Abbreviated Title: Alpha and Pain Sensitivity

Sensorimotor peak alpha frequency is a reliable biomarker of pain sensitivity

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Abstract

We have previously observed that the speed of pain-free, sensorimotor peak alpha frequency (PAF) can predict a healthy individual's sensitivity to prolonged pain. Here we test the reliability and specificity of pain-free, sensorimotor PAF's relationship to prolonged pain sensitivity. We collected PAF at two visits (n = 61 and 46 for visits 1 and 2, respectively), separated on average by eight weeks, where participants completed a series of thermal tests. Included were two tests of prolonged pain, Phasic Heat Pain and Capsaicin Heat Pain, and two acute tests, warmth and heat detection thresholds. We demonstrate that PAF predicts sensitivity to both prolonged pain tests but not to either acute test. Furthermore, we show that this prediction occurs at both short (minute) and long (week) timescales. These results s that pain-free, sensorimotor PAF is a reliable biomarker of prolonged pain sensitivity with potential for prospectively identifying pain sensitivity in the clinic.

Introduction

Chronic pain is a debilitating condition with cognitive, affective, and sensory symptoms that afflicts nearly one fifth of the American population (Kennedy et al., 2014), leading to treatment and work loss costs totaling nearly six hundred billion dollars annually (Gaskin & Richard, 2012). Identifying individuals who present at high risk for developing chronic pain is a crucial avenue for combatting chronic pain and its related economic burdens. At present, prediction of chronic pain development is poor: for example, one of the best predictors of persistent post-surgical pain is pain severity reported directly after surgery (e.g Katz et al., 1996). While useful for case management after surgery, these measures cannot be used to identify, and target prophylactic treatments to, individuals at high risk for developing chronic pain. Biomarkers that predict the amount pain an individual will experience in response to a noxious event thus have important clinical potential.

Recent findings indicate that Peak Alpha Frequency (PAF), often referred to as the frequency within the 8-12 Hz range that displays maximal power, may represent one such marker for pain sensitivity. The alpha rhythm represents the predominant oscillatory activity in the EEG while an individual is quietly resting, and is chiefly observed in primary sensory cortices (e.g. vision, somatosensation, audition). Although previously considered a signature of cortical "idling", significant evidence now suggests that alpha-related processes play a top-down role in gating information transfer across neural ensembles by coordinating cycles of excitation and inhibition (Jensen & Mazaheri, 2010). PAF can be viewed as reflecting the speed of these cycles (Mierau et al., 2017; Van Rullen, 2016) and has been shown to modulate the rate of sampling in multiple sensory domains (e.g. Samaha & Postle, 2015). PAF is a heritable trait that varies considerably between individuals (Bazanova & Vernon, 2014) and this variability has been suggested to contribute to individual differences in multiple psychological and physiological processes (e.g. Klimesch, 2012; Haegens et al., 2014), including those associated with pain.

A number of studies have reported abnormally slow PAF in patients with chronic pain (e.g. Sarnthein et al., 2006), and PAF slowing has long been hypothesized to reflect a pathological process involved in the chronification of pain (Llinás et al., 1999). Recent work from our lab, however, has demonstrated that this slowing may also reflect pain processes not directly tied to the presence of chronic pain. We reported that sensorimotor PAF collected from healthy individuals in the absence of pain could predict the intensity of a prolonged pain experience occurring either 45 minutes (Furman et al., 2018) or 4 to 6 days (Seminowicz et al., 2018) in the future. In both cases, PAF recorded during a baseline, pain-free state was negatively associated with pain intensity; that is, individuals with slower sensorimotor PAF

went on to experience more pain than those with relatively faster sensorimotor PAF. These findings have led us to hypothesize that PAF is a biomarker for prolonged pain sensitivity in healthy individuals. Such an interpretation is not necessarily at odds with findings of PAF slowing in chronic pain patients given that heightened pain sensitivity is a relevant risk factor in determining chronic pain vulnerability (Diatchenko et al., 2005).

While our earlier work has provided preliminary evidence that PAF can serve as a marker of pain sensitivity, a number of questions remain to be answered in order to better evaluate the hypothesis that PAF is a reliable biomarker of prolonged pain sensitivity. First, it is unknown whether sensitivity to the types of prolonged pain paradigms we have previously employed is itself a reliable estimate of individual differences in pain processing. Establishing that sensitivity to prolonged pain events is a trait-like characteristic is a necessary precursor to any argument that PAF is a biomarker of pain sensitivity. Second, it is unknown whether PAF can provide predictions about pain sensitivity at multiple time points or whether it is limited to a single point in time. Demonstrating that PAF can provide cogent predictions about pain sensitivity at multiple time points would strongly reinforce the notion that PAF is a biomarker of pain sensitivity per se. Finally, it is unclear whether PAF shares a relationship with all types of pain events, with a particular type of pain event (i.e. prolonged pain), or whether it has multiple, distinct relationships depending on the type of pain event. For example, separate studies have suggested that PAF has opposite relationships to phasic and prolonged pain sensitivity (Furman et al., 2018; Nir et al., 2010).

In the current study, we collected pain-free, sensorimotor PAF and recorded pain responses to a series of thermal sensory tests. These thermal tests varied in their duration and noxious content, so that participants were exposed to a short, innocuous event, warmth detection thresholds, a short, pain event, heat pain thresholds, as well as two prolonged, pain events, Phasic Heat Pain (PHP) and Capsaicin Heat Pain (CHP), that are known to produce sensitization. This battery of tests allowed us to begin examining whether PAF is specifically related to tests of prolonged thermal pain, to any painful thermal test, or to any test involving thermal stimuli whether they are painful or not. For example, by including a second type of prolonged pain, PHP, we were able to determine whether PAF is related to multiple prolonged pain events or just CHP specifically.

In addition, most participants came back for a second, identical testing visit occurring an average of seven weeks later. By having participants complete multiple testing sessions, we were able to directly test two key predictions of the PAF pain sensitivity biomarker hypothesis; namely, that prolonged pain sensitivity is itself a trait-like quality and, furthermore, that PAF can predict pain sensitivity at more than one time point. In accord with these predictions, we present novel data demonstrating that prolonged-pain sensitivity is stable across two testing sessions, that PAF is related to two types of prolonged pain, and that this relationship remains stable within and across both testing visits.

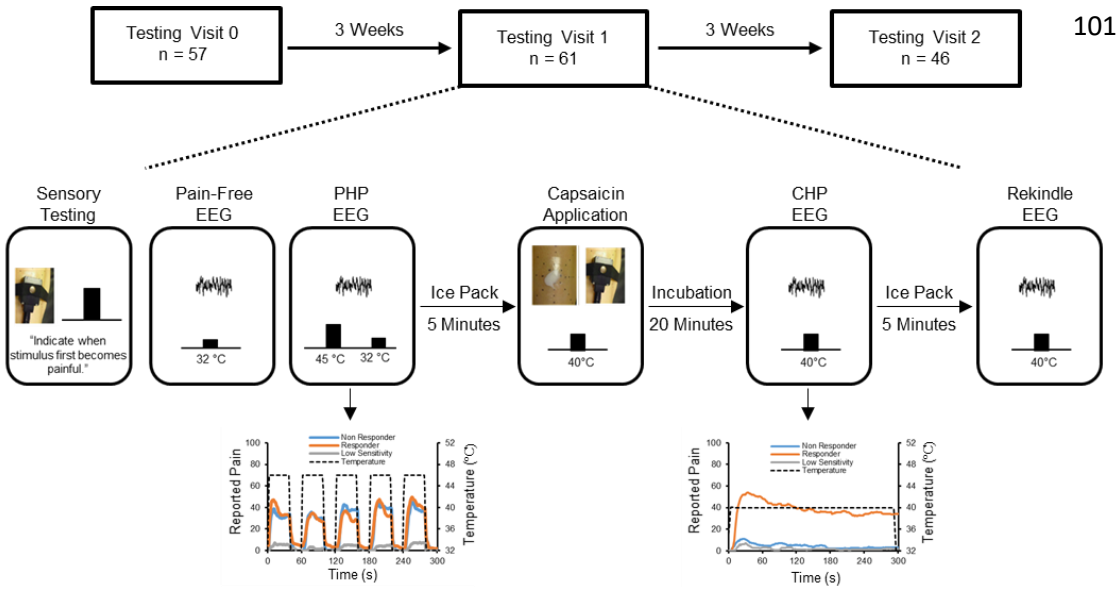

Figure 1. Outline of the experimental timeline and procedures. Participants first completed a Testing Visit 0 where sensory testing occurred. At a minimum of three weeks later, Testing Visit 1 was completed. At Testing Visit 1, participants completed a brief sensory testing session and then completed a 5 minute eyes-closed resting state in the absence of any noxious stimuli. Next, participants completed a 5 minute eyes-closed "Phasic Heat Pain (PHP)" resting state. Average Testing Visit 1 ratings from this session, broken down by pain classification, are shown on the bottom left. After a 5 minute period in which an ice pack was applied to the skin, capsaicin was applied to the forearm and allowed to incubate for twenty minutes. Next, a 5 minute eyes-closed "Capsaicin-Heat Pain (CHP)" resting state was completed while a 40°C thermode was placed on top of the capsaicin. Average V1 ratings from this session, broken down by pain classification, are shown on the bottom right. After a 5 minute period in which an ice pack was applied to the skin, the 40°C thermode was again placed on top of the capsaicin and a five minute eyes-closed "rekindle" resting state was completed. Identical procedures were performed at V2. CHP responders were defined as all individuals with > 10/100 pain with CHP, CHP non-responders were defined as those who had <10/100 pain with CHP, but >10/100 on PHP, while high pain tolerance individuals were defined as subjects who did not have a pain response to either CHP or PHP (<10/100 for both). Testing Visit 2 took place a minimum of three weeks after Testing Visit 1 and was identical to what occurred during Testing Visit 1.

