Date: 6/6/2018
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Application Number: HP-00064214

Development of a reliable neurophysiological pain assessment tool

Short title: Alpha as a Predictive Biomarker (APB)

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Supported by:

Purdue Pharma LP

Version Number: 6/6/2018

1. Tool Revision History

Version Number: 8/25/2016
Summary of Revisions Made: added a screening session, for a total of 3 visits instead of 2.

Version Number: 8/4/2017
Summary of Revisions Made: added brush allodynia test; urine toxicity tests changed to randomly selected (1/10); clarified that the first 60 subjects would complete visits 0, 1, and 2, and the remaining subjects would complete visits 0 and 1.

Version Number: 6/6/2018
Summary of Revisions Made: added quantitative sensory testing on the face and neck. Updated offset analgesia to include simultaneous testing at two body sites.

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2. Lay Summary

Neurophysiological investigations of pain have suggested that electroencephalograpic (EEG) measures of peak alpha frequency might provide a means of objective pain assessment. In healthy subjects, increased peak alpha frequency is strongly correlated with pain self-reports after exposure to acute noxious heat. Conversely, chronic pain patients compared to healthy control display decreased peak alpha frequencies higher in alpha power. Measures of peak alpha frequency also show a negative relationship with disease duration (de Vries et al., 2013), suggesting that peak alpha frequency may not only index ongoing pain but also disease progression.

While a simple EEG-based test could provide a useful diagnostic tool for assessing pain objectively, no study to date has evaluated this potential in a systematic way. Here we propose to build on our ongoing work to develop a reliable and simple measure of pain. Our overall aim is to determine the predictive accuracy, reliability, and specificity of EEG alpha activity in acute pain and a model of neuropathic pain in healthy subjects.

3. Purpose/specific aims/objectives and hypothesis

Our overall aim is to determine the predictive accuracy, reliability, and specificity of EEG alpha activity in acute pain and a model of neuropathic pain in healthy subjects.

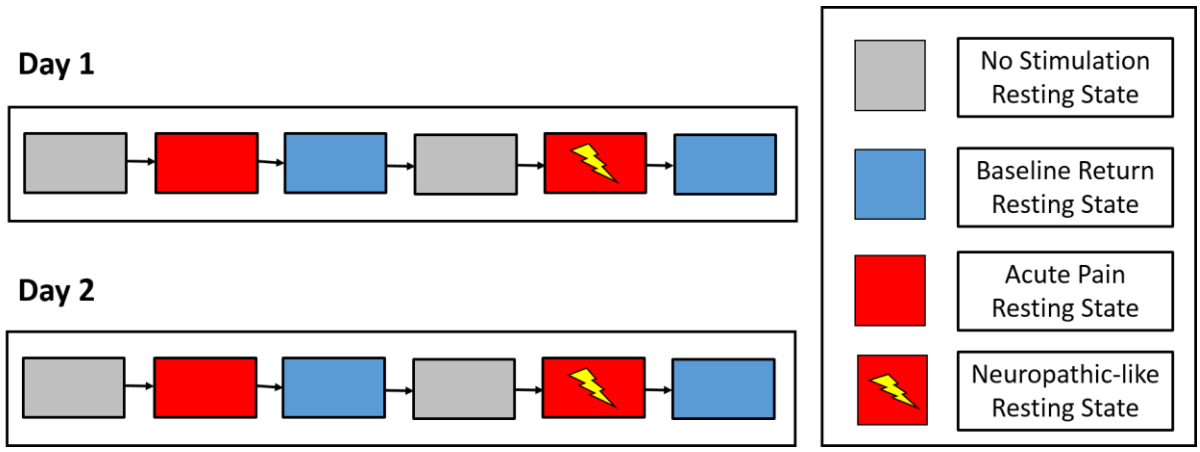
Aim 1. To determine how the experience of innocuous and noxious stimulation affects alpha activity and whether alpha activity can predict acute and neuropathic-like pain.

Aim 2. To assess the test-retest reliability of the alpha frequency as an objective measure of pain in two different experimental pain states (acute and neuropathic-like).

If our approach is successful it would be a major step forward to providing an objective measurement of pain. This approach could be adopted by other laboratories to test on multiple chronic pain populations. Once we have optimized the procedure, the setup could be simplified to only include a few recording electrodes and would thus be a relatively quick method. Future work would also include testing the effects of analgesics versus placebo in healthy subjects, as well as in chronic pain patients, to determine whether this method could provide a sensitive test of drug efficacy that would not rely on subjective ratings.

