

1 Abbreviated Title: Alpha and Pain Sensitivity bio

2 Sensorimotor peak alpha frequency is a reliable biomarker of pain sensitivity

3 Andrew J. Furman<sup>1,2,3</sup>, Mariya Prokhorenko<sup>2</sup>, Michael L. Keaser<sup>2,3</sup>, Jing Zhang<sup>2</sup>, Shuo Chen<sup>4</sup>, Ali Mazaheri<sup>5</sup>,  
4 and David A. Seminowicz<sup>2,3</sup>

5 <sup>1</sup> Program in Neuroscience, University of Maryland School of Medicine, Baltimore, MD, 21201

6 <sup>2</sup> Department of Neural and Pain Sciences, University of Maryland School of Dentistry, Baltimore, MD,  
7 21201

8 <sup>3</sup> Center to Advance Chronic Pain Research, University of Maryland Baltimore, Baltimore, MD, 21201

9 <sup>4</sup> Department of Epidemiology and Public Health, University of Maryland Baltimore, Baltimore, MD,  
10 21201

11 <sup>5</sup> School of Psychology, University of Birmingham, B15 2TT, United Kingdom.

12

13 Please address correspondences to:

14 David A. Seminowicz, PhD

15 Department of Neural & Pain Sciences

16 University of Maryland School of Dentistry

17 650 W. Baltimore Street, 8 South

18 Baltimore, MD 21201 ph.

19 410.706.3476

20 [dseminowicz@umaryland.edu](mailto:dseminowicz@umaryland.edu)

21

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## 26 **Abstract**

27 Previous research has observed that individuals with chronic pain demonstrate slower alpha band  
28 oscillations (8-12 Hz range) during resting electroencephalography (EEG) than do age-matched, healthy  
29 controls. While this slowing may reflect pathological changes within the brain that occur during the  
30 chronification of pain, an alternative explanation is that healthy individuals with slower alpha  
31 frequencies are more sensitive to prolonged pain, and by extension, more susceptible to developing  
32 chronic pain. To formally test this hypothesis, we examined the relationship between the pain-free,  
33 resting alpha frequency of healthy individuals and their subsequent sensitivity to two experimental  
34 models of prolonged pain, Phasic Heat Pain and Capsaicin Heat Pain, at two testing visits separated by 8  
35 weeks on average ( $n = 61$  Visit 1,  $n = 46$  Visit 2). We observed that the speed of an individual's pain-free  
36 alpha oscillations was negatively correlated with sensitivity to both prolonged pain tests and that this  
37 relationship was reliable across short (minutes) and long (weeks) timescales. Furthermore, we used the  
38 speed of pain-free alpha oscillations to successfully identify those individuals most sensitive to  
39 prolonged pain, which we also validated on data from a separate, independent study. These results  
40 suggest that alpha oscillation speed is a reliable biomarker of prolonged pain sensitivity with the  
41 potential to become a tool for prospectively identifying pain sensitivity in the clinic.

## 42 **Introduction**

43 Chronic pain is a debilitating condition with cognitive, affective, and sensory symptoms that afflicts  
44 nearly one fifth of the American population (Kennedy et al., 2014), leading to treatment and work loss  
45 costs totaling nearly six hundred billion dollars annually (Gaskin & Richard, 2012). Identifying individuals  
46 at high risk for developing chronic pain is a crucial, but under-explored, avenue for combatting chronic  
47 pain and its related economic burdens. At present, prediction of chronic pain development is poor: for  
48 example, one of the best predictors of persistent post-surgical pain is the intensity of pain reported  
49 directly after surgery (e.g. Katz et al., 1996). While useful for post-operative case management, these  
50 measures cannot be used to identify, and target prophylactic treatments to, individuals at risk for  
51 developing chronic pain. What is urgently needed is a measure of an individual's sensitivity to prolonged  
52 pain that can be obtained prior to medical intervention. To that end, the objective of the current study  
53 is to systematically investigate the hypothesis that an individual's peak alpha frequency, measured with  
54 resting state electroencephalography (EEG), is a trait-like marker of their sensitivity to prolonged pain.

55 The alpha rhythm (8-12 Hz) is the predominant oscillatory activity observed in scalp-recorded EEG of the  
56 primary sensory cortices (e.g. occipital, somatosensory) while an individual is quietly resting. Across  
57 individuals, there is considerable variability in the alpha band frequency from which the greatest power  
58 is recorded (Haegens et al, 2014, Bazanova & Vernon, 2014). This frequency, often labeled the Peak or  
59 Individual Alpha Frequency (PAF/IAF), has been suggested to contribute to individual differences in  
60 multiple psychological and physiological processes (e.g. Klimesch, 2012; Samaha and Postle, 2015;  
61 Gulbnaite et al., 2017; Mierau et al., 2017; Van Diepen et al., 2019).

62 Previous research has consistently observed abnormally slow PAF in chronic pain patients (Sarnthein et  
63 al., 2005; Walton et al., 2010; Lim et al., 2016), with increasingly slower PAF associated with increasingly  
64 longer durations of chronic pain (de Vries et al., 2013). This apparent slowing of PAF in chronic pain has  
65 been interpreted to reflect pathological changes within the brain that occur during the chronification of  
66 pain (Llinás et al., 1999). Work from our lab has, however, shown that slow PAF, recorded in the absence  
67 of pain (i.e. pain-free PAF), also reflects heightened sensitivity to prolonged pain in healthy individuals  
68 (Furman et al, 2018 & 2019). Given that heightened pain sensitivity is a risk factor for developing chronic  
69 pain (Diatchenko et al., 2005), an alternative interpretation of the aforementioned chronic pain findings

70 is that slow PAF reflects an increased sensitivity to prolonged pain that predates disease onset. Put  
71 another way, slow PAF may reflect a predisposition for developing chronic pain rather than a result of its  
72 development.

73 In the current study, we sought to further characterize the relationship of pain-free PAF to prolonged  
74 pain sensitivity by exposing participants to two experimental models of prolonged pain, Phasic Heat Pain  
75 (PHP) and Capsaicin Heat Pain (CHP), at two testing visits separated by multiple weeks. This study design  
76 allowed us to test two key predictions of the hypothesis that pain-free PAF is a trait-like marker of an  
77 individual's sensitivity to prolonged pain: (1) that pain-free PAF reflects pain sensitivity to multiple  
78 prolonged pain tests; and (2) that an individual's pain-free PAF can predict their sensitivity to prolonged  
79 pain at more than one point in time. In addition to these main aims, and with an eye towards its  
80 potential clinical application, we also examined whether pain-free PAF can be used to successfully  
81 identify high and low pain sensitive individuals.

## 82 **Materials and methods**

### 83 *Participants*

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

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

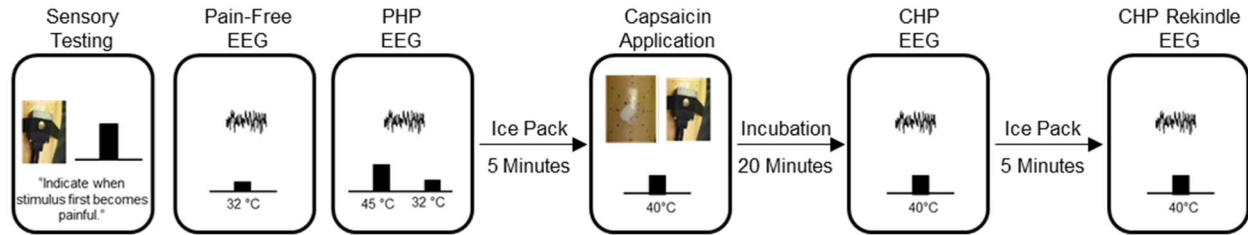
### 90 *EEG*

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

### 99 *Thermal Stimulator and Pain Scale*

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

103 Unless otherwise stated, pain ratings were collected continuously with a manual analog scale consisting  
104 of a physical sliding tab (Medoc Advanced Medical Systems Ltd., Ramat Yishai, Israel). Prior to testing,  
105 participants were instructed that the lower and upper bounds of the scale represented no pain and the  
106 most pain imaginable, respectively, and that they should continuously update the position slider to  
107 indicate the amount of pain currently being experienced. Care was taken by experimenters to avoid  
108 providing numerical anchors when describing the scale and no additional physical landmarks were  
109 present on the scale. Prior to testing, participants were given an opportunity to practice using the device  
110 with their eyes open and closed. During testing, participants were permitted to briefly open their eyes  
111 while rating.



**Figure 1.** Outline of the experimental procedure. After a brief sensory testing session, participants completed a pain-free EEG. Next, participants completed a “Phasic Heat Pain (PHP)” EEG. After a 5-minute period in which an ice pack was applied to the skin, a second pain-free EEG was collected (not shown). Afterwards, capsaicin was applied to the forearm and incubated for twenty minutes. Next, a “Capsaicin-Heat Pain (CHP)” EEG was completed while a 40°C thermode was placed on top of the capsaicin. After a 5-minute period in which an ice pack was applied to the skin and a third pain-free EEG was collected (not shown), the 40°C thermode was again placed on top of the capsaicin and a 5-minute eyes-closed “CHP rekindle” EEG was completed. For each EEG, data was collected for 5 minutes while participants were instructed to keep their eyes closed. Identical procedures were performed at Visit 1 and Visit 2.

112 Pain ratings were collected from the manual analog scale at a rate of 1000 Hz. Manual analog scale data  
113 was transformed by converting the horizontal position of the slider into a continuous value between 0  
114 and 100.

#### 115 *Quantitative Sensory Testing*

116 Participants were asked to complete four threshold tests: 1) to report when they felt a temperature  
117 increase (Warmth Detection Threshold); (2) to report when they felt a temperature decrease (Cool  
118 Detection Threshold); (3) to report when an increasing temperature first became painful (Heat Pain  
119 Threshold); and (4) to report when a decreasing temperature first became painful (Cold Pain Threshold).  
120 A total of three trials were presented for each test with an ISI of 4-6 seconds (randomly determined on a  
121 per trial basis). Participants provided feedback for each test by clicking either the left or right button of a  
122 computer mouse placed in their right hand. For each test, temperatures were applied with a rise rate of  
123 1°C/second and return rate of 2°C/second (initiated on any mouse click).

124 All testing was performed on the volar surface of the left forearm. The distance from the wrist to elbow  
125 joint was measured and the forearm was divided into three equal length zones. For each test, the first  
126 trial was administered to the zone closest to the wrist, the second trial administered to the middle  
127 forearm zone, and the third trial administered to the zone closest to the elbow.

#### 128 *Phasic Heat Pain (PHP) Model*

129 Temperatures used during the PHP model were determined during each participant’s initial screening  
130 visit to the laboratory (Visit 0). During these sessions, participants were exposed to 12, 20 second trials  
131 in which a single temperature (2.5 second rise and fall) was applied to the volar surface of the left  
132 forearm. At the conclusion of each trial, participants reported the average pain they experienced during  
133 temperature application; participants were instructed to report pain ratings on a scale of 0-10, with 0  
134 indicating no pain and 10 the most pain imaginable. Temperatures ranged from 37 to 48°C (intervals of  
135 2°C, starting as if 37°C was 38°C) and each temperature was presented twice in a pseudorandom order.  
136 Trials were separated by 10 seconds and after each trial the thermode was moved to a neighboring  
137 forearm zone in order to minimize sensitization. Using pain reports from these trials, the temperature  
138 that most closely evoked an average pain rating of 5/10 was selected. This level of pain was targeted in  
139 order to best match the intensity of pain evoked by the CHP model (Furman et al., 2018). For a few  
140 participants, none of temperatures were able to evoke a 5/10 pain rating. For these individuals, 48°C  
141 was used during PHP testing.

	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.

142 The PHP model itself consisted of a series of five consecutive stimulus trains. Each train lasted one  
 143 minute and consisted of application of a predetermined temperature for 40 seconds (rise and fall times  
 144 of 2s) followed by application of a neutral skin temperature stimulus (32°C) for 20 seconds. PHP scores  
 145 were calculated by averaging pain ratings from the five, forty second periods in which the temperature  
 146 was present.