Materials and methods

Participants

Sixty-one pain-free, neurotypical adult participants (31 males, mean age = 27.82, age range = 21-42) took part in the experiment between 7/6/2016 and 10/20/2017. This study was approved by the University of Maryland, Baltimore Institutional Review Board, and informed written consent was obtained from each participant prior to any study procedures. The study was pre-registered on ClinicalTrials.gov (NCT02796625).

Table 1 provides information regarding how many participants contributed data to each analysis.

Procedure

An outline of the experimental timeline and procedures is presented in Figure 1. In order to allow sufficient time for any long-term effects of capsaicin administration to subside, all visits were separated by a minimum of 21 days (one subject returned at 19 days because of a scheduling conflict; mean separation of Visit 1 and Visit 2 = 54.74 days, S.D. = 55.92 days, range = 19 – 310 days, Figure S1).

Participants first completed an introductory Visit 0 (V0) session during which they first underwent a brief a brief sensory testing session in which they were asked to report when they felt a change in temperature (for warmth (WDT) and cool detection threshold (CDT)) or when the temperature first became painful (heat pain threshold (HPT) and cold pain threshold (CPT)). Three trials were presented for each test. Also occurring at V0 were tests to ensure that 40°C was rated as non-painful, to identify

	Visit 1			Visit 2		
	Capsaicin Responder	Capsaicin Non-Responder	High Pain Tolerance	Capsaicin Responder	Capsaicin Non-Responder	High Pain Tolerance
Total Participants	35 (19 Female)	15 (6 Female)	11 (5 Female)	27 (14 Female)	11 (4 Female)	8 (3 Female)
Exclusions						
EEG Technical Error	1 (Female)	0	0	0	0	0
Abnormal Pain Ratings	1 (Female)	0	1 (Male)	1 (Female)	0	1 (Male)
Abnormal CHP Change	1 (Female)	0	0	1 (Female)	0	0
Participants Remaining	32 (16 Female)	15 (6 Female)	10 (5 Female)	25 (12 Female)	11 (4 Female)	7 (3 Female)

Table 1. Summary of exclusions and participants contributing data at each testing visit.

the temperature for the Phasic Heat Pain (PHP) stimulus, and to provide initial exposure to the Capsaicin Heat Pain (CHP) model. For all V0 testing, participants were seated in a comfortable chair and EEG was not recorded. For the first four participants, the V0 session was not performed and these procedures, excluding CHP exposure, were performed immediately prior to the V1 session procedures.

A minimum of three weeks after completion of V0, participants returned for Testing Visit 1 (V1). The majority of participants then returned at least three weeks after V1 for Testing Visit 2 (V2). Procedures for the V1 and V2 testing sessions were identical. For the entirety of each testing session, participants were seated in a comfortable chair in a quiet room that was isolated from strong electrical interference. For all sessions in which EEG was recorded, lights in the testing room were turned off and participants were instructed to close their eyes, remain still, relax without falling asleep, and continuously rate any pain they experienced with a manual analog scale placed at their right hand. Testing sessions began with measurement of each participant's WDT, CDT, HPT, and CPT. For the first four participants, this sensory testing was not performed at the V2 sessions. After this brief sensory testing session, the lights in the testing room were turned off and EEG was recorded during a pain-free resting state for a total of five minutes.

Immediately following the pain-free resting state EEG recording, participants were informed that the PHP model would begin. Once the participants acknowledged that they were ready to begin, the PHP model, described below, began. Continuous EEG was recorded during this PHP resting state for a total of five minutes. Upon completion of the PHP resting state, the lights in the testing room were turned on and a disposable ice pack was placed onto the left forearm. The icepack was left in place until the participant reported a complete absence of pain at the site where the thermode had been applied. The icepack was then removed, and the participant was asked again if any pain was present, with procedures continuing only when pain was reported to be absent. The lights in the testing room were then turned off and five minutes of continuous EEG was collected for this return to baseline resting state session (not included in current analyses).

After finishing the return to baseline resting state EEG recording, the lights in the testing room were turned on, and administration of the CHP model began. We followed the procedures described in our previous publication with only a slight modification to the incubation period (Furman 2018). In brief, capsaicin was applied to the participant's left forearm and a 40°C thermode was placed directly on top of the capsaicin application for a total of twenty minutes. Participants were instructed to continuously rate their pain during the incubation period.

Following the 20-minute incubation period, the lights in the testing room were turned off and participants were informed that the prolonged pain model would begin. Once the participants

acknowledged that they were ready to begin, the thermode temperature was again increased to 40°C and 5 minutes of continuous EEG was recorded for the CHP resting state session. A second return to baseline resting state session (not included in current analyses), identical to the one that occurred after the PHP resting state session, was then performed. Finally, a 40°C stimulus was re-applied over the capsaicin and five minutes of eyes closed, continuous EEG was recorded for the "rekindle" resting state session (not included in current analyses).

EEG

Scalp EEG was collected from an EEG cap housing a 63 channel BrainVision actiCAP system (Brain Products GmbH, Munich, Germany) labeled according to an extended international 10–20 system (Oostenveld and Praamstra, 2001). All electrodes were referenced online to the average across all recording sensors and a common ground set at the AFz site. Electrode impendences were maintained below 5 kΩ throughout the experiment. Brain activity was continuously recorded within a 0.01–100 Hz bandpass filter, and with a digital sampling rate of 500 Hz. The EEG signal was amplified and digitized using an actiCHamp DC amplifier (Brain Products GmbH, Munich, Germany) linked to BrainVision Recorder software (version 2.1, Brain Products GmbH, Munich, Germany).

Thermal Stimulator and Pain Scale

Thermal stimuli were delivered to the volar surface of the participant's left forearm using a thermal-contact heat stimulator (27mm diameter Medoc Pathway CHEPS Peltier device; Medoc Advanced Medical Systems Ltd., Ramat Yishai, Israel).

Pain ratings were collected continuously with a manual analog scale consisting of a single sliding tab (Medoc Advanced Medical Systems Ltd., Ramat Yishai, Israel). Prior to testing, participants were instructed that the lower and upper bounds of the scale represented no pain and the most pain imaginable, respectively, and that they should continuously update the slider to indicate how much pain they were experiencing at the current moment in time. Care was taken by experimenters to avoid providing numerical anchors when describing the scale and no additional landmarks were physically present on the scale. Prior to testing, participants were given an opportunity to practice using the analog device with both their eyes open and closed. During testing, participants were permitted to briefly open their eyes while rating.

Cool and Warmth Detection Thresholds (CDT, WDT) and Cold and Heat Pain Thresholds (CPT, HPT)

Tests were always administered in the following order: WDT, CDT, HPT, and finally CPT. Prior to each test, participants were instructed on the required response criteria (i.e. for HPT: you will be presented with an increasingly hot temperature on your forearm, please indicate when the temperature first becomes painful). Participants provided feedback by clicking either the left or right button of a computer mouse placed in their right hand.

A total of three trials were presented for each test with an ISI of 4-6 seconds (randomly determined on a per trial basis). For all tests, temperatures were applied with a rise rate of 1°C/second and return rate of 2°C/second (initiated on any mouse click). All threshold testing was performed on the volar surface of the left forearm. Prior to testing, the distance from the wrist to elbow joint was measured and the forearm was then divided into three equal length zones. For each test, the first trial was administered to

the zone closest to the wrist, the second trial administered to the middle forearm zone, and the third trial administered to the zone closest to the elbow.

