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Pain Related Anxiety and Pain Severity are Associated with Increased Activation of Central Arousal Circuits in Fibromylagia

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A thesis submitted in partial satisfaction of the requirements for the degree Master of Science in Clinical Research

by

Trinh Thi Nhat Truong

2013

ABSTRACT OF THE THESIS

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Robert M Elashoff, Chair

Studies suggest that patients with chronic pain disorders such as fibromyalgia (FM) develop persistent symptom-specific anxiety from previous threatening stimuli, which may contribute to pain-related symptoms. The aim of this study is to determine if pain and symptom-specific anxiety in FM correlates with an objective, non-invasive index of affective response to specific contexts or stimuli (acoustic startle response [ASR]). Women with FM and healthy women underwent somatic perception testing using a dolorimeter and ASR testing. ASR was measured at: 1) baseline, 2) context and 3 cued threat conditions: 3) safe (no stimulation), 4) anticipation of possible threat, and 5) imminent threat of an aversive somatic stimulus. To maintain anticipation, one moderately intense biceps electrical stimulus was given in the middle of the experiment. Pain severity and pain-related anxiety were measured. Compared to controls, FM patients

demonstrated increased somatic pain perception. Both groups showed increasing ASR to progressively increasing threat but there were no group differences. However, in the FM group, pain intensity correlated with ASR to imminent threat ($r=0.52$, $p<0.05$) and pain-related anxiety correlated with ASR to anticipated ($r=0.61$, $p<0.01$) and imminent threat ($r=0.49$, $p<0.05$). Thus, pain-related anxiety and severity were associated with enhanced responsiveness of emotional arousal circuitry in FM.

The thesis of Trinh Thi Nhat Truong is approved.

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2013

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Master of Science in Clinical Research

University of California, Los Angeles, 2013

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PAIN-RELATED ANXIETY AND PAIN SEVERITY ARE ASSOCIATED WITH INCREASED ACTIVATION OF CENTRAL AROUSAL CIRCUITS IN FIBROMYALGIA

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ABSTRACT

Studies suggest that patients with chronic pain disorders such as fibromyalgia (FM) develop persistent symptom-specific anxiety from previous threatening stimuli, which may contribute to pain-related symptoms. The aim of this study is to determine if pain and symptom-specific anxiety in FM correlates with an objective, non-invasive index of affective response to specific contexts or stimuli (acoustic startle response [ASR]). Women with FM and healthy women underwent somatic perception testing using a dolorimeter and ASR testing. ASR was measured at: 1) baseline, 2) context and 3 cued threat conditions: 3) safe (no stimulation), 4) anticipation of possible threat, and 5) imminent threat of an aversive somatic stimulus. To maintain anticipation, one moderately intense biceps electrical stimulus was given in the middle of the experiment. Pain severity and pain-related anxiety were measured. Compared to controls, FM patients demonstrated increased somatic pain perception. Both groups showed increasing ASR to progressively increasing threat but there were no group differences. However, in the FM group, pain intensity correlated with ASR to imminent threat ($r=0.52$, $p<0.05$) and pain-related anxiety correlated with ASR to anticipated ($r=0.61$, $p<0.01$) and imminent threat ($r=0.49$, $p<0.05$). Thus, pain-related anxiety and severity were associated with enhanced responsiveness of emotional arousal circuitry in FM.

PERSPECTIVE

This study suggests that activation of central arousal circuits in FM is associated with increased symptoms of pain-related cognitive and physiologic anxiety which may contribute to enhanced pain severity.

INTRODUCTION

Chronic pain is one of the most prevalent, costly and disabling conditions in clinical practice. Chronic pain disorders commonly overlap and have shared clinical and pathophysiologic characteristics. Fibromyalgia (FM) is a condition characterized by the presence of chronic widespread muscular pain and tenderness[61] and often occurs concomitantly with other pain disorders such as irritable bowel syndrome (IBS) and interstitial cystitis (IC).[51] The lowered pain threshold that can be demonstrated clinically and experimentally in patients with chronic pain syndromes such as fibromyalgia, irritable bowel syndrome, tension headache and temporomandibular disorders is thought to occur primarily as result of stress induced alterations in the central nervous system circuits leading to an inadequate antinococeptive response, altered autonomic response, and an altered hypothalamic-pituitary-adrenal (HPA) axis response.[29]

In our study, we wanted to assess the emotional arousal that affects pain modulation. The acoustic startle response (ASR) is a modality used to measure emotional arousal. Startle is modulated by the extended amygdala, a component of the limbic system involved in modulation of emotional states and physical sensations and is a key part of the pain amplification circuits in the brain. Fear potentiated startle is used to analyze neural networks involved in fear and anxiety and has been shown to be a sensitive measure of anticipatory fear or anxiety.[12] Previous studies by Naliboff and colleagues have shown enhanced startle response to abdominal pain threat in patients with other functional pain disorders such as IBS[45] and IC.[54] Thus, we hypothesized that amygdala-related central pain amplification, as demonstrated by an enhanced startle response, also plays a key role in pain processing and the resultant increased somatic pain perception seen in FM as well.

Prevalence and definition of FM

According to the criteria of the American College of Rheumatology (ACR), FM is defined as chronic widespread pain and tenderness in at least eleven of 18 defined tender points. [61] Population based estimates of the prevalence of FM range from 0.5% to 5.8%.[11,24] FM is frequently associated with fatigue, sleep disorder, other functional somatic syndromes, mental and physical disorders, as well as disability and diminished quality of life (QOL).[30,55] FM patients incur high direct medical costs[4,47,59] and consume significant indirect costs (e.g. sick-leave, disability pension).[31,60]

Overlap with other chronic pain disorders

Fibromyalgia commonly overlaps with other chronic pain disorders including IC/ painful bladder syndrome (PBS) and IBS. IC is a chronic clinical syndrome affecting the lower urinary tract that is associated with urinary frequency and urgency and/or pelvic pain in the absence of any other identifiable pathology and is commonly associated with FM.[1,9] In a study by Alagiri M et al[1] of 2682 community based IC pts, FM was the 5th most common concomitant IC symptom (24.6%).

IBS is also associated with FM. IBS is a chronic functional GI disorder characterized by abdominal pain associated with alterations in defecation or stool frequency and consistency. In a study by Sperber et al. [51] 31.6% (25/79) of IBS patients met criteria for FM while 32% (32/100) of FM patients met criteria for IBS. Patients with both disorders had significantly worse scores for physical functioning and health related quality of life (HRQOL) than patients with either disorder alone, or controls.

Although genitourinary, gastrointestinal and musculoskeletal symptoms predominate in IC, IBS and fibromyalgia respectively, all three disorders share a number of features in common including: pain as the predominant symptom, female predominance (IC:female-to-male 8 : 1),[21] initiation or exacerbation of symptoms associated with stressful life events, disturbed sleep and fatigue, and show beneficial responses to psychologic and behavioral therapies such as antidepressants. All three disorders are characterized physiologic changes including hyperalgesia, pain specific hypervigilance and hyperattentiveness, increased activation of brain regions (i.e. ACC subregion) involved in attention, enhanced basal circadian rhythm of HPA axis, and autonomic dysregulation.[8]

Central mechanisms of chronic pain disorders

With the substantial clinical and physiological overlap between the various chronic pain conditions, it is likely that a common central mechanism contributes to the underlying pain pathogenesis. In particular, the emotional motor system (EMS) is suspected to play a key role in this pain pathophysiology. The EMS is a network of brain regions activated in response to stress and includes the anterior cingulate cortex (ACC), hypothalamus, and the amygdala. Life events create stress that act on the EMS which in turn lead to autonomic and neuroendocrine responses as well as sensory modulation resulting in pain pathophysiology and chronic pain symptomatology. These in turn feedback onto the EMS, which leads to hypervigilance, increased arousal of the CNS, and emotional feelings. These different, overlapping brain networks mediate the effects of cognitions and emotions on the perception of homeostatic feelings, including pain and discomfort. Differential dysregulations of one or several of these

networks could result in altered perception, even in the presence of normal visceral or somatic afferent input to the brain.

Altered activation of these brain regions involved in pain modulation has been demonstrated in brain imaging studies in chronic pain disorders including FM and IBS. In functional MRI (fMRI) studies, FM patients showed aberrant pain responses compared to healthy controls to somatic pain. In an fMRI study by Gracely et al,[22] both FM patients and controls showed activation of the somatosensory cortex to equivalent ratings of somatic pressure. The FM patients, however, had significantly less activation than controls in other brain regions involved in affective pain modulation. The authors speculate that the somatic pain inflicted during the study may not be significantly different enough from the chronic somatic pain felt by FM patients to active other pain modulatory brain regions to the same extent as in controls.

FM disease severity has also been linked to brain perfusion abnormalities. In a brain SPECT perfusion study by Guedi et al[26] higher disease severity (as measured by the Fibromyalgia Impact Score) was associated with greater perfusion of the bilateral superior parietal lobule, the bilateral precuneus, and the left postcentral cortex. Lower disease severity, on the other hand, was associated with greater perfusion of a left anterior temporal cluster, fusiform gyrus; and uncus.

The ACC is also activated to a greater degree in patients with other chronic pain syndromes. In particular, greater activation of the ACC was seen in response to visceral stimulus in patients with IBS, a condition associated with greater GI pain symptom severity, compared to

patients with concomitant IBS and FM. In the IBS+FM group (a group associated with greater somatic pain symptom severity), greater activation of the ACC was seen in response to somatic stimuli. Thus, the ACC is equally activated in response to altered perception of visceral or somatic pain in both syndromes.[6]

The unique location of activation of the contralateral ACC has also been shown to be very close to regions associated with pain catastrophizing in correlational analysis. Pain catastrophizing (i.e. exaggerated negative interpretation of actual or anticipated pain experiences) has been suggested to augment pain perception through enhanced attention to painful stimuli, and heightened emotional responses to pain perception.[22] Vigiliance to pain is strongly related to pain coping and pain-related fear and anxiety.[49] McCracken and colleagues demonstrated a significant overlap between catastrophizing and cognitive and physiologic anxiety to pain in patients with chronic musculoskeletal pain.[40] Relationships between hypervigilance and catastrophizing and amplification of somatic pain have been demonstrated in FM patients.[7,18,33] Thus altered activation of some of the brain regions associated with pain catastrophizing, anticipation and attention to pain and the emotional aspects of pain (e.g. extended amygdala (amygdala and bed nucleus of the stria terminalis [BNST]), may play a key role in fear- and anxiety-related behaviors and has been implicated in pain modulation and stress hormone responses.[57].