4. Research design

120 healthy male and female adult subjects will complete the following procedures on two or three separate visits, separated by at least three weeks. A flow chart of the events can be seen in the figure. Using a 64-channel EEG system, peak alpha frequency will be measured while participants undergo a baseline, pain-free resting state (no stimulation), an acute pain resting state, a return from pain resting state, and a neuropathic-like pain (ongoing pain) resting state.



Experimental paradigm. Subjects are tested on one or two separate visits and undergo multiple types of painful and nonpainful stimulation while EEG is continuously recorded.

Visit 0 is an eligibility screening visit that consists of drug testing (randomly selected with 1/10 probability), a health screening, and quantitative sensory testing (QST) with thermal stimulation by machine and capsaicin cream application. Upon arriving at the imaging site, participants will complete all relevant consent, demographic, and questionnaire forms. Participants will then undergo a brief sensory testing session, using the Medoc Pathway system, to assess the temperature at which each individual first feels pain (pain threshold). All sensory testing will occur with a thermode placed on the left volar forearm.

A subject is eligible to continue to complete one or two EEG study visits once the study team confirms that the subject's warmth detection (WDT) and heat pain thresholds (HPT) are in the range of normal values (WDT of less than 39°C, HPT between 41 and 47°C) and that the subject has a pain reaction to capsaicin cream. The EEG study visit(s) consist of a sequence of resting state EEG sessions. During all resting state sessions, moment to moment fluctuations in pain ratings will be collected in order to test their relationship to peak alpha frequency.

Subjects will then complete two separate EEG visits; all visits (V0-V2) are separated by at least three weeks. Using a 64-channel EEG system, peak alpha frequency will be measured while participants undergo a baseline, pain-free resting state (no stimulation), an acute pain resting state, a return from pain resting state, and a neuropathic-like pain resting state. During all resting state sessions, moment to moment fluctuations in pain ratings will be collected in order to test their relationship to peak alpha frequency.

At the start of Visit 1 and Visit 2, study staff will again randomly conduct a urine toxicity (and take pregnancy test for female subjects) screen and reassess the participant's eligibility. Next, participants complete questionnaire. We will then prepare participants for EEG recordings with a 64-channel EEG cap and auxiliary recordings of skin conductance, respiration, and ECG. To ensure consistent head electrode placement across participants the two frontal electrodes, FP1 and FP2, will be placed a distance from the eyebrows equal to 10% of the total skull diameter (measured from the eyebrows to the inion

landmark). The center array electrode, Cz, will be placed at a distance from the inion and eyebrow that is equal to 50% of the total skull diameter. Once the cap has been placed appropriately, conducting gel will be passed through each head electrode until electrode impedance falls below the 5 mV mark and online assessment of EEG signal at each electrode reveals clear brain signal.

Participants will then undergo a brief sensory testing session, using the Medoc Pathway system, to assess the temperature at which each individual first feels pain (pain threshold). All sensory testing will occur with a thermode placed on the left volar forearm.

Prior to beginning the formal experiment, participants will complete a one minute eyes open resting state and a one minute eyes closed resting state. These two sessions will be used to identify each individual's alpha frequency band.

The following resting state scans are completed in a darkened testing room with subjects instructed to close their eyes, remain still, and avoid falling asleep. All resting state scans are five minutes in length except for the acute pain condition which is comprised of five, one minute resting state scans. During all scans, pain ratings will be captured with an analog response device. Scans are separated by brief rest periods.

Condition 1: innocuous. The thermode is set to a baseline temperature of 32°C (skin temperature).

Condition 2: acute pain. The thermode will be set to the temperature that previously produced moderate pain intensity.

Condition 3: neuropathic-like pain. A topical application of 10% capsaicin cream will be placed on the participant's left volar forearm and covered with a Tegaderm bandage. A 40°C thermode will placed directly on top of the capsaicin, and incubation will occur for twenty minutes. 40°C is confirmed as a temperature below the participant's pain threshold at V0. A five minute resting state EEG session follows incubation.

Condition 4: return to baseline. Following completion of the acute pain and neuropathic-like pain conditions, an ice-pack will be placed directly over the recently stimulated skin. The icepack will be held in place for five minutes. Pain ratings will be assessed to ensure that pain reports return to baseline levels. Following five minutes of icepack application, the ice pack will be removed and the thermode placed on the skin at skin temperature.

5. Relevant prior experience and gaps in current knowledge

While a simple EEG-based test could provide a useful diagnostic tool for assessing pain objectively, no study to date has evaluated this potential in a systematic way. Here we propose to build on our ongoing work to develop a reliable and simple measure of pain.