#### 147 *Capsaicin Heat Pain (CHP) Model*

148 The CHP model lasts for hours to days and recapitulates some cardinal sensory aspects of chronic  
 149 neuropathic pain (Culp et al., 1989; LaMotte RH, et al., 1992; Baron 2009; Lötsch et al., 2015) without  
 150 causing lasting tissue damage (Henriques & Moritz, 1947). CHP procedures were similar to those used in  
 151 our prior study (Furman et al., 2018). In brief, we applied ~1 g 10% capsaicin paste (Professional Arts  
 152 Pharmacy, Baltimore, MD) topically to the volar surface of the left forearm, fixing it in place with a  
 153 Tegaderm bandage. A thermode was then placed over top of the capsaicin application, heated to 40°C  
 154 and held in place for 20 minutes to allow for capsaicin incubation. Given that pain from topically applied  
 155 capsaicin varies as a function of skin temperature (Anderson et al., 2002), the thermode temperature  
 156 was held at 40°C for all participants. This temperature was selected because, in the absence of capsaicin,  
 157 most individuals find it non-painful thereby providing comfort that any pain generated by this  
 158 temperature during capsaicin exposure is likely a consequence of the agent's sensitizing effects. CHP  
 159 scores were calculated by averaging ratings across the entire five-minute CHP test that followed  
 160 incubation.

161 To further test of the reliability of CHP sensitivity, we included a "rekindling" phase (CHP rekindle; Dirks  
 162 et al., 2003). After the initial CHP testing was completed, an icepack (see below for details) was applied  
 163 to the forearm until a complete termination of pain was reported. Afterwards, the thermode was again  
 164 placed over top of the site of capsaicin application, heated to 40°C, and held in place for five minutes.  
 165 CHP rekindle scores were calculated as the average of the pain ratings provided during this five-minute  
 166 period.

#### 167 *Icepack Application*

168 At the conclusion of the PHP and CHP tests, the thermode was removed and a disposable icepack was  
 169 applied the stimulated area of the left forearm. This was done to prevent pain carryover from one test  
 170 to another and to ensure that pain ratings for subsequent tests were captured from a starting state of  
 171 no ongoing pain. The icepack was left in place until the complete absence of pain was reported by the  
 172 participant. No participants indicated that the icepack itself was ever painful. Following each icepack  
 173 application, a 5-minute pain-free, eyes closed EEG session occurred.

#### 174 *Procedure*

175 An outline of the experimental timeline and procedures is presented in Figure 1. In order to allow  
 176 sufficient time for any long-term effects of capsaicin exposure to subside, visits were separated by 21

177 days or more (except for one case where a subject returned at 19 days because of a scheduling conflict;  
178 mean separation of Visit 1 and Visit 2 = 54.74 days, S.D. = 55.92 days, range = 19 – 310 days, Figure S1).

179 Participants first underwent an initial screening visit, Visit 0, that included quantitative sensory testing  
180 as well as additional tests to ensure that 40°C was rated as minimally-painful, to identify the appropriate  
181 PHP temperature, and to provide initial exposure to capsaicin. For the first four participants, these  
182 procedures, excluding capsaicin exposure, were performed during Visit 1.

183 Participants returned for Visit 1 at least three weeks after completing Visit 0. Most participants then  
184 returned at least three weeks after Visit 1 for Visit 2. Procedures for Visits 1 and 2 were identical. For the  
185 entirety of Visits 1 and 2, participants were seated in a comfortable chair in a quiet room that was  
186 isolated from strong electrical interference. For all EEG sessions, lights in the testing room were turned  
187 off and participants were instructed to close their eyes, remain still, relax without falling asleep, and  
188 continuously rate any pain they experienced with the manual analog scale placed at their right hand.  
189 Visits 1 and 2 began with quantitative sensory testing. For the first four participants, this sensory testing  
190 was not performed at Visit 2. After quantitative sensory testing, a brief 2-minute EEG was collected to  
191 ensure the quality of EEG recording. Next, a room temperature thermode was placed onto the left  
192 forearm while eyes closed, pain-free EEG was collected for 5 minutes. The primary objective of the  
193 current study was to use PAF recorded during this pain-free period as a predictor of subsequent pain  
194 sensitivity during CHP and PHP.

195 Following the pain-free EEG, prolonged pain was induced with the PHP model. During the five minutes  
196 of PHP, EEG was collected while participants rested with their eyes closed and continuously rated the  
197 intensity of any perceived pain. Upon completion of the PHP model, a disposable ice pack was placed  
198 onto the participant's left forearm until they reported being completely free of pain after which 5  
199 minutes of eyes closed EEG was collected. Next, the second model of prolonged pain, CHP, was  
200 induced. Participants were instructed to continuously rate the intensity of experienced pain during this  
201 incubation period.

202 Following the 20-minute incubation period, and with the thermode temperature still held at 40°C, 5  
203 minutes of eyes closed, continuous EEG was recorded while participants continuously rated the intensity  
204 of any perceived pain. An icepack was then applied to the forearm and, once pain was reported to be  
205 completely absent, 5 minutes of eyes closed EEG was collected. Afterwards, a 40°C thermode was placed  
206 over the site of capsaicin application to induce CHP rekindling. Five minutes of eyes closed EEG was then  
207 recorded while participants continuously rated the intensity of any perceived pain.

### 208 *Data Processing*

209 Because our primary objective was predicting pain sensitivity, the EEG data of interest were the initial  
210 pain-free EEGs collected at the beginnings of Visits 1 and 2. EEG data were preprocessed with EEGLAB  
211 13.6.5b (Delorme and Makeig, 2004). Preprocessing began with filtering the data between .2 and 100Hz  
212 using a linear FIR filter. Channel data were then visually inspected and overtly noisy channels were  
213 removed from further analysis. Removed channels were not interpolated. On average, 1.64 (S.D. = 1.92,  
214 range: 0 – 8) and 1.79 (S.D. = 1.79, range: 0 – 6) channels were removed per individual from Visit 1 and  
215 Visit 2 datasets, respectively. Finally, Principal Components Analysis (PCA) was performed and  
216 components with spatial topographies and time series resembling blinks and/or saccades were removed  
217 from the data.

218 As opposed to our previous studies which used ICA to isolate alpha sources over visual and  
219 somatosensory regions, we used channel level data to increase the ease with which our methods can be

220 reproduced. Although it may decrease the signal to noise ratio of the data, this approach eliminates the  
221 need to identify ICA components on a participant by participant basis and is equally effective for  
222 capturing the PAF-pain sensitivity relationship (Furman et al., 2019). For channel level analyses, we  
223 focused on channels (C3, Cz, and C4) that most strongly reflected the sensorimotor component  
224 topography observed in our original study (Furman et al., 2018). If a channel from this sensorimotor  
225 region of interest (ROI) was removed due to noise, only the remaining channels were used; this affected  
226 few participants (Visit 1: n = 4; Visit 2: n = 1) and no participant had more than one channel removed. In  
227 order to make the current results easily comparable to previous findings, all main analyses use PAF  
228 calculated from this sensorimotor ROI; use of this ROI is not intended to imply a mechanism or source  
229 for any documented effects.

230 To explore if additional EEG channels capture the PAF-pain sensitivity relationship, the surface Laplacian  
231 was computed following preprocessing (Perrin et al., 1989). Results from analyses using this estimate of  
232 current source density can be found in the Supplemental Data (Figure S6).

### 233 *Quantification of Sensorimotor PAF*

234 The frequency decomposition of the sensorimotor ROI data was performed using routines in FieldTrip  
235 (Oostenveld et al., 2011). Data from each pain-free EEG session was segmented into non-overlapping 5  
236 second epochs and power spectral density in the .2–100 Hz range (0.2 Hz bins) was derived with the  
237 ‘ft\_freqanalysis\_mtmfft’ function. A Hanning taper was applied to the data prior to calculating the  
238 spectra to reduce edge artifacts (e.g. Mazaheri et al., 2014).

239 At every channel and for each epoch, PAF was estimated using a center of gravity (CoG) method  
240 (Klimesch et al., 1993). We defined CoG as follows:

$$241 \quad CoG = \frac{\sum_{i=1}^n f_i * a_i}{\sum_{i=1}^n a_i}$$

242 where  $f_i$  is the  $i$ th frequency bin including and above 9 Hz,  $n$  is the number of frequency bins between 9  
243 and 11 Hz, and  $a_i$  the spectral amplitude for  $f_i$ . From our previous work, we have determined that this  
244 restricted frequency range reduces the influence of 1/f EEG noise on PAF estimation (Furman et al.,  
245 2018). Epoch-level PAF estimates were averaged to yield a single mean PAF estimate for each channel.  
246 Channel-level PAF estimates were further averaged across sensorimotor channels to yield a single  
247 sensorimotor PAF estimate for each participant at each visit.

248 To ensure that results were not an artifact of the range used for PAF estimation, PAF was additionally  
249 calculated with the wider 8-12 Hz range. Results with this wider estimation range are presented  
250 throughout the text and PAF estimates calculated using either the 9-11 or 8-12 ranges were highly  
251 similar (Figure S2).

### 252 *Statistical Analysis*

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

255 Previous work has found that CHP evokes limited pain or hypersensitivity in roughly one third of  
256 individuals (Liu et al., 1998, Walls et al., 2017). While the reasons for this remain unclear, certain  
257 physiological factors, such as genetic polymorphisms (Campbell et al., 2009), appear to play a role in  
258 limiting the effects of the TRPV1 agonist itself. For this reason, it is difficult to determine whether  
259 insensitivity to capsaicin reflects a failure of the CHP model or an individual’s sensitivity to pain. To  
260 address this problem, we separated participants into three pain response classes: 1) individuals who

261 display a clear pain response to CHP (average pain  $\geq 10$ ) at either Visit 1 or Visit 2 (“CHP responder”); 2)  
262 individuals who display a clear pain response to PHP at either Visit 1 or Visit 2 but no response to CHP at  
263 either visit (average pain  $< 10$ ; “CHP non-responder”); and 3) individuals who do not display a clear pain  
264 response to PHP or CHP at either visit (“high tolerance”). For the high tolerance pain class, the presence  
265 of PHP insensitivity provides important evidence that CHP insensitivity is unlikely to reflect model failure  
266 alone. To ensure that results were not confounded by variability associated with an individual’s  
267 physiological ability to experience CHP, we chose to focus our main analyses on CHP responder and high  
268 tolerance individuals. For all tests involving PHP, results when including all three pain classes are also  
269 provided.

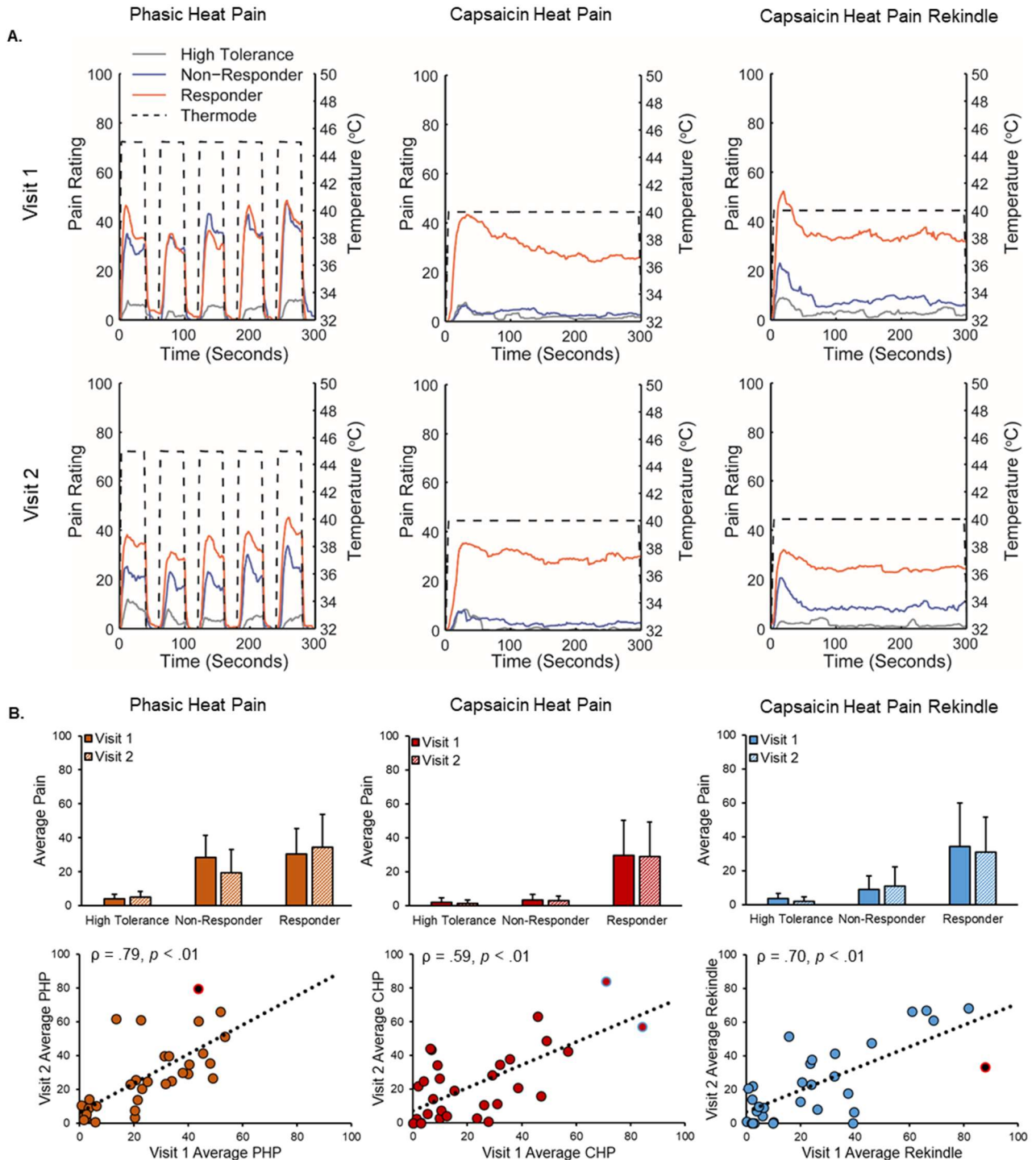
270 To determine if sensitivity to prolonged pain is similar across prolonged pain models, a series of pairwise  
271 correlations was calculated between all possible test pairs at each visit. For these and all other  
272 correlational analyses, Spearman’s rank order correlations were computed, and outliers were defined as  
273 data points greater than 2.5 standard deviations above or below the mean value obtained from Visit 1  
274 data. We further assessed whether sensitivity is reliable across prolonged pain tests using Cronbach’s  $\alpha$ .

