasks, subjects were instructed to begin biting lightly, and then to ramp up to moderate biting magnitudes. At each frequency, subjects produced approximately 10 to 15 dynamic bites.

Ambulatory EMG/ECG recordings:

With respect to self-recording of heart rate (ECG) and masticatory muscle activities (EMG) during sleeping (49), during the first laboratory visit, subjects were trained to apply surface electrodes and use portable recorders. For ECG recordings, subjects were taught to apply adhesive-backed, color-coded bipolar surface electrodes (Bio ProTech T715, Chino, CA) on the chest. For EMG recordings, subjects were shown how to place, bilaterally, bipolar surface electrodes (Ambu Neuroline 720, Columbia, MD) on clean skin over the masseter and temporalis muscles, and on the right mastoid process.

Subjects were taught how to connect all EMG and ECG leads to the portable recorder and were instructed to record for 3 nights at home, where the recording period was to be $\geq$ 6 hours

per night. As well, subjects were instructed to keep a diary of the date of recording, start and stop times, as well as the types of activities that occurred during the recording (ie eating of meals, vigorous kissing).

Second Laboratory Visit:

During the second laboratory visit, subjects returned equipment and recorded data. Recordings were verified for quality and completeness. If noise or other artifacts were present, or recordings were not ≥6 hours in duration, subjects were asked to re-record that session. During the second laboratory visit, subjects repeated the static and dynamic biting tasks performed during laboratory visit #1.

Data Analysis:

Psychosocial Scores and Group Assignment:

Based on the self-reported responses, subjects' individual cumulative scores were tallied for each psychosocial questionnaire according to the guidelines published by the DC/TMD scoring manual (84). No responses were missing for any subject. For assessment of the state of anxiety (GAD-7), a total sum score was computed for each subject. Scores less than 5 were considered normal. Any score equal to or greater than 5 was considered raised. For assessment of depression (PHQ-9), a total sum score was computed for each subject. Scores less than 5 were considered normal. Any score equal to or greater than 5 was considered raised. In assessment of physical symptoms (PHQ-15), a total sum score was computed for each subject. Scores less than 5 were considered normal. Any score greater than or equal to 5 was considered raised.

Ambulatory ECG Data:

The protocol for ECG data analysis began with the identification of peaks and valleys of night-time ultradian cycling of ANS spectral power ratios. Commercial software (MindWare®,

MindWare Technologies, Westerville, OH) was used to analyze heart rate variability (HRV) (49) in order to quantify frequency domain spectral powers of parasympathetic and sympathetic nervous system activity. The software analyzed the spectral powers over successive 5-minute segments of each ECG recording. Fast Fourier transform methods were used to calculate the spectral power (ms^2) within two frequency ranges. Low frequency (LF, 0.04-0.15 Hz) spectral power measured sympathetic nervous system tone. High frequency (HF, 0.15-0.4 Hz) spectral power quantified parasympathetic nervous system activity. The ratio of sympathetic/parasympathetic (LF/HF) spectral powers were plotted versus time for each night-time recording. Time-dependent changes in the LF/HF spectral power ratio during recording periods were characterized by fitting a 9^{th} order polynomial regression to the spectral power ratio data. By this method it was possible to locate the peaks and valleys of ANS ultradian cycling for a given subject and recording (49). Using the polynomial regression characterization of ANS activity (Fig 4), peaks and valleys of ultradian cycles were identified and delineated by 20-minute windows. Within each 20-minute window for two peaks and two valleys, commercial software was used to quantify the standard deviation of the N-N (SDNN) inter-beat interval as a measure of autonomic nervous system activity. SDNN data were normalized, per each night-time recording, by dividing peak and valley SDNN data by peak values per recording.

Ambulatory EMG Data and Jaw Muscle Duty Factors:

Biting task data, which quantified subject specific muscle activity (mV) per N of biting force was used to analyze the ambulatory EMG data. More specifically, masseter and temporalis muscle activities were quantified according to a percentage of muscle activity required, per subject, to produce a bite force of 20 N. The muscle activity per bite force laboratory tasks were used to quantify magnitude ($\%T_{20N}$, μV) and duration (s) of load on the mandible in order to

calculate masticatory muscle duty factors during 20-minute windows associated with the peaks and valleys of ANS ultradian cycling. The inflection points (Fig 4) of the polynomial regression of the spectral power ratio data identified the peaks and valleys. Twenty-minute epochs, defined by ± 10 minutes around each inflection point, were used to sample and analyze nocturnal masseter and temporalis muscle activities. Within each 20-minute epoch, masseter and temporalis EMG data were processed to detect, delimit, and calculate Root Mean Square (RMS) (mV) values for EMG segments defined by 128-ms contiguous rectangular sliding Hamming windows. As previously described (85, 11), and briefly reviewed below, ambulatory EMG data from the 20-minute windows associated with ANS ultradian cycling were filtered to eliminate low-level noise and identify and exclude noisy or blank signals using commercial software (WavePad Sound Editor Master Edition). Customized programs (MATLAB 7.9 R2009b; MathWorks) were used to identify and count the number of 128 ms time windows per 20-minute epoch, where the RMS-EMG (mV) was within 3 thresholds: 1%-9% (1-2 N), 10%-24% (2-5 N), and 25%-49% (5-10 N) of the millivolt activity of given muscle in a subject to produce a 20 N bite force. Duty factors were quantified as a percentage of each 20-minute window. For each of the 3 thresholds, masseter and temporalis muscle duty factors for each recording were determined by adding the number of 128 ms windows, and dividing the cumulative time by each 20-minute window.

Given the limited number of subjects, for each subject, masseter and temporalis muscle duty factors for thresholds from 1 to 24% (1-5 N) and durations of ≤ 5 s, were pooled from all recordings. Within each muscle, duty factors were normalized relative to peak activity per muscle for each subject. For each subject, normalized muscle duty factor data were plotted versus normalized SDNN data from all recordings. Subject-specific linear regressions were used

to characterize the relationships between night-time masseter and temporalis muscle normalized duty factors versus normalized ANS data (Fig 5 as an example. For all linear regressions see Appendix C).

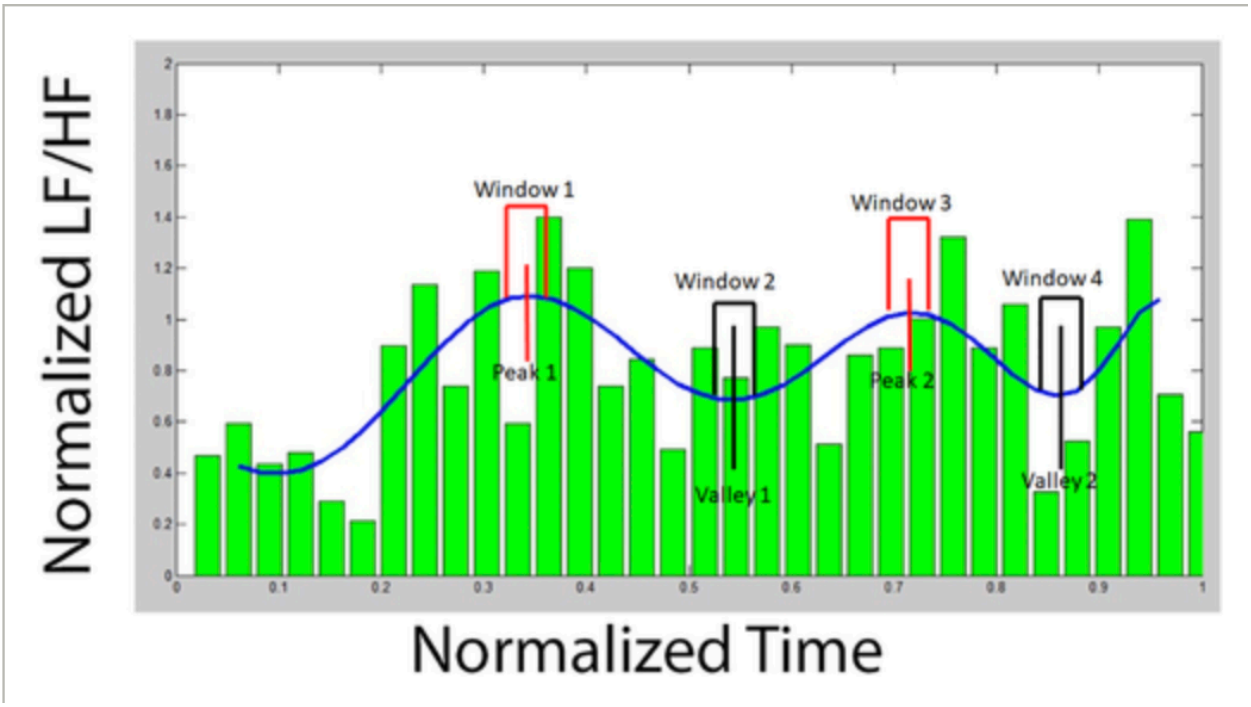

Figure 4 (49): This graph shows an example of sympathetic (low frequency, LF) versus parasympathetic (high frequency, HF) spectral power ratios from a single subject in the study by Iwasaki et al. (49). These data were obtained from a single subject's night-time ECG recording. Throughout the night, the nocturnal electrocardiography (ECG) recordings showed ultradian cycling of the sympathetic versus parasympathetic spectral power ratios obtained from Heart Rate Variability Analysis. This cycling was characterized by a polynomial fit to these data (blue line). Peaks and valleys in the cycles were identified and delineated by 20-minute epochs (Windows 1, 2, 3). Note that normalized data were used in this figure to simplify the illustration (49).

Masticatory Muscle Nocturnal Duty Factors:

Subjects were categorized into Groups A and B, based empirically on magnitudes of nocturnal duty factors, with A being relatively high duty factors and B being relatively low. Subjects with duty factors >1.5 % were assigned to Group A. Subjects with muscle duty factors ≤1.5 % were assigned to Group B (Table 5).

Statistics:

Linear regressions of normalized muscle duty factor versus normalized SDNN were created for each subject for each muscle (left and right masseter and temporalis), thus producing 4 regressions. Right and left data were combined and linear regression slopes and y-intercepts were calculated for the masseter and temporalis muscle in each subject.

Independent samples T-tests with equal variances assumed were used to compare A (High Duty Factor) and B (Low Duty Factor) Group regression slopes and y-intercepts.

Independent samples T-tests with equal variances assumed were used to test for significant differences in Muscle duty factor-ANS regression slopes between subjects with normal and raised psychosocial scores.

4. Results:

Demographics:

Data were collected from 16 subjects, six males and ten females (Table 1). Average age and standard deviation (SD) of males was 53.4 ± 12.7 years with a range from 37.4 to 70.2 years old. Average age and SD of females was 44.7 ± 11.7 years with a range from 32.3 to 66.0 years old. Temporalis EMG data were missing from Subject 11. EMG data were missing for both muscles of Subject 13. GAD-7 responses were missing from Subject 16, hence data from Subject 16 were excluded from the statistical analyses due to incomplete records.