6. Background, rationale, and significance, and supporting literature

Neurophysiological investigations of pain have suggested that peak alpha frequency might provide a means of objective pain assessment. In healthy subjects, increased peak alpha frequency is strongly correlated with pain self-reports after exposure to acute noxious heat (*Nir et al., 2010*). Conversely, chronic pain patients compared to healthy control display decreased peak alpha frequencies higher in alpha power (*Sarnthein et al., 2006*). Measures of peak alpha frequency also show a negative

relationship with disease duration (de Vries et al., 2013), suggesting that peak alpha frequency may not only index ongoing pain but also disease progression. Another important factor is that these outcomes are often observed from temporal and central electrodes overlying the secondary somatosensory/posterior insular region (S2/pINS) (*Dowman et al., 2008*), which strongly tracks nociceptive input and is potentially essential to the experience of pain (*Garcia-Larrea, 2012*). This is important as it indicates that peak alpha frequency not only appears to index pain but originates from a site is both easily accessible with EEG and biologically plausible.

As part of a larger study, our lab recently collected peak alpha frequencies from healthy individuals during exposure to capsaicin and a previously innocuous heat stimulus, which we herein refer to as "neuropathic-like pain." This protocol induces hyperalgesia and allodynia in a large majority of individuals, making it a clinically relevant model since it matches several characteristics of chronic neuropathic pain (*Baron et al., 2009*). Specifically, the capsaicin model induces sustained burning pain, with periods of spontaneous pain, brush allodynia, and heat hyperalgesia. These symptoms are common to several neuropathic pain disorders such as diabetic neuropathy and complex regional pain syndrome. Other models of ongoing pain in experimental settings include infusion of hypertonic saline into the muscle or the use of a prolonged pressure stimulus. The capsaicin method is the model of choice in the current protocol because we can control the pain intensity level for an extended period of time with the application of a warm thermode at a temperature below the pain threshold. The application of a thermode on the capsaicin is required to achieve a consistent pain intensity rating. Some subjects experience mild ongoing (spontaneous) pain even in the absence of a thermode, but the majority of subjects require the application of a warm thermode. Because we use a low temperature thermode, there is no increased risk of damaging the tissue. We use this method in several other ongoing studies in the laboratory and find we can consistently achieve a prolonged pain state in subjects who respond to capsaicin. In direct contrast to what has been found with an acute painful stimulus alone, our analysis revealed a negative relationship between measured peak alpha frequency and reported pain scores at both temporal and central electrodes. The greater pain intensity an individual reported, the lower their observed peak alpha frequency was, mirroring the lower peak alpha frequencies found in chronic pain patients. Furthering this parallel, we found that peak alpha frequency power was higher following capsaicin administration than it was during an otherwise-identical, but pain-free resting state.

While our results suggest peak alpha frequency may distinguish between acute and neuropathic-like pain states, further work is needed to determine how individual differences affect peak alpha frequency across pain states as well as to assess the test-retest reliability of peak alpha frequency during pain states.

7. Inclusion/exclusion criteria

Inclusion Criteria

In order to be eligible to participate in this study, a subject must meet **all** of the following criteria:

- Able to speak, read, and write English
- Between 21 and 44 years of age
- Able to understand and willing to comply with all study procedures and is available for the duration of the study
- Free of an acute or chronic pain condition

- Not history of psychiatric or neurologic condition

Exclusion Criteria

A potential subject who meets any of the following criteria will be excluded from participation in this study:

- Unable to undergo EEG, assessed on an individual basis
- History of unstable major psychiatric disorder (self-report)
- History of chronic pain (self-report)
- More than 14 alcoholic drinks per week on average (self-report)
- Active [within 6 months] substance or alcohol abuse (self-report and urine toxicology)
- Use of opioids (self-report and urine toxicology)
- History of major depressive disorder (self-report)
- Pregnant or Lactating (women only), based on (self-report and urine test)
- Anything that, in the opinion of the investigator, would place the subject at increased risk or preclude the subject's full compliance with or completion of the study

8. Recruitment

The target population for the study is healthy women and men between 18 and 44 years of age. 120 subjects will be enrolled. Subjects will be recruited locally from local campus flyer, website, and email advertisements as well as by Craigslist posting.

In order to support these recruitment goals, research staff will use the following materials.

Flyers. Research staff will post printed IRB-approved flyers at various locations, including, but not limited to the University of Maryland Baltimore Campus. The research team members will obtain permission to post flyers in and around local university campuses (i.e. Johns Hopkins University, Notre Dame University of Maryland, Loyola University, Stevenson University, Towson University, University of Maryland Baltimore County, and Goucher College).