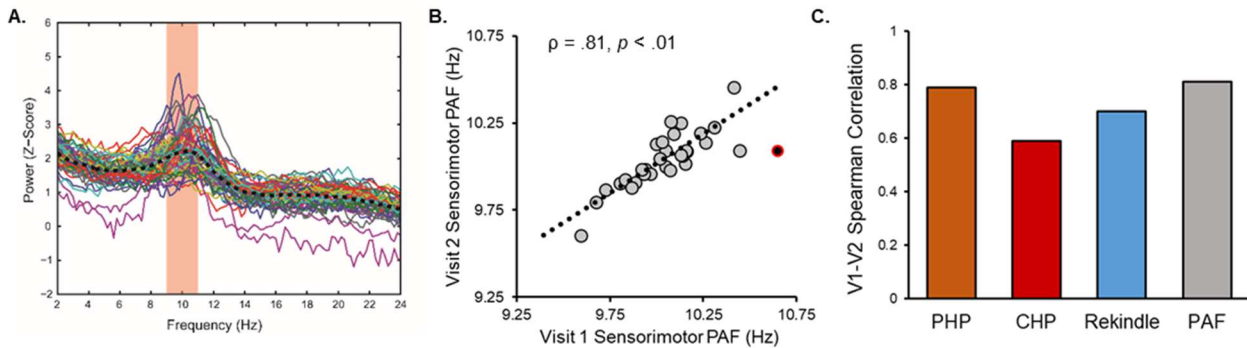
275 To begin testing whether pain-free, sensorimotor PAF is related to prolonged pain sensitivity, we  
276 performed a series of pairwise correlations between pain-free, sensorimotor PAF and each pain test  
277 (PHP, CHP, and CHP rekindle) at each visit. Bonferroni corrections for multiple tests were applied to  
278 data from each visit (3 tests; one per prolonged pain test) yielding a corrected significance threshold of  
279  $p = .017$ . For each test, we also investigated the effect of sex by performing correlations separately for  
280 males and females. To ensure that our results were not an artifact of our PAF estimation algorithm, we  
281 correlated pain sensitivity scores to the average, pain-free estimate of spectral power at each 0.2 Hz  
282 element within the 8-12 Hz range. For this analysis, spectra were z-scored in order to normalize total  
283 spectral power across individuals.

284 Next, we determined whether pain-free, sensorimotor PAF can accurately identify the most or least pain  
285 sensitive individuals. In the first analysis, we used a series of linear support vector machines (SVM) to  
286 perform leave-one-out, within-study classification (internal validation). To do so, pain scores from PHP,  
287 CHP, and CHP rekindle were averaged and, in separate tests, the top or bottom 10% of averaged pain  
288 scores were labelled as targets. A series of SVMs were then trained to identify targets based on Visit 1  
289 baseline, pain-free PAF estimates from all but one individual (training set). Trained support vector  
290 machines were then used to predict whether the withheld participant was a target. Visit 1 data was  
291 used in order to maximize the size of the available dataset. Each participant served as the test exactly  
292 once and predictions were evaluated using  $F_1$  scores (harmonic mean of precision and recall; Sokolova &  
293 Lapalme, 2009; Lipton et al., 2014).  $F_1$  scores are often used when the proportions of two classes are  
294 uneven. To determine the full scope of prediction, we repeated this analysis by increasing the  
295 percentage of data labelled as a target in increments of 10% up to a maximum of 50% (i.e. median split  
296 of data). To evaluate  $F_1$  scores, we generated a distribution of null  $F_1$  scores by assigning targets at  
297 random and then performing the analysis described above. This procedure was carried out 10,000 times  
298 and obtained  $F_1$  scores were evaluated as significant if they were equal to or surpassed the 95<sup>th</sup>  
299 percentile of the null distribution.

300 In the second analysis, we used a single linear SVM to perform cross-study classification using data from  
301 the current study as the training set and data from an earlier study on CHP sensitivity as the test set  
302 (external validation; Furman et al., 2018). Prior to analysis, PAF estimates within each study were  
303 normalized to z-scores. Otherwise, details of this analysis were identical to those of the within-study  
304 classification analysis.







**Figure 3.** A. Pain-free, sensorimotor ROI spectra collected from all participants Visit 1. Colored lines reflect individual participants, and the black dashed line reflects the average spectra across all participants. The red zone reflects the frequency range (9-11Hz) used to calculate PAF according to the center of gravity method. B. Pain-free, sensorimotor PAF estimates are strongly correlated across Visits. Note that Visit 2 occurred, on average, 7.8 weeks after Visit 1. Off-color data points represent statistical outliers not included in analyses and the dotted line represents the linear regression line of best fit. C. Pain-free, sensorimotor PAF and prolonged pain models are stable across Visits.

306 To examine whether pain-free, sensorimotor PAF is reliable across Visits 1 and 2, estimates from each  
307 visit were compared using a paired *t*-test. Bayes factor analysis was used to determine whether the null  
308 hypothesis could be accepted (i.e. no change in PAF between visits). Bayes factor analysis provides a  
309 method for assessing the relative evidence in favor of either the null or alternative hypothesis with a

310 Bayes factor less than .33 or greater than 3 are taken as strong evidence in favor of the null and  
311 alternative hypotheses, respectively (Rouder et al., 2009); Bayes factor scores in-between these values  
312 are considered to provide no evidence in favor of either hypothesis. As an additional test of stability,  
313 PAF estimates at Visits 1 and 2 were correlated with one another.

314 The stability of prolonged pain scores was assessed using a linear mixed effects model with subjects as  
315 random effects (intercept included) and Visit (Visit 1 vs Visit 2), Type (WDT vs. HPT vs. Phasic vs. CHP),  
316 and the Visit X Type interaction as fixed effects. We were specifically interested in determining whether  
317 scores change over time (main effect of Visit) and whether these changes were specific to individual  
318 tests (Visit X Type interaction). For each prolonged pain test, Bayes factor analysis was used to  
319 determine whether the null hypothesis could be accepted (i.e. no change in pain score between visits).  
320 Additionally, the stability of pain scores from each test was analyzed by correlating Visit 1 and Visit 2  
321 pain scores.

322 To further test of the stability of pain-free, sensorimotor PAF and prolonged pain scores, we examined  
323 the correlation between pain-free, sensorimotor PAF at Visit 1 and Visit 2 pain sensitivity. To ensure that  
324 results were not an artifact of our PAF estimation algorithm, we also correlated pain sensitivity scores to  
325 the average, pain-free estimate of spectral power at each 0.2 Hz element within the 8-12 Hz range.  
326 Finally, we tested whether pain-free, sensorimotor PAF at Visit 1 could accurately identify the least and  
327 most pain sensitive individuals at Visit 2. As before, a series of leave-one-out SVMs were trained to  
328 identify the least or most pain sensitive individuals and then tested on the withheld participant.  
329 Performance was quantified by comparing the observed  $F_1$  score to a bootstrapped, null distribution of  
330  $F_1$  scores.

## 331 Results

332 From our initial cohort of 61 individuals, two individuals were removed due to abnormal pain ratings:  
333 one participant fell asleep during ratings while another participant provided extremely high pain ratings  
334 in the absence of any noxious stimuli indicating that they may have been confused by the rating scheme. We

	Visit 1			Visit 2			
	PHP	CHP	CHP Rekindle	CHP Rekindle	CHP	PHP	
PHP		.52 (<.016)	.50 (<.016)	.58 (<.016)	.77 (<.016)		PHP
CHP			.88 (<.016)	.78 (<.016)			CHP
CHP Rekindle							CHP Rekindle

**Table 2.** Spearman correlation coefficients ( $p$  values) between sensory tests at each testing visit.

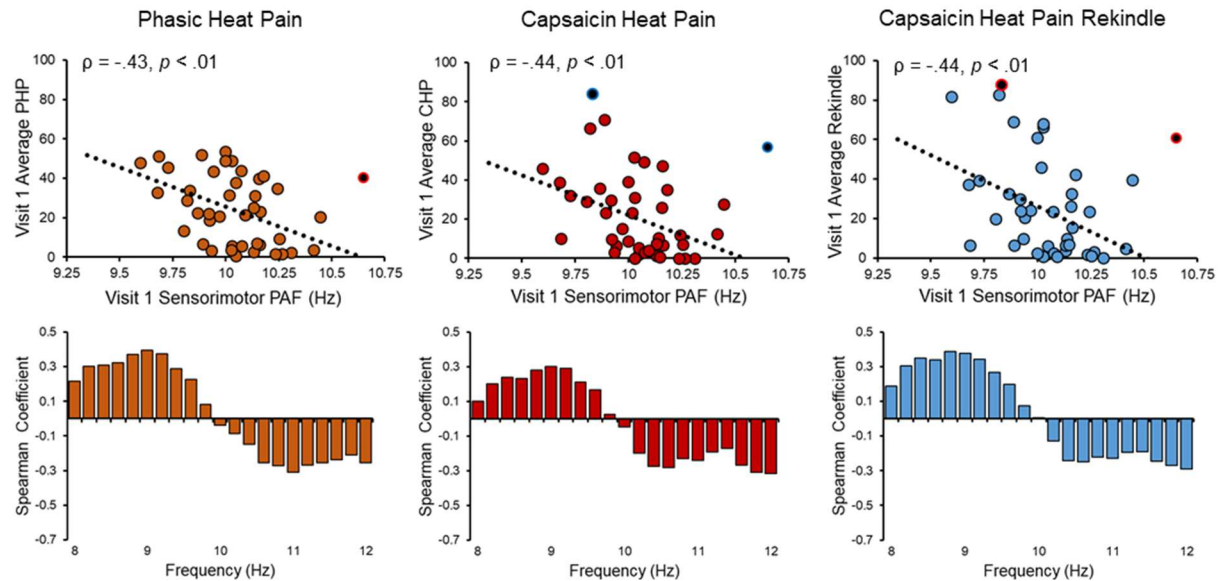
335 excluded one additional participant who experienced a change in CHP score, + 69.26, that was 3.82  
 336 standard deviations greater than the average CHP change (average change = 1.76, S.D. = 17.64). No  
 337 other change in CHP scores was greater than 2.05 standard deviations above the mean (range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

339 From the remaining 58 participants (Table 1), 33 participants were classified as CHP responders (CHP  
 340 score > 10), 10 participants were classified as high tolerance individuals (CHP and PHP scores < 10), and  
 341 15 participants were classified as CHP non-responders (PHP score > 10 & CHP score < 10). Due to a  
 342 technical error, EEG data was lost for one CHP responder at Visit 1; Visit 1 data for this individual was  
 343 only included in prolonged pain analyses. Of the 58 individuals providing data at Visit 1, a total of 43  
 344 individuals also provided data at Visit 2, of which 32 had been classified as a CHP responder or high  
 345 tolerance individual. CHP rekindle data for one participant at Visit 2 was not collected. Unless otherwise  
 346 stated, analyses only include data from high tolerance and CHP responder individuals.