Subject	Gender	Age	GAD-7	PHQ-9	PHQ-15	ANS-Mass Slope	ANS-Temp Slope	NT DF
1	M	55.8	Normal	Normal	Normal	0.53	0.45	A
2	F	40.7	Normal	Normal	Normal	1.0	0.85	A
3	F	66.0	Raised	Normal	Normal	0.72	0.77	A
4	F	34.7	Raised	Normal	Raised	0.45	0.55	A
5	F	60.9	Normal	Normal	Normal	0.74	0.82	A
6	F	54.4	Normal	Normal	Normal	1.23	1.12	B
7	F	33.5	Normal	Normal	Normal	1.71	1.14	B
8	F	37.5	Normal	Normal	Raised	1.00	0.65	B
9	F	51.3	Raised	Raised	Raised	0.79	0.76	B
10	F	36.0	Raised	Raised	Raised	0.84	0.94	B
11	M	40.3	Normal	Normal	Raised	0.83		B
12	M	37.4	Raised	Normal	Raised	0.76	0.69	A
13	M	48.6	Normal	Normal	Normal			
14	F	32.3	Normal	Normal	Raised	1.29	1.14	B
15	M	68.2	Normal	Normal	Raised	1.13	1.11	B
16	M	70.2		Normal	Normal	N/A	N/A	N/A

Table 1: Data by subject, gender (M =6 , F= 10), age (years) including Generalized Anxiety Disorders 7-item scale (GAD-7), Patient Health Questionaire-9 (PHQ-9), and Patient Health Questionaire-15 (PHQ-15), slope of masseter (Mass) and temporalis (Temp) muscle activity versus normalized SDNN (ANS) regressions and night-time duty factor Group (NT DF) where A = >1.5, B = ≤1.5, N/A = insufficient data.

Psychosocial Scores and Group Assignment:

Ten subjects (Subjects 1, 2, 5, 6, 7, 8, 11, 13, 14, 15) were classified as having normal anxiety based on their responses to the GAD-7 questionnaire, where scores where <5 (Table 2). Five subjects (Subjects 3, 4, 9, 10, 12) were classified as having raised anxiety, where scores were ≥5 (Table 2). Two subjects (Subjects 9 and 10) were classified as having raised depression based on the sum of their responses to the PHQ-9 questionnaire being ≥5 (Table 3). The other 12 subjects (Subjects 1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 16) were classified as normal because the sum of their responses to the PHQ-9 was <5. Eight subjects (Subjects 4, 8, 9, 10, 11, 12, 14, 15) were classified as having raised physical symptoms where scores were ≥5. Eight subjects (Subjects 1, 2, 3, 5, 6, 7, 13, 16) were classified as having normal physical symptoms based on their responses to the PHQ-15 questionnaire being <5 (Table 4).

Subject	GAD-7 Q1	GAD-7 Q2	GAD-7 Q3	GAD-7 Q4	GAD-7 Q5	GAD-7 Q6	GAD-7 Q7	GAD-7 SUM
1	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0
3	3	2	2	0	0	0	1	8
4	3	2	2	1	0	0	0	8
5	0	0	0	0	0	0	0	0
6	0	0	1	0	0	0	0	0
7	0	0	0	1	0	1	0	2
8	0	0	0	1	0	1	0	2
9	2	1	2	3	1	1	0	10
10	3	2	2	2	0	1	1	11
11	0	0	0	0	0	0	0	0
12	1	0	1	1	0	1	1	5
13	0	0	0	0	0	0	0	0
14	0	0	0	1	0	1	0	2
15	1	0	1	0	0	0	0	2
16								N/A

Table 2: Raw data obtained from Generalized Anxiety Disorders (GAD) 7-item questionnaire used to screen patients for anxiety and used in the diagnoses of TMD from the Diagnostic

Criteria of Temporomandibular Disorders (DC/TMD). Responses to all seven questions (for specific questions, see Appendix B) were summed for each subject.

Subject	PHQ-9 Q1	PHQ-9 Q2	PHQ-9 Q3	PHQ-9 Q4	PHQ-9 Q5	PHQ-9 Q6	PHQ-9 Q7	PHQ-9 Q8	PHQ-9 Q9	PHQ-9 SUM
1	0	0	0	0	0	0	0	0	0	0
2	0	0	0	0	0	0	0	0	0	0
3	0	1	0	1	1	1	0	0	0	4
4	0	0	1	1	0	1	0	0	0	3
5	0	0	0	0	0	0	0	0	0	0
6	0	0	0	0	0	0	0	0	0	0
7	0	0	0	0	0	0	0	0	0	0
8	0	1	0	2	0	0	0	0	0	3
9	0	0	3	1	1	0	0	0	0	5
10	1	0	2	2	1	0	3	0	0	9
11	0	0	0	0	0	0	0	0	0	0
12	0	0	1	1	0	1	0	0	0	3
13	0	0	0	0	0	1	1	0	0	2
14	0	0	0	1	1	0	0	0	0	2
15	0	0	1	1	0	0	0	0	0	2
16	0	0	0	0	0	0	0	0	0	0

Table 3: Raw data obtained from Patient Health Questionnaire 9, a 9-item questionnaire used to screen patients for depression and used in the diagnoses of TMD from the Diagnostic Criteria of Temporomandibular Disorders (DC/TMD). Responses to all seven questions (for specific questions, see Appendix B) were summed for each subject.

Subject	PHQ-15 Q1	PHQ-15 Q2	PHQ-15 Q3	PHQ-15 Q4	PHQ-15 Q5	PHQ-15 Q6	PHQ-15 Q7	PHQ-15 Q8	PHQ-15 Q9	PHQ-15 Q10	PHQ-15 Q11	PHQ-15 Q12	PHQ-15 Q13	PHQ-15 Q14	PHQ-15 Q15	PHQ-15 SUM
1	0	1	1	0	0	0	0	0	0	0	0	0	0	0	0	2
2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	2
4	0	0	0	1	1	0	0	0	0	0	0	1	1	1	1	6
5	0	1	2	0	0	0	0	0	0	1	0	0	0	0	0	4
6	0	1	0	0	1	0	0	0	0	0	0	0	0	0	1	3
7	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	1
8	1	2	0	0	1	0	0	0	0	0	0	0	2	0	2	8
9	0	1	1	0	1	0	0	0	0	0	0	2	2	2	2	11
10	2	2	0	1	0	0	0	0	0	0	0	2	2	1	2	12
11	1	1	1	0	1	0	0	0	0	0	0	0	1	0	0	5
12	1	0	0	0	0	0	0	0	0	0	0	1	1	1	1	5
13	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	2	1	1	2	1	0	0	0	0	0	0	2	1	1	0	11
15	0	1	1	0	1	0	0	0	0	0	0	0	0	0	1	4
16	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

Table 4: Raw data obtained from Patient Health Questionnaire 15, a 15-item questionnaire used to screen patients for physical symptoms and used in the diagnoses of TMD from the Diagnostic

Criteria of Temporomandibular Disorders (DC/TMD). Responses to all seven questions (for specific questions, see Appendix B) were summed for each subject.

EMG Data and Jaw Muscle Duty Factors by Subject:

Based on EMG muscle recordings, 6 subjects were assigned to Group A, or relatively high muscle duty factors, and 8 subjects were assigned to Group B, or relatively low muscle duty factors (Table 5).

Subject	Overall Average Masseter Muscle Duty Factor (%)		
	Per Night (7 hours)	Per Window (20 minutes)	Diagnostic Category
1	0.09	1.8	A
2	0.76	15.2	A
3	0.17	3.4	A
4	0.11	2.2	A
5	0.18	3.6	A
6	0.04	0.8	B
7	0.01	0.2	B
8	0.00	0.0	B
9	0.00	0.1	B
10	0.08	1.5	B

11	0.06	1.2	B
12	0.15	3.0	A
13	N/A	N/A	N/A
14	0.02	0.4	B
15	0.00	0.0	B
16	N/A	N/A	N/A

Table 5: Masseter night-time EMG data used to categorize subjects into Group A or Group B based empirically on magnitudes of nocturnal duty factors. Group A subjects had high muscle duty factors (>1.5%) and Group B had low muscle duty factors (≤1.5%) based on 20-minute windows.

Muscle Duty Factor versus Normalized SDNN:

R^2 values, Slopes, and Y-intercepts were obtained from muscle duty factor versus normalized SDNN regressions. See Appendix C for regressions for each subject and muscle.

Subject	Masseter R2	Masseter slope	Masseter Y int	Temporalis R2	Temporalis Slope	Temporlais Y int
1	0.689	0.53	0.26	0.520	0.45	0.23
2	0.571	0.95	-0.03	0.696	0.85	0.08
3	0.569	0.72	0.25	0.648	0.77	0.28
4	0.224	0.45	0.35	0.279	0.55	0.02
5	0.678	0.74	0.06	0.772	0.82	-0.06
6	0.467	1.23	0.09	0.366	1.12	0.10
7	0.538	1.71	-0.71	0.402	1.14	-0.18
8	0.731	1.00	-0.24	0.485	0.65	-0.01
9	0.438	0.79	-0.07	0.576	0.76	0.03
10	0.654	0.84	0.08	0.802	0.94	-0.08
11	0.797	0.83	0.12			
12	0.643	0.76	0.08	0.710	0.69	0.14
13						
14	0.774	1.29	-0.38	0.745	1.14	-0.33
15	0.618	1.13	-0.24	0.691	1.11	-0.22
16						

Table 6: R square (R2), slope, and Y-intercept of the muscle duty factor versus normalized SDNN regressions for each muscle group, by subject.

As examples of two temporalis and two masseter muscle regressions of normalized muscle duty factor versus normalized SDNN, Subject 15 is characteristic of the relatively low muscle duty factor Group B, with high masseter and temporalis regression slopes (1.13 and 1.11, respectively; Fig 5). Subject 12 is characteristic of the relatively high muscle duty Group A, with low masseter and temporalis slopes (0.76 and 0.69, respectively; Fig 5). Subject 15 also demonstrates lower y-intercepts of the regression, for both the masseter and temporalis muscles (-0.24 and -0.22, respectively). Subject 12 demonstrates higher y-intercepts for both the masseter and temporalis muscles (0.08 and 0.14, respectively).