Phasic Heat Pain (PHP) Model

Temperatures used during the PHP model were determined at the V0 visit or, for the first four participants, during V1 sensory testing. During these sessions, participants were exposed to a series of 12, 20 second trials in which a single temperature (2.5 second rise and fall) was applied and then asked to provide an average pain rating at the conclusion of each trial. Temperatures ranged from 37 to 48°C (intervals of 2°C, starting as if 37°C was 38°C) and each temperature was presented twice in a pseudo-random order. Trials were separated by 10 seconds and after each trial the thermode was moved to a neighboring forearm zone in order to minimize sensitization. From these trials, the temperature that most closely evoked an average pain rating of 5/10 was selected. This level of pain was targeted in order to best match the amount of pain evoked by the CHP model (Furman et al., 2018).

The PHP model consisted of a series of five consecutive stimulus trains each lasting one minute in total. Within each train, the PHP stimulus was applied for 40 seconds (rise and fall times of 2s) followed by a neutral skin temperature stimulus (32°C) applied for 20 seconds. PHP scores were calculated by averaging ratings during the five, forty second periods in which the PHP stimulus was present. Previous studies using similar durations of stimulation have reported that this procedure results in sensitization of pain ratings in healthy participants (e.g. Granot et al., 2006).

Capsaicin Heat Pain (CHP) Model

CHP procedures were similar to the one used in our prior study (Furman et al., 2018). The CHP model lasts for hours to days and recapitulates some cardinal sensory aspects of chronic neuropathic pain (Culp et al., 1989; LaMotte RH, et al.,1992; Baron 2009; Lötsch et al., 2015). In brief, we applied ~1 g 10% capsaicin paste (Professional Arts Pharmacy, Baltimore, MD) topically to the volar surface of the left forearm, fixing it in place with a Tegaderm bandage. A thermode was then placed over top of the capsaicin application. This procedure does not cause lasting tissue damage (Moritz and Henriques, 1947). CHP scores were generated by averaging ratings across the entire five-minute session that followed incubation.

To restrict analyses to those individuals with clear capsaicin responses, we classified participants based on their pain scores. Previous work has found that CHP evokes no pain or hypersensitivity in roughly one third of individuals (Liu et al., 1998, Walls et al., 2017). The reasons for this remain unclear, making it difficult to determine whether low levels of pain in response to CHP model are due to a failure of the model or lower pain sensitivity in those individuals. To address this problem, we only include participants in the full analysis if they meet one of the two following conditions: 1) average pain greater than 10 out of 100 in response to the CHP paradigm at either V1 or V2 (“CHP responder”), or, 2) average reported pain at V1 less than 10 out of 100 in response to both the CHP and PHP paradigms (“high tolerance individuals”). Given their tolerance of the PHP model, we interpret the low CHP response of this latter group as truly reflecting low pain sensitivity. In order to provide a more complete picture of the PHP data, we also present data from those individuals with no or very weak capsaicin responses (average CHP response at both visits < 10, “CHP non-responders”) when direct comparisons to prolonged pain data are not made.

We excluded one participant who experienced a change in CHP score, + 69.26, that was 3.82 standard deviations greater than the average CHP change (average change = 1.76, S.D. = 17.64). No other change in CHP scores was greater than 2.05 standard deviations above the mean (range = +37.96 to -31.05).

Data Processing

Pain ratings were collected from the manual analog scale at a rate of 1000 Hz. Manual analog scale data was transformed by converting the horizontal position of the slider into a continuous value between 0 and 100. Pain ratings were aligned to the EEG recording through a parallel port connecting the rating device and EEG acquisition computer.

The EEG data of interest were the pain-free resting state EEG sessions collected during V1 and V2 sessions. Initial processing of EEG data was performed using EEGLAB 13.6.5b (Delorme and Makeig, 2004). Processing began with filtering the data between 2 and 100Hz using a linear FIR filter. Channel data were then visually inspected and overtly noisy channels were removed from further analysis. Removed channels were not interpolated. On average, 1.64 (S.D. = 1.92, range: 0 – 8) and 1.79 (S.D. = 1.79, range: 0 – 6) channels were removed per individual from V1 and V2 datasets, respectively.

As opposed to our previous studies which used ICA to identify sensorimotor PAF, we used channel level data to increase the ease of reproducibility of the methods. This approach, while decreasing the signal to noise of the data, eliminates the need to identify ICA components on a participant by participant basis. For channel level analyses, we focused on channels that most strongly contributed to the sensorimotor component from our first study on sensorimotor PAF (Furman et al., 2018). Thus, the sensorimotor ROI included the C3, Cz, and C4 channels. In the event that a channel was removed due to noise, the remaining sensors were used; this affected only a few participants (V1: n = 4; V2: n = 1), and no participant had more than one sensor removed that belong to this ROI.

Quantification of Sensorimotor PAF

The frequency decomposition of the sensorimotor ROI data was performed using routines in FieldTrip (Oostenveld et al., 2011). The data for each resting state session was segmented into 5-s epochs and power spectral density in the 2–50 Hz range was derived for each epoch in 0.2 Hz bins using the 'ft_freqanalysis_mtmfft' function. A Hanning taper was applied to the data prior to calculating the spectra to reduce any edge artifacts (e.g. Mazaheri et al., 2014).

PAF for each 5 second epoch at every sensor was estimated using a center of gravity (CoG) method (Klimesch et al., 1993). We defined CoG as follows:

$$CoG = \frac{\sum_{i=1}^{n} f_i * a_i}{\sum_{i=1}^{n} a_i}$$

where f_i is the ith frequency bin including and above 9 Hz, n is the number of frequency bins between 9 and 11 Hz, and a_i the spectral amplitude for f_i. From our previous work, we have determined that this narrow analysis band reduces the influence of $1/f$ EEG noise on the estimation of PAF (Furman et al., 2018). PAF was estimated for every 5 second epoch and then averaged to yield a single mean PAF estimate for each sensor. Average PAF estimates for sensorimotor ROI sensors were further averaged to yield a grand mean sensorimotor PAF estimate for each participant at each visit.

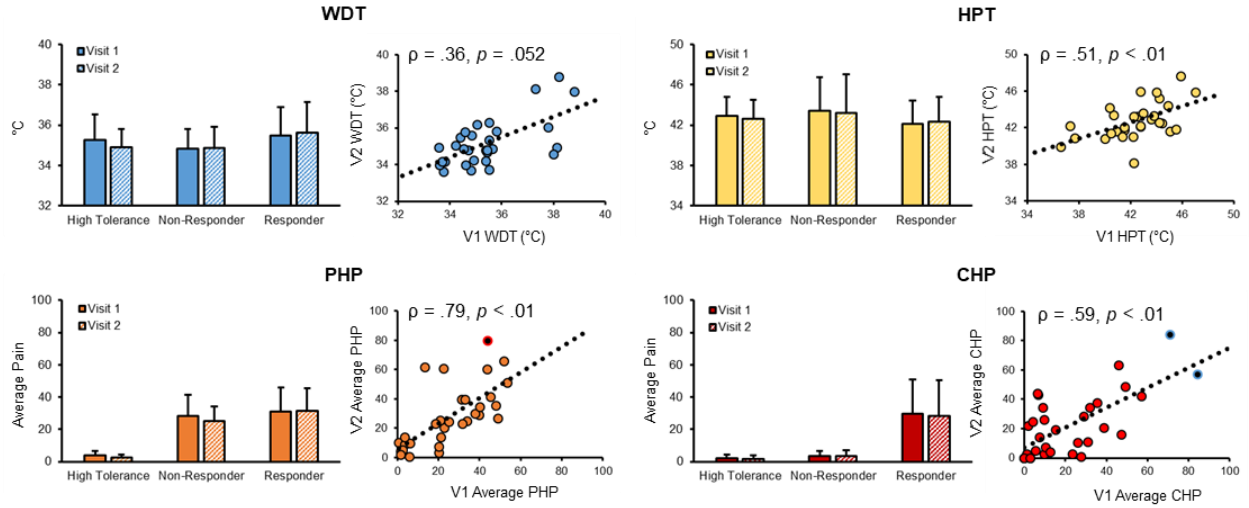

Figure 2. Sensory scores were stable across visits for all pain classifications. Pain ratings (mean + 1 S.D.) broken down by sensory test, pain classification, and visit. Scatter plots only include data from CHP-responders and high tolerance individuals. Off-color data points represent statistical outliers not included in analyses and dotted lines represent the linear regression line of best fit.

Statistical Analysis

All analyses were performed using custom scripts implemented in the Matlab environment (version R2013A). Statistical tests were conducted in Matlab or SPSS (Version 25).