347 A summary of prolonged pain scores for each pain response classification is presented in Figure 2. Both  
 348 PHP and CHP produced sensitization, a hallmark of prolonged pain (see Supplemental Data), and similar  
 349 amounts of pain in males and females (Supplemental Figure S3). Correlations between all possible pairs  
 350 of tests were significant (Table 2) and this conclusion held when analyses were repeated while including  
 351 all participants regardless of pain response classification (Supplemental Figure S1B). Reliability analysis  
 352 further revealed that sensitivity was consistent across prolonged pain tests, Chronbach's  $\alpha = .91$  (Visit 1  
 353 alone,  $\alpha = .82$ , Visit 2 alone,  $\alpha = .83$ ). Including all subjects, regardless of pain response classification, did  
 354 not alter this finding, Chronbach's  $\alpha = .90$  (Visit 1 alone,  $\alpha = .77$ , Visit 2 alone,  $\alpha = .83$ ). Thus, CHP and  
 355 PHP appear to sample similar prolonged pain processes.

### 356 *Sensorimotor PAF is Reliably Predicts Thermal, Prolonged Pain Sensitivity*

357 At Visit 1, pain-free, sensorimotor PAF predicted pain sensitivity to all three prolonged pain tests, PHP:  
 358 Spearman  $\rho = -.43$ ,  $p < .01$ ; CHP: Spearman  $\rho = -.44$ ,  $p < .01$ ; CHP rekindle sensitivity, Spearman  $\rho = -.44$ ,  
 359  $p < .01$  (Figure 4). Similar results were obtained for PHP when we used a partial correlation to account  
 360 for variability in the thermode temperature used during PHP, Spearman  $\rho = -.40$ ,  $p = .01$ , or when we  
 361 included all participants regardless of pain response classification, Spearman  $\rho = -.34$ ,  $p = .01$ . Expanding  
 362 the PAF calculation range to 8 – 12 Hz did not greatly impact the relationship for any prolonged pain  
 363 test, PHP: Spearman  $\rho = -.38$ ,  $p = .01$ ; CHP: Spearman  $\rho = -.34$ ,  $p = .03$ , CHP rekindle:  $-.38$ ,  $p = .01$   
 364 (Supplemental Figure S2E). Furthermore, inspection of the relationship between pain sensitivity and  
 365 power at each frequency element within the alpha range demonstrate that these results are not an  
 366 artifact of our PAF calculation method: for each test, slower (8-9.5 Hz) elements were positively  
 367 associated with pain sensitivity while faster (10.5-12 Hz) elements were negatively associated with pain  
 368 sensitivity (Figure 4 Lower Panels). We found no evidence of sex effects on the relationship of PAF to  
 369 either PHP, CHP, or CHP rekindle (Supplemental Figure S4A). Interestingly, the relationship between PAF



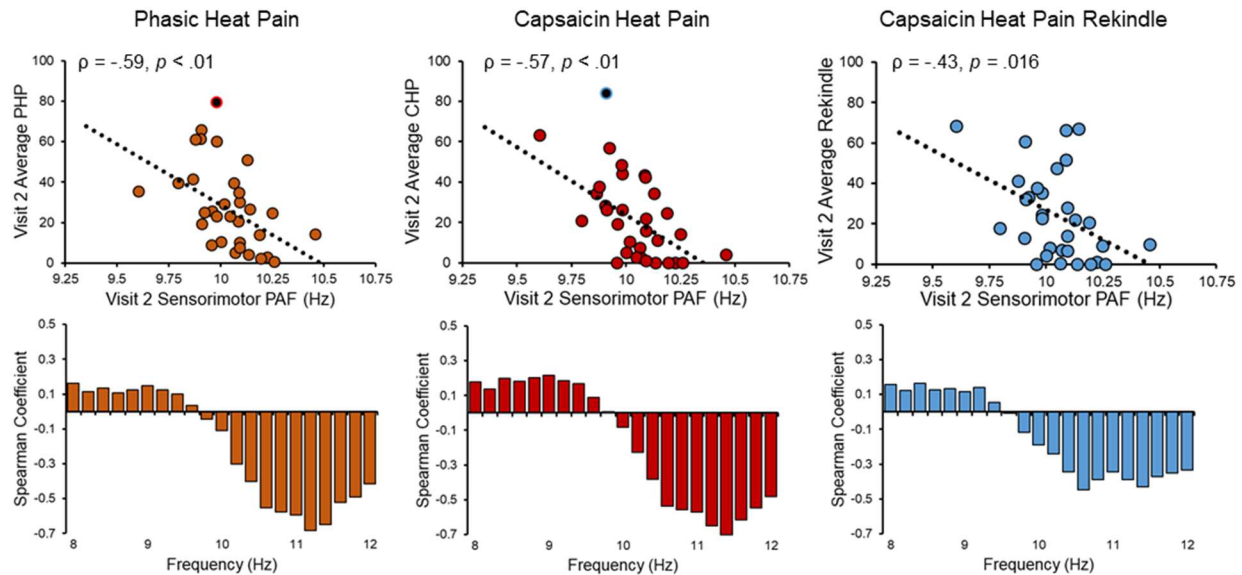
**Figure 4.** Visit 1 pain-free, sensorimotor PAF is correlated with sensitivity to all three Visit 1 prolonged pain tests. Off-color data points represent statistical outliers not included in analyses and dotted lines represent the linear regression line of best fit. Bar graphs below each scatter plot reflect Spearman correlation coefficients between Visit 1 pain scores and Visit 1 estimates of pain-free power at each 0.2 Hz bin within the 8-12 Hz range. For all three tests, frequency elements below 10 Hz are positively associated with pain sensitivity while frequency elements above 10 Hz are negatively associated with pain sensitivity.

370 and prolonged pain sensitivity was apparent at nearly every scalp channel even when volume  
371 conduction was accounted for with a surface Laplacian transformation (Supplemental Figures S5 & S6).

372 At Visit 2, pain-free, sensorimotor PAF again predicted pain sensitivity to all three prolonged pain tests,  
373 PHP: Spearman  $\rho = -.59, p < .01$ ; CHP: Spearman  $\rho = -.57, p < .01$ ; CHP rekindle sensitivity, Spearman  $\rho =$   
374  $-.43, p = .016$  (Figure 5). As before, PHP outcomes remained stable when either accounting for thermode  
375 temperature with a partial correlation, Spearman  $\rho = -.55, p < .01$ , or including all 43 participants  
376 regardless of pain response classification, Spearman  $\rho = -.37, p = .02$ . Expanding the PAF calculation  
377 range to 8-12 Hz did not impact PAF's relationship to any test, PHP: Spearman  $\rho = -.51, p < .01$ ; CHP:  
378 Spearman  $\rho = -.58, p < .01$ , CHP rekindle: Spearman  $\rho = -.44, p = .01$  (Supplemental Figure S2E), and  
379 correlations between pain and power across the alpha range once again revealed an association of slow  
380 and fast ranges with heightened and decreased pain sensitivity, respectively (Figure 5 Lower Panels). As  
381 in Visit 1, there did not appear to be an influence of sex on the relationship between PAF and any of our  
382 prolonged pain tests (Figure S4A) and this relationship was evident across the entire scalp (Figures S5 &  
383 S6).

#### 384 *Sensorimotor PAF Can Identify the Most Pain Sensitive Individuals*

385 Given the high sensitivity of classification analyses to outliers, one participant with an extreme PAF  
386 estimate was not included in either analysis (PAF = 10.65, 3.20 S.D. above the mean). In order to make  
387 the classification analysis generalizable to other datasets, and to take advantage of the strong  
388 correlation between prolonged pain tests, we created a composite pain sensitivity score by averaging  
389 scores from all three prolonged pain tests (PHP, CHP, CHP Rekindle). This pain sensitivity score was  
390 significantly correlated with PAF at both Visit 1, Spearman  $\rho = -.51, p < .01$ , and Visit 2, Spearman  $\rho = -$   
391  $.60, p < .01$  (Supplemental Figure S7A). This relationship remained evident when we included all



**Figure 5.** Visit 2 pain-free, sensorimotor PAF is significantly correlated with sensitivity to all three Visit 2 prolonged pain tests. Off-color data points represent statistical outliers not included in analyses and dotted lines represent the linear regression line of best fit. Bar graphs below each scatter plot reflect Spearman correlation coefficients between Visit 2 pain scores and Visit 2 estimates of pain-free power at each 0.2 Hz bin within the 8-12 Hz range. For all three tests, frequency elements below 10 Hz are positively associated with pain sensitivity while frequency elements above 10 Hz are negatively associated with pain sensitivity.

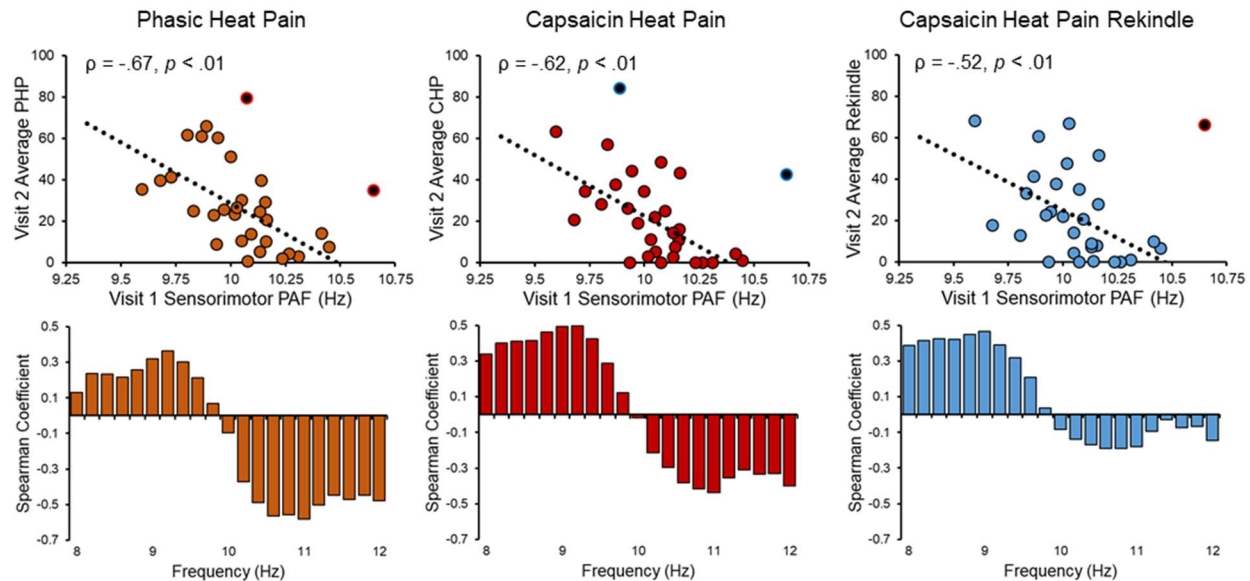
392 participants regardless of classification, Visit 1: Spearman  $\rho = -.42, p < .01$ ; Visit 2: Spearman  $\rho = -.33, p =$   
 393  $.03$  (Supplemental Figure S7A).

394 Support vector machines (SVM) trained and tested on the current dataset were able to identify both the  
 395 least and most sensitive individuals using just pain-free PAF estimates (internal validation; details found  
 396 in the Statistics section). Compared to a simulated null distribution of  $F_1$  scores, the least pain sensitive  
 397 individuals were identified at above chance levels at all labelling intervals but the 20% one (Figure 7B).  
 398 Similarly, the most sensitive individuals were identified at above chance levels at all labelling intervals  
 399 but the 30% one. When including all participants, regardless of classification, PAF significantly identified  
 400 the least sensitive individuals at all labelling intervals but only the most sensitive individuals at the 10%  
 401 and 50% intervals (Supplemental Figure S7B). This latter result likely reflects that the composite pain  
 402 sensitivity score fails to capture the mixed sensitivity of CHP non-responders to CHP and PHP.