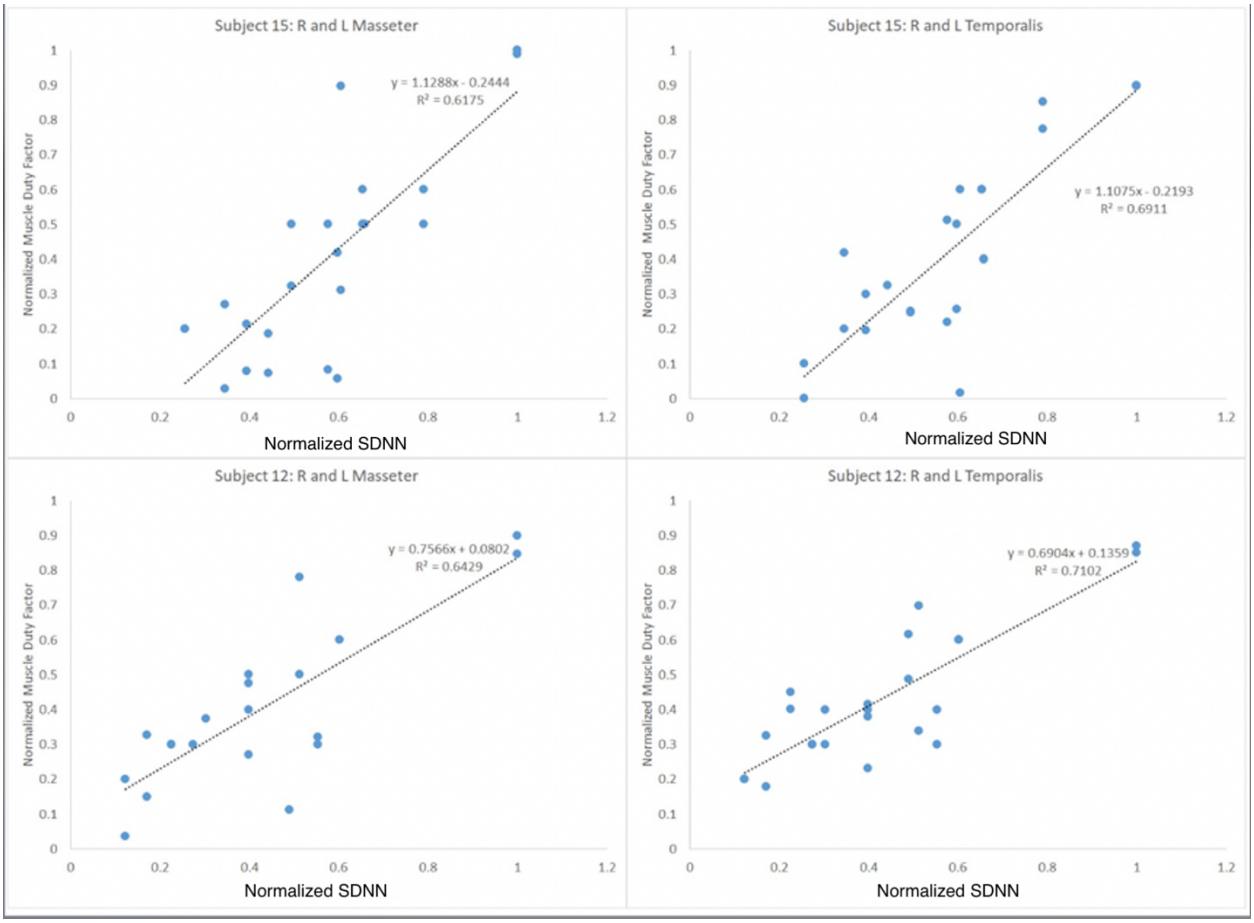

Figure 5: Example of two masseter and two temporalis muscle regressions of muscle duty factor versus normalized heart rate variability (Normalized SDNN). These data came from two subjects' night time electromyography (EMG) and electrocardiography (ECG) recordings. EMG and ECG data were used to build regressions with muscle duty factor on the vertical axis as the dependent variable and autonomic data (Normalized SDNN) was on the horizontal axis as the independent variable. Subjects were categorized into Groups A or B, based empirically on magnitudes of nocturnal duty factors, with A being relatively high duty factors and B being relatively low. Subjects with duty factors > 1.5 % were assigned to Group A and those ≤ 1.0 % were assigned to Group B. Subject 15 is characteristic of diagnostic Group B, with high masseter and temporalis ANS-Muscle Duty Factor slopes (1.13 and 1.11, respectively). Subject 12 is characteristic of diagnostic Group B with low masseter and temporalis ANS-Muscle Duty Factor slopes (0.76 and 0.69, respectively). Subject 15 also demonstrates lower y-intercepts of the regression, at -0.24 for the masseter and 0.22 for the temporalis muscle. Subject 12 demonstrates higher y-intercepts, at 0.08 for the masseter and 0.14 for the temporalis muscle.

Differences in Muscle duty Factor versus SDNN Regression Slopes Between Diagnostic Groups:

Group B subjects with relatively lower duty factors (lower muscle activity) were found to have significantly higher muscle duty factor versus normalized SDNN regression slopes for both the masseter ($p=0.01$) and temporalis ($p=0.01$) muscles when compared to Group A subjects with relatively higher muscle activities (Figure 6).

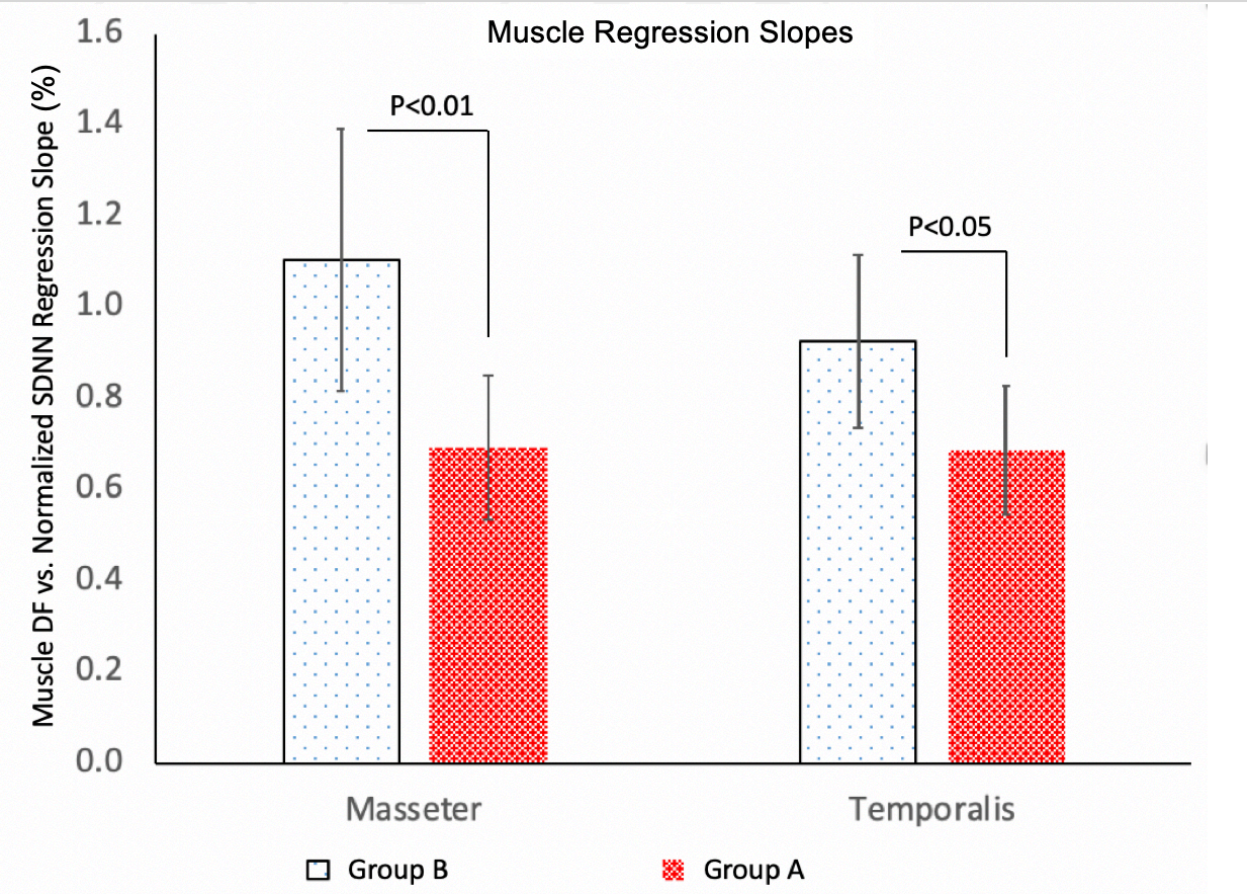

Figure 6: Independent samples T-tests with equal variances assumed were used to compare Muscle Duty Factor versus Autonomic Nervous System (Normalized SDNN) regression slopes by Groups A (Higher Duty Factor) versus B (Lower Duty Factor) for masseter and temporalis muscles (Appendix C and Table 5).

Differences in Muscle Duty Factor versus Normalized SDNN Regression Intercepts Between Diagnostic Groups:

Group B subjects with lower duty factors were found to have significantly lower masseter and temporalis muscle duty factor versus normalized SDNN regression y-intercepts compared to Group A subjects for both the masseter and temporalis muscles ($p<0.02$ for both muscle groups).

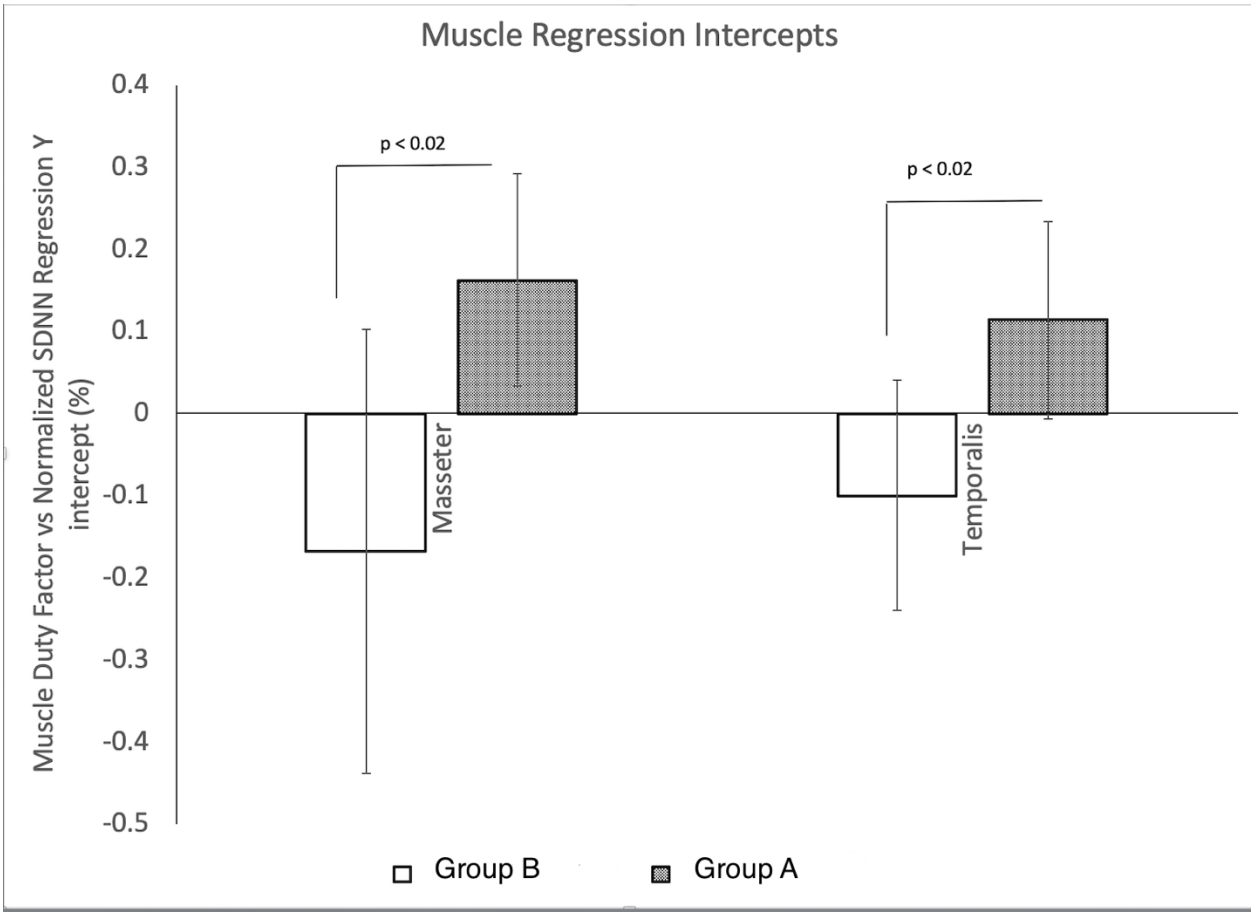

Figure 7: Independent samples T-tests with equal variances assumed were used to compare Muscle Duty Factor versus Normalized SDNN Regression y-intercepts by Groups A (Higher Duty Factor) versus B (Lower Duty Factor) for masseter and temporalis muscles (Appendix C and Table 5).