We first investigated whether estimates of WDT, HPT, PHP, and CHP are reliable over time, and as such reflect reliable estimates of individual differences in thermal heat sensory processing. Sensory tests were compared using a linear mixed effects model with subjects as random effects (intercept included) and Visit (V1 vs V2), Type (WDT vs. HPT vs. Phasic vs. CHP), and the Visit X Type interaction as fixed effects. We were specifically interested in determining whether scores change over time (main effect of Visit) and whether these changes were specific to particular tests (Visit X Type interaction). In cases where we needed to determine whether the null hypothesis could be accepted (i.e. no change in scores between visits), we used Bayes factor analysis. Bayes factor analysis provides a method for assessing the relative evidence in favor of either the null or alternative hypothesis. A Bayes factor less than .33 or greater than 3 are taken as strong evidence in favor of the null and alternative hypotheses, respectively; Bayes factor scores in-between these values are considered to provide no evidence in favor of either hypothesis. Additionally, the stability of sensory tests was analyzing by correlating scores at V1 and V2 for each sensory test. For these and all correlational analyses, Spearman's rank order correlations were used and outliers were identified as data points 2.5 standard deviations greater than the mean based on values obtained from V1 data. Finally, to assess the relationship of scores across testing type, we performed a series of pair-wise correlations between all possible pairs of sensory tests. Corrections for the 6 total tests were made according to the Bonferroni method, yielding a significance threshold of p = .008.

Similarly, we determined whether pain-free, sensorimotor PAF was reliable across the two testing visits. This was accomplished by comparing V1 and V2 estimates of pain-free, sensorimotor PAF using a paired t-test and by correlating estimates at each visit with one another. Finally, to determine whether the null hypothesis could be accepted (i.e. no change in PAF between visits), we used Bayes factor analysis.

	Visit 1				Visit 2				
	WDT	HPT	PHP	CHP	CHP	PHP	HPT	WDT	
WDT		0.22(.16)	.11(.48)	-.10(.48)	.14(.46)	.20(.29)	0.23(.22)		WDT
HPT			.05(.75)	-.29(.06)	-.40(.03)	-.29(.06)			HPT
PHP				.52(<.008)	.77(<.008)				Phasic Pain
CHP									CHP

Table 2. Spearman correlation coefficients (*p* values) between sensory tests at each testing visit. Cells highlighted in red denote significant correlations after correction for multiple comparisons.

In order to identify how pain-free, sensorimotor PAF is related to our sensory tests, we performed a series of pairwise correlations between sensorimotor PAF and each sensory test. Corrections for multiple tests at each visit (4 tests; one per sensory test) were made according to the Bonferonni method, yielding a significance threshold of $p = .0125$.

Finally, we median-split our data according to V1 estimates of sensorimotor PAF to yield "slow" and "fast" PAF groups. Sensory test scores were then compared using separate linear mixed effects model with subjects as random effects (intercept included) and Visit (V1 vs. V2), PAF Speed (Slow vs. Fast), and the Visit X Speed interaction as fixed effects.

Results

From our initial cohort of 61 individuals, two individuals were removed due to abnormal pain ratings: one participant fell asleep during ratings while another participant provided abnormally high pain ratings in the absence of any noxious stimuli suggesting that they may been confused by the rating scheme. One additional participant was removed due to an extreme change in CHP score at V2.

From the remaining 58 participants (Table 1), 33 participants were classified as CHP responders (average CHP > 10), 10 participants were classified as high tolerance individuals (average CHP and PHP < 10), and 14 participants were classified as CHP non-responders (only average CHP < 10). Due to a technical error, EEG data was lost for one CHP responder at V1; V1 data for this individual was only included in sensory test analyses. Of the 58 individuals providing data at V1, a total of 43 individuals provided data at Visit 2, of which 32 had been classified as capsaicin responders or high tolerance individuals at V1. In total, 31 of 32 these participants provided complete V1 and V2 datasets.

A summary of average sensory test scores for each pain classification is presented in Figure 2. Additionally, the average V1 PHP and CHP time courses for all three pain classifications are presented in Figure 1; data from V2 were qualitatively similar (data not shown). In line with what has been previously reported, both PHP and CHP produced sensitization (see Supplemental Data).

In order to determine whether pain levels were stable over time, we submitted sensory test scores from CHP responders and high tolerance individuals to a linear mixed effects model with participants as random effects and Visit (V1 vs. V2), Type (WDT vs. HPT vs. Phasic vs. CHP), and the Visit X Type interaction as fixed effects. The absence of a significant main effect of Visit, $F_{(1,128.30)} = .33$, $p = .57$, or Visit x Pain Type interaction, $F_{(3,69.62)} = .83$, $p = .48$, indicates that sensory test scores were generally stable over time. Bayes factor analysis (Rouder et al., 2009) supported the null hypothesis that V1 and

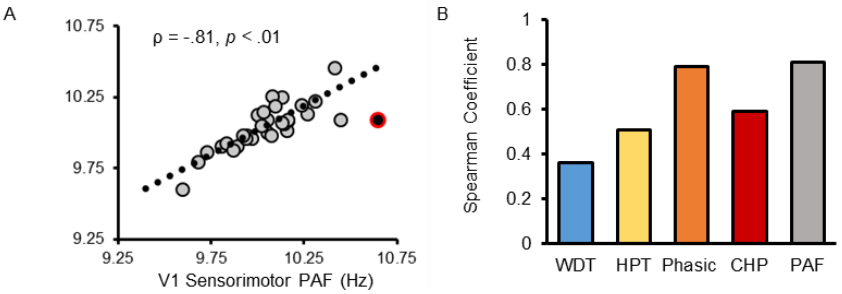

Figure 3. A. Pain-free, sensorimotor PAF estimates are strongly correlated across Visits. Scatter plot only includes data from CHP responders and high tolerance individuals. Off-color data points represent statistical outliers not included in analyses and dotted lines represent the linear regression line of best fit.. B. PAF and sensory tests are stable across Visits. Prolonged pain paradigms are the most stable sensory tests while sensorimotor PAF is the most stable of our metrics.

V2 scores were the same for WDT (Bayes Factor = .10) and HPT (Bayes Factor = .19) but were insensitive with respect to PHP (Bayes Factor = 1.12) and CHP (Bayes Factor = .71)

As another demonstration of stability, scores at V1 and V2 were significantly correlated for HPT, $\rho = .51$, $p < .01$, PHP, $\rho = .79$, $p < .01$, and CHP: $\rho = .59$, $p < .01$ (Figure 2). WDT scores at V1 and V2 trended towards significance, $\rho = .36$, $p = .052$. When we expanded the dataset to also include CHP non-responders, the correlations for all sensory tests became significant (WDT: $\rho = .42$, $p < .01$; HPT: $\rho = .61$, $p < .01$; PHP: $\rho = .74$, $p < .01$; CHP: $\rho = .69$, $p < .01$). Surprisingly, scores for the CHP and PHP paradigm were qualitatively more reliable than either WDT or HPT (Fig 3B). Despite the ubiquity of WDT and HPT in pain research, this finding suggests that our CHP and PHP paradigms may be equally capable at capturing variability in pain sensitivity. Above all else, these results demonstrate that sensory scores from each test are stable over time and that prolonged pain sensitivity is a trait-like characteristic within our sample.

Finally, we investigated inter-test relationships by performing correlations between all possible pairs of sensory tests (Table 2). The only sensory tests that were significantly correlated were CHP and PHP. This finding suggests that CHP and PHP sample the same form of sensitivity, prolonged pain sensitivity, and that other tests are largely distinct from one another, thereby allowing us to determine whether PAF has different relationships with different types of thermal sensitivity.

Sensorimotor PAF is Stable over Time

Given the stability of sensory tests, we expected that sensorimotor PAF should be equally stable if it is indeed a reliable predictor of pain sensitivity. Two observations strongly suggest that sensorimotor PAF was stable across the 7-week (on average, Figure S1) period separating V1 and V2. First, a paired t-test found no significant difference between V1 (mean = 10.04, S.D. = .20) and V2 (mean = 10.04, S.D. = .16) estimates of Sensorimotor PAF, $t_{(29)} = .32$, $p = .75$, and Bayes factor analysis showed that our findings supported the null hypothesis that there are no differences between PAF estimates at V1 and V2 (Bayes Factor < .01). These results did not change when we included all participants regardless of pain classification $t_{(40)} = .34$, $p = .73$, Bayes Factor < .01. Second, V1 and V2 sensorimotor PAF were strongly correlated, Spearman $\rho = .81$, $p < .01$ (Figure 3A). Inclusion of all participants regardless of pain classification did not change the magnitude of this relationship, Spearman $\rho = .82$, $p < .01$. These results clearly demonstrate that sensorimotor PAF is highly stable over time, indeed the most stable measure recorded in this study (Fig 3B), and thus represents a reliable individual trait characteristic.