403 A linear SVM trained on the current dataset could identify high and low pain sensitive individuals in a  
 404 separate, independent study (external validation). Using a similar procedure to one used for within-  
 405 study classification, a single linear SVM trained on data from the current study was used to predict the  
 406 identity of 21 participants from a previous study on CHP sensitivity (Furman et al., 2018). Compared to a  
 407 simulated null distribution of  $F_1$  scores, we found that PAF estimates identified the most pain sensitive  
 408 individuals at above chance levels for all labelling intervals and identified the least pain sensitive  
 409 individuals at above chance levels only at the two largest, 40% & 50%, intervals (Figure 7D). Rerunning  
 410 the analysis with all participants, regardless of pain response classification, included in the training set  
 411 yielded identical results (Supplemental Figure S7B)

#### 412 *Sensorimotor PAF and Prolonged Pain Sensitivity Are Stable over Time*

413 One possible explanation for the presence of a reliable relationship between pain-free, sensorimotor  
 414 PAF and prolonged pain sensitivity at Visits 1 and 2 is that both measures are themselves stable over



**Figure 6.** Visit 1 pain-free, sensorimotor PAF predicts sensitivity to all three Visit 2 prolonged pain tests. Note that Visit 2 occurred, on average, 7.8 weeks after Visit 1. Off-color data points represent statistical outliers not included in analyses and dotted lines represent the linear regression line of best fit. Bar graphs below each scatter plot reflect Spearman correlation coefficients between Visit 2 pain scores and Visit 1 estimates of pain-free power at each 0.2 Hz bin within the 8-12 Hz range. For all three tests, frequency elements below 10 Hz are positively associated with pain sensitivity while frequency elements above 10 Hz are negatively associated with pain sensitivity.

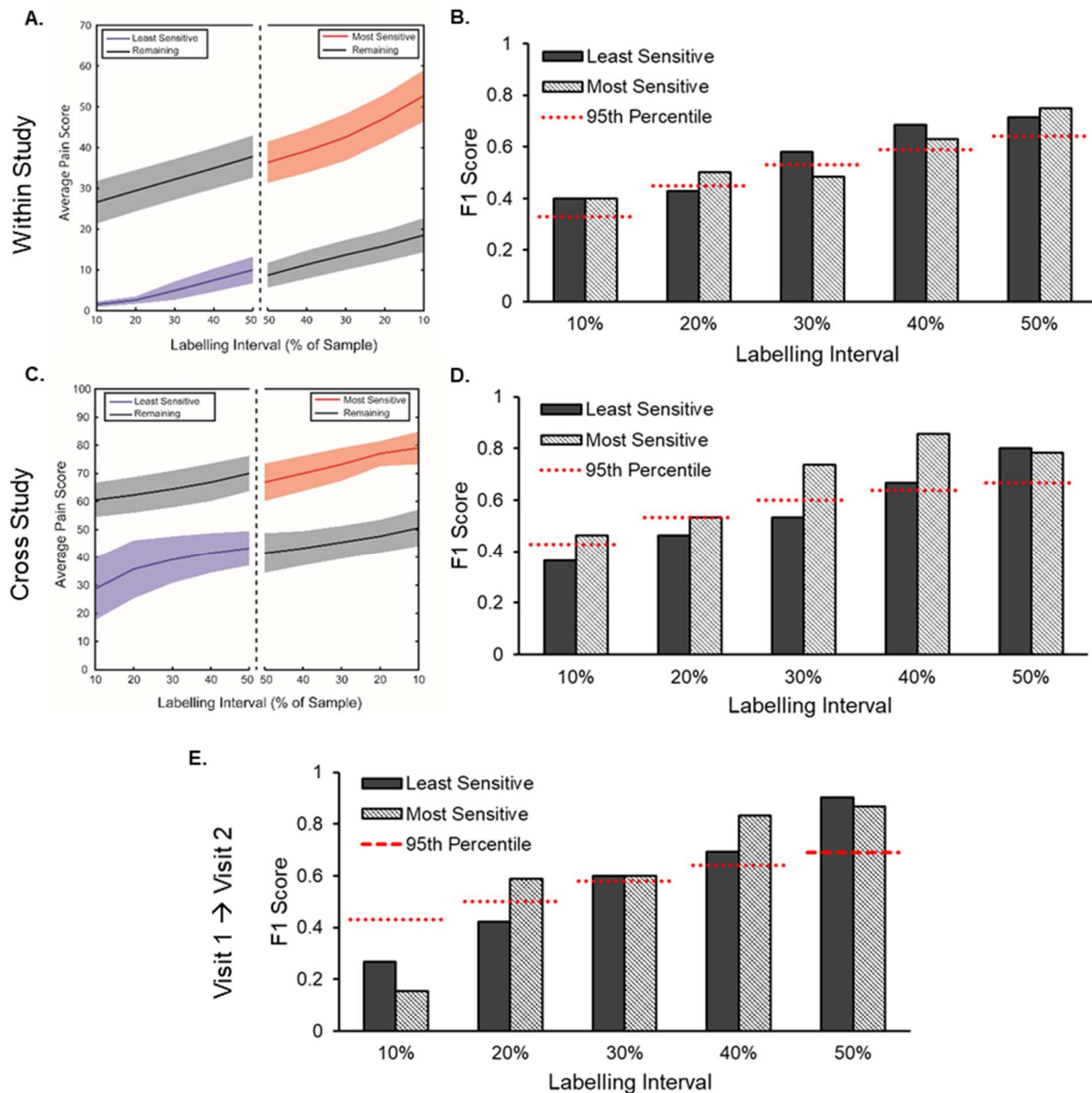
415 time. In line with this premise, Visit 1 (mean = 10.04, S.D. = .20) and Visit 2 (mean = 10.04, S.D. = .16)  
 416 estimates of pain-free, sensorimotor PAF were not significantly different,  $t(29) = .32$ ,  $p = .75$ , and Bayes  
 417 factor analysis supported the null hypothesis of no differences between the two, Bayes Factor < .01.  
 418 These results did not change when we included all participants regardless of pain response classification  
 419  $t(40) = .34$ ,  $p = .73$ , Bayes Factor < .01. What's more, Visit 1 and Visit 2 estimates of pain-free,  
 420 sensorimotor PAF were strongly correlated, Spearman  $\rho = .81$ ,  $p < .01$  (Figure 3A); this finding did not  
 421 change when we including all participants regardless of pain response, Spearman  $\rho = .82$ ,  $p < .01$ , or  
 422 expanded the PAF calculation range to 8-12 Hz, Spearman  $\rho = .86$ ,  $p < .01$ .

423 Similarly, a linear mixed effects model revealed that prolonged pain sensitivity did not change over time  
 424 with neither the main effect of Visit,  $F_{(1,161.32)} = .13$ ,  $p = .72$ , nor the Visit x Pain Type interaction,  $F_{(2,113.244)}$   
 425  $= .26$ ,  $p = .77$ , reaching significance. Bayes factor analysis failed, however, to support either the null or  
 426 alternative hypothesis for any prolonged pain test, PHP: Bayes Factor = 1.19, CHP: Bayes Factor = .71,  
 427 CHP Rekindle: Bayes Factor = .74. Visit 1 and Visit 2 pain scores were correlated for all three prolonged  
 428 pain tests, PHP,  $\rho = .79$ ,  $p < .01$ , CHP,  $\rho = .59$ ,  $p < .01$ , and CHP rekindle,  $\rho = .70$ ,  $p < .01$  (Figure 2), and  
 429 remained so when we expanded the dataset to include CHP non-responders, PHP:  $\rho = .74$ ,  $p < .01$ ; CHP:  
 430  $\rho = .69$ ,  $p < .01$ , CHP Rekindle,  $\rho = .68$ ,  $p < .01$ .

#### 431 *Sensorimotor PAF Can Predict Thermal, Prolonged Pain Sensitivity Occurring 8 Weeks Later*

432 If pain-free, sensorimotor PAF and prolonged pain sensitivity are stable traits then Visit 1 PAF should be  
 433 able to predict Visit 2 pain scores collected, on average, 8 weeks later. Indeed, we found that Visit 1  
 434 pain-free, sensorimotor PAF and Visit 2 pain scores were strongly correlated, PHP: Spearman  $\rho = -.67$ ,  $p$   
 435  $< .01$ ; CHP: Spearman  $\rho = -.62$ ,  $p < .01$ ; CHP rekindle: Spearman  $\rho = -.52$ ,  $p < .01$  (Figure 6). For PHP, this  
 436 relationship remained when we controlled for variability in thermode temperature, Spearman  $\rho = -.66$ ,  $p$   
 437  $< .01$ , or included all participants regardless of pain response classification, Spearman  $\rho = -.44$ ,  $p < .01$ .

438



**Figure 7.** Visit 1 pain-free, sensorimotor PAF can accurately predict the identity of the most pain sensitive individuals and a support vector machine trained on this data can identify the most pain sensitive in an independent study. A. Visit 1 pain scores from the three prolonged pain models were averaged and a relevant percentage of the sample, ranging from 10% to 50% (i.e. median split), was identified as high or low pain sensitive. Colored lines (shading = 95% confidence interval) reflect the average sensitivity for identified participants and those not classified (black lines; “Remaining”). B. A support vector machine trained on Visit 1 pain-free, sensorimotor PAF predicts the identity of high and low pain sensitive individuals from the same study at almost all labelling intervals. An  $F_1$  score of 1 indicates perfect classifier performance and the dashed red lines reflect the 95<sup>th</sup> % of a null distribution of  $F_1$  scores. C. Same as in A., except pain scores were taken from an independent study on PAF and CHP (Furman et al., 2018). D. A support vector machine trained on Visit 1 pain-free, sensorimotor PAF predicts the identity of high pain sensitive individuals from an independent study at all labelling intervals. E. A support vector machine trained on Visit 1 pain-free, sensorimotor PAF predicts the identity of Visit 2 high pain sensitive individuals. Note that pain scores for this test are not provided but are nearly identical to those present in C.

439 Expanding the PAF calculation range to 8-12 Hz did not impact PAF's relationship to any test, PHP:  
440 Spearman  $\rho = -.57$ ,  $p < .01$ ; CHP: Spearman  $\rho = -.52$ ,  $p < .01$ , CHP rekindle: Spearman  $\rho = -.45$ ,  $p = .015$ ,  
441 and correlations between pain and power across the alpha range again demonstrated that the slow and  
442 fast ranges were associated with heightened and decreased pain sensitivity, respectively (Figure 6 Lower  
443 Panels).

444 What's more, the least and most pain sensitive individuals at Visit 2 could be identified using Visit 1  
445 pain-free, sensorimotor PAF. Visit 2 pain sensitivity, represented as the average pain score across tests,  
446 was strongly correlated to Visit 1 pain-free, sensorimotor PAF, Spearman  $\rho = -.66$ ,  $p < .01$ , and remained  
447 so when including all participants regardless of pain classification, Spearman  $\rho = -.45$ ,  $p < .01$   
448 (Supplemental Figure S7A). Compared to a null distribution of  $F_1$  scores, pain-free, sensorimotor PAF  
449 identified the most sensitive individuals at all but the smallest labelling interval and the least sensitive  
450 individuals at all but the two smallest labelling intervals (Figure 7E). Classification failure at the smallest  
451 labelling intervals was likely due to the relatively low number of targets available (sample = 30; targets =  
452 3 and targets = 6 at the 10% and 20% labelling intervals, respectively). Rerunning the analysis with all  
453 participants, regardless of pain response classification, again demonstrated that pain-free, sensorimotor  
454 PAF could identify the most sensitive individuals at all labelling intervals but the smallest one. For the  
455 least pain sensitive individuals, pain-free, sensorimotor PAF failed to yield significant predictions at any  
456 labelling interval (Supplemental Figure S7B).

## 457 Discussion

458 Cycles of the 8 – 12 Hz Alpha oscillation are thought to reflect rhythmic, inhibitory processes that  
459 control the temporal dynamics of sensory processing (Jensen & Mazaheri, 2010; Van Rullen 2016). Peak  
460 Alpha Frequency (PAF), the individual-specific frequency at which these rhythms are dominantly  
461 expressed, is thought to reflect the speed at which sensory information is sampled (e.g. Samaha &  
462 Postle, 2015; Cecere et al., 2015; Wutz et al., 2018). PAF abnormalities are evident in several chronic  
463 pain conditions, with patients often demonstrating slowed PAFs relative to age-matched controls (e.g.  
464 Sarnthein et al., 2006; de Vries et al., 2013; Lim et al., 2016). These findings have led to proposals that  
465 PAF disturbances reflect ongoing, pathological processes such as Thalamocortical Dysrhythmia (e.g.  
466 Llinás et al., 1999). PAF, however, also appears to play a role in shaping the sensitivity of healthy  
467 individuals to prolonged pain (Nir et al., 2010; Furman et al., 2018; Furman et al., 2019). We have  
468 previously shown that the speed of PAF collected in the absence of a noxious stimulus is negatively  
469 related to an individual's sensitivity to future prolonged pain events (i.e. slower PAF = greater pain  
470 sensitivity). This has led us to propose that pain-free PAF is a biomarker of prolonged pain sensitivity  
471 and, furthermore, that chronic-pain related disturbances of PAF may reflect differences in pain  
472 sensitivity that predate disease onset.