Websites. Research staff will post the IRB-approved web ad on the online classified website (www.craigslist.org) once a week. Advertisements are free to post and are listed for 10 days. The ad will be posted in the "Community" category under "Volunteers" for the Baltimore-Metro area. Additionally, the IRB-approved online advertisement will be placed in The Elm, the UMB community website once a month. Advertisements are free to post and run for 4 weeks. The content of the advertisement will be available on the website (www.elm.umaryland.edu) and in weekly emails to UMB staff, students, and faculty.

Interested potential participants will contact the study team and be screened by telephone (see telephone screen attachment). Names, phone numbers, and email addresses will be kept for eligible subjects for contact purposes only. The screening forms will be destroyed if a subject is enrolled in the study or is ineligible for participation.

Avoiding participant coercion or undue influence

A copy of the Informed Consent will be handed or mailed to the individual before the actual screening takes place. An in-depth discussion of the Informed Consent will take place before the screening visit or at the beginning of that visit before any testing commences.

The participant will be given ample time to ask questions and consider the answers that we will provide. Only after all questions are answered and all concerns are addressed will the subject be asked to sign the witnessed Informed Consent. Participants will be determined to be competent with the use of an Evaluation to Sign Informed Consent assessment questionnaire. A copy of the questionnaire is attached.

Participants will be assured that participation in or withdrawal from the study in no way affects their access to care.

Students will undergo the same recruitment and informed consent procedures as all other volunteers. There will be no requirement for student participants to identify themselves as students. Students will be assured that their participation is strictly voluntary and their decision to participate or withdraw will in no way affect them academically or otherwise. Student participant confidentiality will be protected by the same mechanisms as for all participants, including using identification codes in place of names.

9. Detailed procedures

Individuals who meet the inclusion criteria will be scheduled for Visit 0. Study enrollment occurs when a participant arrives at Visit 0. The first 60 participants will complete Visits 1 and 2, while the remaining participants will only complete Visit 2. Visit 1 occurs at least 3 weeks after Visit0 and Visit 2 occurs at least 3 weeks after Visit 1.

Visit 0:

The following procedures and assessments will be conducted at Visit 0:

1. Obtain informed consent
2. Perform urine screening for toxicology (random selection)
3. Urine pregnancy test (females only)
4. Administer health history form and review eligibility criteria, complete and sign eligibility form
5. Administer questionnaires (STAI-T, STAI-S, SCQ, BDI, EHI, BIS-11)
6. Pain scale training on COVAS
7. Quantitative Sensory Testing (QST) thermal threshold/supra-threshold testing (see below for details)
8. Offset analgesia test (see below for details)
9. Administer post-pain questionnaires (PCS, SFMPQ)
10. Ice pack application – total of 5 minutes or until pain = 0 for 60 seconds
11. Capsaicin application and incubation
12. Brush allodynia test and flare mapping
13. Ice pack application – total of 5 minutes or until pain = 0 for 60 seconds
14. Payment form completion
15. Schedule Visit 1

Visit 1:

The following procedures and assessments will be conducted at Visit 1:

1. Perform urine screening for toxicology (random selection)
2. Urine pregnancy test (females only)
3. Review eligibility criteria, complete and sign eligibility form

4. Administer questionnaires (STAI-T, STAI-S, SCQ, BDI, EHI, BIS-11)
5. Pain scale training on COVAS
6. EEG cap setup
7. Quantitative Sensory Testing (QST) thermal threshold/supra-threshold testing (see below for details)
8. Offset analgesia test (see below for details)
9. Ice pack application – total of 5 minutes or until pain = 0 for 60 seconds
10. Resting state EEG runs (acute series):
    a. 1 minute eyes open resting state
    b. 1 minute eyes closed resting state
    c. Baseline-acute resting state – 5 minutes (baseline thermode on)
    d. Painful-acute resting state – 5 minutes
    e. Administer post-pain questionnaires (PCS, SFMPQ)
    f. Ice pack application – total of 5 minutes or until pain = 0 for 60 seconds (no EEG recorded)
    g. Acute-return baseline resting state – 5 minutes
11. 5 minute break
12. Resting state EEG runs (chronic series):
    a. Capsaicin application and incubation (EEG recorded) – 20 minutes
    b. Painful-chronic resting state – 5 minutes
    c. Administer post-pain questionnaires (PCS, SFMPQ)
    d. Ice pack application – total of 5 minutes or until pain = 0 for 60 seconds (no EEG recorded)
    e. Acute-return baseline resting state – 5 minutes (baseline thermode on)
    f. Rekindle. Painful-chronic resting state –5minutes
13. Capsaicin removal and brush allodynia test and flare mapping
14. EEG cap removal
15. Payment form completion
16. Schedule Visit 2

Visit 2:

The following procedures and assessments will be conducted at Visit 2:

1. Perform urine screening for toxicology (random selection)
2. Urine pregnancy test (females only)
3. Review eligibility criteria, complete and sign eligibility form
4. Administer questionnaires (STAI-T, STAI-S, SCQ, BDI, EHI, BIS-11)
5. Pain scale training on COVAS
6. EEG cap setup
7. Quantitative Sensory Testing (QST) thermal threshold/supra-threshold testing (see below for details)
8. Offset analgesia test (see below for details)
9. Ice pack application – total of 5 minutes or until pain = 0 for 60 seconds
10. Resting state EEG runs (acute series):
    a. 1 minute eyes open resting state
    b. 1 minute eyes closed resting state
    c. Baseline-acute resting state – 5 minutes (baseline thermode on)
    d. Painful-acute resting state – 5 minutes

e. Administer post-pain questionnaires (PCS, SFMPQ)
f. Ice pack application – total of 5 minutes or until pain = 0 for 60 seconds (no EEG recorded)
g. Acute-return baseline resting state – 5 minutes

11. 5 minute break
12. Resting state EEG runs (chronic series):
    a. Baseline-chronic resting state – 5 minutes (baseline thermode on)
    b. Capsaicin application and incubation (EEG recorded) – 20 minutes
    c. Painful-chronic resting state – 5 minutes
    d. Administer post-pain questionnaires (PCS, SFMPQ)
    e. Ice pack application – total of 5 minutes or until pain = 0 for 60 seconds (no EEG recorded)
    f. Acute-return baseline resting state – 5 minutes (baseline thermode on)
    g. Rekindle. Painful-chronic resting state –5minutes
13. Capsaicin removal and brush allodynia test and flare mapping
14. EEG cap removal
15. Payment form completion

Quantitative Sensory Testing (QST)

All quantitative sensory testing will be performed on the face, arm, and neck.

Test for Warmth, Cool, and Heat Pain Detection Thresholds, Heat Pain Ratings. Trained study staff will perform thermal warm, cool, and heat pain testing procedures using a computer controlled Medoc Pathway system. To familiarize participants with the stimuli, there will be an initial trial in which the temperature of the thermode is slowly decreased or increased to the maximum tolerable levels. The participant will hold the thermode and remove it from the skin when it becomes intolerable. Thresholds are determined by raising or dropping the temperature slowly from baseline skin temperature until the participant reports feeling warmth or cool, or until the participant reports pain. The experimenter will then determine the temperature that is consistently rated as moderately painful by applying a temperature above the threshold level and asking the participant to rate the pain from 0 (no pain) to 100 (most intense pain imaginable) on a continuous visual analog scale (COVAS). The testing for this part will be performed on the participant's left volar forearm. The temperature that gives a consistent rating of about 50 to 60 out of 100 will be used in EEG resting state with acute pain portion. Participants will also be asked to rate the pain unpleasantness of some stimuli.

Test for Offset Analgesia. Offset analgesia involves applying a temperature of 47°C to the arm, briefly increasing the temperature to 48°C, then returning back to 47°C. A dramatic reliable effect is seen when the temperature is lowered: the pain decreases well below the level previously experienced at 47°C (Martucci et al., 2012;Grill and Coghill, 2002). The thermode will be strapped to the volar forearm, with a baseline temperature of 32°C. The subject will use the COVAS to continuously rate pain on a 0 – 100 VAS. Once the subject is ready, the experimenter will begin the trial. The thermal probe rises to 47°C with a ramp rate of 10°C/s. the temperature remains at 48°C for 4-7 s (T1), then increases to 48°C for 4-6 s (T2), then returns back to 47°C for 30 s. The following metrics are recorded, according to (Martucci et al., 2012;Grill and Coghill, 2002): Max T2 (the maximum intensity rating following the increase to 50°C), minimum offset latency, min offset, VAS end latency. The procedure is repeated at least three times,

with at least 30 seconds between trials, and the thermode is moved to a different position on the forearm/face/neck for each trial. The T1 and T2 durations for the three trials are: 4s/5s, 7s/4s, 5s/6s. We will subsequently test for offset analgesia at each body site while a second stimulus is simultaneously applied to another body site. This will be done for the following combinations of body sites: arm/face, left arm/right arm. Participants will be instructed to rate the pain intensity at one of the two sites using the COVAS.