Psychosocial Scores and Muscle Duty Factor versus Normalized SDNN Regression Slopes:

GAD-7 (Anxiety):

Subjects with raised anxiety (n=5), had significantly lower muscle duty factor versus normalized SDNN regression slopes for the masseter muscle (p=0.02) compared to subjects with normal anxiety (n=10). No significant difference was found between subjects with raised and normal anxiety for the temporalis muscle duty factor versus normalized SDNN regression slopes (p=0.16). No subjects were found to have severe anxiety.

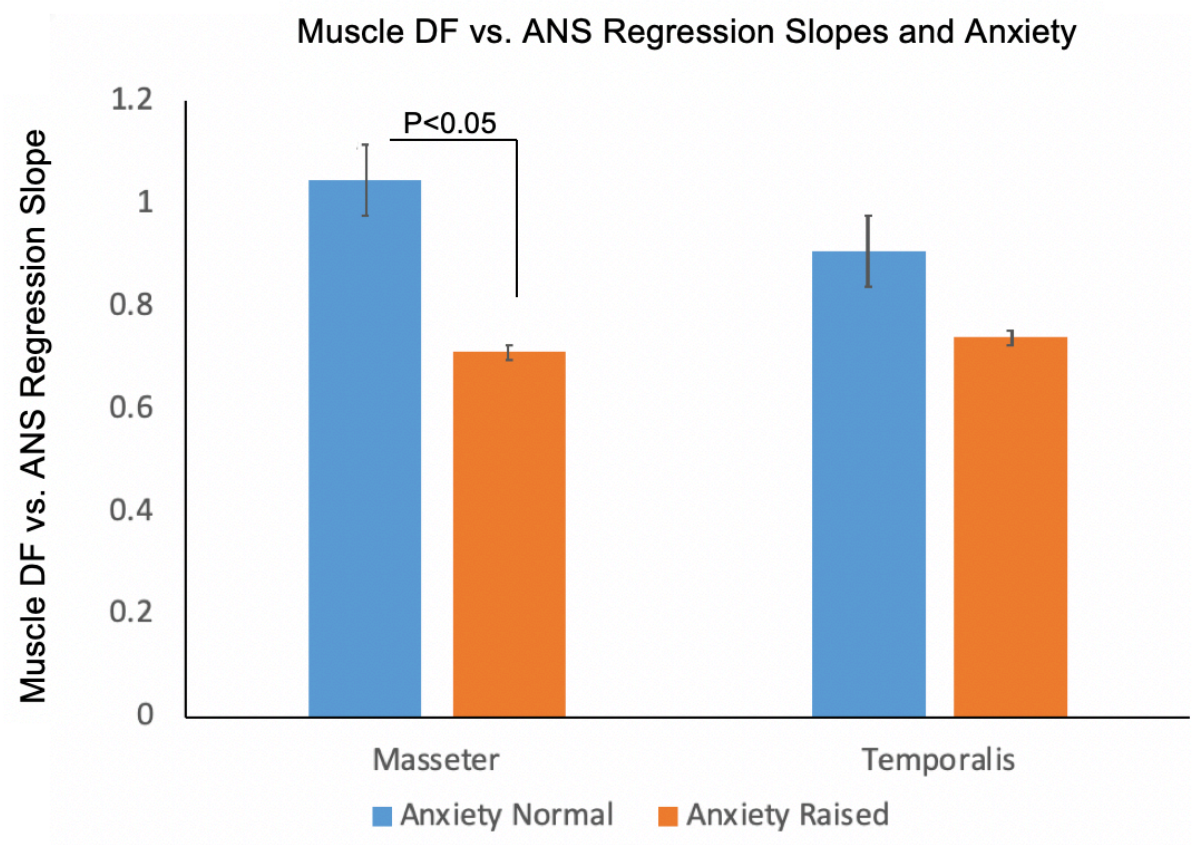

Figure 8: Independent samples T-tests with equal variances assumed were used to test for significant differences in masseter and temporalis muscle duty factor versus normalized SDNN regression slopes between subjects with normal (n=10) and raised (n=5) anxiety based on responses to Generalized Anxiety Disorders 7-item questionnaire (GAD-7).

PHQ-9 (Depression):

No significant difference was found between those with raised depression (n=2) and those with no depression (n=12) with respect to muscle duty factor versus normalized SDNN slope for either the masseter (p=0.3) or the temporalis (p=0.2) muscles. No subjects were found to have moderate or severe depression.

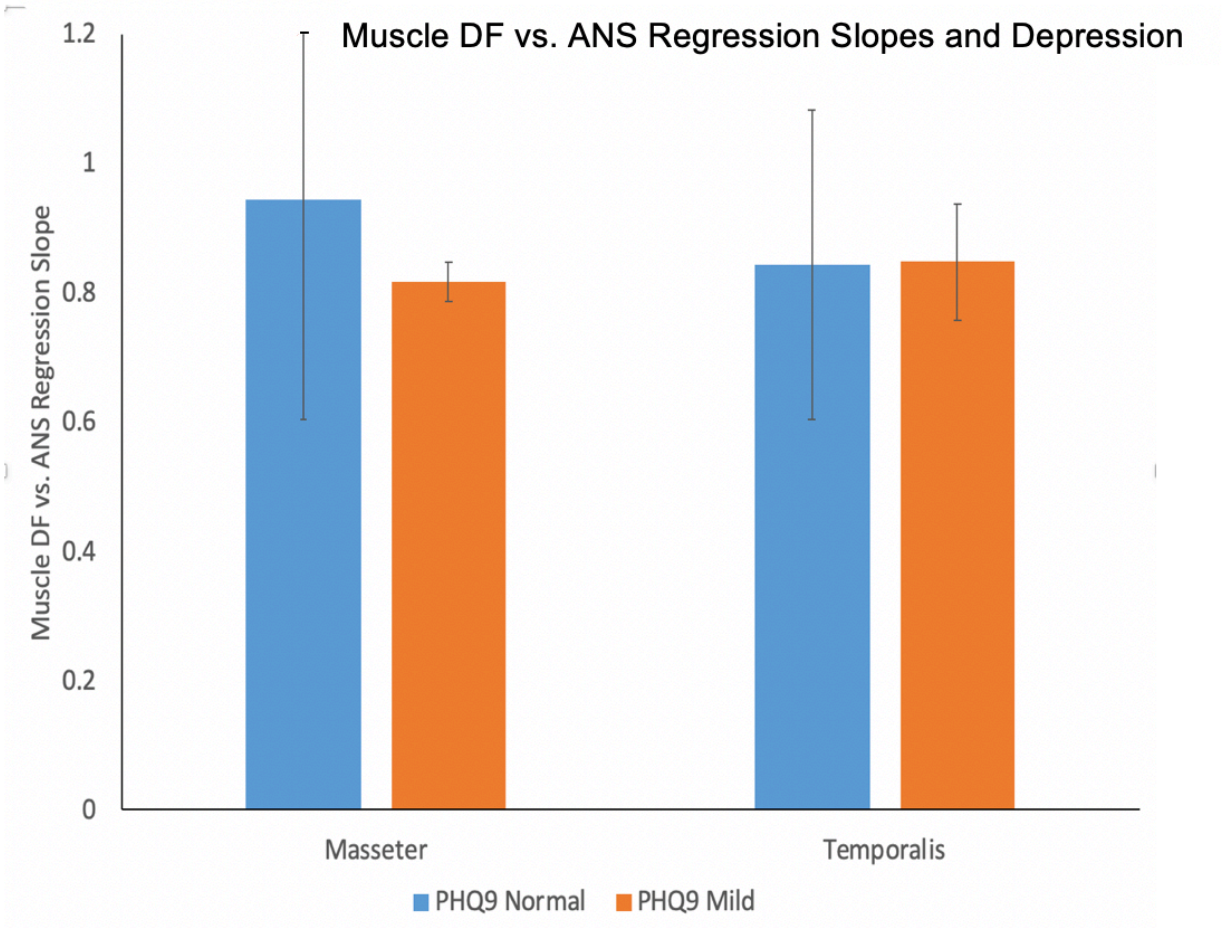

Figure 9: Independent samples T-tests with equal variances assumed were used to test for significant differences ($p \leq 0.05$) in muscle duty factor versus normalized SDNN regression slopes between subject with normal and raised depression based on responses to the Patient

Health Questionnaire 9-item questionnaire (PHQ-9). Two subjects were classified as having mild depression and the other 12 were classified as normal. No significant difference was found between those with mild depression and those with no depression with respect to muscle duty factor versus normalized SDNN regression slope.

PHQ-15 (Physical Symptoms):

No significant difference was found between subjects with increased physical symptoms (n=8) and those without (n=8) physical symptoms with respect to muscle duty factor versus normalized SDNN regression slopes for both the masseter (p=0.16) and temporalis (p=0.10) muscles. No subjects were found to have high physical symptoms.