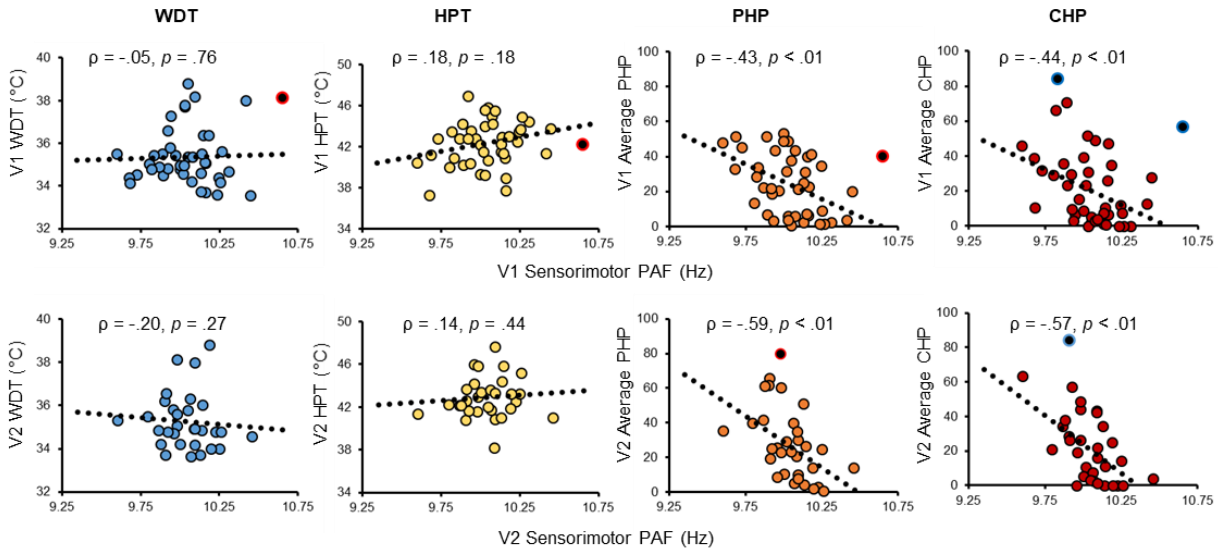

Figure 4. Phasic Pain and CHP are the only sensory tests showing significant correlations to pain-free Sensorimotor PAF at both testing visits. Scatter plots only include data from CHP-responders and high tolerance individuals Off-color data points represent statistical outliers not included in analyses and dotted lines represent the linear regression line of best fit.

Sensorimotor PAF Specifically and Reliably Predicts Thermal Prolonged Pain Paradigms

We next performed a series of correlations to determine which sensory tests are related to sensorimotor PAF. Specifically, we were interested in replicating our previous finding that pain-free, sensorimotor PAF is related to CHP scores and also determining whether PAF would share a similar a relationship with a second prolonged pain test, PHP. At V1, significant relationships emerged between pain-free, sensorimotor PAF and both prolonged pain paradigms, PHP: Spearman ρ = -.43, p < .01; CHP: Spearman ρ = -.44, p < .01 (Figure 4). Similar results were obtained for PHP when we either used a partial correlation to account for the thermode temperature used during PHP, Spearman ρ = -.40, p = .01, or included all participants regardless of pain classification, Spearman ρ = -.34, p = .01; the former result suggests the relationship between PAF and PHP is not a function of stimulus intensity but instead how sensitive the individual is to the stimulus. In comparison, we could not identify a significant relationship between pain-free, sensorimotor PAF and either WDT, Spearman ρ = -.05, p =.76, or HPT, Spearman ρ = .25, p =.11. These results did not change when we expanded our analyses to include all participants regardless of pain classification, WDT: Spearman ρ = .06, p = .66; HPT: Spearman ρ = .18, p = .18. Importantly, we could not find any evidence of sex effects in the relationship of PAF to either PHP or CHP (Figure S3A). Inspection of the sensor level correlation distributions, the distribution of correlations between sensory test scores and PAF calculated at each individual sensor, clearly demonstrates the robustness of these results (Figure S4A).

An identical analysis performed on V2 data similarly revealed a significant relationship between pain-free, sensorimotor PAF and both prolonged pain paradigms, PHP: Spearman ρ = -.59, p < .01; CHP: Spearman ρ = -.57, p < .01 (Figure 4); PHP outcomes remained stable when either accounting for thermode temperature with a partial correlation, Spearman ρ = -.55, p < .01, or including all participants regardless of pain classification, Spearman ρ = -.37, p = .02. Similarly, we were unable to identify a significant relationship between pain-free, sensorimotor PAF and WDT or HPT, WDT: Spearman ρ = -.20, p = .27; HPT: Spearman ρ = .14, p = .44. These results did not change when we considered all participants regardless of pain classification, WDT: Spearman ρ = -.16, p = .31; HPT: Spearman ρ = .03, p

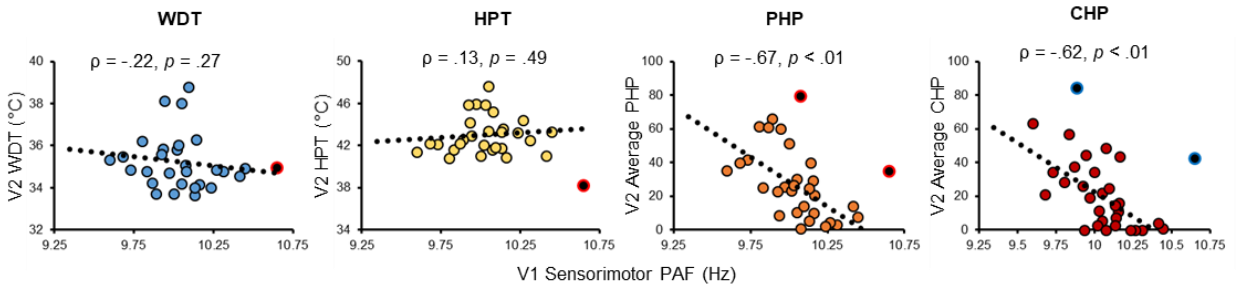

Figure 5. Pain-free, sensorimotor PAF at V1 is a significant predictor of Phasic Pain and C-HP scores occurring, on average, 7 weeks later at V2. Scatter plots only include data from CHP-responders and high tolerance individuals. Off-color data points represent statistical outliers not included in analyses and dotted lines represent the linear regression line of best fit.

= .85. As in V1, there did not appear to be an influence of sex on the relationship between PAF and either of our prolonged pain tests (Figure S4B). As before, sensor level distributions of PAF-sensory score correlations revealed that these findings were evident across the entire scalp (Figure S4B).

Finally, we determined whether V1 estimates of sensorimotor PAF could predict V2 sensory scores occurring, on average, 7 weeks later. Indeed, V1 pain-free, sensorimotor PAF was negatively related to both V2 PHP scores, Spearman ρ = -.67, p < .01, and V2 CHP scores, Spearman ρ = -.62, p < .01 (Figure 5). This relationship between V1 pain-free, sensorimotor PAF and V2 PHP remained when we controlled for thermode temperature, Spearman ρ = -.66, p < .01, or included all participants regardless of pain classification, Spearman ρ = -.44, p < .01. In contrast, we could find no evidence of a relationship between V1 pain-free, sensorimotor PAF and either V2 WDT, Spearman ρ = -.22, p = .27, or V2 HPT, Spearman ρ = .13, p = 49.; these results did not change when we included all participants regardless of pain classification, WDT: Spearman ρ = -.14, p = .38; HPT: Spearman ρ = .11, p = .49. This set of findings provide an important insight into the apparent stability of the PAF-prolonged pain relationship at both V1 and V2. Specifically, the strong relationship of V1 PAF to V2 pain scores suggest that, rather than the appearance of a *de novo* relationship at each time point, V2 correlations are a direct recapitulation of the relationship shared by PAF and prolonged pain scores at V1. In summary, these and the findings presented above suggest that sensorimotor PAF is a reliable predictor or prolonged pain sensitivity both within and across our two visits.

"Slow" and "Fast" Sensorimotor PAF Individuals Experience Different Amounts of Prolonged Pain

In our previous work (Furman et al., 2018), we demonstrated there are differences in PAF speed between low and high pain sensitive groups. We now take the complementary approach to determine whether there are differences between "Slow" and "Fast" PAF individuals in their sensory test scores. To do so, we first performed a median split of our data based on PAF estimates obtained at V1 (labels were carried over to V2). This yielded 21 "Slow" (mean = 9.88 Hz, S.D. = .13) and 21 "Fast" (mean = 10.21 Hz, S.D. = .15) PAF individuals at V1, and 14 "Slow" (mean = 9.87 Hz, S.D. = .13) and 17 "Fast" (mean = 10.21 Hz, S.D. = .16) individuals at V2.