Altered activation of brain regions involved in pain modulation has also been demonstrated in IBS patients as well. In brain imaging studies of IBS patients, healthy controls show extensive activation of the brain stem, including the periaqueductal grey (PAG) region, in response to painful rectal balloon distension, while IBS patients show only minimal responses in

thalamus and brain stem and do not activate the other regions. Rather IBS patients activate the rostral ACC and posterior cingulate/retrosplenial cortex. The reduced responses in the medial and lateral PFC as well as areas of the insula, lentiform nucleus, and thalamus have been interpreted as reflecting an altered cognitive and evaluative processing of acute pain based on experience of coping with chronic pain or adaptation-level effects. The PAG is one of the main brain regions involved in descending pain inhibition, thus the reduced responses in these areas may reflect an inability of IBS patients to adequately inhibit pain processing.[44,56] Rectal pressure distension in IBS patients also increased regional cerebral blood flow (rCBF) in areas commonly associated with somatic pain, including the anterior cingulate, insula, prefrontal cortex, thalamus, and cerebellum. In particular, insula activation was associated most strongly with objective visceral pressure, whereas anterior cingulate activation was associated more with correlated ratings of subjective discomfort.[3] Furthermore, during anticipation of uncomfortable visceral distention, healthy women, but not IBS patients, were able to deactivate the bilateral locus coeruleus and left amygdala, areas also associated with cognitive coping. Thus the inability of patients with chronic pain syndromes to deactivate these brain regions may lead to poor cognitive coping and altered pain.

Acoustic startle response

The acoustic startle response (ASR), a measure emotional arousal, has also shown to be enhanced in chronic pain syndromes. ASR is a simple reflex characterized by a fast twitch of facial and body muscles (e.g., eye blink in humans) reliably evoked by a sudden and intense tactile, visual or acoustic stimulus and is mediated by the amygdala complex[57] and has been shown to be a sensitive measure of anticipatory fear or anxiety.[12] In particular, patients with

IBS[45] and IC[54] have show enhanced ASR to visceral stimuli. IBS subjects had significantly higher startle responses compared to controls to the threat of a painful visceral stimulation, consistent with greater excitability of the limbic regions to a visceral pain threat.[45] IC likewise showed increased startle in response to anticipation of a painful stimuli supporting the notion that IC patients over-respond to ambiguous, potentially threatening situations.[54]

In our study, we wanted to assess the emotional arousal that affects pain modulation. Using the acoustic startle response (ASR) to measure emotional arousal, we hypothesize that similar to IBS and IC patients, amygdala-related central pain amplification also plays a key role in pain processing and the resultant increased somatic pain perception seen in FM.

Specific aims

In the present study, the aims are to: 1) compare ASR to a somatic threat in FM patients and healthy controls, 2) determine if ASR correlates with subjective pain intensity or pain-related anxiety in FM, 3) determine if ASR correlates with somatic pain thresholds and sensory ratings to mechanical stimuli in FM, and 4) determine if ASR correlates with general anxiety and depression symptoms in FM patients.

MATERIALS AND METHODS

Subjects

Healthy controls

Twenty-two healthy women were recruited by advertisement. None of the control subjects had a history of FM, IBS or an acute or chronic illness or pain syndrome by symptom questionnaire, medical history and physical examination.

Subjects with Fibromyalgia (FM)

Twenty-one women with FM were recruited from advertisement, the University of California at Los Angeles (UCLA) Center for Neurobiology of Stress, and the UCLA East-West (complementary alternative medicine) Center. All subjects had previously been diagnosed with FM by a rheumatologist and met the 1990 American College of Rheumatology (ACR) diagnostic criteria at the screening visit.[61] Patients were also screened for IBS using Rome diagnostic criteria.[36]

Screening Visit

All potential subjects first underwent an initial screening visit. A study physician or a nurse practitioner performed the medical history and physical examination. A urine pregnancy test was taken and if positive, the subject was excluded. All subjects completed symptom questionnaires including: 1) Hospital Anxiety and Depression (HAD) questionnaire[62] which measures the presence of anxiety or depression symptoms, and 2) Pain Vigilance and Awareness scale (PVAQ)[49] which assesses hypervigilance and attention to pain. The FM group also completed pain-specific validated questionnaires including: 1) Pain Anxiety Symptom Scale (PASS)[41] which assesses pain related fear and anxiety, and 2) Short form McGill Pain Scale[42] which measures the intensity and quality of pain. All subjects then underwent a Structured Clinical Interview for DSM-IV (SCID)[19] by a trained therapist (MM) to assess for the presence of DSM-IV Axis I disorders, childhood (≤ 13 years of age) and lifetime (any age) physical, sexual, and verbal abuse, and early adverse life events. Early adverse life events included abuse, history of

divorce, death, alcoholism, or mental illness of a parent, and distant relationship with the mother. Control subjects were excluded only for a current history of a SCID diagnosis.

Test session visits

Subjects underwent two separate test sessions: 1) somatic perception testing and 2) ASR testing. With the exception of two subjects (1 FM patient and 1 control), somatic perception testing occurred on the first test session day and startle testing occurred on the second test session day. All visits were done during the follicular phase of the menstrual cycle in premenopausal patients or within the first 14 days of the menstrual cycle if taking an oral contraceptive pill (OCP). Every attempt was made to schedule the two test sessions on separate days within the follicular phase of the same menstrual cycle or separated by one menstrual cycle. Only two FM subjects had their two test sessions separated by two menstrual cycles. All test sessions were completed in the follicular phase.

Somatic perception testing

Somatic pain perception testing was conducted as described in our previous study.[7] Briefly, two somatic sites (1 "active" tender point and 1 "control" point) on the non-dominant side were selected to test for perception by dolorimetry. A variable pressure dolorimeter (John Chatillon and Sons, Kew gardens, New York) with a spring loaded gauge and contact head whose surface area was equal to 1.54 cm^2 was used to apply pressure between 0-9 kg. The "active" point site was located 2 cm distal to the lateral epicondyle (LE), one of the tender points of the FM

diagnostic examination.[61] The second "control" point was located at the dorsal aspect of the distal third of the forearm. The order of testing of the two somatic sites was randomized.

Ascending series. Two separate, somatic stimuli were applied using increasing pressure to measure the somatic pain threshold and maximal tolerated pressure. During the advancement of the dolorimeter at the somatic site at a rate of 1 kg/second, the *somatic pain threshold* was defined as the pressure (kg/1.54 cm^2) at which the subject first felt that the stimulus became painful. The *maximal tolerated pressure* was defined as the pressure (kg/1.54 cm^2) at which the subject could no longer tolerate the pain. Subjects were asked to rate the sensory intensity of the somatic stimuli using validated verbal descriptor visual analog scales (VDVAS).[23]

Fixed stimulus protocol. Sensory ratings to a randomized series of varying pressure stimuli were measured during the fixed stimulus protocol similar to that described in our previous FM study.[7]. A total of 15 stimuli were given in random order (three each of 2, 3, 4, 5, and 6 kg/1.54cm^2). After each pressure stimuli, subjects rated the sensory intensity using VDVAS.[23] The fixed stimulus protocol always followed the ascending series.

Acoustic startle response testing

Subjects underwent testing at the Human Psychobiology Laboratory at UCLA between 12PM and 2:30PM to minimize diurnal variability in startle magnitude and stress hormones.

Material and methods. The study methods are similar to those used in our previous studies.[54] Auditory startle stimuli (105dB, zero rise time, 50ms white noise bursts) were presented binaurally through stereophonic headphones. The stimulation to the non-dominant biceps muscle was delivered by a digital 807 Electrical Muscle Stimulation Device (Everyway

Medical Instruments Co.). Stimulations consisted of a 1 sec duration, 20.4 mA peak current passing between two pads (50 V peak), based on pilot testing to represent an uncomfortable but not painful intensity of muscle contraction for most subjects. The electromyogram activity of the orbicularis oculi (ooEMG) was used to index startle response and was recorded from electrodes placed beneath the right eye.

Startle Procedure. The startle session consisted of a baseline and context condition, followed by cued threat conditions. After the startle electrodes were attached to the orbicularis oculi muscles, startle response was measured (baseline period). Immediately following baseline startle response, the electrical stimulation pad was then attached to the subject's biceps muscle on the non-dominant arm. Startle response was again measured (context period). During the baseline and context periods, 7 startle stimuli were delivered during each period with a mean inter-stimulus interval of 22 seconds while subjects focused on a fixation cross in the center of the computer monitor. The baseline and context periods were similar except that a stimulation pad was placed on the subject's arm during the context period. The context condition was generated by informing subjects that the pad would be used for stimulation but no stimulations would be given during this period.

The baseline and context periods were then followed by cued threat conditions that consisted of safe or danger periods (anticipation and imminent threat). During the cued threat conditions, 6 safe and 6 threat periods (70 sec duration each) were presented in alternating order. Subjects were told that no uncomfortable stimulations would be given during the "safe" periods, but that during "danger" periods (anticipation and imminent threat) they might receive a stimulation to their arm. These periods were divided into two blocks and a single biceps

stimulation was administered at 65 sec during the last threat period of the first block. A computer monitor informed subjects of which period they were in both in words and color in the form of a progressing bar. Two startle stimuli were presented within each of the safe and danger periods (1 during anticipation, 1 during imminent threat). ASRs were recorded twice during each safe period (i.e. when the bar is green and the subject knows that they are safe and no stimulations will be given.), once during the anticipation period (i.e., when the bar is still pink and the subjects know that no electrical stimulation will be given), and once during each imminent threat period (i.e., when the bar is red and subjects know that an electrical stimulation, if one will be given, will occur during this condition: between 45 and 70 s.). ASRs recorded during the 19-24 second and the 54-59 second intervals of safe periods were used to represent the safe condition. ASRs elicited during the 19-24 second intervals of danger periods are referred to as the anticipation condition. ASRs elicited during the 54-59 second interval of the danger periods are labeled as the imminent threat condition, which is much longer than the interval during which the stimulation is given.