473 In the current study we examined the relationship of pain-free PAF to two models of prolonged pain,  
474 Capsaicin Heat Pain (CHP) and Phasic Heat Pain (PHP), within the same group of participants at two  
475 separate timepoints. From these experiments, we present two key pieces of evidence supporting the  
476 hypothesis that pain-free PAF is a prolonged pain sensitivity biomarker. First, pain-free PAF shares a  
477 near identical, negative relationship to CHP and PHP sensitivity, with increasingly slower PAF associated  
478 with increasingly greater pain intensity during each test. While we have previously reported a  
479 relationship between pain-free PAF and CHP sensitivity (Furman et al., 2018), the described relationship  
480 to PHP sensitivity is entirely novel. Reproduction of this relationship across models, despite differences  
481 in their length of application, the temperatures used, and the presence of a sensitizing agent, provides  
482 important evidence that PAF is a marker of prolonged pain sensitivity per se and not specific portions of



483 either model. This interpretation is also supported by the replication of our earlier CHP findings despite  
484 large procedural differences between the two studies (i.e. CHP preceded by a cognitive or separate pain  
485 task). Preservation of the PAF-pain sensitivity relationship through the rekindling phase of the CHP  
486 model provides yet another piece of evidence that PAF captures an element of the prolonged pain  
487 experience that is independent of the local context (i.e. continuous vs. interrupted pain). Although the  
488 association of pain-free PAF with non-thermal forms of prolonged pain was not tested in the current  
489 study, similar findings in a musculoskeletal model of prolonged pain provide some assurance that pain-  
490 free PAF is likely to apply to a wide range of prolonged pain modalities (Furman et al., 2019).

491 Second, the relationship between pain-free, PAF and prolonged pain sensitivity is reliable over time.  
492 Within the same set of individuals, we show that the relationship between pain-free PAF and prolonged  
493 pain sensitivity is present at two separate testing visits. It should be acknowledged that this relationship  
494 was qualitatively stronger at Visit 2, which could be interpreted as evidence that factors that change  
495 with repeated testing, such as participant familiarity and/or vigilance, mediate the connection between  
496 pain-free PAF and prolonged pain sensitivity. While these effects cannot be entirely discounted, a  
497 separate explanation centers on the limited participant sample available at Visit 2; restricting Visit 1  
498 analyses to only those participants completing both visits revealed relationship magnitudes, PHP:  $\rho = -$   
499  $.50$ ; CHP:  $\rho = -.50$ ; CHP rekindle:  $-.52$ , closer to those found at Visit 2.

500 This temporally stable association of pain-free PAF and prolonged pain sensitivity appears to be a  
501 consequence of the temporal stability of the measures themselves. For both pain-free PAF and  
502 prolonged pain sensitivity, we found that Visit 1 and Visit 2 estimates were strongly correlated and did  
503 not significantly differ from one another. These findings fit well with previous studies of PAF and  
504 prolonged pain sensitivity that demonstrate each are trait-like measures (e.g. Grandy et al., 2013; Naert  
505 et al., 2008; Koenig et al., 2014). Importantly, the average length of time separating visits ( $\sim 8$  weeks), as  
506 well as the absence of visual and haptic feedback during rating, provides comfort that the reliability of  
507 pain scores is not simply the result of participant's explicit recollection of previous pain. From a broader  
508 perspective, these findings suggest the that relationship between pain-free PAF and prolonged pain is  
509 not uniquely determined at each visit but is instead an association that remains consistent *across* time;  
510 put another way, the *same* pain-free, PAF and the *same* prolonged pain sensitivity are sampled from  
511 individuals at each visit. Indeed, the ability of Visit 1 pain-free PAF to predict prolonged pain sensitivity  
512 at Visit 2 provides strong evidence in favor of this conclusion. Thus, these findings clearly show that  
513 pain-free PAF can provide cogent information about prolonged pain sensitivity at both short (i.e.  
514 minutes/hours separating PAF acquisition and pain testing) and long (weeks/months separating visits)  
515 timescales.

516 Considering its apparent reliability as a pain sensitivity biomarker, as well as its ease of obtainment,  
517 pain-free PAF holds real promise as a pain management and prophylaxis tool. This may be especially  
518 true in cases of planned surgery, where post-operative pain sensitivity is consistently found to be an  
519 important risk factor for chronic pain development (Hah et al., 2019). For example, identification of high  
520 pain sensitivity with PAF could be used to inform clinician decision making about surgical alternatives.  
521 To evaluate this possible real-world application, we examined whether pain-free PAF can predict the  
522 identify of high or low pain sensitive individuals. In almost all cases, a support vector machine trained on  
523 pain-free PAF was able predict the identity of the most pain sensitive individuals. This held true when  
524 the test data came from the current study or when it originated from an entirely separate study  
525 (Furman et al., 2018). In contrast, identification of the least pain sensitive individuals occurred when  
526 classification was applied to data from the current study but not when applied to outside data. These  
527 results suggest that pain-free PAF is particularly well suited for identifying high pain sensitive individuals.

528 Importantly, Visit 1 pain-free PAF could be used to predict high pain sensitivity at Visit 2 suggesting that  
529 pain sensitivity prediction remains relevant across clinically relevant periods of time. Prospective  
530 collection of pain-free PAF at routine check-ups may therefore prove an effective strategy for ensuring  
531 information about an individual's pain sensitivity is available to clinicians in cases of unplanned surgical  
532 intervention.

533 Despite the promise of the current findings, some potential limitations must be acknowledged. First, a  
534 subset of individuals demonstrating insensitivity to CHP were not included in the main set of analyses.  
535 Although a wide range of factors can render an individual less sensitive to capsaicin, at least some cases  
536 appear to be determined by physiological factors, like genetic polymorphisms (Campbell et al., 2009),  
537 that limit the effects of the TRPV1 agonist itself (i.e. model failure). The sources of pain sensitivity for  
538 these individuals and for those susceptible to the full range of capsaicin effects are thus fundamentally  
539 different and not comparable. This represents a limitation of the CHP model and not, in our opinion, a  
540 limitation of PAF's ability to reflect pain sensitivity. To overcome this potential pitfall, we only included  
541 CHP-insensitive individuals if they also reported minimal pain in response to PHP. In these cases, the  
542 presence of PHP insensitivity provided important evidence that CHP insensitivity was at least partly  
543 attributable to an individual's high tolerance of pain and not just model failure. While this decision could  
544 be interpreted as a confound to analyses of PHP, where sensitivity to capsaicin is not a relevant factor,  
545 supplementary results when all participants were included are provided for each test and, in all cases,  
546 conclusions regarding the relationship of pain-free PAF and PHP remained unchanged. Similarly,  
547 averaging pain scores across tests revealed that, even when including all participants, this broader  
548 description of pain sensitivity was well described by pain-free PAF. As a result, we feel confident that  
549 pain-free PAF's relationship to pain sensitivity holds broadly across individuals.

550 Additionally, the current study is unable to provide concrete information about PAF's source or identity.  
551 For the sole purpose of remaining consistent with our earlier methods, we chose to explicitly focus on  
552 PAF recorded from sensorimotor channels. As we have noted previously (Furman et al., 2019), PAF's  
553 relationship to pain sensitivity is not restricted to sensorimotor channels and instead appears to  
554 encompass nearly every scalp channel. This continued to hold true in the current study even when  
555 possible volume conduction effects were controlled with a Laplacian transform. Although considered a  
556 limitation here, the widespread nature of PAF's relationship to pain sensitivity may provide an  
557 important clue to its identity. In line with findings that the alpha rhythm travels across the cortex in  
558 "waves" (Zhang et al., 2018; Lozano-Soldevilla et al., 2019), PAF may reflect processes or sources whose  
559 actions are distributed across the brain. The thalamus represents one obvious candidate given its  
560 extensive cortical projections (e.g. Behrens et al., 2003) and central role in generating the alpha rhythm  
561 (Hughes and Crunelli, 2005). Large-scale, functional networks like those involved in attention also  
562 represent promising possibilities. Among these, the frontoparietal network is particularly interesting  
563 given that its relationship to the alpha rhythm is speed dependent (Sauseng et al., 2005; Sadaghiani et  
564 al., 2012) and has itself been implicated in individual differences in pain sensitivity (Kong et al., 2013; Tu  
565 et al., 2019). Resolution of this question will ultimately require both spatially sensitive methods, like  
566 EEG-fMRI, and careful behavioral testing to determine the brain regions and processes which mediate  
567 the relationship between pain-free PAF and pain sensitivity.

568 Some readers may also be concerned with the limited, 9-11 Hz frequency range that was used to  
569 calculate PAF. Alpha activity is not limited to 9-11 Hz range and has even been suggested to extend  
570 beyond the canonical 8-12 Hz range (Haegens et al., 2014). One advantage of the restricted calculation  
571 range we employed is that it most effectively negates the impact of the  $1/f$  aperiodic signal on PAF  
572 estimation (Furman et al., 2018). While methods for isolating narrowband signal from aperiodic signal

573 are advancing quickly (i.e. Haller et al., 2018), we found that they were unable to generate adequate  
574 solutions for all participants. As a result, we chose to focus on the 9-11 Hz range in order to provide the  
575 cleanest possible estimate of PAF. Importantly, results for all analyses were unchanged when PAF was  
576 calculated using the full 8-12 Hz range. Similarly, correlations of pain with estimates of spectral power at  
577 each 0.2 Hz element within the 8-12 Hz range confirmed that this relationship is not an artifact of either  
578 the range or method used to calculate PAF. Frequency elements below 10 Hz showed a consistent,  
579 positive relationship to pain sensitivity whereas elements above 10 Hz were negatively associated with  
580 pain. This finding reinforces that *where* power is expressed within the alpha range is relevant to pain  
581 sensitivity and, furthermore, suggests that different elements of the alpha range represent distinct  
582 processes (e.g. Klimesch et al., 1998).

583 In summary, our results clearly demonstrate that pain-free PAF is a reliable predictor of prolonged pain  
584 sensitivity. In addition to demonstrating that pain-free PAF is related to multiple models of prolonged  
585 pain, we provide compelling evidence that this relationship is stable over both immediate, i.e.  
586 minutes/hours, and more extended, i.e. weeks/months, periods of time. Furthermore, we demonstrate  
587 that pain-free PAF can be used to accurately identify high pain sensitive individuals in multiple datasets.  
588 These findings now firmly position pain-free PAF as a biomarker of pain sensitivity with untapped  
589 potential in clinical settings.

590 **References**

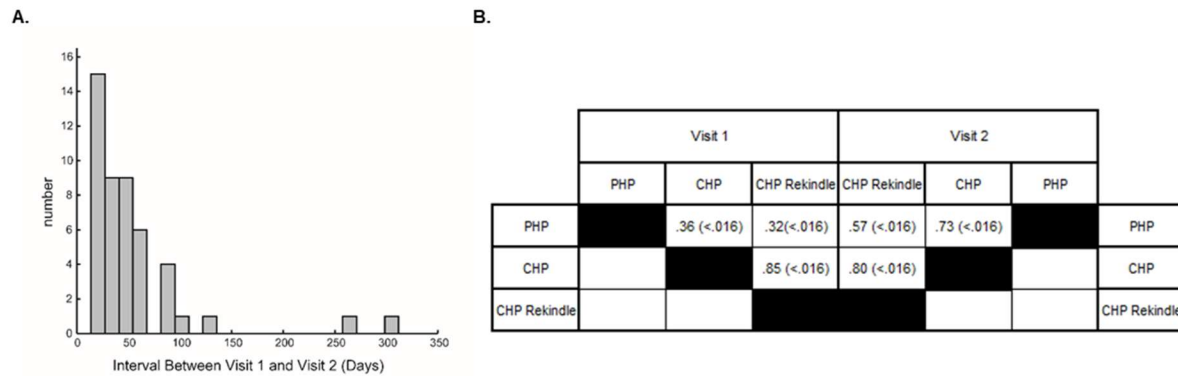
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737 **Supplemental Data**



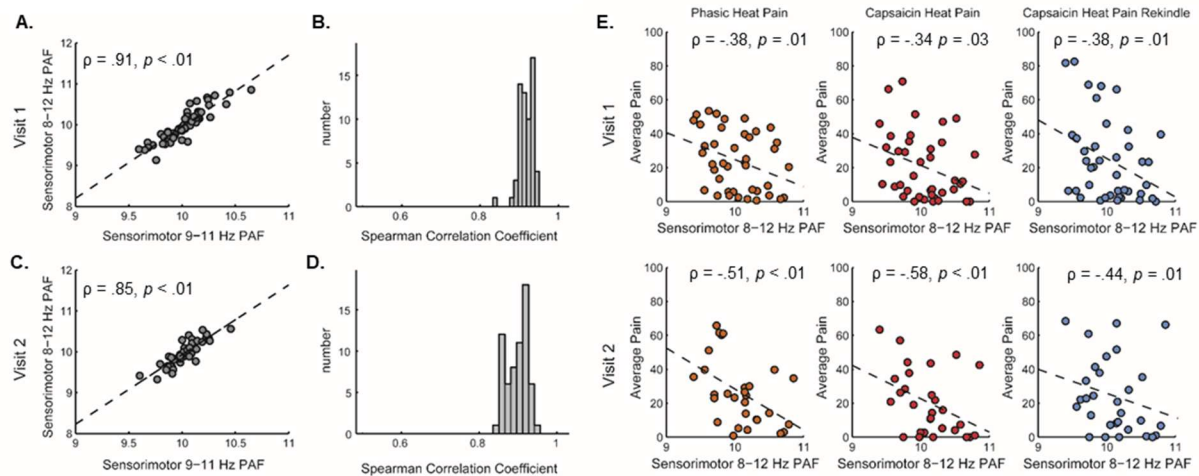
**Figure S1.** A. Histogram of days separating Visit 1 and Visit 2. On average visits were separated by 54.7 days (7.8 weeks). B. Correlations between prolonged pain tests when all participants, regardless of pain response classification, are included.