Test for Brush Allodynia. This test is performed last, after incubation at V0 and after rekindle at V1 and V2. The capsaicin cream and film cover are removed, and the arm area is cleaned with alcohol and the original 1 inch square site is marked. The area of secondary hyperalgesia on the participant's arm is measured with a foam paintbrush dragged at 1 cm per second along four linear paths arranged vertically and horizontally around the marked capsaicin site. Brush stimulation from each direction is started outside the hyperalgesic area, and continued towards the marked square until the participant reports a change in sensation (`burning', `tenderness', `pain'). The border is marked on the skin with a marker from each direction. Finally the flare outside the capsaicin site is traced on transparency paper.

EEG

The EEG session will last about 2 hours and will include the following runs: 1 minute open eyes resting state, 1 minute closed eyes resting state, 5 minutes run of baseline-acute resting state, 5 minute run of acute-painful resting state, 5 minute run of acute-return baseline resting state, 15 minute run of capsaicin cream incubation, , 5 minute run of chronic-painful resting state, and 5 minute run of chronic-return baseline resting state, 5 minute run of chronic-painful resting state (rekindle).

Urine Screening

Urine screening will be decided randomly, by allowing the participant to choose 1 of 10 envelopes that s/he opens to reveal his or her assignment. 9 of the envelopes contain a paper that says 'No' to indicate no urine test and 1 envelope contains a paper that says 'Yes' to indicate a urine test will be conducted. Urine samples will be collected and immediately discarded after screening for drug toxicology. A urine sample for toxicology screening testing will be collected in a urine collection cup. The toxicology screening will be performed using Instant-View 12 Panel Drug Tests.
Trained personnel will collect the urine sample, read and record the results. When collecting urine samples, research staff will escort the participant to the designated bathroom and instruct them to leave the sample on the counter of the bathroom. The staff should examine the urine sample and make note if there is anything suspicious about the sample, such as a cold temperature, which might indicate that the participant has tampered with the sample. Both the drug and pregnancy screenings are dipstick tests, which involve placing the end of the test into the sample for 10 seconds and then waiting for no less than 4 minutes and no more than 7 minutes for the results to appear on the test. Urine samples are collected for screening purposes only and will be sealed in their collection cups and disposed of in biohazard bins immediately following processing. If the participant fails the drug screening test, the staff will ask the participant about any medications she/he might be taking that could affect the results of the test. Participants who fail the drug screening will not be eligible to continue with the study. Results from the urine test will be recorded by study staff in the subject's study flow form.

10. List of questionnaires (with brief description and duration of test)

Health history form. This custom form will assess a participant's self-reported medical history.

State-Trait Anxiety Inventory (STAI-S, STAI-T). This scale is commonly used to assess anxiety in clinical and research settings. There are 20 questions for state anxiety and 20 for trait anxiety. State anxiety items include: "I am tense; I am worried" and "I feel calm; I feel secure." Trait anxiety items include: "I worry too much over something that really doesn't matter" and "I am content; I am a steady person." All items are rated on a 4-point scale (e.g., from "Almost Never" to "Almost Always") (Spielberger et al., 1970).

Situational Catastrophizing Questionnaire (SCQ). This is a short 6-item questionnaire rated on a 5-point scale ranging from 0 = "not at all" to 4 = "all the time" administered immediately following the Acute-painful resting state and Chronic-painful resting state EEG runs. The SCQ assesses the subject's thoughts and feelings during the pain session.

Pain Catastrophizing Scale (PCS). This questionnaire consists of 13 items rated on a 5-point scale ranging from 0 = "not at all" to 4 = "all the time". Subjects indicate the degree to which they have specified thoughts and feelings when experiencing pain. This instrument assesses 3 domains of catastrophizing: rumination, magnification, and helplessness (Sullivan, 1995;Sullivan et al., 1995).

Short form McGill pain (SFMPQ). In this brief questionnaire, the subject rates a pain descriptor word such as "stabbing" on a scale of 0 (none) to 3 (severe). Subjects complete the scale with respect to their usual headache pain and their current headache pain. The SFMPQ-2 will assess the type of pain the patient is experiencing, which can be compared to databases of many pain disorders (Melzack, 1987;Dworkin et al., 2009).

Beck Depression Inventory (BDI-II). This is a 21-question multiple-choice self-report inventory that assesses the severity of depression in adults (Beck et al., 1988). If the participant's score on this scale is 29 or above, or if the participant scores greater than 0 on the suicide item, we will take the following steps: 1) ask the participant if these feelings have changed recently; 2) ask the participant if he or she has a plan if these feelings have been changing (i.e. a plan to seek care); 3) ask the participant if it is possible that they might cause harm to themselves or to others because of these feelings. In the case that the participant shows the potential to harm themselves or others or a lack of self care, the participant will be escorted to psychiatric services or an emergency department. In the case that the patient seems unlikely to harm themselves or others, the participant will be asked to follow-up with a primary care physician or counselor. We will offer to help identify these services if necessary. Additionally, if the participant scores greater than 0 on the suicide item, they will also complete the Columbia-Suicide Severity Rating Scale (C-SSRS), which is an industry standard for assessment of suicide risk severity. The study team member will then decide on the appropriate action, erring on the side of a psychiatric consult at the emergency department when in doubt. In the event of potential immediate risk for suicidal behavior the participant will be accompanied to the ER.