Lastly, subjects were informed that during the entire procedure they may get the stimulation up to three times, with subsequent stimulations stronger than the first. In reality, each subject received only one bicep stimulation between the two blocks to maintain anticipation.

Startle testing outcome measures. Amplitudes of EMG were defined as the difference between the mean amplitude of the 200ms of EMG preceding the startle stimulus and the peak of the response, expressed in microvolts (μV).[46] Subjects with 20 or more rejected trials (excessive baseline EMG, movement during the trial, spontaneous blink detected on an EOG channel) or 16 or more zero trials (no observable blink activity) were excluded from analyses.

Laboratory measures. Salivary cortisol, plasma adrenocorticotropin hormone (ACTH), norepinephrine (NE), and epinephrine (EPI) were taken at the beginning (after a 30 minute resting baseline period in a sitting position) and at the end of the startle session. Salivary cortisol (High Sensitivity Salivary Cortisol Enzyme Immunoassay Kit, Salimetrics LLC) and serum ACTH (chemiluminescent immunoassay) was processed through the UCLA General Clinical Research Center (GCRC) Core Laboratory. Serum EPI and NE was sent to an outside professional laboratory (ARUP Laboratories at the University of Utah) and measured via high performance liquid chromatography.

Subjective ratings. At completion of the experiment, subjects rated the intensity of the bicep stimulation, on the VDVAS.[23]

Statistical Analysis

T-tests were used to compare means of subject variables between FM and controls (i.e. age, BMI, etc). Chi square tests were used to test group differences for nominal variables.
Due to a positive skew, ASRs were square root transformed, ACTH and cortisol were log transformed and EPI was inverse transformed to best fit a normal distribution. For the primary outcomes (VDVAS intensity, EMG, neuroendocrine hormone levels), FM and controls were compared across conditions using repeated measures ANOVA (Proc mixed procedure in SAS [SAS Institute Incorporated, Carey, NC]. Post hoc pairwise mean comparisons were made under the ANOVA model using the usual Tukey-Fisher criteria. Residual errors were examined to confirm normality of the data. The varying somatic pain ratings obtained were analyzed as a function of the five experimental conditions using a 2 Group (FM, Control) x 5 Condition (2, 3,

4, 5, and 6 kg/1.54cm²) design. ASRs elicited during the five startle conditions were analyzed using separate ANOVAs for a 2 Group x 2 Condition (baseline, context) and a 2 Group x 3 Condition (safe, anticipation, imminent threat) design. This ANOVA study design was used to compare context to baseline to evaluate the contextual anxiety effect and independently to compare the cued threat conditions (safe, anticipation, imminent threat) to each other to evaluate the more specific anxiety and fear. Data from subjects with excessively high baseline EMG and/or with more than 16 zero trials were excluded. Neuroendocrine levels were analyzed using a 2 Group x 2 Condition (baseline, end) design.

To account for the age difference between groups, age was controlled for in the model as a covariate. We attempted to control for psychological differences between groups (anxiety, depression) but found that they were not statistically significant in the model and thus were not included in the final model.

Exploratory correlation analyses were performed to assess relationships between ASRs and other outcome measures (McGill, PASS, HAD scores and somatic pain thresholds and ratings). For the correlation analyses, ASRs during anticipation to threat and imminent threat conditions were calculated as % anticipation change in ASRs (i.e., anticipation-safe/safe) and % threat change (imminent threat-safe/safe). Correlations were analyzed separately for the FM and control groups due to the differences in variability of data. The lack of variability in the control group was due to the fact that controls had little if any anxiety or depression symptoms and lacked pain, which were the main clinical outcome measures that were assessed. Additionally, some of the outcome measures that were analyzed in the correlation analyses (e.g., pain-related measures) could only be assessed in the FM group. The predictability of various variables to

each other was examined using the correlate function in SPSS [Pearson Education, Inc. Boston, MA].

All study protocols were performed after approval by the UCLA Health care system Institutional Review Boards. Verbal and written informed consent was obtained from all subjects.

RESULTS

Subject characteristics

A total of forty-six women (25 controls and 21 FM patients) were screened. Three healthy controls screen failed due to positive SCID testing. Twenty-two healthy women and twenty-one women with FM underwent physiologic testing. All 22 healthy subjects completed both the somatic and startle studies Two FM patients did not complete both studies. One FM subject completed only startle testing, while another FM subject completed only somatic testing. Regarding the startle studies, three subjects (2 controls and 1 FM) were excluded because one control subject had excessively high baseline EMG with more than 16 zero trials and an additional control and FM subject had unusable EMG data from technical difficulties. Thus for the startle studies, usable data was obtained from 19 FM patients and 20 controls. The clinical characteristics of the study subjects are shown in **Table 1**. FM patients were significantly older than controls ($p=0.05$). FM patients had significantly higher HAD and PVAQ scores than controls. In addition, 43% of FM patients were on an antidepressant.

Thirteen of the FM patients met diagnostic Rome III criteria for IBS.[36] but reported that their FM symptom severity far outweighed their IBS symptoms. Other than lower depression

scores (FM+IBS: 4.7±1.0 and FM alone: 9.3 ±1.5, p=0.02), patients with coexistent FM and IBS had similar clinical characteristics to FM patients without IBS.

Somatic perception testing

Pain thresholds at the LE and forearm sites

During the ascending series, mean pain thresholds and maximal tolerated pressure thresholds were significantly lower at both the active and control sites in FM patients compared to controls (**Figure 1)**.

Pain severity ratings to varying somatic pressures

During the fixed stimulus series, mean intensity ratings in response to all somatic stimulus pressures were overall significantly higher in FM patients than controls at both sites (forearm: F(4.23), p= 0.04; LE: F(4.09), p=0.04, **Table 2**). However, group differences at some of the individual lower pressure levels did not meet statistical significance.

Acoustic startle response (ASR) testing

ASR responses

All subjects showed increasing ASRs with increasing imminence of the biceps stimulation particularly after an actual electrical shock was given (**Figure 2**). ASRs were greatest during the imminent threat conditions measured both before and after an actual shock was given. These values were higher than during the anticipation conditions and ASRs were smallest during the

safe periods. However, there were no statistically significant differences in ASR responses between the FM and control groups (F(0.17), p= 0.68).

Subjective response to biceps stimulus

FM subjects rated the electrical biceps stimulus as significantly more intense than healthy controls (15.7±0.5 vs. 12.7±1.0 cm, p=0.01).

Neuroendocrine measures

Cortisol levels significantly decreased from baseline to the end of the startle study in both the FM and control groups (p<0.01) (**Figure 3**). Baseline and post-procedure salivary cortisol levels were significantly lower in the FM vs. control group (p<0.05). Plasma ACTH levels also significantly decreased after the experimental procedure in both FM (0.14±0.02mcg/dl to 0.7±0.02) and controls (0.19±0.04 to 0.11±0.03mcg/dl) (p<0.02) but there was no group difference.

Plasma NE levels were higher at the beginning and end of the startle procedure in the FM patients than the controls (p=0.05) (**Figure 3**). However, there was no significant change in either group from baseline to the post-procedure period. EPI levels did not differ between groups but decreased after the startle study, mainly in the FM patients (13.1±1.8 to 9.0±10.7pg/ml) (p<0.02) but not controls (10.4±2.9 to 9.5±3.9pg/ml).

Correlation of ASRs with somatic, psychological, neuroendocrine, and pain measures

Significant correlations were found in the FM patient group (see Appendix for all correlations). ASRs to context (after placement of biceps stimulation pads) negatively correlated with symptom scores for depression ($r=-0.46$, $p=0.04$). The overall McGill pain intensity scores positively correlated to the % threat change in ASRs *before* the biceps stimulation ($r=0.52$, $p<0.05$). Both PASS physiologic ($r=0.62$, $p<0.01$) and cognitive ($r=0.61$, $p<0.01$) pain-related anxiety ratings positively correlated with the % anticipation change in ASRs *after* having already received the electrical stimulus **(Figure 4).** PASS physiologic scores also correlated with the % threat change in ASRs post-shock ($r=0.49$, $p=0.04$). Centrally acting medication did not have a significant impact on post-shock ASRs in the FM patients (**Table 3)**. There were no significant correlations between somatic perception measurements and ASR.

In controls, but not FM subjects, cortisol levels negatively correlated with ASRs during the context condition ($r=-0.52$, $p<0.01$) and the % threat change prior to receiving a biceps stimulation ($r=-0.63$, $p<0.01$). Conversely, in FM subjects, cortisol levels positively correlated with ASRs during context ($r=0.51$, $p=0.03$) and to physiologic (but not cognitive) anxiety to pain as measured by the PASS ($r=0.54$, $p<0.03$). There were no significant correlations between startle responses and catecholamine levels.

DISCUSSION

While both FM patients and healthy individuals showed greater startle magnitude to increasing threat of an aversive somatic stimulus, no group differences in this response were found. In addition, ASR failed to correlate with experimental measures of somatic pain perception in FM patients. The lack of significant differences in the magnitude of the ASR between FM patients

and controls may be due to conflicting effects of various cognitive, emotional, and physiologic factors on ASR. This is particularly evident in the FM patients because, unlike the control subjects, they have symptoms of anxiety, depression and pain which have been shown to impact ASR. In the FM group, depression symptoms *negatively* correlated with startle response, while measures of cognitive and physiologic anxiety related to pain, clinical pain intensity, and cortisol levels *positively* correlated with ASRs. Thus, our findings suggest that in patients with FM, pain-related anxiety and pain-severity are associated with increased activation of central emotional arousal circuits as measured by the ASR, but these effects may be counteracted by increasing levels of depression. Enhanced startle response has been demonstrated in patients with other chronic pain disorders such as IBS and interstitial cystitis (IC), which frequently coexist with FM, but the level of depression as not been examined as a mediating variable.[1,48]

Association of pain-related anxiety and intensity and ASR in FM

The current study is based on the general hypothesis that FM patients have developed persistent symptom-specific anxiety that can amplify the perception of visceral and somatic afferent input to the brain, thereby contributing to pain-related symptoms. Based on previous studies, the anxiety-related amplification of pain can be explained by an altered responsiveness of the extended amygdala as measured by the ASR. The ASR pattern is suggestive of a protective function from a threatening condition.[34,35] The startle response is robustly increased when it is elicited in association with a stress- or anxiety-related context. Therefore, in our study, the acoustic startle paradigm involves the elicitation of startle responses in the anticipation and imminent threat periods and not during or after the somatic pain stimulation is given.