738 *PHP and CHP Produce Sensitization*

739 We first sought to determine whether sensitization, a putative hallmark of prolonged pain, is present in  
 740 our two prolonged pain paradigms. Inspection of the PHP time course suggest that following a decrease  
 741 in pain ratings from the first to the second PHP trial, which may reflect the enhanced salience of the first  
 742 stimulus train (Iannetti et al., 2008), ratings increased linearly from the second to fifth PHP stimulus train  
 743 (Figure 1B). To formally test this observation, we calculated each participant’s average pain rating during  
 744 PHP stimulus trains 2 and 5. Scores for all participants, regardless of pain response classification, were  
 745 submitted to a linear mixed model with participants as random effects (slope included) and Visit (V1 vs.  
 746 V2), Trial (2 vs. 5) and the Visit X Trial interaction as fixed effects. If PHP scores sensitize over time, then  
 747 a significant main effect of Trial should be present. This analysis revealed a significant main effect of  
 748 Trial,  $F_{(1,88.14)} = 19.06, p < .01$ , without a significant main effect of Visit,  $F_{(1,103.08)} = .35, p = .55$ , or  
 749 significant Visit X Trial interaction,  $F_{(1,88.18)} = .02, p = .89$ . The estimated effect of Trial on PHP scores  
 750 8.61(95% Confidence Intervals: 2.13 – 15.10). This increase in scores from trial 2 (mean = 20.81, S.D. =  
 751 17.88) to trial 5 (mean = 29.31, S.D. = 21.43) in response to the same noxious stimulus is evidence of  
 752 sensitization.

753 Two findings support the presence of sensitization during CHP. First, across all participants a pair of one-  
 754 sample t-tests revealed that CHP scores were significantly greater than 0 at both V1,  $t_{(57)} = 6.63, p < .01$ ,  
 755 and V2,  $t_{(42)} = 5.72, p < .01$ . Second, another pair of one-sample t-tests indicated that HPTs were  
 756 significantly greater than the CHP temperature, 40°C, at both V1,  $t_{(57)} = 7.90, p < .01$ , and V2,  $t_{(39)} = 8.89$ ,  
 757  $p < .01$ . Pain in response to a temperature below an individual’s WDT is a strong indicator of  
 758 sensitization given that we have previously demonstrated that presentation of a similar temperature  
 759 without capsaicin does not produce pain (Furman et al., 2018).





**Figure S2.** Calculating PAF using the wider 8-12 Hz range does not alter the main study conclusions. A & C. Estimates of 8-12 Hz, sensorimotor PAF and 9-11 Hz, sensorimotor PAF at both Visit 1 (A) and Visit 2 (C) are highly similar. Data comes from all participants, regardless of pain response classification, and dotted lines reflect the linear regression line of best fit. B & D. Estimates of PAF calculated using the 9-11 or 8-12 Hz range are similarly correlated at all EEG sensors. Plots reflect the distribution of Spearman correlations for 9-11 and 8-12 Hz PAF estimates across all 63 EEG channels at Visit 1 (B) and Visit 2 (D). E. 8-12 Hz, sensorimotor PAF is correlated to sensitivity to each prolonged pain test at each visit. Upper panels reflect Visit 1 pain scores and pain-free PAF; lower panels reflect Visit 2 pain scores and pain-free PAF. Presented data comes from CHP responders and High Tolerance individuals only. Dotted lines reflect the linear regression line of best fit and outliers presented in Figures 4 & 5 are omitted.

760 *The Frequency Range Used to Calculate PAF Does not Alter the PAF-Pain Sensitivity Relationship*

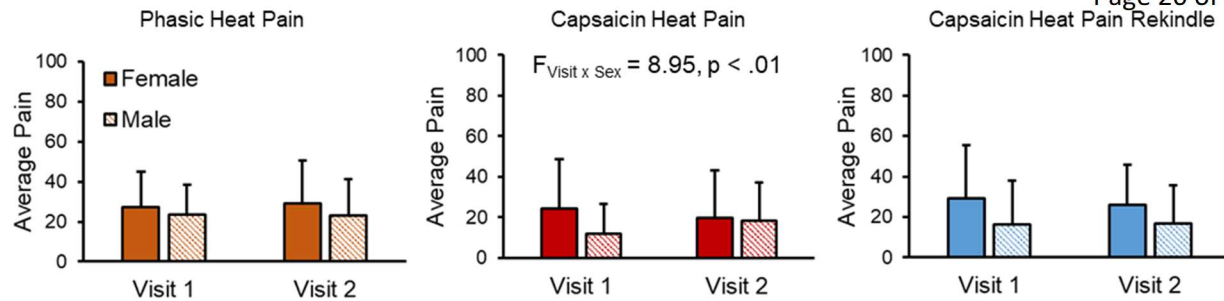
761 Estimates of pain-free, sensorimotor PAF calculated with either a 9-11 or 8-12 Hz frequency range were  
 762 highly correlated with one another whether we included all participants (V1:  $\rho = .91$ ; V2:  $\rho = .85$ ;  
 763 Supplemental Figure S2A & S2C) or only CHP responders and high tolerance individuals (V1:  $\rho = .92$ ; V2:  
 764  $\rho = .84$ ). Similar correlation magnitudes between PAF estimates were evident at all scalp channels  
 765 (Supplemental Figure S2B & S2D).

766 Correlations between pain-free, sensorimotor PAF and sensitivity to each prolonged pain test were not  
 767 dramatically altered when estimating PAF with the wider 8-12 Hz range (Supplemental Figure S2E).

768 *Prolonged Pain Sensitivity is Similar for Men and Women*

769 Previous studies have reported that sex may be an important variable in determining pain sensitivity (i.e.  
 770 Dao & LeResche, 2000). Average pain scores (+ 1 S.D) for the sexes on each test at each visit can be seen  
 771 in Supplementary Figure S3.

772 To determine whether sex impacted pain scores, we performed a linear mixed model for scores from  
 773 each prolonged pain test with subjects as random effects and Visit (V1 vs V2), Sex (Male vs. Female), and  
 774 the Visit X Sex interaction as fixed effects. Given that our study was not powered with respect to sex  
 775 effects, analyses were performed on all participants regardless of pain response classification in order to  
 776 maximize available statistical power. For PHP scores, this analysis revealed no significant effects of Visit,  
 777  $F_{(1,41.34)} = .37, p = .54$ , Sex,  $F_{(1,53.35)} = 1.72, p = .20$ , or Visit X Sex interaction,  $F_{(1,41.34)} = .76, p = .39$ . For CHP  
 778 scores, this analysis revealed a significant Visit by Sex interaction,  $F_{(1,41.86)} = 8.95, p < .01$ , but no  
 779 significant main effects of Visit,  $F_{(1,41.86)} = .06, p = .80$ , or Sex,  $F_{(1,54.53)} = 1.38, p = .25$ . For CHP rekindle



**Figure S3.** Prolonged pain scores broken by sex and visit. The only significant effect is a Visit X Sex interaction for CHP scores – scores increase for men from Visit 1 to Visit 2 and decrease for women from Visit 1 to Visit 2. Data reflect means and error bars reflect +1 standard deviation.

780 scores, this analysis revealed no significant effects of Visit,  $F_{(1,41.88)} = .11, p = .74$ , Sex,  $F_{(1,53.87)} = 3.00, p =$   
781  $.09$ , or Visit X Sex interaction,  $F_{(1,41.88)} = 1.97, p > .17$ .

782

783 For CHP, the Visit X Sex interaction reflects the fact that males experience increases in CHP scores from  
784 V1 (mean = 12.10, S.D. = 14.50) to V2 (mean = 16.12, S.D. = 18.44), whereas females experience  
785 decreases in CHP scores from V1 (mean = 24.37, S.D. = 24.45) to V2 (mean = 20.01, S.D. = 23.06).

786

787 *The PAF-Pain Sensitivity Relationship is Similar for Both Sexes*

788 One important consideration for any pain biomarker is whether it applies equally to both sexes.

789 Correlation magnitudes for pain-free, sensorimotor PAF and CHP or PHP sensitivity were similar for both  
790 sexes; for CHP rekindle, this relationship was larger for males than females at both visits.

791 (Supplementary Figure 4). We do not provide  $p$  values for these tests as our study was not powered to  
792 investigate sex differences directly.

793 To more formally test whether sex influences the relationship of PAF to pain sensitivity, we performed

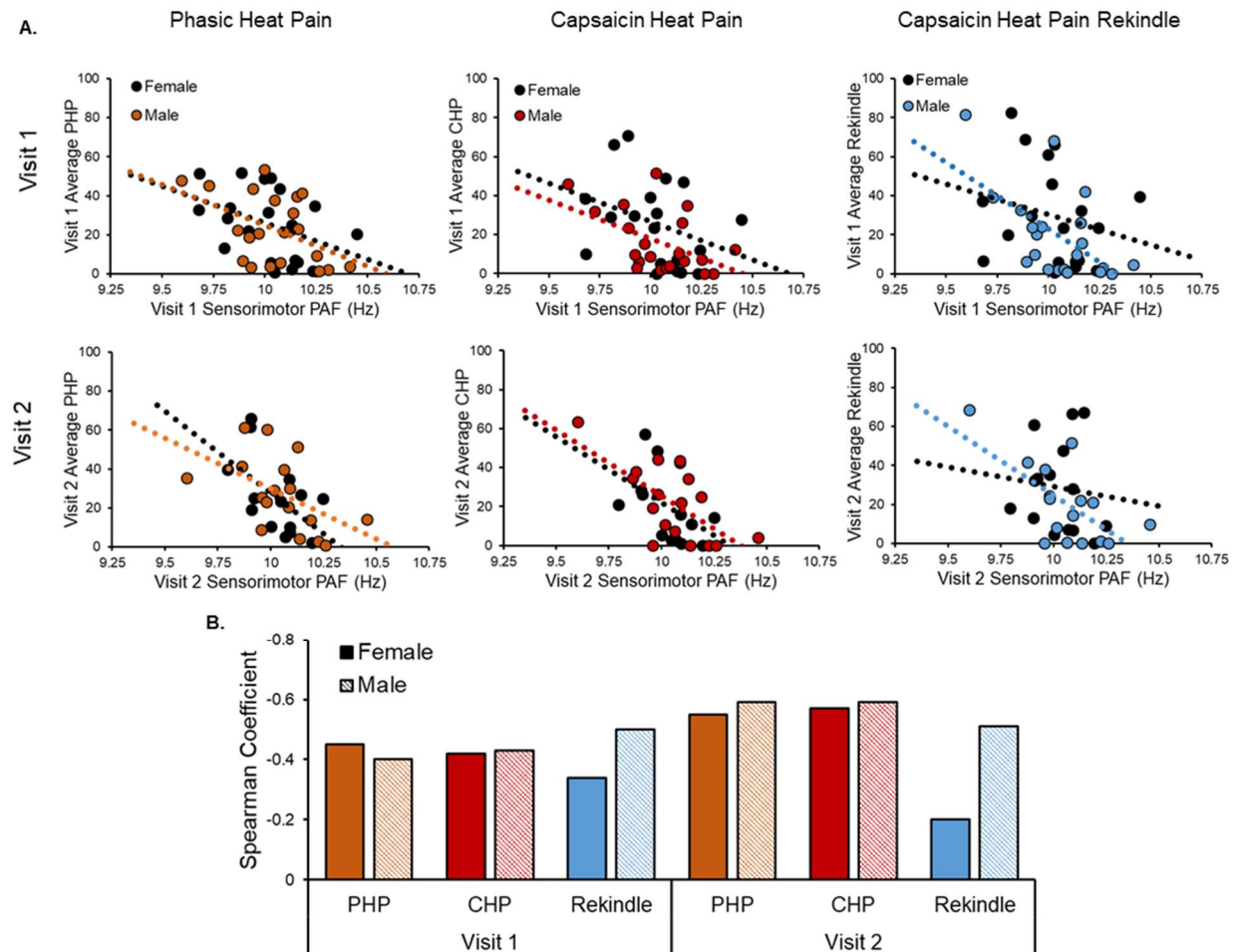
794 six separate moderation analyses (one for PHP, CHP, and CHP Rekindle at each visit) using PROCESS