Next, we performed four, separate linear mixed models, one for each sensory test, with subjects as random effects and Visit (V1 vs V2), Speed (Slow vs. Fast), and the Visit X Speed interaction as fixed effects. Average sensory test scores for each speed group at each visit can be seen in Figure 6. For PHP scores, this analysis revealed a significant main effect of Speed, $F_{(1,37.42)}$ = 7.04, p = .01, but neither a significant main effect of Visit, $F_{(1,29.61)}$ = 2.01, p = .17, nor a significant Visit X Speed interaction, $F_{(1,29.61)}$ = .98, p =.33. According to this analysis, the estimated effect of PAF speed on PHP scores was 16.67 (95%

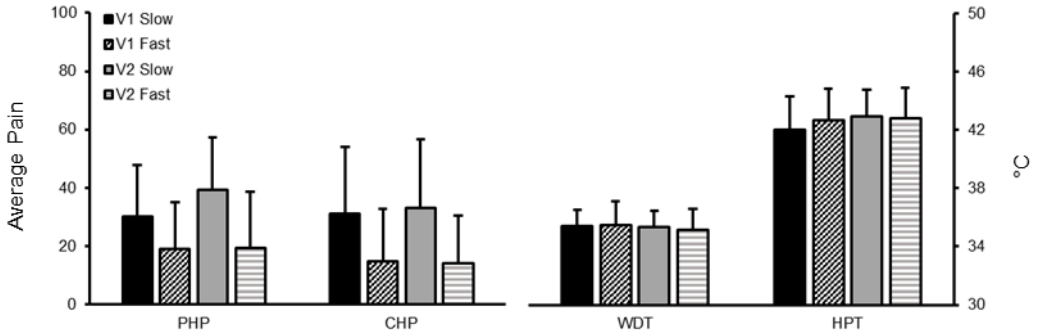

Figure 6. "Fast" and "Slow" PAF individuals vary only in the amount of average Phasic Pain and C-HP that they experience. Speed groups were generated by performing a median split on V1 sensorimotor PAF estimates. Bars reflect mean scores (+1 S.D.).

Confidence Intervals: 3.22 – 30.11). This difference emerged despite the fact that PHP thermode temperatures were nearly identical for the Slow PAF group (mean = 45.52°C, S.D. = 1.47°C) and Fast PAF group (mean = 46.23°C, S.D. = 1.34°C, $t_{(40)} = 1.65$, $p = 11$). Indeed, the fact that thermode temperatures were qualitatively higher for Fast PAF individuals suggests that we may, if anything, be underestimating the effect of PAF speed on pain sensitivity. The same pattern of results emerged for CHP scores with a significant main effect of Speed, $F_{(1,39.96)} = 9.34$, $p < .01$, and without a significant main effect of Visit, $F_{(1,32.27)} = .05$, $p = .83$, or a significant Visit X Speed interaction, $F_{(1,32.27)} = .23$, $p = .64$. The estimated effect of PAF speed on CHP scores was 19.13 (95% Confidence Intervals: 5.48 – 32.79). In contrast, we could not identify any significant main effects or interaction for either WDT (all $p > .29$) or HPT (all $p > .23$).

To determine whether these group differences were specific to particular portions of the prolonged pain tests or present across the entire test, we computed the average pain time course separately for each speed group on each prolonged pain test. This revealed differences between Slow PAF and Fast PAF groups that were evident across the entirety of the PHP and CHP paradigms (Figure 7); differences in pain ratings appeared almost immediately and persisted stably throughout testing without any apparent interaction between group and time. In line with this observation, we could find no evidence that "Slow" and "Fast" PAF groups differed in the amount of sensitization they experienced in the PHP Paradigm (Supplementary Data). This suggests that PAF's impact on the pain experience is likely manifest in processes that organize an individual's general, or "trait-like", pain sensitivity rather than those processes that modify the amount of ongoing pain (i.e. sensitization).

Discussion

In the current study we set out to test whether pain-free, Sensorimotor Peak Alpha Frequency (PAF) is a specific and reliable predictor of prolonged pain. We found that pain-free, Sensorimotor PAF could predict Capsaicin Heat-Pain sensitivity (CHP), with increasingly slower PAF being associated with

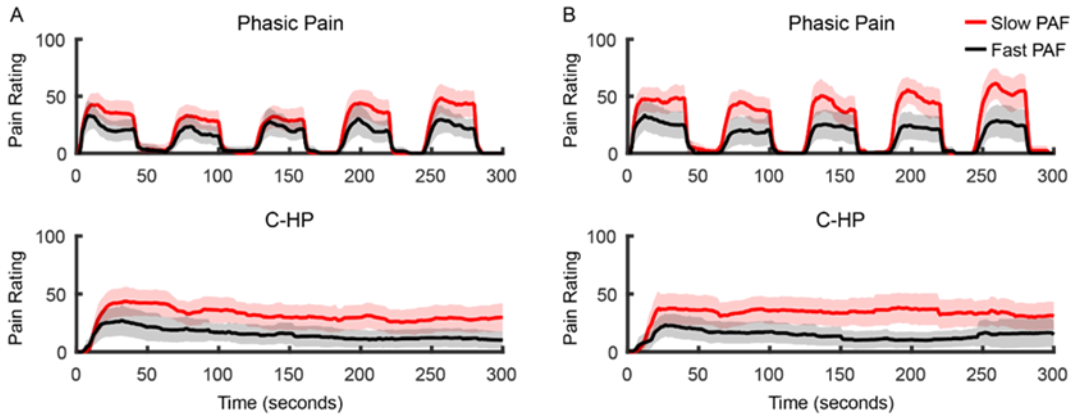

Figure 7. Differences between "Slow" And "Fast" PAF groups are present immediately across the entire prolonged pain time-course during V1 (A) and V2 (B). Solid lines represent the group average and shaded lines the +/- 95% confidence interval.

increasingly greater CHP intensity, at both short (45 minutes) and long (8 week) timescales. We also found that pain-free, sensorimotor PAF shares a near identical relationship with a second test of prolonged pain, Phasic Heat Pain (PHP). While both CHP and PHP produce sensitization and similar amounts of pain, the actual procedures used differ in the length of application, the temperatures used, and the presence of sensitizing agent. In light of the fact that PAF was not related to two standard tests of acute thermal processing, warmth detection threshold (WDT) and heat pain threshold (HPT), these differences between the tasks provides an important measure of comfort that PAF is related to the characteristic shared by each task, perhaps the prolonged nature of the pain, rather than non-pain variables that are unique to each task.

Peak Alpha Frequency (PAF) is an alpha rhythm characteristic hypothesized to represent processes that control the speed of perceptual cycles (e.g. Samaha & Postle, 2015; Cecere et al., 2015; Wutz et al., 2018). Previous investigations of PAF and chronic pain (e.g. Sarnthein et al., 2006; de Vries et al., 2013; Lim et al., 2016) have reliably found that PAF is slowed in patients, leading many to suggest that disturbances in PAF reflect ongoing, pathological processes. Earlier hypotheses about the role of disrupted communication between the thalamus and cortex in chronic pain, so called Thalamocortical Dysrhythmias (e.g. Llinás et al., 1999), have proven particularly influential in providing context for these interpretations. Alongside these findings, work from both our lab and others has demonstrated that PAF recorded in the absence of pain can serve to distinguish high and low pain sensitive individuals in the healthy population (Nir et al., 2010; Furman et al., 2018). These findings have led us to hypothesize that PAF is a biomarker of pain sensitivity in healthy individuals and to propose that apparent chronic pain disturbances of PAF may, at least in part, reflect differences in prolonged pain sensitivity that predate disease onset.

We were able to replicate our previous finding that sensorimotor PAF collected during a pain-free resting state can predict future pain sensitivity to the CHP model. This replication is notable because the sample size used in the current study was nearly double that used previously thereby giving us confidence that our previous results were not a consequence of spurious effects associated with small sample sizes. Furthermore, our results extend these prior findings by clearly establishing that prolonged pain sensitivity is a trait-like characteristic. Three key points justify this conclusion. First, we were unable to identify a significant effect of Visit or Visit X Type in our linear mixed model suggesting that pain intensities for both CHP and PHP do not change across the two testing visits. Second, pain intensities for

both prolonged pain tests are strongly correlated across visits. Indeed, we found these tests were generally more reliable than either warmth detection (WDT) or heat pain thresholds (HPT). This is somewhat surprising given that WDT and HPT are considered gold standards of quantitative sensory testing (e.g. Rolke et al., 2006) while prolonged pain paradigms are used less often. Third, the average interval between visits was more than 7 weeks making it unlikely that the reliability of pain scores is biased by the participant's memory of earlier pain ratings. The fact that participants completed these tasks without any haptic or visual feedback of their ratings also argue against this interpretation. Instead, the most parsimonious explanation of these results is that prolonged pain sensitivity is a stable individual characteristic.