In FM, pain-related anxiety and intensity were found to be associated with ASR, which is a physiological indicator of activation of central fear and anxiety circuits (i.e. the extended amygdala), which are typically activated in response to actual stressors. Stressors such as physical or emotional trauma (e.g., musculoskeletal injury, emotional stress) may be important in the initiation and maintenance of FM.[52] These threatening events, which are attached to their symptoms, may be involved in the development of anticipatory or anxiety-related responses[13] related to symptom recurrence. Symptom-specific anxiety-related responses may also impact FM symptoms through similar mechanisms by amplifying the perception of somatic afferent input to the brain, thereby contributing to pain-related symptoms. In healthy controls, negative affect induced by viewing unpleasant pictures led to increased startle responses as well as enhanced RIII peripheral nociceptive reflex, a surrogate marker of diminished descending pain inhibition.[5,48]

While this is the first study that we are aware of to evaluate the effect of pain-specific anxiety on startle response, generalized anxiety has been associated with startle potentiation.[17] Fear potentiated startle to painful electrical finger stimulation was significantly greater for high-anxiety than for low-anxiety subjects during simple visual tasks. Although we did not measure neuroticism (state anxiety) in this study, we have previously demonstrated that neuroticism positively correlated with startle in IBS patients during non-imminent threat (e.g., anticipation) conditions to an abdominal shock stimulus.[45] This finding is consistent with studies of anxiety and post-traumatic stress disorder (PTSD) where there was an increased startle response to non-imminent threat conditions.[25]

Brain regions such as the amygdala (which modulates the startle response), anterior

cingulate cortex (ACC), and ventral striatum have been implicated in pain and its emotional modulation.[50] Studies have identified the amygdala and related regions of emotional arousal circuits to be important in the brain's preparatory response to both expected aversive stimuli and to the delivered stimulus.[39] Altered activation of these areas has been demonstrated in patients with FM. In a fMRI study evaluating brain activation patterns to blunt somatic pressure in FM patients, Graceley and colleagues showed that pain catastrophizing was significantly associated with increased activation of brain regions concerned with anticipation and attention to pain (medial and dorsolateral prefrontal cortex and ACC), as well as the emotional aspects of pain (claustrum, closely connected to the amygdala) independent of the influence of depression.[22]

Association of startle with depression symptoms

We found that depression symptoms negatively correlated with ASRs during the context threat condition in FM. This association is supported by previous depression studies which demonstrated a lack of affective startle modulation.[2,14,15,20,33,43] It is conceivable that startle modulation in depression, like anxiety, may be due to dissociation between the judgments of affective stimuli and their psychobiologic responses to such stimuli. Allen et al.[2] suggested that depressed subjects may also have abnormal allocation of attention across affective stimuli, with extra-attentional resources used to process extraneous stimuli, such as the startle probe, that are presented in other sensory modalities and less attention to visual stimuli, cues, or processing of affective properties of a stimulus. Mneimne et al.[43] found similar results in a non-medicated depressed group of patients, and thus it is unlikely that depression-induced startle modulation is medication related. We, likewise, did not find a relationship of antidepressants to startle response

in our study. Overall, it appears that the presence of depression symptoms blunts startle reactivity in contrast to the potentiating effect of anxiety on startle responses. This suggests that an inherent dysregulation in the adaptive context modulation of emotional response occurs in patients with symptoms of depression or anxiety.

Neuroendocrine alterations and relation to ASR in FM

The hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS) are traditionally considered as the two branches of the central stress response system, and alterations in both have been reported in several stress-sensitive disorders, including anxiety, depression and FM.[10] The central nucleus of the amygdala is involved in the regulation of corticotropin releasing factor (CRF) neurons in the paraventricular nucleus (PVN) of the hypothalamus and is thought to increase the secretion of HPA axis hormones, including cortisol, under stress conditions.[32] In FM patients, the positive correlation of cortisol levels with startle response suggests that those patients with greater fear-elicited responses to an aversive somatic stimulus have greater amygdala activation and HPA axis hormone secretion. However, the lack of significant group differences in ASR is likely due to the fact that the correlation with cortisol was only moderate and that there are other factors that modulate startle response. Studies evaluating HPA axis activity in FM have demonstrated differing results but a recent review found that most studies showed reduced activity.[53] Similarly, we found lowered salivary (free) cortisol levels in FM patients compared to controls. This may be in part due to a greater prevalence of depression symptoms in the FM group which has been reported to be associated with lower cortisol levels in FM patients.[27,28] The decline in cortisol levels seen in both groups during the experimental

protocol likely occurred because of the normal circadian rhythm of cortisol secretion although the rate of decline is influenced by the experimental conditions. In contrast, the patients with FM had significantly higher NE levels compared to controls although there was no association with the startle response. Our findings are consistent with previous studies that have shown increased basal sympathetic tone in FM.[10,37,38]

Limitations

Several limitations of the current study should be mentioned. First, subjects were pre-warned during potential shock conditions and thus, it is possible that we may have had a greater startle response if this shock predictability was reduced. Second, although the vast majority of our subjects rated the stimulus intensity to be in the moderate to high range, the stimulus may not have been perceived to be sufficiently threatening to elicit group differences. Third, although we did our best to match characteristics between groups, confounding factors such as age and psychological comorbidities may have also affected results. However, the prevalence of psychological symptoms and McGill pain scores in our patient population were similar to that reported in community samples of FM[16,18] although their FIQ scores were lower.[58,59] Thus, while our patient sample fairly closely represents the typical FM patient population, our results may not be applicable to all FM patients. Fourth, our sample size may not have been large enough to detect a significant difference between groups, however sample size calculations from our previous startle studies in IBS[45] and IC[54] yielded a sample size of at least 21 per group to detect significant group differences. Lastly, neuroendocrine responses were not drawn immediately

following shock but at end of experiment. Thus, post-procedure levels may not reflect responsiveness to the somatic stimulus.

In summary, FM patients demonstrate that central activation of arousal circuits as measured by the acoustic startle response is associated with persistent symptom-specific anxiety, enhanced pain intensity and abnormal stress hormone responses. As pain related anxiety and intensity were not similarly assessed in our IC or IBS acoustic startle studies, it is not known whether the relationship of these pain-related measures and central excitability is true for other chronic or acute pain conditions.

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FIGURE LEGENDS

Figure 1. The somatic thresholds for pain and maximal tolerated pressure for both the active point (LE) and control point (forearm) measured during the ascending series for the FM patients and healthy controls are shown (mean values ± SEM). FM patients had significantly lower somatic perceptual thresholds than controls.

Figure 2. Acoustic startle responses (ASRs) to the varying threat conditions before and after an actual electrical shock was applied to the biceps muscle. The square root transformed values measured in microvolts (μV) are shown. Overall, both groups showed increasing ASRs with increasing imminence of the biceps stimulation particularly after an actual electrical shock was given ($p<0.01$). There was, however, no overall difference between groups (p=NS).

Figure 3. Salivary cortisol (A) and plasma norepinephrine (B) levels at baseline (time period 1) and at the end of the study (time period 2) are shown. (A) Cortisol levels significantly decreased from baseline to the end of the startle study in both the FM and control groups ($p<0.02$). Baseline and post-procedure salivary cortisol levels were significantly lower in FM patients ($p<0.05$). (B) Plasma NE levels were higher at the beginning and end of the startle procedure in the FM patients than the controls ($p=0.05$); however, there was no significant change in either group from baseline to the post-procedure period.

Figure 4. The percent change in ASRs from the safe to anticipation conditions significantly correlated with physiologic anxiety scores (PASS) in the FM patients. ($r=.615$, $p<.01$). PASS

physiologic pain-related anxiety ratings positively correlated with the % anticipation change in ASRs *after* having already received the electrical stimulus ($r=0.62$, $p<0.01$).

Table 1. Clinical characteristics of women with FM and healthy women

Variables	Controls (n=22)	Fibromyalgia (n=21)	P value
Age (yrs ± SD)	30.7 ± 7.0	37.2 ± 3.7	.05
BMI (± SD)	24.2 ± 4.2	24.4 ± 6.9	.9
Duration of FM symptoms (yrs ± SD)	0	7.6±7.1	<0.01
Menopausal- Pre	22(100%)	14 (67%)	0.01
Peri	0	2 (10%)	0.01
Post	0	5 (24%)	0.01
OCP/ HRT	7 (32%)	5 (24%)	.62
Antidepressant use	0	9 (43%)	<0.01
Low dose	0	5 (24%)	<0.01
High dose	0	4 (19%)	<0.01
HAD- Anxiety (0-21)	3.1 (2.4)	8.5 (5.3)	<0.01

HAD- Depression (0-21)	0.6 (.74)	6.4 (4.3)	<0.01
History of childhood abuse	3 (14%)	7 (33%)	.14
Reported lifetime abuse	4 (18%)	8 (38%)	.14
Early adverse life events	9 (41%)	10 (48%)	.75
Positive SCID*	3/20(15%)	17/20 (85%)	<0.01
PVAQ (mean ± SD) (0-80)	27±3.4	40.9±3.2	<0.01
PASS- Cognitive (0-25)	-----	14.5±5	-----
Physiologic (0-25)	-----	8.1±4	-----
Total (0-100)	-----	40.2±13	-----
FM- 24 hr S (mean± SD) (1-20)	-----	9.4±3.5	-----
FM- 24 hr A (mean± SD) (1-20)	-----	11±3.7	-----
FM- 1 mo. S (mean± SD) (1-20)	-----	11.8±4	-----
FM- 1 mo. A (mean± SD) (1-20)	-----	10±3.5	-----
FIQ- Anxiety (0-10)	-----	5±3.28	-----
Depression (0-10)	-----	3.7±3	-----
Overall Score (0-100)	-----	46.8±14.3	-----

McGill- Present pain intensity (0-10)	-----	5.6±1.3	-----
Total pain (0-45)	-----	20.6±7.3	-----
Overall intensity (0-5)	-----	2.6±0.78	-----

* FM group: past history of depression: 6 (29%), current history of depression: 3 (14%), psychosis 1 (5%), lifetime history of panic disorder: 5 (24%), obsessive compulsive disorder: 5 (24%), post-traumatic stress disorder: 3 (14%), lifetime history of general anxiety: 5 (24%), alcohol abuse: 1 (5%), past history of substance abuse: 1 (5%). Control group: lifetime history of panic disorder: 1 (5%), past history of alcohol abuse: 2 (10%), past history of substance abuse: 1 (5%).