Edinburgh Handedness Inventory (EHI). This is a 10-item questionnaire used to determine used the subject's hand dominance.

Barratt Impulsiveness Scale (BIS-11). This 30-item questionnaire measures impulsive personality traits by self-report. The BIS score is determined using first order factors (attention, motor, self-control, cognitive complexity, perseverance, cognitive instability) and second order factors (attentional, motor, nonplanning) (Patton et al., 1995).

11. Risks and benefits

Research-related risk, likelihood/seriousness of the risk, and provisions for minimizing the risk

Some of the heat stimuli will be painful to participants. Participants will be able to stop the stimulation (the heat or cold on the skin) at any time if participants find it too uncomfortable. The stimulation equipment is designed not to exceed safe temperatures, but there is a small chance of short-lasting tissue injury such as redness, tenderness, and/or in very rare cases minor blistering at the site where heat has been applied. Any effects should go away within 24 hours. No reports of serious injuries from this stimulator have been made to the manufacturers or the FDA. (The FDA is the government agency that oversees the safety of drugs, vaccines, and medical devices.)

During each visit participants will be asked to endure controlled experimental pain. For the temporary pain, participants will be exposed to a hot temperature for a total of 5 minutes. For the capsaicin pain, participants will be exposed to capsaicin for up to 40 minutes. Each type of pain will only occur once per visit and will be well controlled. If participants want to stop this portion of the experiment at any time we will stop the pain with no prejudice. Participants will be reminded they may elect to end the experiment at any time at each session. Any residual pain can be eliminated by applying a cold wet cloth.

From wearing the EEG cap, participants may experience slight sensations and irritation as the scalp is lightly rubbed at the recording sites. There is some risk that the skin may be broken during the EEG cap preparation, however this is rare in our lab. Also, a small number of people may be allergic to the conducting gel and/or adhesive used to attach the other sensors on the skin, but this is rare. Please let us know if participants are experiencing irritation around sensor placement areas. Lastly, if participants experience a temporary reddening of the skin around any of the sensor sites, this typically goes away within an hour or so.

Participants may feel at unease when reading or responding to personal questions. Participants can refuse to answer any question and stop at any time.

There is a very small risk for breach of confidentiality of the information that participants provide for this study. We will do the most we can to keep their information secure and confidential. Loss of confidentiality will be minimized by storing data in a locked cabinet and on password-protected computers.

Participants will be asked to contact a study team member to report unanticipated events or potential adverse events (see Data and safety monitoring plan).

Potential direct benefit(s) to participants

There is no direct benefit to participants.

Importance of the knowledge expected to result from the study

The study will provide critical knowledge about the brain mechanisms of pain. We study this by stimulating the arm with thermal stimuli and examining EEG activity before and during the experience of pain. This could lead to an objective test of sensitivity to pain and could guide preventive and therapeutic approaches to chronic pain in future work.

How the potential risks to participants are reasonable in relationship to the potential benefits

There are minimal risks to the subject, but potentially great gains for the pain research community from the outcomes of the present study. The potential risks to participants associated with sensory testing and EEG are minimal. The thermal stimulation device delivers temperatures that produce the perception of pain without creating any lasting effects such as a burn to the skin, unlike situations commonly encountered in daily life (burning of the mouth on hot coffee or pizza, burning a hand on a stove). The benefits of identifying brain mechanisms associated with different types of pain are potentially very great for the pain research community, as the information may lead to developing novel treatments for chronic pain and a biomarker for pain sensitivity.

Alternatives to participation in this study

Participation is voluntary and the alternative to participating is to not participate.

12. Withdrawal or Removal

Subjects are free to withdraw from participation in the study at any time upon request, and the investigator may withdraw a subject from participation for any of the following reasons:

- Withdrawal of consent.
- Subject noncompliance with the protocol that results in inability to assess primary outcomes.
- Any event, condition, or situation that would make continued participation in the study not in the best interest of the subject, as determined by the investigator.
- Investigator discretion.

Whether a participant has completed all the requirements of the study or decides to terminate his or her participation before all the requirements are met, we ask nothing further of that participant. If the participant has completed a partial session, the data collected in that time may be used in the group analyses.