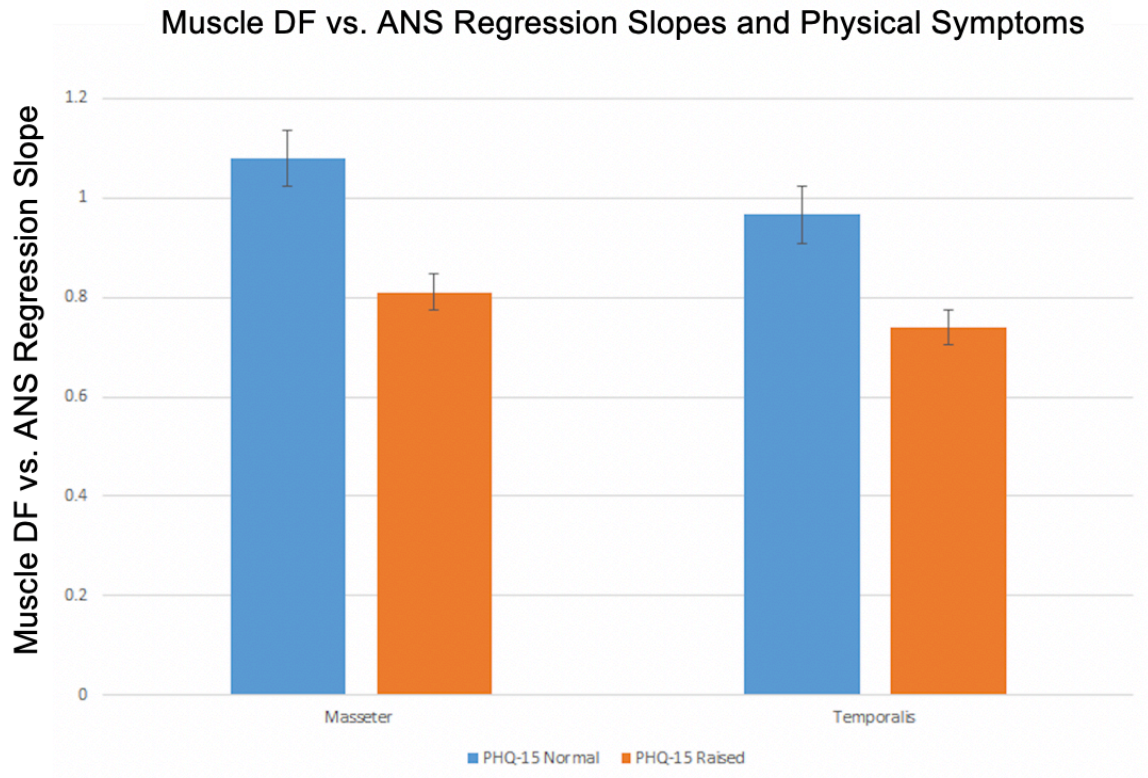

Figure 10: Independent samples T-tests with equal variances assumed were used to test for significant differences ($p \leq 0.05$) in muscle duty factor versus normalized SDNN regression

slopes between subjects with normal and raised physical symptoms based on responses to the Patient Health Questionnaire 15-item questionnaire (PHQ-15). Eight subjects were classified as having raised physical symptoms and eight were classified as having no physical symptoms based on their responses to the PHQ-15 questionnaire (Table 1). No significant difference was found between subjects with normal and raised physical symptoms with respect to muscle duty factor versus normalized SDNN regression slopes for both the masseter ($p=0.16$) and temporalis ($p=0.07$) muscles.

5. Discussion:

Group A subjects with higher night-time muscle duty factors had significantly lower muscle duty factor versus normalized SDNN regression slopes. A lower muscle duty factor versus normalized SDNN regression slope may be due to the maintenance of a higher sympathetic tone and higher muscle activity throughout ANS ultradian cycles. Group A patients also had significantly higher y-intercepts of the muscle duty factor versus normalized SDNN regressions and may be due to increased night-time sympathetic nervous system tone.

Central sympathetic activity has a direct impact on the production inflammatory cytokines (86). Similarly, stress responses that modulate sympathetic nervous system activity have a great impact on inflammation (86). Increased sympathetic activity affects both the motor and sensory branches of the autonomic nervous system.

Concerning the role of the sympathetic nervous system and masticatory muscle motor control, muscle spindles are specialized sensory receptors in most skeletal muscles and are sensitive to changes in muscle length (87). They receive both sensory and motor innervation. It has been shown in animal models that sympathetic activation influences spindle sensitivity and may affect motor control under stress conditions (87). Muscle spindles have been hypothesized

to be involved in the pathophysiology of chronic muscle pain syndromes. Radovanovic et al. were the first to show that human muscle spindles are innervated by sympathetic neurons and that they stimulate intrafusal fibers (88). Roatta et al. in 2002 suggested that sympathetic innervation to spindle afferents may be one of the mechanisms involved in adjusting a motor activity during states of physical and emotional stress (89). An increase in sympathetic outflow depresses the feedback control of muscle length. During the fight or flight response, precision and fine control of movements can be sacrificed temporarily for stability and fast running or fighting movements (89). Enhanced sympathetic outflow may cause inefficient muscle use, and lead to work-related myalgia that develops under states of psychosocial stress (90-92).

On the sensory side, the masticatory muscles of individuals displaying higher muscle duty factors produce more afferent output from the muscle spindles. Muscle spindles send primary afferent information to the mesencephalic nucleus (93). Higher muscle duty factors result in increased sensory output which increases the barrage of information imposed on the mesencephalic and spinal tract nuclei. This increased afferent barrage, combined with increased subclinical inflammation of central nervous system due to psychosocial state, creates an increased risk for the development of chronic pain. Chronic stress causes glial cells to release Interleukin 1-beta, Calcitonin gene-related peptide (CGRP), and prostaglandins, which in turn result in primary and secondary nociceptive neuron membrane instability. This, plus the increased afferent barrage due to higher muscle duty factors, could create central and peripheral pain responses to non-noxious stimuli.

In 2020, Slade et al. conducted a randomized prospective clinical trial to investigate the effects of Propranolol, a nonselective β-adrenergic receptor antagonist, in 143 patients with painful TMD (94). Propranolol has been found to be effective for migraine prevention (95).

Propranolol inhibits pain processes that amplify trigeminal nociception by reducing trigeminovascular responses in the thalamic ventroposteromedial nucleus. It has been suggested that the drug mitigates migraine headaches by blocking the thalamic β_1 adrenoceptor (94, 96). Slade et al. found no significant pain reduction in TMD patients taking propranolol. Propranolol blocks both the β_1 and β_2 adrenergic receptor subtypes, however α_1 and α_2 adrenergic receptor subtypes are also present on arteries, but they are absent in the blood vessels in skeletal muscles. α_1 adrenergic antagonists have different centrally and peripherally mediated effects (97). Centrally, sympathetic activity is increased, whereas peripherally, it is decreased. Future clinical trials are needed to investigate other potential therapies. Combining non-selective β-blockers with other pharmaceuticals that are known to reduce increased sympathetic signaling might be necessary to diminish the increased sympathetic response present in TMD pain as long as cardiovascular side effects are well understood.

Nitric oxide, a neuromodulator, modulates the excitability of peripheral sensory and motor neurons of cardiovascular reflexes and of the central neurons that integrate their function (98). Its influence acts to restrain sympathetic outflow and facilitate parasympathetic outflow, both centrally and peripherally (97, 98). Similarly, the administration of nitric oxide has cardiovascular implications and therefore it must be used carefully if successful modulation of sympathetic nervous system activity is achieved.

Parasympathetic activity is an inhibitor of pain transmission in the subnucleus caudalis via cholinergic receptors (99). When the LF/HF spectral power ratio is high, reduced parasympathetic output may be a contributing element in addition to increased sympathetic drive. A multi-drug cocktail may be necessary to both decrease sympathetic drive and to increase

parasympathetic drive, by incorporating pharmacologic agents such as Pilocarpine, a cholinergic parasympathomimetic agent.

It has been shown that the effects of 5- Hydroxytryptophan (5-HTP), a precursor of serotonin, along with noradrenaline in the subnucleus caudalis, can work as either inhibitory or faciliatory, depending on the receptor subtype. 5-HTP exerts a suppressive effect on nociceptive neurons (100). Additionally, 5-HTP, when imbalanced, is implicated in depression and anxiety (100). It has been shown that administration of 5-HTP effectively increases central concentrations of 5-HTP and serotonin and increases parasympathetic nervous system tone (101). Autonomic regulation is complex and multifactorial, and more studies are needed on how to regulate sympathetic nervous system tone to treat TMD-related pain.

Future studies to investigate TMD-related pain therapies should consider the approach used by Burgess et al. (2008). These investigators focused on the neural structures responsible for inducing REM atonia. Skeletal muscle tone, which is suppressed during REM sleep, is modulated in a stereotypical pattern across the sleep-wake cycle, and where abnormalities in this modulation contributed to major sleep disorders (77). They found that functional glutamatergic drive onto motoneurons regulates levels of muscle tone during wakefulness and phasic REM sleep. The source of this drive appeared to be the medial reticular formation, a brainstem region that plays a pivotal role in coupling arousal state and motor activity (77, 102). If the REM-specific inhibitory mechanism could be pharmacologically reproduced, it could serve as a powerful therapeutic tool to suppress the pathological movements associated with motor disorders, and possibly be used in the treatment of increased masticatory duty factors associated with TMD-related pain (77).

The current study has several limitations that should be considered for future investigations. The study was limited in size, with only six subjects in Group A and eight subjects in Group B. Future analyses should include a statistical estimation of effect size (ie. Cohens D; Partial eta^2), and where indicated, a power analysis performed to determine the sample size required to allow for post-hoc analysis. In particular, PHQ-9 data were especially limited, as only two subjects were classified as having elevated depression scores. Future studies are needed to further investigate the relationship between duty factors and ANS tone, as well as duty factors and anxiety, depression, and physical symptoms, as this could lead to potential therapeutic targets aimed at regulating sympathetic or parasympathetic tone to decrease nighttime muscle duty factors in the treatment of TMD-related pain.

A further limitation of the study was a lack of an analysis of awake-state recordings for comparison with nighttime data. There is evidence that awake-state muscle duty factors are significantly higher than at night, and reflect psychosocial state (11, 103). It is also unknown whether or not awake-state muscle activities reflect ANS activity.

Another limitation of the study was the lack of data to determine whether or not subjects had sleep apnea. Obstructive sleep apnea could significantly impact ECG and EMG recordings. Additionally, no questions were asked of patients regarding if they had consumed alcohol, a depressant, or other substances that could either be depressants or stimulants. If consumed, these agents could also significantly impact ECG and EMG recordings.

6. Conclusions:

This study set out to test whether or not there was a correlation between nocturnal ANS activity and night-time masticatory muscle activity. A significant difference in the slopes and y-intercepts of regressions of night-time masticatory muscle activity, as measured by duty factors, and nocturnal ANS activity, as measured by the standard deviation of ECG inter-beat intervals (SNDD), was found between those with relatively high (Group A) and low (Group B) masticatory muscle duty factors. Additionally, this study showed a correlation between masticatory muscle versus normalized SDNN regression slopes and increased anxiety, as measured by the GAD-7 questionnaire from the DC/TMD. Subjects with increased anxiety had significantly lower duty factor versus normalized SDNN regression slopes for the masseter muscle only. No significant difference was found between subjects with increased depression and those with no depression with respect to muscle duty factor versus normalized SDNN regression slope. No significant difference was found between subjects with and without increased physical symptoms with respect to muscle duty factor versus normalized SDNN regression slopes for both muscles.