Abbreviations: SD: standard deviation, BMI: body mass index, FM: fibromyalgia, yrs: years, pre: pre-menopausal, post: post-menopausal, peri-menopausal, OCP: oral contraceptive pills, HRT: hormone replacement therapy, HAD: Hospital Anxiety and Depression questionnaire, PVAQ: Pain Vigilance and Awareness scale, PASS: Pain Anxiety Symptom Scale, McGill: Short form McGill Pain Scale, SCID: Structured Clinical Interview for DSM-IV, FM- 24 hr S: sensory intensity rating of muscle pain over the past 24 hours, FM- 24 hr A: unpleasantness rating of muscle pain over the past 24 hours, FM- 1 mo S: sensory intensity rating of muscle pain over the past 1 month, FM- 24 hr A: unpleasantness rating of muscle pain over the past 1 month, FIQ: Fibromyalgia Impact Questionnaire.

Table 2. Mean sensory intensity ratings to varying somatic pressures

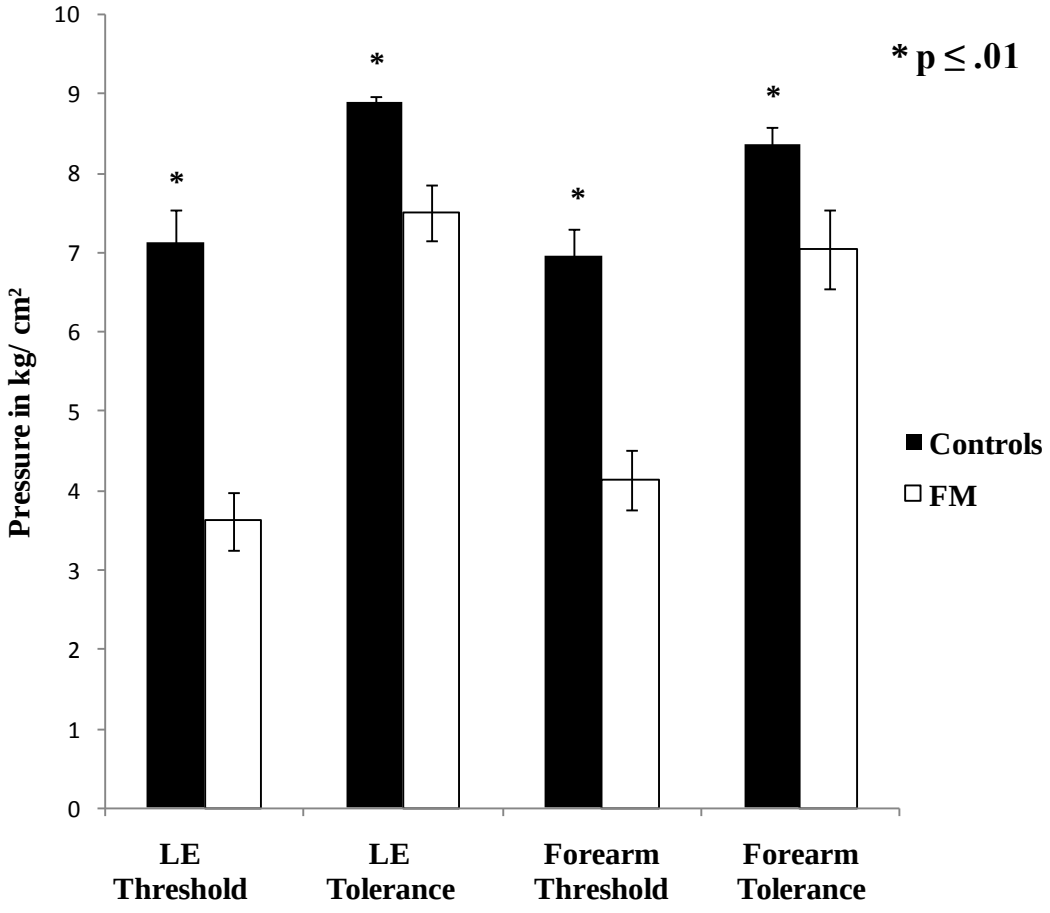
Somatic Pressures	**Controls (n=22)** intensity± SEM	**Fibromyalgia (n=20)** intensity± SEM	**P value**
LE site			0.04
2kg/1.54cm²	3.0±0.5	4.8±0.6	0.07
3kg/1.54cm²	5.0±0.7	7.4±0.6	0.02
4kg/1.54cm²	6.6±0.8	9.3±0.7	0.01
5kg/1.54cm²	7.5±0.9	10.2±0.7	<0.01
6kg/1.54cm²	9.2±1.0	11.6±0.8	0.02
Forearm site			0.04
2kg/1.54cm²	4.1±0.8	5.4±0.6	0.26
3kg/1.54cm²	6.1±0.9	8.2±0.8	0.07
4kg/1.54cm²	7.5±0.9	10.2±0.6	0.02
5kg/1.54cm²	9.5± 1.0	12.0±0.7	0.03
6kg/1.54cm²	10.8±1.0	12.9±0.8	0.07

Abbreviations: SEM: standard error of the mean, LE: lateral epicondyle

Table 3. Acoustic startle responses (sqrt μV) and medication use in the FM patients

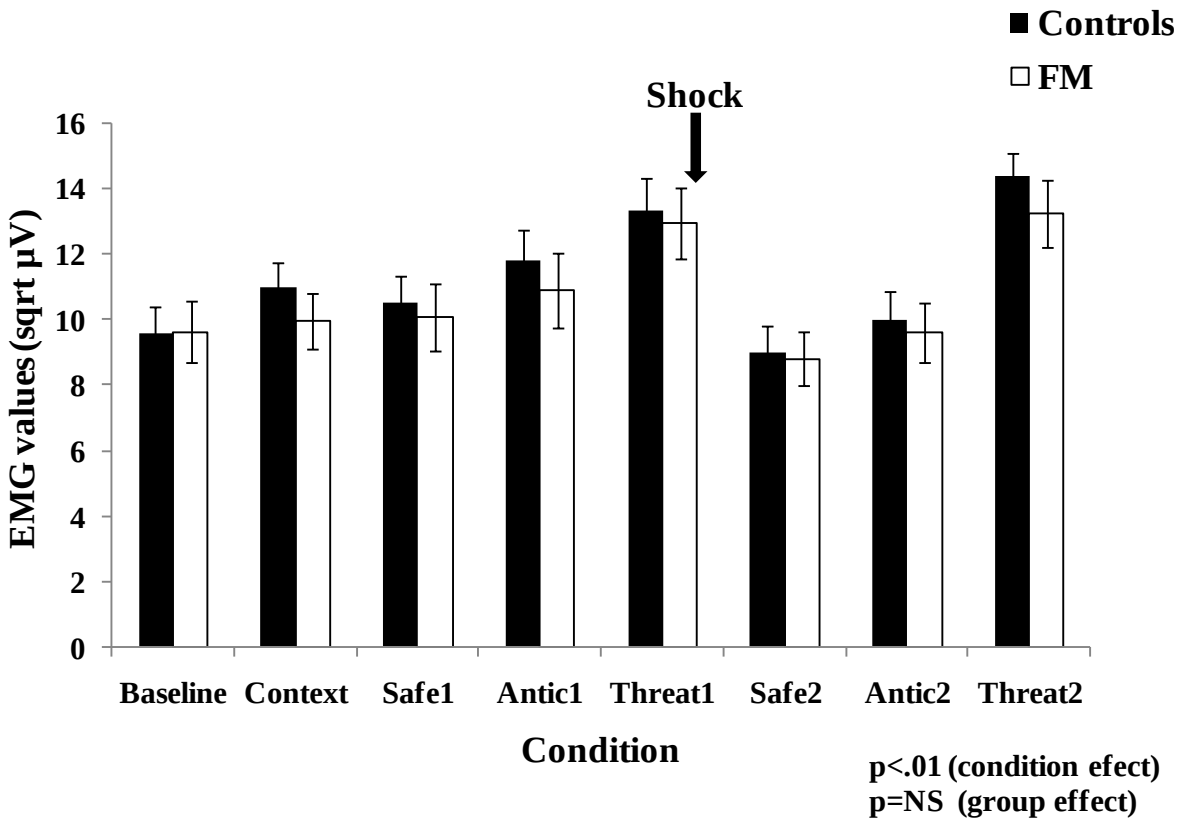
	Startle Condition (post-shock)			**Class of Medication**					
FM Subject #	Safe	Anticipation	Threat	Anxiolytics/ Sedatives	Antidepressants	Opioids	Anti-convulsants	Tryptans	NSAIDs/ Aspirin
1	4.1	**3.2**	5.2		X				
2	4.6	**4.5**	6.2	X					
3	6.1	**4.8**	6.4	X			X		
4	2.5	**5.1**	12.0						
5	6.7	**5.5**	12.9						
6	4.8	**6.0**	7.3						X
7	10.7	**7.4**	13.7	X	X	X		X	
8	8.1	**9.8**	9.7		X				
9	7.6	**10.4**	18.3		X	X			
10	10.3	**10.6**	15.3		X			X	X
11	8.2	**10.8**	11.8						
12	10.4	**11.2**	12.7						
13	11.5	**11.6**	19.1		X				X
14	12.8	**11.9**	17.7						
15	14.7	**12.0**	17.3	X	X				
16	6.3	**12.3**	15.1						
17	13.7	**12.7**	16.5	X	X				
18	11.5	**15.0**	18.2						
19	12.7	**17.7**	16.3						X
Percentage of Use for Each Drug Class				**26%**	**42%**	**11%**	**5%**	**11%**	**21%**