Beyond showing that Sensorimotor PAF is similarly related to two different tests of prolonged pain, we also demonstrate that this relationship is reliable across multiple time points. At both V1 and V2, pain-free, sensorimotor PAF was negatively related to CHP and PHP intensities; for both visits, individuals with increasingly slower PAF experienced increasingly greater CHP or PHP intensities. It should be noted that this relationship was qualitatively stronger at V2, which may suggest that a portion of the PAF's predictive ability is related to factors associated with a participant's familiarity with the sensory tests. The fact that this relationship was present at V1, where participants experienced the PHP paradigm for the very first time, argues against familiarity as the sole factor responsible, however.

One of the most intriguing results of the current study is that V1 estimates of sensorimotor PAF can predict V2 prolonged pain scores. This finding not only demonstrates that, in addition to providing separate predictions at each time point, a single estimate of sensorimotor PAF can also provide cogent predictions of pain sensitivity at multiple time points. This appears to be a result of the fact that both sensorimotor PAF and prolonged pain sensitivity do not change much over time in healthy individuals.

Exposing participants to thermal tests that varied in their noxious content (innocuous vs. noxious) and their duration (acute vs. prolonged) allowed us to begin probing the boundaries of the sensorimotor PAF-pain sensitivity relationship. While we were able to find reliable relationships between prolonged pain and sensorimotor PAF, we could not find any evidence of a similar correlation to either WDT or HPT. These absences provide preliminary evidence that PAF is not related to either innocuous heat processing or to transient pain events. It should be cautioned, however, that this apparent specificity may reflect differences in the rating procedures used between tests. Whereas both prolonged pain paradigms require participants to make continuous, magnitude judgements, WDT and HPT involve a single "stop" decision and are thus influenced by additional factors like reaction time. Additional studies that can control for this discrepancy are needed to ensure that the current results are due to the type of stimulation rather than the type of response. Nonetheless, the WDT results appear to argue against a purely perceptual role for PAF in thermal processing, similar to what has been shown for tactile discrimination (Baumgarten et al., 2017); in theory, faster PAF should be associated with lower WDT since shorter intervals between sampling bouts should promote more rapid change detection. Ultimately, multi-modal imaging (i.e. EEG-fMRI) is still needed to firmly assess whether PAF exerts its effects in or outside of the sensorimotor system.

As in our first study, median split analysis suggests that pain-free, Sensorimotor PAF may be a useful tool for separating individuals into "high" and "low" prolonged pain sensitivity groups. A crucial difference from our previous approach, however, is that we performed our median split on Sensorimotor PAF estimates rather than pain scores. We did this to more directly simulate what might happen in a clinical

setting where only PAF estimates are available. Indeed, our findings suggest that clinicians could potentially use pre-intervention PAF to inform pain management decisions so that post-surgical treatment is personalized to both the intervention and the participant's expected pain sensitivity. Such an approach could pay dividends in reducing opiate burdens by helping practitioners identify patients who are unlikely to need opiate pain relief. An unexpected result of our median split is that differences between PAF speed groups were present immediately and persisted throughout the entire prolonged pain time course. This suggests that PAF's relationship to pain is mediated by processes that organize general responses to pain, such as attention (i.e. Miron et al., 1989; Klimesch, 2012; Guilbinaite et al., 2017), rather than specific pain processes, like sensitization, that determine particular elements of the pain experience.

In summary, our results clearly demonstrate that sensorimotor PAF is a reliable predictor of prolonged pain sensitivity. In addition to replicating the relationship between sensorimotor PAF and prolonged pain sensitivity, we now provide compelling evidence that this relationship is stable over both immediate, i.e. minutes/hours, and more extended, i.e. weeks/months, periods of time. Furthermore, we provide preliminary findings that sensorimotor PAF is a specific biomarker for prolonged pain sensitivity and that splitting participants based on pain-free PAF can provide meaningful information for identifying high and low pain sensitivity individuals. These findings now firmly position sensorimotor PAF as a biomarker of pain sensitivity with untapped potential in clinical settings.

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Supplemental Data

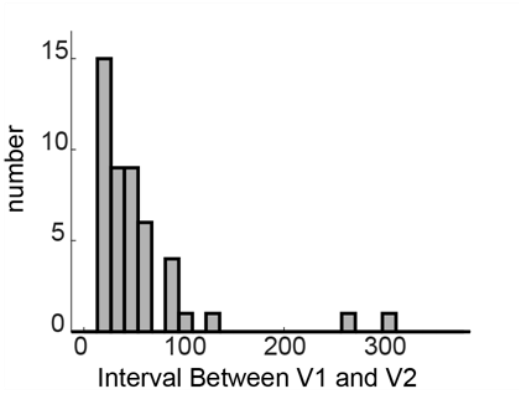

Figure S1. Histogram of days separating Visit 1 and Visit 2 sessions. On average visits were separated by 54.74 days (7.82 weeks).

PHP and CHP Produce Sensitization

We first sought to determine whether sensitization, a putative hallmark of prolonged pain, is present in our two prolonged pain paradigms. Inspection of the PHP time course suggest that following a decrease in pain ratings from the first to the second PHP trial, which may reflect the enhanced salience of the first stimulus (Ianetti et al., 2008), ratings increased linearly from the second to fifth PHP trial (Figure 1B). To formally test this observation, we calculated the average pain rating for PHP trials 2 and 5 for all participants, regardless of pain classification, and submitted these scores to a linear mixed model with participants as random effects (slope included) and Visit (V1 vs. V2), Trial (2 vs. 5) and the Visit X Trial interaction as fixed effects. If PHP scores sensitize over time, then a significant main effect of Trial should be present. This analysis revealed a significant main effect of Trial, $F_{(1,88.14)} = 19.06$, $p < .01$, without a significant main effect of Visit, $F_{(1,103.08)} = .35$, $p = .55$, or significant Visit X Trial interaction, $F_{(1,88.18)} = .02$, $p = .89$. The estimated effect of Trial on PHP scores 8.61(95% Confidence Intervals: 2.13 – 15.10). This increase in scores from trial 2 (mean = 20.81, S.D. = 17.88) to trial 5 (mean = 29.31, S.D. = 21.43) in response to the same noxious stimulus is evidence of sensitization.

Two findings support the presence of sensitization during CHP. First, across all participants, a pair of one-sample t-tests revealed that CHP scores were significantly greater than 0 at both V1, $t_{(57)} = 6.63$, $p < .01$, and V2, $t_{(42)} = 5.72$, $p < .01$. Second, another pair of one-sample t-tests indicated that HPTs were significantly greater than the CHP temperature, 40°C, at both V1, $t_{(57)} = 7.90$, $p < .01$, and V2, $t_{(39)} = 8.89$, $p < .01$. We believe that this pain response to a sub-WDT temperature is a strong indicator of sensitization given that we have previously demonstrated that a similar temperature in the absence of capsaicin does not produce pain (Furman et al., 2018).

CHP Scores Vary Across Visits as a Function of Sex

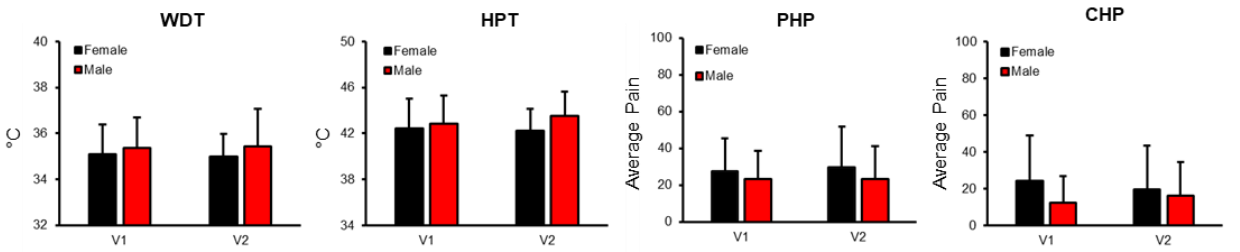

Figure S2. Sensory test scores broken by sex and visit. CHP scores show a significant Visit X Sex interaction – scores increase for men from V1 to V2 and decrease for women from V1 to V2.

Average scores (+ 1 S.D) for the sexes on each test at each visit can be seen in Supplementary Figure 2. Previous studies have reported that sex may be an important variable in determining pain sensitivity (i.e. Dao & LeResche, 2000).