795 (V3.2; Hayes, 2012) implemented in SPSS. For these regression analyses, sensory test scores served as

796 the dependent variable with pain-free, sensorimotor PAF as the independent variable and sex as a

797 dichotomous moderator variable. As with other correlational analyses, we excluded PAF or sensory test

798 scores greater than 2.5 SD above or below the mean value obtained at Visit 1. To account for possible

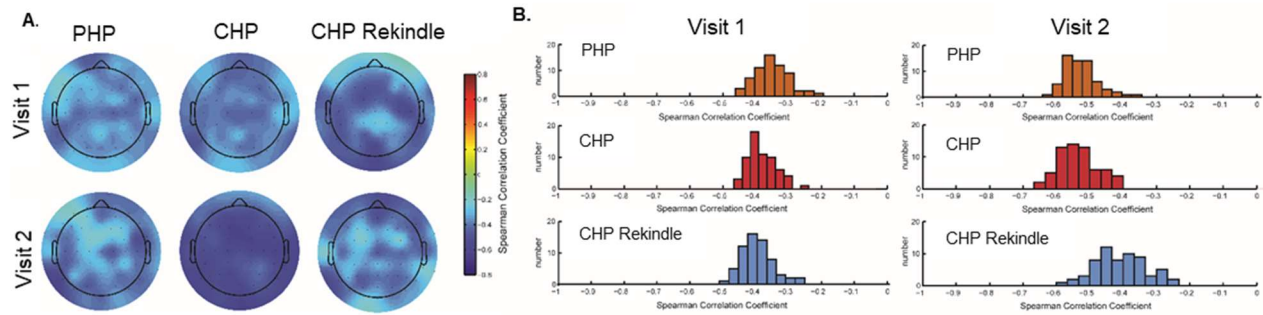


**Figure S4.** PAF bears a similar relationship to pain sensitivity for both sexes. A. Correlations between pain-free, sensorimotor PAF and prolonged pain tests are similar for both sexes at both Visit 1 and Visit 2. Note that statistical outliers presented in Figures 4 and 5 are omitted for the purpose of visual clarity. Dotted lines reflect the linear regression lines of best fit. B. Spearman correlation coefficients for the PAF-pain sensitivity relationship broken down by visit, prolonged pain test, and sex.

799 multi-collinearity, independent variables and moderators were mean centered. In our moderation  
 800 analyses, a significant interaction of sex and PAF would indicate that the relationship between PAF and  
 801 pain sensitivity is different for the two sexes. The PAF x Sex interaction failed to reach significance for  
 802 PHP scores at either V1,  $t = -.16, p = .87$ , or V2,  $t = .74, p = .47$  or for CHP scores at either V1,  $t = -.01, p =$   
 803  $.99$ , or V2,  $t < .01, p > .99$ . For CHP rekindle, the PAF x Sex interaction was not significant at either V1,  $t =$   
 804  $-1.01, p = .32$ , or V2,  $t = -.98, p = .34$ . According to our moderation analyses we can conclude that the  
 805 PAF-pain sensitivity relationship is not different for the two sexes.

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

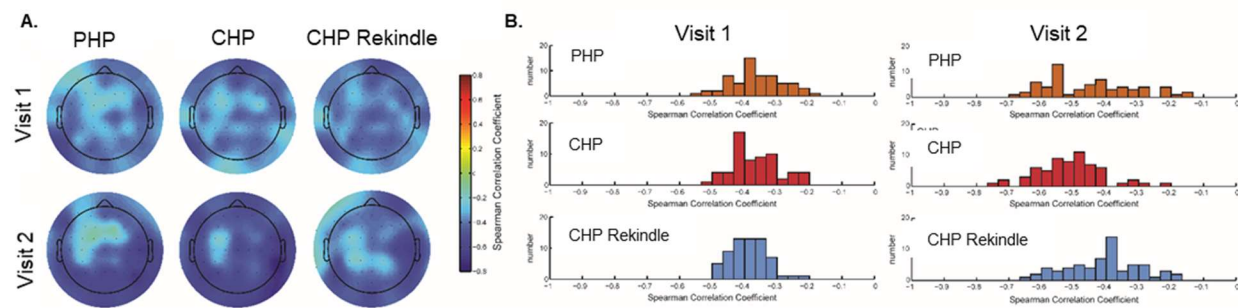
807 Previously unpublished findings from our lab have suggested that the PAF-pain sensitivity relationship is  
 808 not privileged to channels that putatively sample the sensorimotor cortex. To determine whether similar  
 809 conclusions can be drawn from the current dataset, we first calculated pain-free PAF separately at each  
 810 of the 63 EEG channels. Next, we correlated PAF estimates from each channel with scores on each  
 811 sensory test to yield a total of 63 correlation values for each prolonged pain test. As can be seen in  
 812 Supplementary Figure S5, the distribution of sensor correlations largely recapitulated what we found



**Figure S5.** Relationship between pain-free PAF and prolonged pain sensitivity is observable across the entire EEG montage. For each prolonged pain test, and each of the 63 individual EEG sensors, the Spearman correlation between channel-level PAF and pain scores was computed to yield a total of 63 correlations coefficients for each test. Results for Visits 1 and 2 are presented either on the scalp (A) or as distributions (B).

813 when we focused only on our sensorimotor ROI. Specifically, we found that correlations between PAF  
 814 and PHP, CHP and CHP rekindle were moderately large and in the negative direction. Accounting for the  
 815 possible effects of volume conduction with a surface Laplacian transformation did not change these  
 816 conclusions (Supplemental Figure S6).

817



**Figure S6.** Relationship between pain-free PAF and prolonged pain sensitivity is observable across the entire EEG montage even after accounting for possible effects of volume conduction. Prior to estimation of spectral power, a surface Laplacian transformation was applied to the preprocessed EEG data. For each prolonged pain test, and each of the 63 individual EEG channels, the Spearman correlation between channel PAF and pain scores was calculated to yield a total of 63 correlations coefficients for each test. Results for Visits 1 and 2 are presented either on the scalp (A) or as distributions (B).

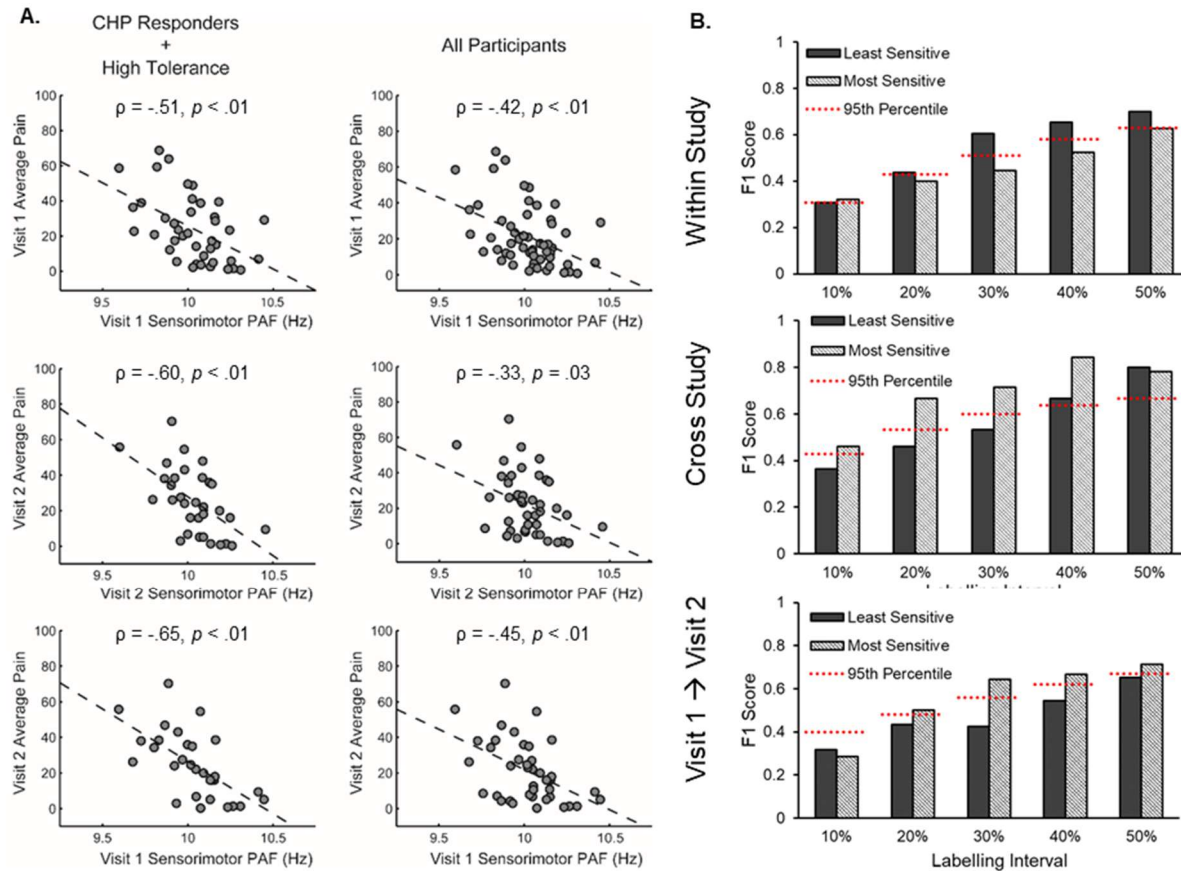
818 *Classification of Pain Sensitivity Using PAF: Results When Including All Participants*

819

820 Correlations between average pain sensitivity to all tests and pain-free, sensorimotor PAF are presented  
 821 in Figure S7. Whether considering only CHP responders and high tolerance individuals (left panels), or all  
 822 participants (right panels), pain-free, sensorimotor PAF was significantly related to this composite  
 823 measure of pain sensitivity.

824

825 A series of within-study linear support vector machines trained on all available data from the current  
 826 study identified the least sensitive individuals at above chance levels for all labelling intervals. When  
 827 trying to identify the most sensitive individuals, the support vector machine was only able to do so at  
 828 the smallest (10%) and largest (50%; i.e. median-split) labelling intervals. This latter result likely reflects  
 829 that our composite score is an inaccurate description of the mixed sensitivity of CHP non-responders  
 830 (i.e. insensitive to CHP but sensitive to PHP).



**Figure S7.** A. Pain sensitivity averaged across all prolonged pain tests is related to pain-free, Sensorimotor PAF when including only CHP Responders and High Tolerance individuals (left panels) or when including all participants regardless of pain response classification (right panels) B. Same as Figures 7B and 7D, except analyses performed while including all participants regardless of pain response classification. In brief, a support vector machine trained on Visit 1 pain-free, sensorimotor PAF predicts the identity of low pain sensitive individuals from the same study at almost all labelling intervals (top panel). A support vector machine trained on Visit 1 pain-free, sensorimotor PAF predicts the identity of high pain sensitive individuals from an independent study at all labelling intervals (Furman et al., 2018; middle panel). A support vector machine trained on Visit 1 pain-free, sensorimotor PAF predicts the identity of Visit 2 high pain sensitive individuals at almost all labelling intervals (bottom panel). An  $F_1$  score of 1 indicates perfect classifier performance and the dashed red lines reflect the 95<sup>th</sup> % of a null distribution of  $F_1$  scores.

831 A cross-study linear support vector machine trained on all available data was able to identify the most  
 832 sensitive individuals from a separate dataset (Furman et al, 2018) at all labelling intervals. For the least  
 833 sensitive individuals, this support vector machine only performed at above chance levels for the two  
 834 largest labelling intervals.

### 835 Supplemental References

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