Figure 1



Abbreviations: LE: lateral epicondyle

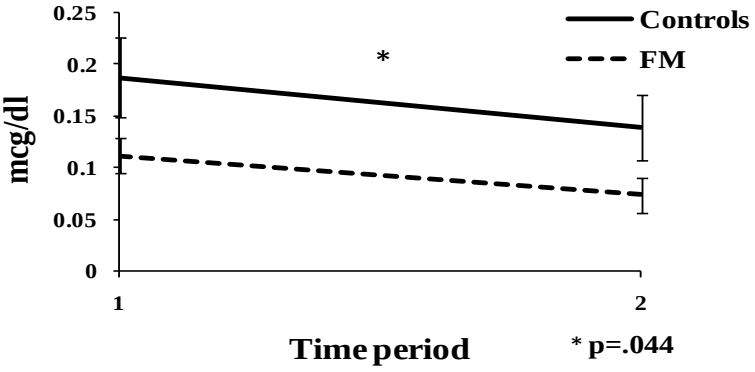
Figure 2



Abbreviations: sqrt µV: square root microvolt, EMG: electromyogram, Safe1: Safe condition prior to receiving shock, Antic1: anticipation condition prior to receiving shock, Threat1: threat condition prior to receiving shock, Safe2: safe condition after having already received a shock, Antic2: anticipation condition after having already received a shock, Threat2: threat condition after having already received a shock, NS: not significant

Figure 3

A. Cortisol



B. Norepinephrine

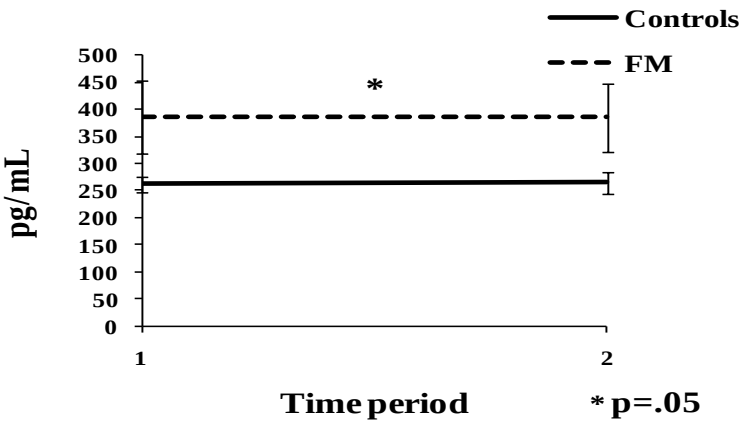
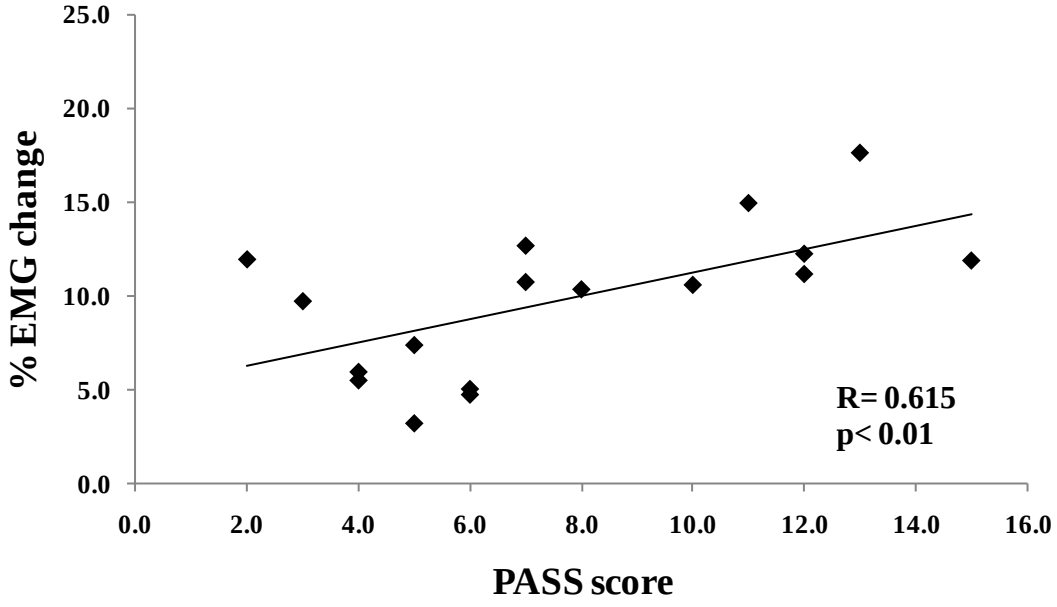


Figure 4



Abbreviations: PASS: Pain Anxiety Symptom Scale, sqrt µV: square root microvolt, EMG: electromyogram

Appendix: Statistical Analysis

Standard T tests were used to compare means of baseline variables between FM and healthy controls (i.e. age, BMI, etc). Chi square tests were used to test group differences for nominal variables. For the primary outcomes (VAS intensity, EMG, catecholamine measurements) mean values for FM and controls were compared across conditions using repeated measures ANOVA (Proc mixed procedure in SAS [SAS Institute Incorporated, Carey, NC]. Post hoc pairwise mean comparisons were made under the ANOVA model using the usual Tukey-Fisher criteria. Residual errors were examined to confirm normality of the data (see table 1). Repeated measures ANOVAs were used instead of factoral ANOVAs to compare means of continuous variables where all groups were related with the same individual(s) being repeatedly measured under different conditions. Means values across conditions were used rather than incorporating all data points in an attempt to increase data reliability. For example, EMG values were obtained seven times across the initial baseline condition. Instead of using all seven values, however, the mean value was used to try to obtain a more accurate EMG reading as well as minimize the effect of time across the condition.

The varying somatic pain ratings obtained were analyzed as a function of the five experimental blocks using a 2 Group (FM, Control) x 5 Condition (2, 3, 4, 5, and 6 kg/1.54cm^2) design.

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Catecholamines were also analyzed using a 2 Group (FM, Control) x 2 Condition (baseline and at the end of the experiment) design on raw data (norepinephrine [NE]) as well as log transformed (cortisol, ACTH) or inverse transformed (epinephrine [EPI]) data due to positive skew.

To account for the age difference between groups, age was controlled for in the model. We attempted to control for psychological differences between groups (anxiety, depression) but found that they were not statistically significant to the model and thus were not included in the

calculations used in the final model. Although age was also not significant to the model, it was kept in the model, as we felt our readers would expect to see age controlled for even though it mathematically made no difference.

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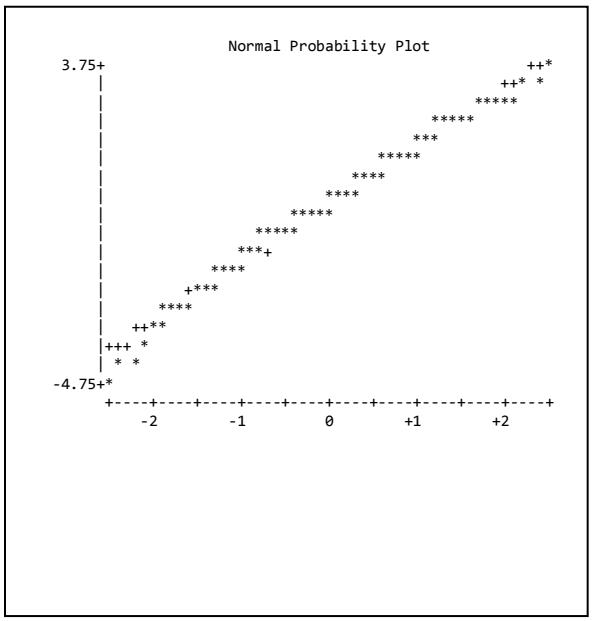
variables to each other was examined using the correlate function in SPSS [Pearson Education, Inc. Boston, MA]. We did not control for multiple correlations, however, even though these analyses were admittedly exploratory, we did place a lot of forethought into the selection of variables being correlated and did not just correlate all variables aimlessly (see Table 2 for a list of all correlations made).

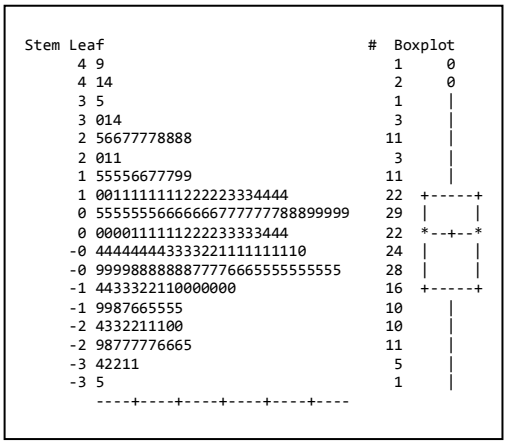
Table 1. Plot of Residual Errors & Normal Probability Plot

A: Somatic Data: Forearm Intensity

```
Stem Leaf                              #  Boxplot
      4 9                              1     0
      4 14                             2     0
      3 5                              1     |
      3 014                            3     |
      2 56677778888                   11     |
      2 011                            3     |
      1 55556677799                   11     |
      1 0011111111222223334444        22  +-----+
      0 555555566666667777777888999999 29  |     |
      0 000011111122223333444         22  *--+--*
     -0 444444443333221111111110      24  |     |
     -0 999988888887777666555555555   28  |     |
     -1 4433322110000000              16  +-----+
     -1 9987665555                    10     |
     -2 4332211100                    10     |
     -2 98777776665                   11     |
     -3 42211                          5     |
     -3 5                              1     |
        ----+----+----+----+----+----
```