13. Privacy

Screening, consent, and testing will be done in private rooms. Study team members who have access to participant names will not talk about participants using names, whether in private or public. When contacting participants by phone, information about the study will only be given directly to the participant.

Potential participants will receive research information from recruitment posters around the Baltimore area and university campuses, including UMB, UMMS, UMBC, and Johns Hopkins. Further information will be given via telephone when the potential participant calls the phone number on the recruitment letter or poster.

14. Confidentiality

Participants will be numerically coded upon enrollment. Only the team members directly involved with the participants will have access to their names, and once a participant has completed the study, only numerical codes will be used. The original list with the codes and names will be securely locked.

Identifiable data will be kept in the PI's office. Coded data will be kept on a central server that can only be accessed by research team members (password protected).

Identifiable data will be kept in a locked filing cabinet.

The PI will have access to identifiable data. Only research team members will have access to coded data.

15. Data and safety monitoring plan

Data Safety Monitoring by the PI and reported to the IRB semi-annually.

The PI will take note of any unexpected risks or harm to the participants and report them to the IRB and the sponsor, Purdue Pharma LP.

Data to be reviewed include enrollment, adverse events, unanticipated events, and preliminary analyses.

Participants will be asked to contact a study team member to report unanticipated events or potential adverse events.

16. Payment

For their time, participants will be compensated $25 for the eligibility screening visit and $75 per EEG visit. Payments will be made by check.

17. Sample size calculation

The target sample size for this study is 120 healthy male and female subjects. This number is based on our preliminary data and accounts for variability in the response to capsaicin (about 10% of people do not react to topical capsaicin). Additionally, this sample size is sufficiently powered to capture a moderately size relationship ($r > .3$) between pain and peak alpha frequency, according to a correlation power analysis with a Type I error rate of .05 and a Type II error rate of .2. The sample size is also sufficient for performing multivariate analyses, which will allow us to develop data-driven algorithms for pain sensitivity.

18. Data analysis plan

Data Analysis Approach

Data preprocessing for all resting state scans will proceed as follows: data will be re-referenced to the average of the two ear electrodes, band-pass filtered, removed of obvious non-brain (i.e. movement) artifacts, and epoched into 4 second segments. Following preprocessing, a fast fourier transform will be applied to each epoch. The resulting spectral information will then be averaged across epochs. This spectral information will then be exported to Matlab where it will be analyzed with custom scripts developed in our laboratory. Activity in the alpha frequency band (8-12hz) will serve as the primary frequency band of interest.

Interim Data Analysis

Following acquisition of five full data sets, preliminary analyses will occur to assess the primary study outcomes and provide an opportunity to modify the protocol if necessary.

Aim 1. To determine how the experience of innocuous and noxious stimulation affects alpha activity.

First, to identify the potential mechanisms that may drive modulations of alpha frequency under painful conditions, we will compare both the acute pain and neuropathic-like pain state scans against the most temporally proximate innocuous state scans. Each pain state will be compared to the baseline scan directly preceding it, and the differences between acute pain and warm and neuropathic-like pain and warm will be compared.

Second, we will compare the spectral characteristics of acute pain and neuropathic-like pain in the alpha frequency band and determine the ability of alpha frequency measures distinguish these two pain states. Each pain state will be compared to the baseline scan directly preceding it, and the differences between acute pain and warm and neuropathic-like pain and warm will be compared.

Aim 2. To assess the test-retest reliability of the alpha frequency as an objective measure of pain in two different experimental pain states (acute and neuropathic-like).

Here, we will identify the degree to which alpha frequency under various pain states remains constant across this time. This will be accomplished by comparing the resting states, and the differences between them, across testing sessions. If alpha frequency observations remain stable, within an individual, across testing sessions, this will provide important evidence that alpha frequency, and how it shifts, may represent an individually based characteristic or biomarker.

19. Informed Consent Process

Process: Once participants have received the initial telephone screening and approval, as described above, they will be invited to participate in an experiment. The study date will be arranged and the participant will arrive on that date. Before any research procedures begin, the participant will receive the consent form and will be given an opportunity to read the forms and ask questions before agreeing or refusing to consent. If the participant refuses consent, they will not be enrolled in the study.

Who will obtain consent: A research team member will obtain consent.

Setting: Consent will be attained in a private testing room, where only the research team member and participant will be present.

Provisions for understanding: Before the participant signs the consent form, the research team member will ask the participant if he or she understands what he or she has read in the consent form and if he or she has any questions. The research team member will then proceed with the Evaluation to Sign Consent form (attached) consisting of several questions. Upon successful completion of this evaluation, the consent form can be signed.

20. References

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