To determine whether sex may play a role in our sensory tests, we performed four, separate linear mixed models, one for each sensory test, with subjects as random effects and Visit (V1 vs V2), Sex (Male vs. Female), and the Visit X Sex interaction as fixed effects. Given that our study was not powered with respect to gender effects, analyses were performed on all participants regardless of pain classification in order to maximize available statistical power. For PHP scores, this analysis revealed no significant effects of Visit, $F_{(1,41.34)} = .37$, $p = .54$, Gender, $F_{(1,53.35)} = 1.72$, $p = .20$, or Visit X Gender interaction, $F_{(1,41.34)} = .76$, $p = .39$. For CHP scores, this analysis revealed a significant Visit by Gender interaction, $F_{(1,41.86)} = 8.95$, $p < .01$, but no significant main effects of Visit, $F_{(1,41.86)} = .06$, $p = .80$, or Gender, $F_{(1,54.53)} = 1.38$, $p = .25$. For WDT scores, this analysis revealed no significant effects of Visit, $F_{(1,44.39)} = .004$, $p = .95$, Gender, $F_{(1,56.14)} = 1.08$, $p = .30$, or Visit X Gender interaction, $F_{(1,44.39)} = .00$, $p > .99$. For HPT scores, this analysis revealed no significant effects of Visit, $F_{(1,47.64)} = .81$, $p = .37$, Gender, $F_{(1,56.16)} = 1.53$, $p = .22$, or Visit X Gender interaction, $F_{(1,47.54)} = .81$, $p = .37$.

Other than CHP it appears that there are no clear sex differences in sensory test scores. For CHP, the Visit X Gender interaction reflects the fact that males experience increases in CHP scores from V1 (mean = 12.10, S.D. = 14.50) to V2 (mean = 16.12, S.D. = 18.44), whereas females experience decreases in CHP scores from V1 (mean = 24.37, S.D. = 24.45) to V2 (mean = 20.01, S.D. = 23.06).

The PAF-Pain Sensitivity Relationship is Similar for Both Sexes

One important consideration for any pain biomarker is whether it applies equally to both sexes. Inspection of correlation magnitudes for each sex revealed that PAF-pain sensitivity relationships were roughly equivalent between the sexes on both tests at each visit (Supplementary Figure 3). We do not provide *p* values for these tests as our study was not powered to investigate sex differences directly.

To more formally test whether sex influences the relationship of PAF to pain sensitivity, we performed four, separate moderation analyses (one for PHP and CHP at each visit) using PROCESS (V3.2; Hayes, 2012) implemented in SPSS. In these regression analyses, sensory test scores served as the dependent variable with PAF as the independent variable and sex as a dichotomous moderator variable. As with other correlational analyses, we excluded PAF or sensory test scores greater than 2.5 SD above the mean. To account for possible multi-collinearity, independent variables and moderators were mean-centered. In our moderation analyses, a significant interaction of sex and PAF would indicate that the relationship between PAF and pain sensitivity is different for the two sexes. We were unable, however, to identify a significant PAF x Sex interaction for PHP scores at either V1, $t = -.16$, $p = .87$, or V2, $t = .74$, $p = .47$. Similarly, there was not a significant effect of PAF x Sex for CHP scores at either V1, $t = -.01$, $p =$

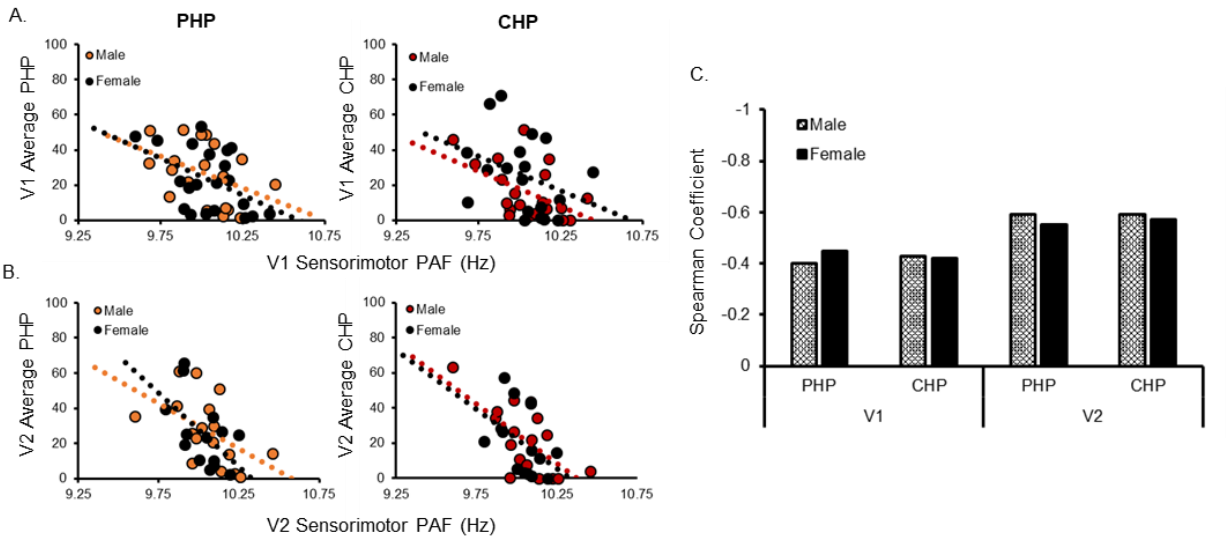

Figure S3. Correlations between PAF and prolonged pain paradigms are statistically identical at both Visit 1 (A) and Visit 2 (B). Note that statistical outliers are not presented in the figure but were not included in any analyses. Spearman correlation coefficients for each gender, prolonged pain paradigm, and visit are presented in C.

.99, or V2, $t < .01$, $p > .99$. According to our moderation analyses we can conclude that the PAF-pain sensitivity relationship is not different for the two sexes.

The PAF-Pain Sensitivity Relationship is Evident Across the Entire Scalp

Previously unpublished findings from our lab have suggested that the PAF-pain sensitivity relationship is not privileged to sensors overlying the sensorimotor cortex. To determine whether similar conclusions can be drawn from the current dataset, we first calculated PAF separately for each individual at each of the 63 EEG sensors. Next, we correlated PAF estimates from each sensor with scores on each sensory test to yield a total of 63 correlation values for each test. To visualize these results, we then plotted the distribution of correlation values for each sensory test with a histogram. As can be seen in Supplementary Figure 4, the distribution of sensor correlations largely recapitulated what we found when we focused only on our sensorimotor ROI. Specifically, we found that correlations between PAF and either CHP or PHP were moderately large and in the negative direction. In comparison, correlations between PAF and either WDT or PHP were much smaller across the board.

PAF Does Not Reflect Sensitivity to Sensitization Processes

Qualitative differences in pain ratings are evident across the entire time courses of both the Phasic Pain and C-HP paradigms (Figure 7). As noted above, both CHP and PHP produce sensitization which might suggest that are differences in sensitization between PAF speed groups. To test whether PAF may be related to the amount of sensitization experienced by an individual, we analyzed PHP sensitization scores, calculated by subtracting trial 5 from trial 2 pain scores, using a linear mixed model with subjects as subjects as random effects (intercept included) and Visit (V1 vs V2), Speed (Slow vs. Fast), and the Visit X Speed interaction as fixed effects. We could not find any evidence of a relationship between PAF and sensitization as neither the main effect of Speed, $F_{(1,32.24)} = 2.82$, $p = .10$, nor the Speed x Visit interaction, $F_{(1,29.21)} = .16$, $p = .69$, were significant. Similarly, there were no differences between sensitization scores as a function of Visit, $F_{(1,29.21)} = .06$, $p = .81$.

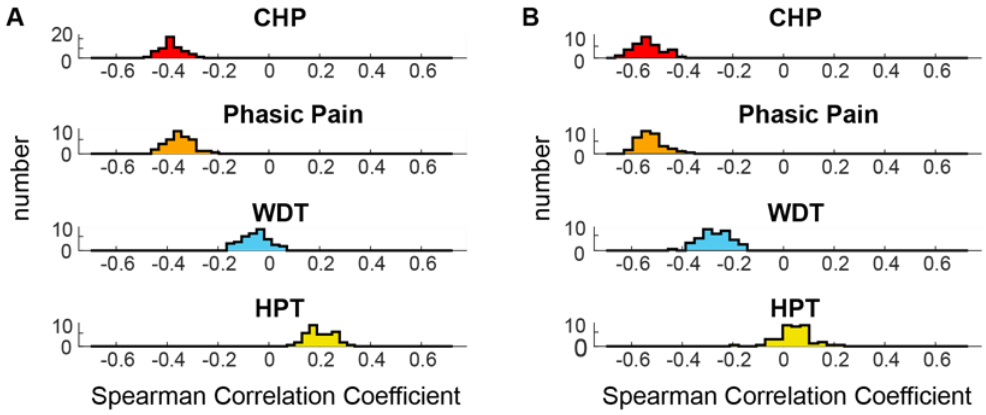

Figure S4. Pattern of sensorimotor PAF-sensory test scores is observable across the entire EEG montage. For each sensory test, and each of the 63 individual EEG sensors, we computed the Spearman correlation between sensor-level PAF and sensory test scores yielded a total of 63 correlations for each sensory test. Graphs display the histograms of these correlations at V1 (A) and V2 (B).

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716 **Supplemental References**

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