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Appendix A: OHSU IRB Approval

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

June 28, 2019

Dear Investigator:

On June 27, 2019, the IRB reviewed the following submission:

IRB ID:	STUDY00018800	MOD or CR ID:	MODCR00010019
Type of Review:	Modification and Continuing Review, Study Closure or Check-in		
Title of Study:	Contribution of Mechanobehavioral, Psychosocial, and Physiological Domains in the Progression of Temporomandibular Disorders		
Title of modification	Contribution of Mechanobehavioral, Psychosocial, and Physiological Domains in the		
Principal Investigator:	Laura Iwasaki		
Funding:	Name: American Association of Orthodontists Foundation, PPQ #: 1014638		
IND, IDE, or HDE:	None		

Documents Reviewed:	• Radiology Review - Dr Kharta • Memo - UB Radiation Safety Office (18SEP2018) • Subject Diary - Biorecorder • Memo - Response to IRB Review (18SEP2018) • Questionnaire 1 - Patient History • DCF - DC/TMD Examination Form • Questionnaire 2 - GAD-7 • Questionnaire 4 - Jaw Functional Limitation Scale • Data Safety and Monitoring Plan • Questionnaire 3 - Graded Chronic Pain Scale • Questionnaire 9 - PSQI • Subject Diary - Saliva Sample • Questionnaire 7 - Patient Health Questionnaire 9 • Questionnaire 6 - Oral Behavior Checklist • DCF - Subject Behavior Log Form • Questionnaire 8 - Patient Health Questionnaire 15 Somatic Symptom Severity Scale • Smart IRB LOA - UB cede to OHSU (29AUG2018) • Questionnaire 5 - Medical History • DCF - Recording Log for Jaw Tracking with OPTIS • Protocol • Consent and Authorization Form • Telephone Script • HIPAA Prep to Research Form • Use-of-Ionizing-Radiation-in-Humans-Form_071618.docx • Award notification • Award application • Demographics_POTmj.docx • POTmj_pregnacy_form.doc • DC-TMD-pain-drawing_2013_05_12-1.pdf • Biorecorder instruction manual • Saliva collection instruction manual

The IRB granted final approval on 6/27/2019. The study is approved until 6/26/2020.

Review Category: Full Board

Copies of all approved documents are available in the study's **Final** Documents (far right column under the documents tab) list in the eIRB. Any additional documents that require an IRB signature (e.g. IIAs and IAAs) will be posted when signed. If this applies to your study, you will receive a notification when these additional signed documents are available.

The IRB does not require re-consent with this submission.

Ongoing IRB submission requirements:

- Six to ten weeks before the expiration date, you are to submit a continuing review to request continuing approval.
- Any changes to the project must be submitted for IRB approval prior to implementation.
- Reportable New Information must be submitted per OHSU policy.
- You must submit a continuing review to close the study when your research is completed.

Guidelines for Study Conduct

In conducting this study, you are required to follow the guidelines in the document entitled, "Roles and Responsibilities in the Conduct of Research and Administration of Sponsored Projects," as well as all other applicable OHSU IRB Policies and Procedures.

Requirements under HIPAA

If your study involves the collection, use, or disclosure of Protected Health Information (PHI), you must comply with all applicable requirements under HIPAA. See the HIPAA and Research website and the Information Privacy and Security website for more information.

IRB Compliance

The OHSU IRB (FWA00000161; IRB00000471) complies with 45 CFR Part 46, 21 CFR Parts 50 and 56, and other federal and Oregon laws and regulations, as applicable, as well as ICH-GCP codes 3.1-3.4, which outline Responsibilities, Composition, Functions, and Operations, Procedures, and Records of the IRB.

Sincerely,

The OHSU IRB Office

Appendix B: GAD-7, PHQ-9, PHQ-15 Questionnaires

GAD-7 :

Generalized Anxiety Disorder 7-item (GAD-7) scale

Over the last 2 weeks, how often have you been bothered by the following problems?	Not at all sure	Several days	Over half the days	Nearly every day
1. Feeling nervous, anxious, or on edge	0	1	2	3
2. Not being able to stop or control worrying	0	1	2	3
3. Worrying too much about different things	0	1	2	3
4. Trouble relaxing	0	1	2	3
5. Being so restless that it's hard to sit still	0	1	2	3
6. Becoming easily annoyed or irritable	0	1	2	3
7. Feeling afraid as if something awful might happen	0	1	2	3
Add the score for each column	+	+	+	
Total Score *(add your column scores)* =				

PHQ-9 :

Patient Health Questionnaire (PHQ-9)

Patient Name: _____ **Date:** _____

	Not at all	Several days	More than half the days	Nearly every day
1. Over the *last 2 weeks,* how often have you been bothered by any of the following problems?				
a. Little interest or pleasure in doing things	☐	☐	☐	☐
b. Feeling down, depressed, or hopeless	☐	☐	☐	☐
c. Trouble falling/staying asleep, sleeping too much	☐	☐	☐	☐
d. Feeling tired or having little energy	☐	☐	☐	☐
e. Poor appetite or overeating	☐	☐	☐	☐
f. Feeling bad about yourself or that you are a failure or have let yourself or your family down	☐	☐	☐	☐
g. Trouble concentrating on things, such as reading the newspaper or watching television.	☐	☐	☐	☐
h. Moving or speaking so slowly that other people could have noticed. Or the opposite; being so fidgety or restless that you have been moving around a lot more than usual.	☐	☐	☐	☐
i. Thoughts that you would be better off dead or of hurting yourself in some way.	☐	☐	☐	☐

PHQ-15 :

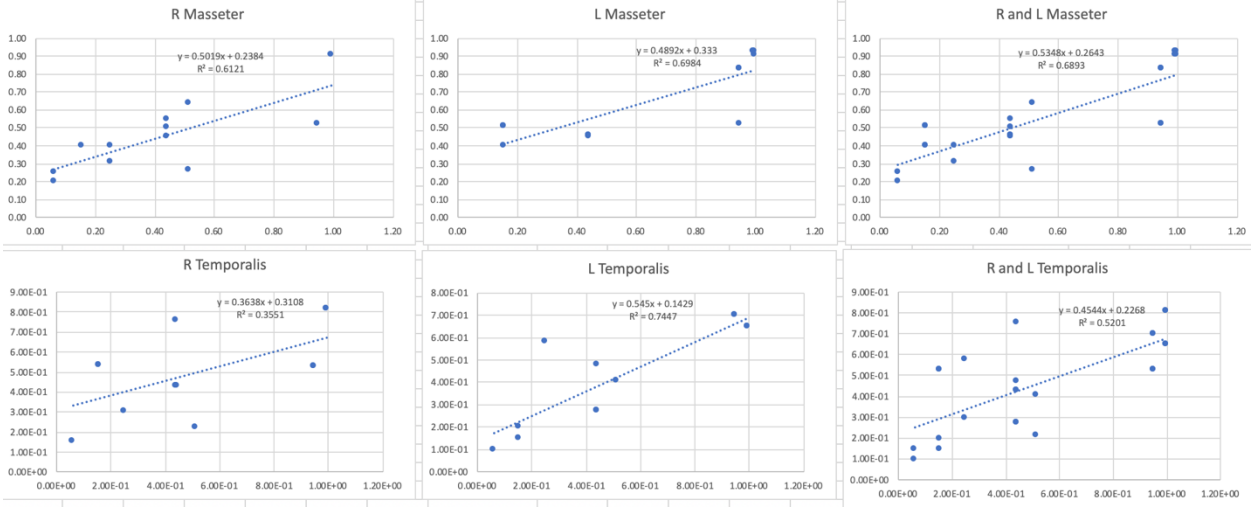
Physical Symptoms (PHQ-15)

During the past 4 weeks, how much have you been bothered by any of the following problems?

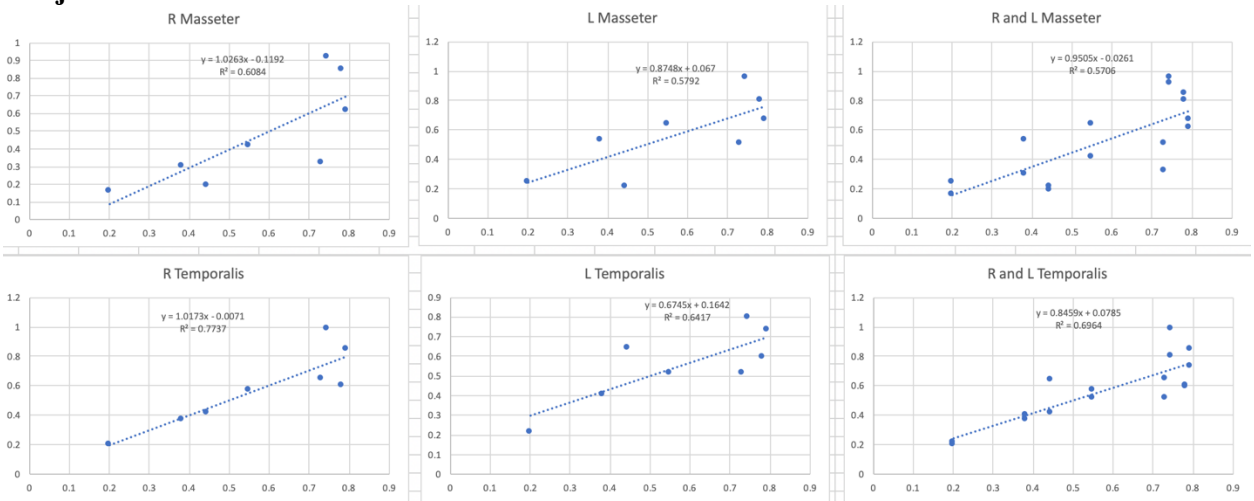
	Not bothered at all [0]	Bothered a little [1]	Bothered a lot [2]
a. Stomach pain	☐	☐	☐
b. Back pain	☐	☐	☐
c. Pain in your arms, legs, or joints (knees, hips, etc.)	☐	☐	☐
d. Menstrual cramps or other problems with your periods [***Women only***]	☐	☐	☐
e. Headaches	☐	☐	☐
f. Chest pain	☐	☐	☐
g. Dizziness	☐	☐	☐
h. Fainting spells	☐	☐	☐
i. Feeling your heart pound or race	☐	☐	☐
j. Shortness of breath	☐	☐	☐
k. Pain or problems during sexual intercourse	☐	☐	☐
l. Constipation, loose bowels, or diarrhea	☐	☐	☐
m. Nausea, gas, or indigestion	☐	☐	☐
n. Feeling tired or having low energy	☐	☐	☐
o. Trouble sleeping	☐	☐	☐

Appendix C: Regressions for each subject - Horizontal Axis: Normalized SDNN. Vertical Axis: Normalized Muscle Duty Factor

Subject 1:



Subject 2:



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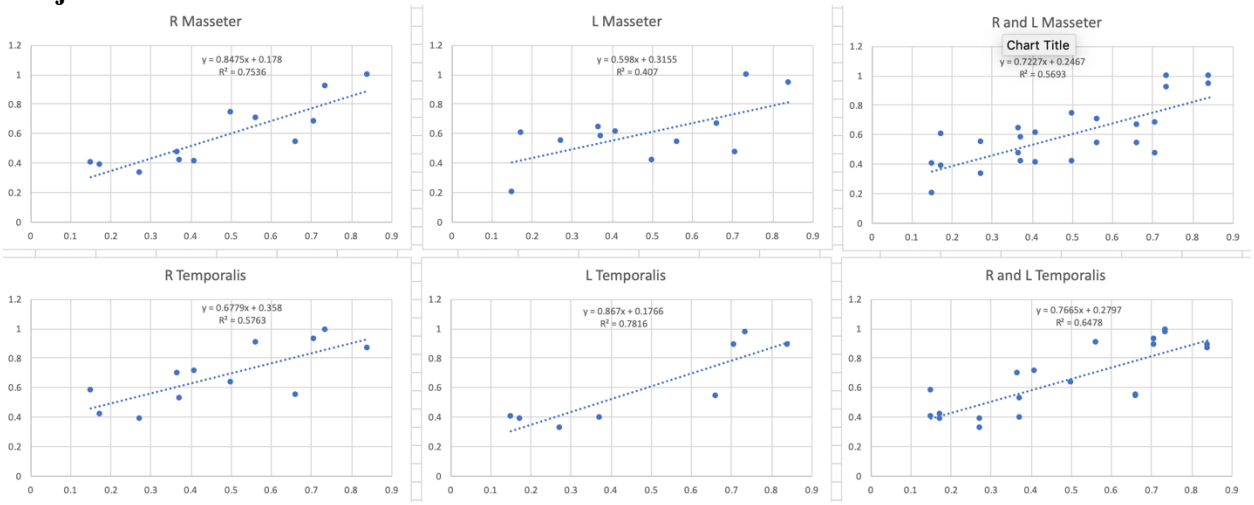

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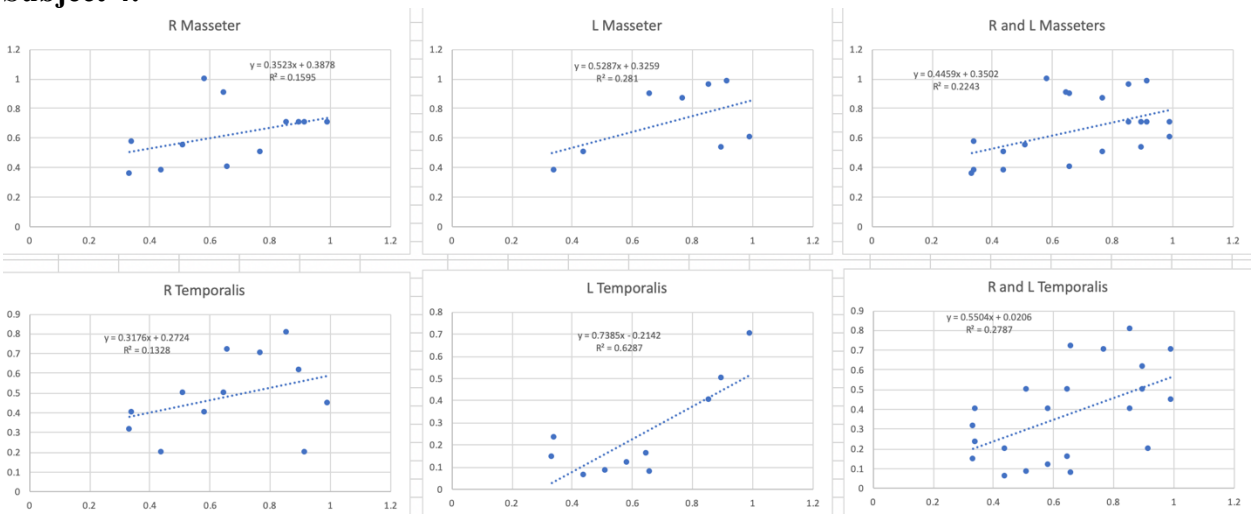

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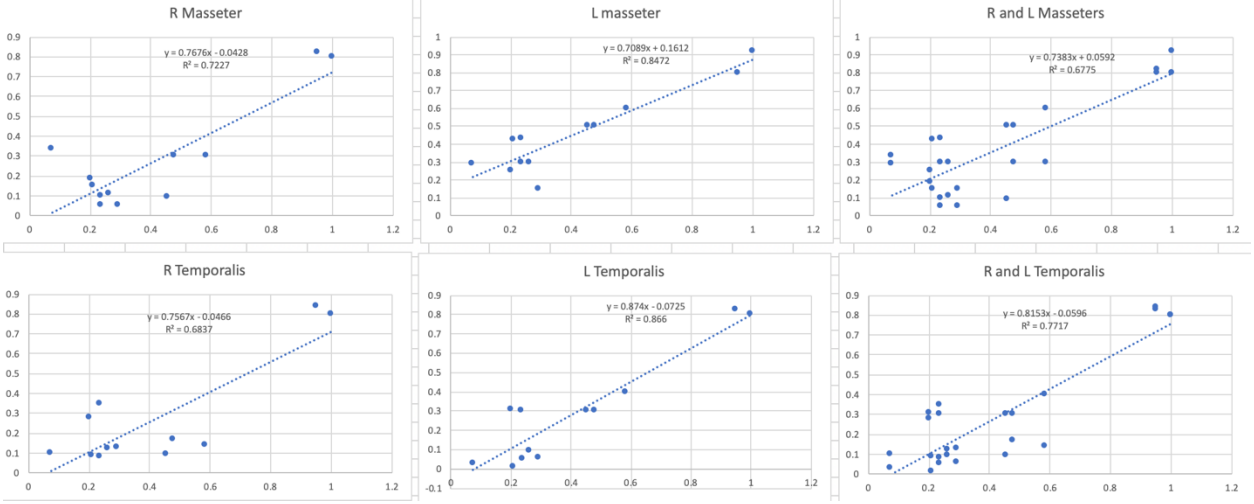

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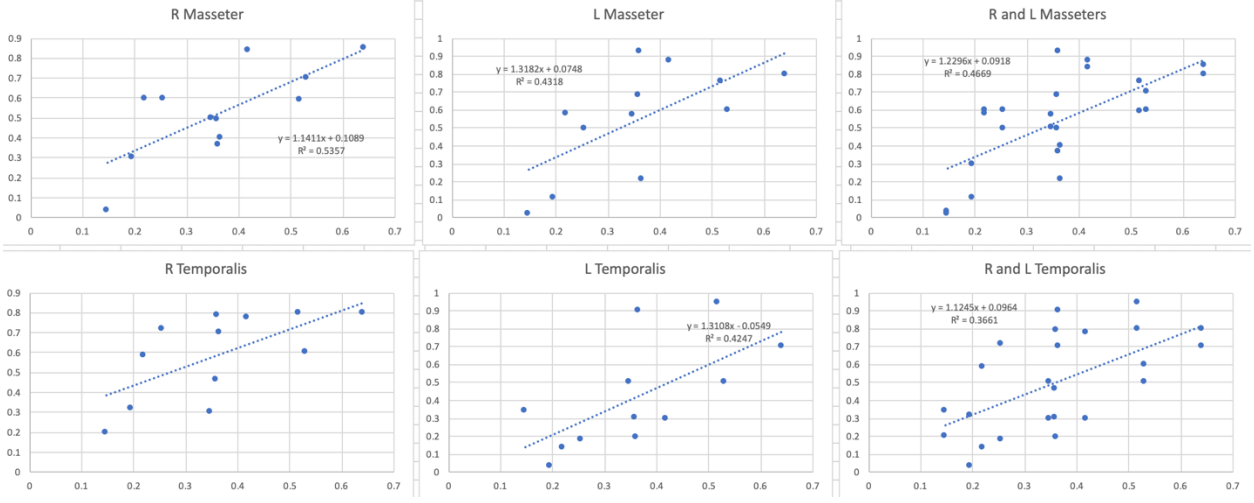

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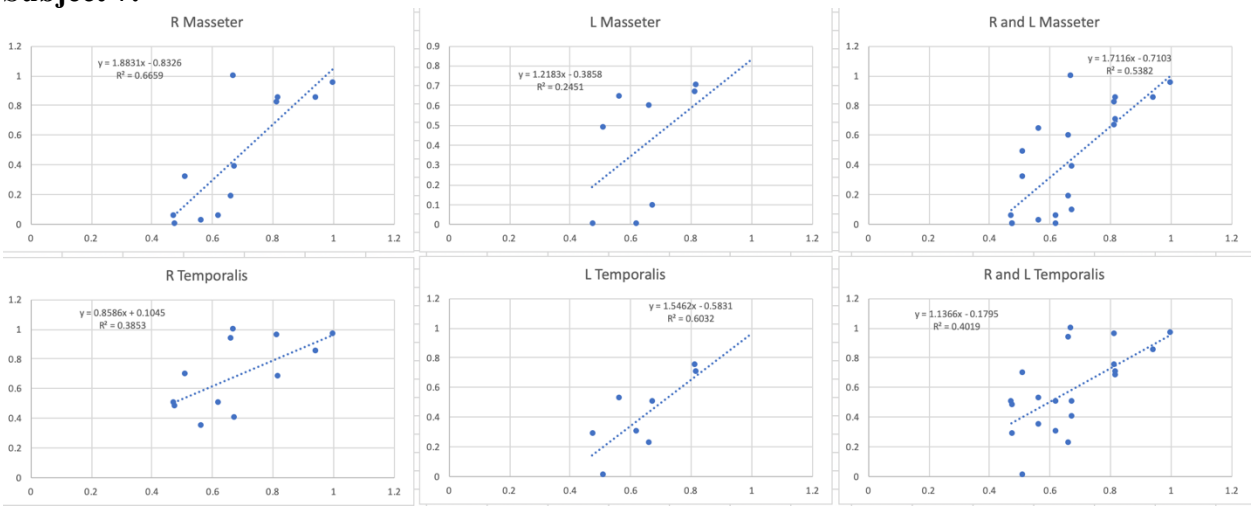