B. Somatic Data: Lateral Epicondyle Intensity



```
Stem Leaf                              #  Boxplot
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      4 14                             2     0
      3 5                              1     |
      3 014                            3     |
      2 56677778888                   11     |
      2 011                            3     |
      1 55556677799                   11     |
      1 00111111112222233344 44       22  +-----+
      0 555555566666667777777888999999 29  |     |
      0 00001111112222333344 44       22  *--+--*
     -0 444444443333221111111110      24  |     |
     -0 99998888888777766655555555555 28  |     |
     -1 4433322110000000              16  +-----+
     -1 9987665555                    10     |
     -2 4332211100                    10     |
     -2 98777776665                   11     |
     -3 42211                          5     |
     -3 5                              1     |
        ----+----+----+----+----+----
```
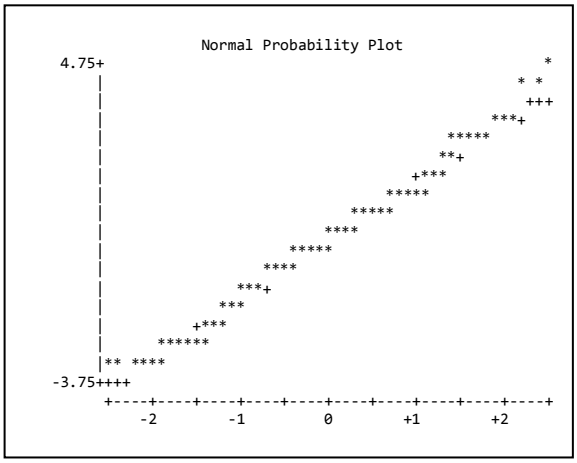
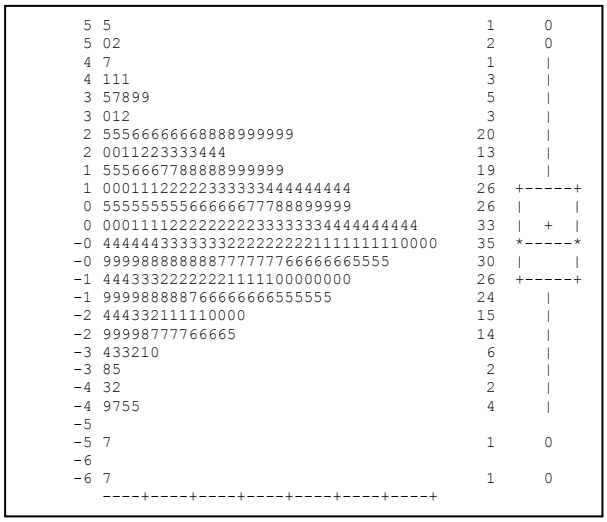



C. Startle Data



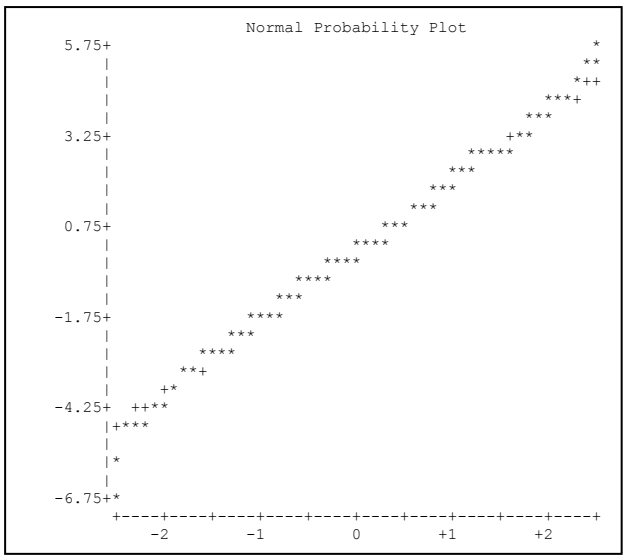


Table 2: Correlations

All Correlation with startle measures									
		Baseline	**Context**	**Safe 1**	**Safe 2**	**% Anticipation 1**	**% Anticipation 2**	**% Threat 1**	**% Threat 2**
PASS-cognitive	r	0.36	0.14	0.20	0.27	0.18	0.61**	0.42	0.43
	p	0.14	0.59	0.45	0.29	0.48	0.01	0.09	0.09
PASS-physiologic	r	0.38	0.26	0.38	0.32	0.34	0.62**	0.36	0.49*
	p	0.12	0.29	0.13	0.22	0.18	0.01	0.15	0.04
McGill	r	0.45	0.42	0.39	0.29	0.25	0.08	0.52*	0.35
	p	0.07	0.09	0.13	0.28	0.36	0.78	0.04	0.19
HAD-Anxiety	r	-0.08	-0.13	-0.07	0.15	-0.05	0.08	-0.02	0.04
	p	0.74	0.58	0.79	0.53	0.85	0.76	0.95	0.86
HAD-Depression	r	-0.42	-0.46*	-0.54*	-0.37	-0.32	-0.28	-0.43	-0.15
	p	0.07	0.04	0.02	0.12	0.18	0.25	0.07	0.54
PVAQ	r	0.09	0.06	0.18	0.03	0.18	0.47	0.34	0.23
	P	0.71	0.80	0.50	0.89	0.49	0.06	0.18	0.38
FIQ	r	-0.17	-0.19	-0.21	-0.10	-0.04	0.09	-0.01	-0.11
	p	0.47	0.43	0.40	0.70	0.86	0.72	0.98	0.66
Forearm Threshold	r	0.19	0.27	0.29	0.36	0.17	0.26	0.40	0.39
	p	0.44	0.27	0.25	0.15	0.49	0.31	0.10	0.11
Forearm Tolerance	r	0.33	0.23	0.24	0.24	0.28	-0.04	0.19	0.19
	p	0.17	0.34	0.34	0.33	0.27	0.87	0.46	0.46
LE Threshold	r	0.14	0.12	0.10	0.10	0.12	0.11	0.13	0.27
	P	0.57	0.62	0.69	0.68	0.64	0.67	0.59	0.27
LE Tolerance	r	-0.07	-0.16	-0.14	-0.10	-0.05	-0.39	-0.31	-0.10

	p	0.76	0.50	0.58	0.70	0.83	0.11	0.21	0.69
Cortisol1	r	-0.23	-0.20	-0.26	-0.30	-0.29	-0.11	-0.23	-0.12
	P	0.35	0.41	0.30	0.22	0.23	0.66	0.35	0.63
Cortisol2	r	.573*	.510*	0.45	0.30	0.23	0.25	0.23	0.38
	p	0.01	0.03	0.07	0.24	0.37	0.33	0.37	0.14
ACTH1	r	-0.27	-0.14	-0.18	-0.02	-0.07	0.28	0.08	0.12
	p	0.27	0.59	0.50	0.95	0.79	0.28	0.75	0.63
ACTH2	r	-0.26	-0.19	-0.22	0.00	-0.07	0.18	0.11	0.19
	p	0.28	0.44	0.38	1.00	0.78	0.46	0.66	0.46
NE1	r	-0.03	-0.15	-0.10	-0.05	0.32	0.02	0.01	0.26
	p	0.93	0.60	0.73	0.86	0.24	0.95	0.97	0.35
NE2	r	0.00	-0.17	-0.04	-0.04	0.38	0.21	0.16	0.40
	p	1.00	0.53	0.88	0.88	0.16	0.45	0.58	0.13
EPI1	r	0.15	0.34	0.22	0.29	0.04	0.21	0.17	0.27
	p	0.58	0.22	0.43	0.29	0.90	0.46	0.54	0.33
EPI2	r	0.00	0.00	0.09	0.16	0.01	-0.07	0.27	0.10
	p	0.99	1.00	0.76	0.58	0.96	0.81	0.32	0.71

** Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Abbreviations: Baseline: baseline EMG, Context: Context EMG, Safe 1: EMG during the safe condition during the first block, Safe 2: EMG during the safe condition during the second block, % Anticipation 1: percent change EMG during the anticipation condition during the first block, % Anticipation 2: percent change EMG during the anticipation condition during the second block, % Threat 1: percent change EMG during the threat condition during the first block, % Threat 2: percent change EMG during the threat condition during the second block, PASS-Cognitive: Pain Anxiety Symptom Scale cognitive anxiety score, PASS-Physiologic: Pain Anxiety Symptom Scale physiologic anxiety score, McGill: Short form McGill Pain Scale , HAD-Anxiety: Hospital

Anxiety and Depression questionnaire- Anxiety rating, HAD-Depression: Hospital Anxiety and Depression questionnaire- Depression rating, Forearm Threshold: forearm somatic threshold for pain, Forearm Tolerance: forearm somatic maximal tolerated pressure for pain, LE Threat: lateral epicondyle somatic threshold for pain, LE Tolerance: lateral epicondyle somatic maximal tolerated pressure for pain, PVAQ: Pain Vigilance and Awareness scale, FIQ: Fibromyalgia Impact Questionnaire, Cortisol 1: baseline cortisol level (period 1), Cortisol 2: cortisol level at the end of the study (period 2), ACTH 1: Baseline adrenocorticotropic hormone level (period 1), ACTH 2: adrenocorticotropic hormone level at the end of the study (period 2), NE 1: baseline norepinephrine level (period 1), NE 2: norepinephrine level at the end of the study (period 2), EPI 1: baseline epinephrine level (period 1), EPI 2: epinephrine level at the end of the study (period 2).

CHAPTER TWO: APPENDIX OF THE THESIS

Pain Related Anxiety and Pain Severity are Associated with Increased Activation of Central Arousal Circuits in Fibromylagia

by

Trinh Thi Nhat Truong

Master of Science in Clinical Research

University of California, Los Angeles, 2013

Robert M Elashoff, Chair

Standard T tests were used to compare means of baseline variables between FM and healthy controls (i.e. age, BMI, etc). Chi square tests were used to test group differences for nominal variables. For the primary outcomes (VAS intensity, EMG, catecholamine measurements) mean values for FM and controls were compared across conditions using repeated measures ANOVA (Proc mixed procedure in SAS [SAS Institute Incorporated, Carey, NC]. Post hoc pairwise mean comparisons were made under the ANOVA model using the usual Tukey-Fisher criteria. Residual errors were examined to confirm normality of the data (see table 1). Repeated measures ANOVAs were used instead of factoral ANOVAs to compare means of continuous variables where all groups were related with the same individual(s) being repeatedly measured under different conditions. Means values across conditions were used rather than incorporating all data points in an attempt to increase data reliability. For example, EMG values

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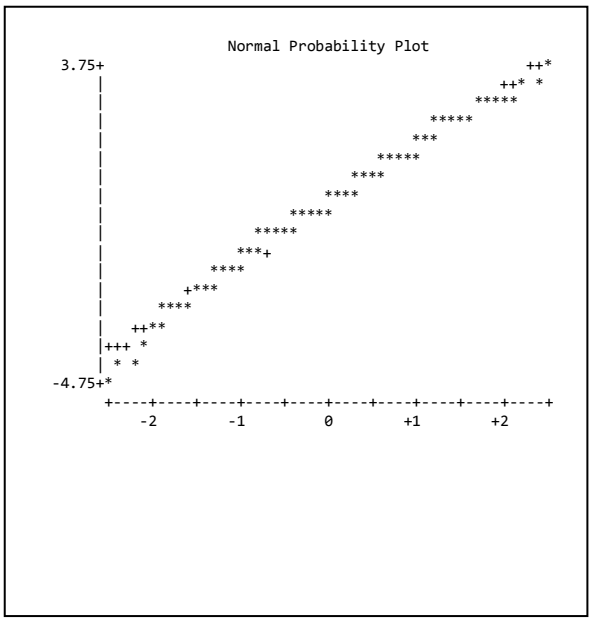
```
Stem Leaf                              #  Boxplot
      4 9                              1     0
      4 14                             2     0
      3 5                              1     |
      3 014                            3     |
      2 56677778888                   11     |
      2 011                            3     |
      1 55556677799                   11     |
      1 0011111111222223334444        22  +-----+
      0 555555566666667777777888999999 29  |     |
      0 000011111122223333344         22  *--+--*
     -0 444444443333221111111110      24  |     |
     -0 999988888887777666555555555   28  |     |
     -1 4433322110000000              16  +-----+
     -1 9987665555                    10     |
     -2 4332211100                    10     |
     -2 98777776665                   11     |
     -3 42211                          5     |
     -3 5                              1     |
        ----+----+----+----+----+----
```



B. Somatic Data: Lateral Epicondyle Intensity

```
Stem Leaf                              #  Boxplot
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      4 14                             2     0
      3 5                              1     |
      3 014                            3     |
      2 56677778888                   11     |
      2 011                            3     |
      1 55556677799                   11     |
      1 001111111222223334444         22  +-----+
      0 555555566666667777777888999999 29  |     |
      0 000011111122223333444         22  *--+--*
     -0 444444443333221111111110      24  |     |
     -0 999988888887777666555555555   28  |     |
     -1 4433322110000000              16  +-----+
     -1 9987665555                    10     |
     -2 4332211100                    10     |
     -2 98777776665                   11     |
     -3 42211                          5     |
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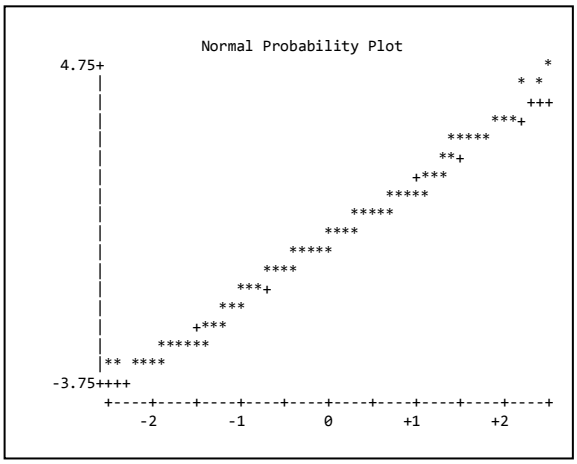

C. Startle Data

```
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  3 57899                                      5     |
  3 012                                        3     |
  2 55566666668888999999                      20     |
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  1 5556667788888999999                       19     |
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  0 000111122222222233333334444444444         33  |  +  |
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 -3 433210                                     6     |
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 -4 32                                         2     |
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 -6
 -6 7                                          1     0
    ----+----+----+----+----+----+----+
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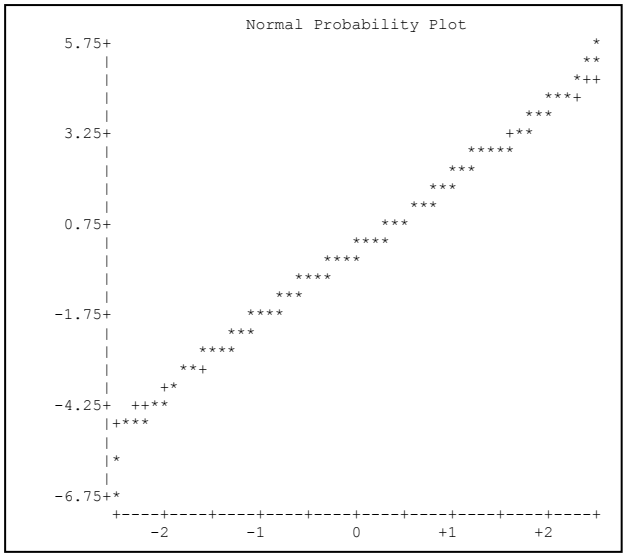

Table 2: Correlations

All Correlation with startle measures									
		Baseline	**Context**	**Safe 1**	**Safe 2**	**% Anticipation 1**	**% Anticipation 2**	**% Threat 1**	**% Threat 2**
PASS-cognitive	r	0.36	0.14	0.20	0.27	0.18	0.61**	0.42	0.43
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	p	0.12	0.29	0.13	0.22	0.18	0.01	0.15	0.04
McGill	r	0.45	0.42	0.39	0.29	0.25	0.08	0.52*	0.35
	p	0.07	0.09	0.13	0.28	0.36	0.78	0.04	0.19
HAD-Anxiety	r	-0.08	-0.13	-0.07	0.15	-0.05	0.08	-0.02	0.04
	p	0.74	0.58	0.79	0.53	0.85	0.76	0.95	0.86
HAD-Depression	r	-0.42	-0.46*	-0.54*	-0.37	-0.32	-0.28	-0.43	-0.15
	p	0.07	0.04	0.02	0.12	0.18	0.25	0.07	0.54
PVAQ	r	0.09	0.06	0.18	0.03	0.18	0.47	0.34	0.23
	P	0.71	0.80	0.50	0.89	0.49	0.06	0.18	0.38
FIQ	r	-0.17	-0.19	-0.21	-0.10	-0.04	0.09	-0.01	-0.11
	p	0.47	0.43	0.40	0.70	0.86	0.72	0.98	0.66
Forearm Threshold	r	0.19	0.27	0.29	0.36	0.17	0.26	0.40	0.39
	p	0.44	0.27	0.25	0.15	0.49	0.31	0.10	0.11
Forearm Tolerance	r	0.33	0.23	0.24	0.24	0.28	-0.04	0.19	0.19
	p	0.17	0.34	0.34	0.33	0.27	0.87	0.46	0.46
LE Threshold	r	0.14	0.12	0.10	0.10	0.12	0.11	0.13	0.27
	P	0.57	0.62	0.69	0.68	0.64	0.67	0.59	0.27

LE Tolerance	r	-0.07	-0.16	-0.14	-0.10	-0.05	-0.39	-0.31	-0.10
	p	0.76	0.50	0.58	0.70	0.83	0.11	0.21	0.69
Cortisol1	r	-0.23	-0.20	-0.26	-0.30	-0.29	-0.11	-0.23	-0.12
	P	0.35	0.41	0.30	0.22	0.23	0.66	0.35	0.63
Cortisol2	r	.573*	.510*	0.45	0.30	0.23	0.25	0.23	0.38
	p	0.01	0.03	0.07	0.24	0.37	0.33	0.37	0.14
ACTH1	r	-0.27	-0.14	-0.18	-0.02	-0.07	0.28	0.08	0.12
	p	0.27	0.59	0.50	0.95	0.79	0.28	0.75	0.63
ACTH2	r	-0.26	-0.19	-0.22	0.00	-0.07	0.18	0.11	0.19
	p	0.28	0.44	0.38	1.00	0.78	0.46	0.66	0.46
NE1	r	-0.03	-0.15	-0.10	-0.05	0.32	0.02	0.01	0.26
	p	0.93	0.60	0.73	0.86	0.24	0.95	0.97	0.35
NE2	r	0.00	-0.17	-0.04	-0.04	0.38	0.21	0.16	0.40
	p	1.00	0.53	0.88	0.88	0.16	0.45	0.58	0.13
EPI1	r	0.15	0.34	0.22	0.29	0.04	0.21	0.17	0.27
	p	0.58	0.22	0.43	0.29	0.90	0.46	0.54	0.33
EPI2	r	0.00	0.00	0.09	0.16	0.01	-0.07	0.27	0.10
	p	0.99	1.00	0.76	0.58	0.96	0.81	0.32	0.71

** Correlation is significant at the 0.01 level (2-tailed)

*Correlation is significant at the 0.05 level (2-tailed)

Abbreviations: Baseline: baseline EMG, Context: Context EMG, Safe 1: EMG during the safe condition during the first block, Safe 2: EMG during the safe condition during the second block, % Anticipation 1: percent change EMG during the anticipation condition during the first block, % Anticipation 2: percent change EMG during the anticipation condition during the second block, % Threat 1: percent change EMG during the threat condition during the first block, % Threat 2: percent change EMG during the threat condition during the second block, PASS-Cognitive: Pain Anxiety Symptom Scale cognitive anxiety score, PASS-Physiologic: Pain Anxiety

Symptom Scale physiologic anxiety score, McGill: Short form McGill Pain Scale , HAD-Anxiety: Hospital Anxiety and Depression questionnaire- Anxiety rating, HAD-Depression: Hospital Anxiety and Depression questionnaire- Depression rating, Forearm Threshold: forearm somatic threshold for pain, Forearm Tolerance: forearm somatic maximal tolerated pressure for pain, LE Threat: lateral epicondyle somatic threshold for pain, LE Tolerance: lateral epicondyle somatic maximal tolerated pressure for pain, PVAQ: Pain Vigilance and Awareness scale, FIQ: Fibromyalgia Impact Questionnaire, Cortisol 1: baseline cortisol level (period 1), Cortisol 2: cortisol level at the end of the study (period 2), ACTH 1: Baseline adrenocorticotropic hormone level (period 1), ACTH 2: adrenocorticotropic hormone level at the end of the study (period 2), NE 1: baseline norepinephrine level (period 1), NE 2: norepinephrine level at the end of the study (period 2), EPI 1: baseline epinephrine level (period 1), EPI 2: epinephrine level at the end of the study (period 2).