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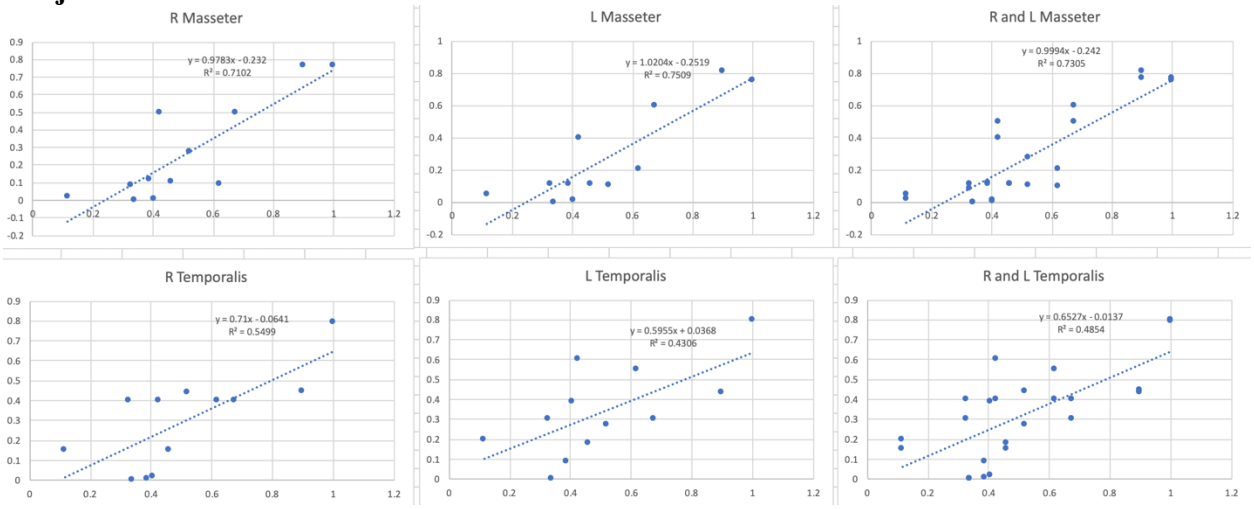
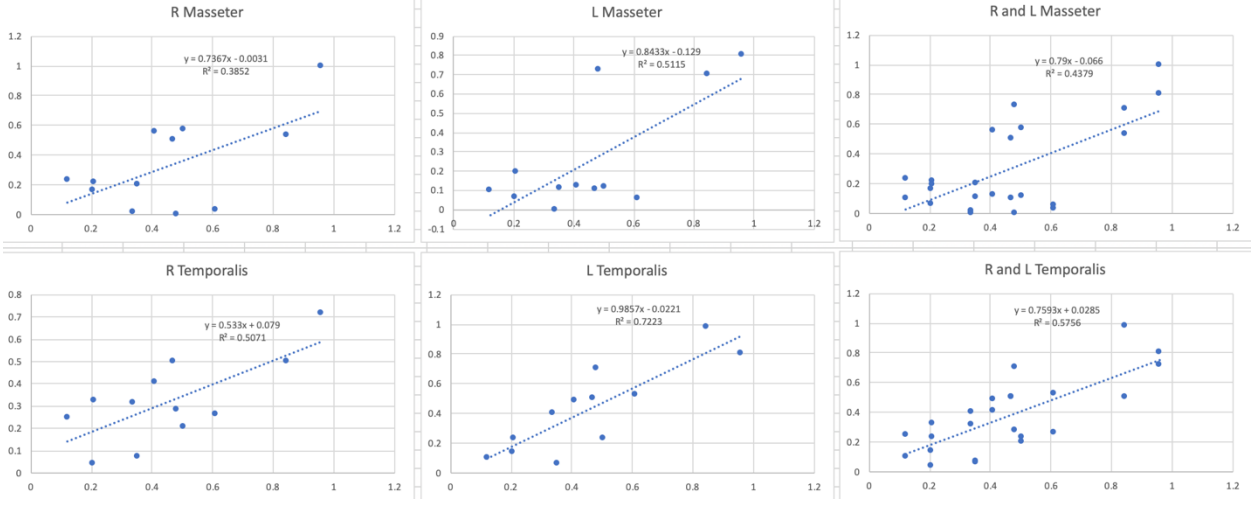


Subject 9:



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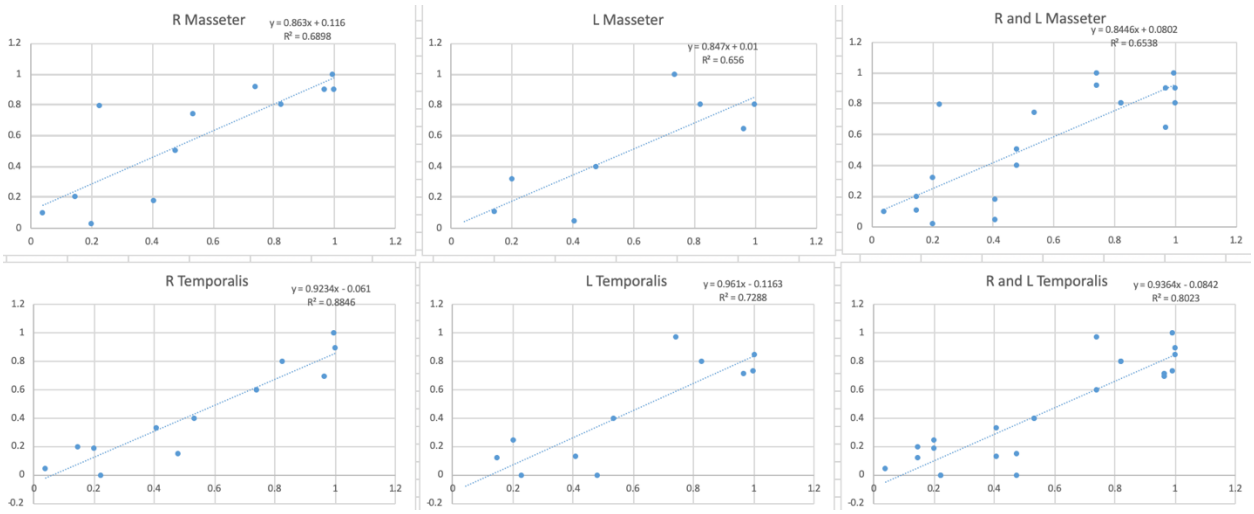

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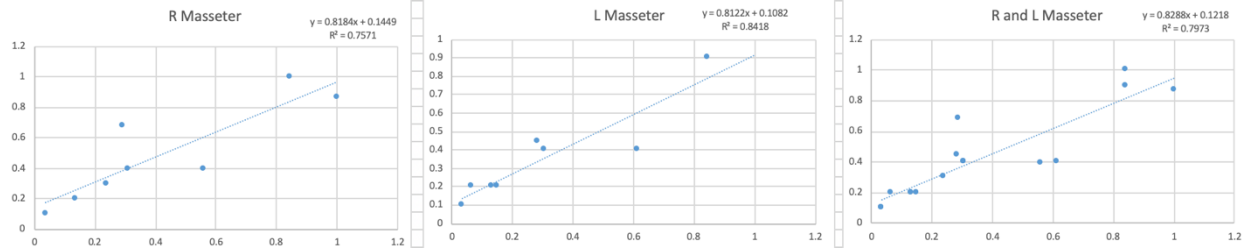

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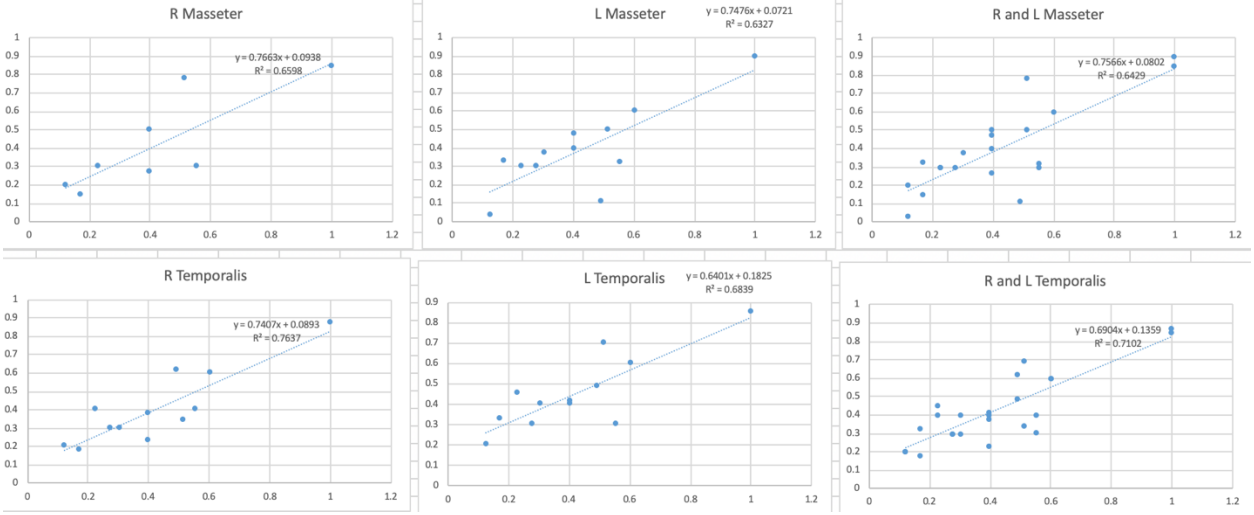
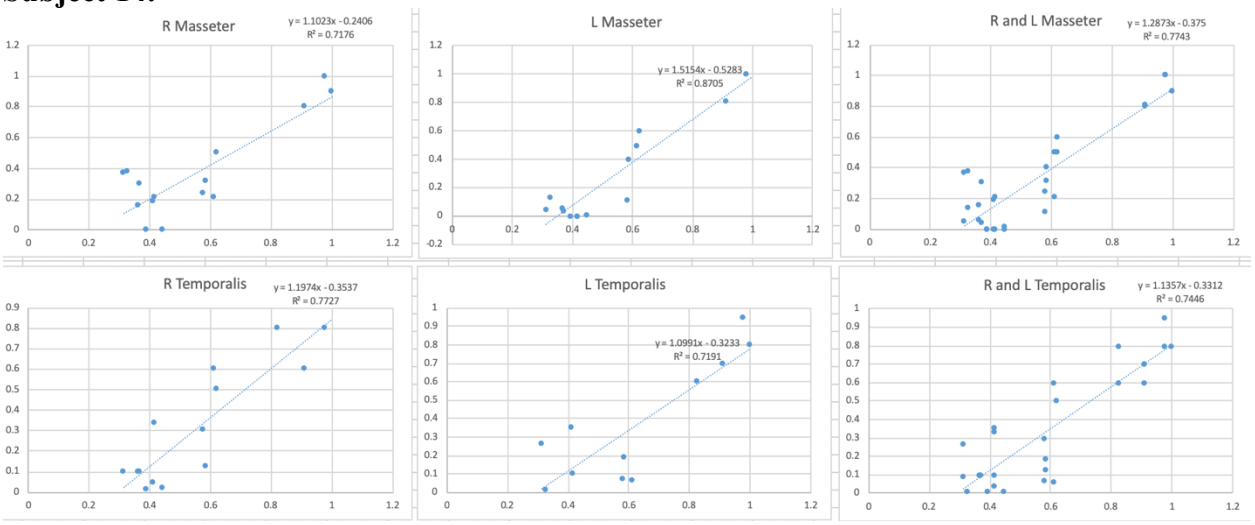


Subject 13: DATA MISSING

Subject 14:



Subject 15: