Running Head

PAF and HFS

Title

Interindividual variations in peak alpha frequency do not predict the magnitude or extent of secondary hyperalgesia induced by high-frequency stimulation

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Abstract (242 words)

Background

Previous studies have shown an association between interindividual variations in the frequency of alpha-band EEG oscillations such as estimates of peak alpha frequency (PAF) and pain sensitivity. Whether differences in PAF also influence the susceptibility to develop central sensitization (CS) is unknown.

Objective

This study aimed to determine if the PAF of vision- and sensorimotor-related alpha-band activity is associated with the magnitude and extent of secondary mechanical hyperalgesia induced by high-frequency stimulation (HFS) of the skin, a surrogate marker of CS.

Methods

The EEG was recorded in 32 healthy participants at rest during eyes open and eyes closed conditions, and during bilateral finger movements. Two methods were used to isolate vision- and sensorimotor-related alpha-band activity based on sensitivity to eye closure and movement: one based on an independent component analysis, the other on spectral subtraction. PAF was assessed using a center-of-gravity approach or the Fitting Oscillations and One-Over-F (FOOOF) method, accounting for the aperiodic EEG component. HFS was applied to the right forearm, and pinprick sensitivity was assessed at both forearms, before and 40 minutes after HFS.

Results

Neither sensorimotor- nor vision-related PAF were significantly correlated with the magnitude or extent of HFS-induced secondary hyperalgesia. Interestingly, at the non-sensitized forearm, participants with a higher vision-related PAF exhibited greater habituation to pinprick stimuli, suggesting that variations in PAF may relate to variations in sensory habituation.

Conclusion

Interindividual variations of PAF were not significantly associated with the susceptibility to develop HFS-induced secondary hyperalgesia.

Keywords

High Frequency Stimulation (HFS), Peak Alpha Frequency, EEG Spectrum, alpha-band, Secondary Hyperalgesia, Central Sensitization

New and Noteworthy (44 words):

Using several methods to estimate vision- and sensorimotor-related peak alpha frequency (PAF) in the EEG frequency spectrum, we found no significant association between interindividual variations in PAF and the susceptibility to develop secondary hyperalgesia following high-frequency stimulation (HFS) of the skin in healthy participants.

INTRODUCTION

Alpha-band oscillatory activity (8-12 Hz) is the most prominent feature of the EEG recorded during rest in awake humans. The alpha rhythm was first described by Hans Berger, nearly a century ago (1). A distinctive feature of that rhythm is its predominance over posterior areas of the brain, and the fact that its magnitude markedly increases when participants close their eyes. The sensitivity to eye closure of posterior alpha oscillations has led most authors to consider that this oscillatory activity reflects a default state of brain areas "resting" or "idling" in the absence of input (2). However, alpha-band oscillations are not restricted to the visual cortex. Gastaut et al. (1952) described the Rolandic mu rhythm oscillating between 8-12 Hz over the scalp vertex and bilateral sensorimotor areas (3). Present at rest, the mu rhythm decreases during movement execution (3–5), most pronouncedly in the hemisphere contralateral to the executed movement (5). It is also suppressed during motor imagery (6,7), modulated by mental effort (8), attention and fatigue (9), and relatively unaffected by opening/closing of the eyes (10). Studies comparing the effects of upper and lower limb movements found that the movement-related suppression of the mu rhythm follows the mototopic and somatotopic organization of primary motor (M1) and somatosensory (S1) cortices (5).

Several studies have reported that the frequency at which the EEG spectrogram peaks within the alpha-band, referred to as the "peak alpha frequency" (PAF), tends to be slower in patients with chronic pain (11–16). Other studies have found that a slower PAF, recorded in healthy participants before they experienced experimental pain is associated with a greater sensitivity to phasic heat pain (17), tonic pain produced by topical capsaicin (18) and deep tissue pain produced by nerve growth factor injection (19,20), whereas an opposite trend has been observed in a previous study with tonic heat pain (17). The PAF has been shown to vary across individuals, but to remain relatively stable over time (17,20,22), suggesting that PAF may reflect an individual trait somehow related to pain sensitivity.

82 An important property of the nociceptive system is its propensity to sensitize when exposed to
83 repeated nociceptive input or following tissue injury (23–27). Sensitization of the nociceptive system
84 involves changes at the level of peripheral nociceptors leading to an increased sensitivity to noxious
85 stimuli (peripheral sensitization) (28) and changes at the level of the central nervous system leading
86 to an increased synaptic transmission of nociceptive input (central sensitization) described
87 extensively at the level of the dorsal horn in animal studies (29,30), but most likely also involving
88 higher-order synapses (31). A perceptual consequence of sensitization is hyperalgesia, i.e., an
89 increased sensitivity to pain (32). Following skin injury, hyperalgesia develops both within the injured
90 area (primary hyperalgesia) and in the surrounding uninjured skin (secondary hyperalgesia) (33,34).
91 While both peripheral and central sensitization may contribute to the development of primary
92 hyperalgesia, secondary hyperalgesia is thought to result exclusively from central sensitization
93 (34,35). A hallmark feature of secondary hyperalgesia is a prominent increase in sensitivity to
94 mechano-nociceptive input as compared to thermo-nociceptive input, enabling its characterization
95 by testing sensitivity to mechanical pinprick stimulation of the skin (36,37).

96 In a recent study conducted in patients scheduled for lateral thoracotomy, we found that
97 interindividual variations in the pre-operative susceptibility to develop secondary hyperalgesia
98 following high-frequency electrical stimulation of the skin (HFS) – a procedure used to induce central
99 sensitization experimentally – was associated with a greater likelihood post-thoracotomy pain at 2
100 months (38). The primary aim of the present study was to test whether interindividual variations in
101 PAF may be related to variations in this susceptibility to develop HFS-induced secondary
102 hyperalgesia.

103 Supporting this hypothesis, several studies have suggested that non-invasive brain stimulation of the
104 sensorimotor cortex can reduce pain sensitivity (39,40), and that this effect may be mediated
105 through a modulation of M1-connected brain structures involved in descending pain modulation
106 (41). Therefore, if variations of sensorimotor-related alpha band activities index variations in M1
107 function, interindividual differences in sensorimotor PAF could be associated with changes in the

108 state of descending pain modulation and the susceptibility to develop central sensitization. Finally,
109 since most previous reports of a relationship between PAF and sensitivity to pain have used models
110 of sustained pain which are known to induce some amount of sensitization, these observations might
111 be explained in part by a relationship between PAF and susceptibility to sensitize.

112 To test this hypothesis, the EEG was recorded in healthy participants with their eyes open at rest,
113 their eyes closed at rest, and with their eyes open while performing repetitive bilateral finger
114 movements. These three EEG recording conditions were used to functionally isolate, vision-related
115 alpha-band oscillations whose magnitude is known to increase following closure of the eyes and
116 sensorimotor-related alpha-band oscillations whose magnitude reduces during finger movements. As
117 in a multiverse analysis (42), several methods were used to (1) isolate vision- and sensorimotor-
118 related alpha-band activity and (2) estimate PAF. After the EEG recording, central sensitization was
119 induced in each participant using HFS delivered to the right volar forearm skin. The magnitude and
120 extent of HFS-induced secondary hyperalgesia was estimated by assessing the sensitivity to
121 mechanical pinprick stimuli delivered to the sensitized and non-sensitized forearms before and 40
122 minutes after HFS. The collected data allowed us to examine whether interindividual variations in
123 vision- and/or sensorimotor-related PAF predicts the susceptibility to develop HFS-induced
124 secondary mechanical hyperalgesia.

125

126 **METHODS**

127 **Participants**

128 Descriptive statistics are presented as mean ± standard deviation (SD), unless otherwise specified.

129 A total of 32 right-handed healthy volunteers (16 women and 16 men aged 25.3 ± 4.5 years [range:
130 19-38]) were included in this study between June and July 2020. Hand dominance was confirmed
131 using the Flinders Handedness Survey (43). The study was approved by the local ethics committee
132 (Comité d'Ethique Hospitalo-Facultaire des Cliniques universitaires Saint-Luc-UCLouvain;

133 B403201316436). All participants provided written informed consent and received financial
134 compensation. The experiment was conducted in agreement with the Declaration of Helsinki. The
135 decision to include 32 participants was guided by the correlation coefficients reported in previous
136 studies investigating the relationship between PAF and pain ratings in healthy volunteers (r = 0.74 in
137 (21) and r = 0.5 in (18). Based on these two studies, the estimated required sample sizes range from
138 12 to 38 participants to achieve a power of 0.90 with an alpha level of 0.05 (44).

139 Inclusion criteria were being healthy and aged 18-40 years. Exclusion criteria were (1) any history of
140 cardiac or neurological pathology, (2) regular practice of activities involving repeated stimulation of
141 the volar forearm skin (e.g., volleyball and handball players), (3) a dermatological condition involving
142 the forearm, (4) a history of traumatic injury of the upper limb and (5) regular tobacco consumption,
143 (6) having taken part in a previous experiment involving stimulation of the volar forearm(s).
144 Participants were asked to sleep a minimum of 8 hours the night before the experiment, to refrain
145 from using recreational drugs, psychotropics, analgesics, or other medications in the days leading up
146 to the study.

147 **Experimental design**

148 The time course of the experiment is summarized in Figure 1A. Participants were seated on a
149 comfortable chair with their arms resting on a desk, volar forearms facing upwards. Secondary
150 hyperalgesia was induced at the right volar forearm using HFS regardless of hand dominance. EEG
151 recordings were performed 25 minutes before HFS (T-25), 5 minutes before HFS (T-5), one minute
152 after HFS (T+1) and approximatively 35 minutes after HFS (T+35). Each EEG recording lasted 5
153 minutes and included three conditions: eyes open at rest, eyes closed at rest and eyes open while
154 performing repetitive bilateral flexion-extension movements with their index fingers. During the 30
155 minutes pause separating T+1 and T+35 EEG recordings, participants were instructed to read a book
156 or magazine of their choice. Sensitivity to mechanical pinprick stimuli was evaluated at both volar
157 forearms 20 minutes before (T-20) and approximatively 40 minutes after (T+40) HFS. The extent of

158 the HFS-induced area of secondary hyperalgesia was evaluated 40 minutes after HFS (T+40), at the
159 HFS-treated arm.

160

161 **Figure 1. A.** Timeline of the experiment. HFS was applied to the right volar forearm. EEG segments
162 lasting 5 minutes and including three conditions (eyes open at rest, eyes closed at rest, eyes open
163 while performing bilateral finger movements) were recorded 25 and 5 minutes before HFS (T-25 and
164 T-5), immediately after HFS, and approximately 35 minutes after HFS (T+35). Pinprick sensitivity of
165 the left and right volar forearms was assessed before HFS (T-20) and approximately 40 minutes after
166 HFS (T+40). The spatial extent of the HFS-induced area of secondary hyperalgesia was assessed
167 approximately 40 minutes after HFS (T+40). **B.** HFS was delivered to the forearm skin using a cathode
168 consisting of 10 blunt tungsten pins arranged on a circle with a diameter of 5 mm and a 24x20 mm
169 anode conductive gel pad placed next to the cathode.

170

171 **Induction of secondary hyperalgesia using HFS**

172 HFS was applied to the right volar forearm, 10 cm distal from the cubital fossa using an electrode
173 designed to preferentially activate cutaneous free nerve endings (EPS-P10, MRC Systems GmbH,
174 Heidelberg, Germany). The electrode consisted of a multi-pin cathode and a flat large-surface anode
175 (Figure 1B). The cathode consisted of 10 blunt tungsten pins arranged on a circle with a diameter of 5
176 mm. Each pin had a diameter of 0.25 mm and protruded by 0.65 mm over the base of the electrode.
177 The anode was a 24 x 20 mm conductive gel pad placed next to the cathode. The stimulation
178 consisted of 5 trains of 100 Hz charge-compensated biphasic electrical pulses (45) delivered using a
179 constant-current electrical stimulator (DS5; Digitimer, Welwyn Garden City, United Kingdom)
180 triggered by a National Instruments digital-analogue interface (NI6343, National Instruments, Austin,
181 Texas, USA). Each train lasted 1 second (100 pulses/train). The time interval between the onset of
182 each train was 10 s. The intensity of stimulation was set to 20 the detection threshold to a single

183 pulse, which was estimated in each participant at the beginning of the experiment using the method
184 of limits (0.126 ± 0.045 mA).

185 **Assessment of mechanical pinprick sensitivity at the volar forearms**

186 Before HFS (T-20) and after HFS (T+40), the sensitivity to mechanical pinprick stimuli was assessed at
187 the left and right volar forearms using a custom-made pinprick stimulator (0.25 mm probe diameter,
188 CATL, UCLouvain, Belgium) exerting a 128 mN force (26,27,45). At each time-point, three pinprick
189 stimuli were applied perpendicularly to the skin surrounding the area of HFS stimulation at the right
190 volar forearm and on the same location at the left volar forearm. After each stimulus, participants
191 were requested to report the intensity of the elicited sensation using a numerical rating scale ranging
192 from 0 (no perception) to 100 (maximal imaginable pain), with 50 marking the limit between non-
193 painful and painful sensations as used in several previous experiments (46–49). To avoid sensitization
194 of the skin by repeated mechanical pinprick stimulation, the probe was not applied twice on the
195 same location. The order of the testing (HFS-treated arm first or control arm tested first) was
196 counterbalanced across participants and remained constant across the two time points. The mean of
197 the three ratings obtained at each time point and arm was used as an index of pinprick .

198 **Assessment of the area of HFS-induced secondary hyperalgesia**

199 The same 128 mN pinprick probe was used to map, after HFS (T+40), the area of increased
200 mechanical pinprick sensitivity surrounding the site of HFS stimulation at the HFS-treated volar
201 forearm. The stimuli were applied along 4 axes and 8 directions separated by a 45-degrees angle and
202 each originating from skin location corresponding to the center of the HFS electrode and.
203 Participants were asked to close their eyes during the mapping procedure. Starting away from the
204 HFS-treated skin and moving towards the center, stimuli were applied every 5 mm. Participants were
205 asked to report when the sensation increased, and the corresponding stimulation location was
206 marked with a felt-tip pen (26,47,49–51). For each of the 8 directions, the distance between the
207 center of the HFS electrode location and the mark was measured, and the area of secondary

208 hyperalgesia was computed as the area of a piecewise polynomial fit of a 2D closed curve linking the
209 8 marked locations ('interpclosed' function; Santiago Benito 2023, Matlab Central File Exchange)
210 (26,27). Since this function cannot process null values, we assigned a value of 0.1 when no secondary
211 hyperalgesia area was present in this direction. The radius of the area of increased pinprick
212 sensitivity was then computed as the square root of the estimated area divided by π, as if the area
213 was in the shape of a circle. This transformation was performed prior to any analyses.

214 **EEG recording**

215 The EEG was recorded using 64 scalp electrodes with active shielding, placed according to the
216 International 10-20 system (ANT Neuro, Enschede, The Netherlands). The signals were digitized at
217 1,000 Hz using an average reference. All impedances were kept under 20 kΩ. At each time point (T-
218 25, T-5, T+1 and T+36), the recording consisted of 3 minutes at rest with eyes open (participants
219 were asked to relax and stare at a cross displayed 1 meter in front of them, at a 30° angle below eye
220 level), followed by 1 minute at rest with eyes closed, and 1 minute with eyes open while performing
221 repeated bilateral index finger movements at a rate of approximately 1 Hz while fixing their gaze on
222 the cross. These three conditions (EYES-OPEN-REST, EYES-CLOSED-REST, EYES-OPEN-MVT) were used
223 to differentially modulate vision-related alpha-band activity (which should be enhanced in the EYES-
224 CLOSED-REST condition as compared to the other conditions) and sensorimotor-related alpha-band
225 activity (which should be attenuated when performing finger movements in the EYES-OPEN-MVT
226 condition as compared to the other conditions).

227 **EEG preprocessing**

228 The EEG signals collected before HFS (T-25) were analyzed offline using the Letswave 6 toolbox
229 (http://letswave.org) running on MATLAB 2018a (Mathworks, USA). The EEG data collected at the
230 other time points was not analyzed in the present study.

231 First, the EEG recordings were cut in three segments: a 180-s EYES-OPEN-REST segment, a 60 s
232 segment EYES-CLOSED-REST and a 60 s segment EYES-OPEN-MVT (6). The three segments were then

233 filtered using a 0.5 - 47 Hz zero-phase Butterworth band-pass filter (order 4) and further segmented
234 into half-overlapping 5-s epochs. Each segment was demeaned, and a linear detrend was applied to
235 remove slow drifts of the signal. Noisy electrodes (1-2 electrodes in 10 out of 32 recordings) were
236 interpolated using the average of the three nearest electrodes. Epochs with clear artifacts (absolute
237 amplitude ≥200 μV) were rejected (2.4% of epochs from EYES-OPEN-REST condition, 2.8% of epochs
238 from EYES-CLOSED-REST condition and 2.8% of epochs from EYES-OPEN-MVT condition). Signals were
239 re-referenced to the average of all channels excluding the two mastoid electrodes (M1, M2).

240 **Functional separation of vision- and sensorimotor-related alpha-band activity**

241 In previous studies, PAF has been measured using various methods (17,18,21,52–54) and at different
242 scalp locations (17,55–59), with some studies interpreting it as a marker of sensorimotor activity
243 even when recorded over central or occipital regions or when clearly responding to closure of the
244 eyes (11,17–19). In this work, we aimed to explore multiple approaches to separate vision- and
245 sensorimotor-related alpha-band activity and estimate their PAF.

246 *ICA-based method*

247 In a first approach, an independent component analysis (ICA) was used to dissociate visual alpha-
248 band activity (VISUAL-ALPHA) and sensorimotor alpha-band activity (SM-ALPHA) based on their
249 sensitivity to closure of the eyes and movement of the hands.

250 For each participant, the 'runica' algorithm was used to separate the 62 EEG scalp signals into 61
251 independent components (ICs) having a fixed topographical distribution, and maximally independent
252 time courses. In the case channels were interpolated during preprocessing, the number of ICs was
253 lowered by the number of interpolated channels (60). Because the amplitude of VISUAL-ALPHA
254 should be selectively increased during the EYES-CLOSED-REST condition while SM-ALPHA should be
255 selectively decreased during bilateral moving of the fingers in the EYES-OPEN-MVT condition, ICA can
256 be expected to capture visual- and sensorimotor-related alpha-band activity into separate ICs.

For each participant and each IC time course, a Fast-Fourier Transform (FFT) was used to compute the amplitude frequency spectra of all 5-s half-overlapping epochs, windowed using a Hanning function. The spectra were then averaged per participant. The obtained frequency spectra were used to identify ICs capturing alpha-band activity, and to categorize these ICs into the following three categories: VISUAL-ALPHA (ICs capturing vision-related alpha-band activity that was increased in the EYES-CLOSED-REST condition as compared to EYES-OPEN-REST condition and the EYES-OPEN-MVT condition), SM-ALPHA (ICs capturing sensorimotor-related alpha-band activity that was decreased in the EYES-OPEN-MVT condition as compared to the EYES-OPEN-REST condition and was not increased in the EYES-CLOSED-REST condition as compared to the EYES-OPEN-REST condition) and MIXED-ALPHA (ICs capturing alpha-band activity that was both sensitive to closure of the eyes – greater activity in the EYES-CLOSED-REST condition compared to the EYES-OPEN-REST condition – and sensitive to finger movements – reduced activity in the EYES-OPEN-MVT condition compared to the EYES-OPEN-REST condition).

The classification was achieved for each IC of each participant as follows. First, using the average of the frequency spectra obtained in all three conditions, the peak of potential alpha-band activity captured by the IC was computed using the center of gravity (CoG) formula (19,61)

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

where f_i is the ith frequency bin between 9 and 11 Hz, a_i is the spectral amplitude for f_i, and n is the number of frequency bins between 9 and 11 Hz. This value was then used to define a 6 Hz frequency window of interest centered on the estimated CoG. Second, the mean amplitude and standard error of the mean (SEM: standard deviation of amplitudes divided by the square root of the number of bins) were computed separately for each of the three EEG recording conditions using all amplitude bins located within the selected window of interest. The mean and SEM were used to compute a confidence interval corresponding to the mean ± 1.97*SEM, and the lower and upper bounds of these confidence intervals (CI) were used to perform the categorization. ICs were classified as

capturing vision-related alpha-band activity (VISUAL-ALPHA) when the lower bound of the CI in the EYES-CLOSED-REST condition exceeded the upper bound of the CI in the EYES-OPEN-REST condition (indicating an increase of alpha during eye closure), whereas the CI in the EYES-OPEN-REST condition overlapped the CI in the EYES-OPEN-MVT condition (indicating no strong effect of movement on alpha-band amplitude). Similarly, ICs were classified as capturing movement-related alpha (SM-ALPHA) when the lower bound of the CI in the EYES-OPEN-REST condition exceeded the upper bound of the CI in the EYES-OPEN-MVT condition (indicating a movement-induced reduction of alpha), whereas the CI in the EYES-OPEN-REST and EYES-CLOSED-REST conditions overlapped (indicating low sensitivity to closure of the eyes). Finally, ICs were classified as MIXED-ALPHA when they were both sensitive to closure of the eyes (lower bound of the CI in the EYES-CLOSED-REST condition > upper bound of the CI in the EYES-OPEN-REST condition) and sensitive to the finger movements (lower bound of the CI in the EYES-OPEN-REST condition > upper bound of the CI in the EYES-OPEN-MVT condition). Examples of ICs capturing vision-related and sensorimotor-related alpha-band activity are shown in Figure 2.

For each participant, the VISUAL-ALPHA, SM-ALPHA and MIXED-ALPHA ICs were then used to reconstruct three separate EEG datasets maximally capturing vision-related, sensorimotor-related and mixed alpha-band activity, respectively.

Figure 2. **A.** EEG frequency spectrum of a representative independent component (IC) categorized as capturing vision-related alpha-band activity (VIS-ALPHA) that was increased during closure of the eyes (EYES-CLOSED-REST condition) as compared to the eyes open conditions (EYES-OPEN-REST and EYES-OPEN-MOVEMENT). **B.** EEG frequency spectrum of a representative IC categorized as capturing sensorimotor-related alpha-band activity (SM-ALPHA) that was decreased during finger movements (EYES-OPEN-MVT condition) as compared to rest (EYES-OPEN-REST and EYES-CLOSED-REST

305 conditions). Y-axis: arbitrary units. X-axis: frequency in Hz. The vertical grey lines represent the 6 Hz
306 frequency window used to assess the presence of alpha-band activity.

307 *Subtraction-based method*

308 Suppression of movement-related activity in the EYES-OPEN-MVT condition may be expected to
309 constitute the main difference between that condition and the EYES-OPEN-REST condition.
310 Therefore, computing the difference between the EYES-OPEN-REST and EYES-OPEN-MVT spectra
311 could constitute a simple alternative to efficiently isolate movement-related alpha-band activity.
312 Similarly, because closure of the eyes selectively enhances vision-related alpha oscillations,
313 subtraction of the EYES-OPEN-REST and EYES-CLOSED-REST spectra could constitute a means to
314 isolate vision-related alpha-band activity. As compared to the ICA-based approach, a possible
315 advantage of this alternative approach is that the subtraction can be expected to cancel out the
316 contribution of activity that is equally present in both of the subtracted spectra, including the 1/f
317 aperiodic component of the EEG power spectrum that can bias estimation of PAF (18).

318 **Estimation of peak alpha frequency (PAF)**

319 Vision-related alpha oscillations can be expected to predominate over occipital electrodes, whereas
320 sensorimotor-related alpha oscillations modulated by bilateral movements of the fingers can be
321 expected to spread over left and right central electrodes (5,10). Therefore, the PAF of vision-related
322 alpha-band activity was estimated in the average of the frequency spectra of occipital electrodes O1,
323 Oz and O2, using the eyes-closed derived VISUAL-ALPHA spectra of the ICA-based method and the
324 EYES-CLOSED-REST minus EYES-OPEN-REST spectra of the subtraction-based method. Similarly, the
325 PAF of movement-related alpha-band activity was estimated in the average of the frequency spectra
326 of central electrodes C3, Cz and C4, using the eyes open-derived SM-ALPHA spectra of the ICA-based
327 method and the EYES-OPEN-REST minus EYES-OPEN-MVT spectra of the subtraction-based method.
328 Finally, because the MIXED-ALPHA signals generated by the ICA-based method might have included

329 both vision- and sensorimotor-related alpha-band activity, its PAF was estimated both at occipital
330 and at central channels.

331 Two different methods were used to estimate the PAF: the center of gravity (CoG) method and the
332 Fitting Oscillations and One Over F method (FOOOF (62)).

333 For the CoG method, we used the formula described above and a 9-11 Hz interval as was used in
334 previous studies assessing the relationship between PAF and sensitivity to pain (18,19). As visual
335 inspection of the EEG frequency spectra indicated that the PAF of some individuals was located
336 outside the 9-11 Hz interval, the CoG was also estimated using a larger 7-13 Hz window. In the
337 subtracted spectra, some amplitude values within the 9-11 or 7-13 Hz windows were negative. Only
338 positive values were used for the CoG estimation.

339 For the FOOOF method, the spectrum was decomposed in its periodic and aperiodic components, as
340 described in Donoghue et al. (62). In this case, the alpha band was defined as the range from 7 to 14
341 Hz and default algorithm parameters were employed, in coherence with other studies that
342 performed similar analyses (63). When using this approach a larger frequency range is advised, as the
343 overlapping of the frequency borders with a spectral peak is associated with higher parameter
344 estimation errors (64). After the decomposition, only the periodic component was considered, as it is
345 expected to contain alpha-band oscillatory activities. A gaussian curve was used to fit the periodic
346 component of the spectrum, and its parameters were used to characterize the PAF (defined as the
347 center of the gaussian curve). As the FOOOF method is intended to suppress the 1/f aperiodic
348 component of the EEG, it was not applied to the subtracted spectra.

349 **Statistical analysis**

350 Statistical analyses were performed using JASP (JASP Version 0.14.1.0 for Windows). Statistical tests
351 were chosen depending on the distribution of the dependent variables, which was assessed by visual
352 examination of the histograms for skewness and kurtosis, and the Q-Q plots of the residuals.

353 *HFS-induced change in pinprick sensitivity*. Mechanical pinprick ratings followed a close-to-normal
354 distribution and we thus conducted a repeated-measures ANOVA with two within-subjects factors:
355 TIME (before vs. after HFS) and ARM (HFS-treated vs. control arm), and one between-subjects factor:
356 ORDER (whether the HFS-treated or control arm was tested first). Data were averaged per condition
357 for each participant prior to analysis.

358 *Peak-alpha frequency*. A correlation analysis was used to evaluate the relationship between
359 sensorimotor- and vision-related PAF and the changes in pinprick sensitivity after HFS (change in
360 pinprick sensitivity at the sensitized HFS-treated forearm: $\Delta NRS_{HFS}=NRS_{HFS}(T+40) - NRS_{HFS}(T-20)$,
361 change in pinprick sensitivity at the non-sensitized forearm: $\Delta NRS_{NS}=NRS_{NS}(T+40) - NRS_{NS}(T-20)$,
362 difference in the change in pinprick sensitivity at the two forearms: $\Delta\Delta NRS = \Delta NRS_{HFS} - \Delta NRS_{NS}$), and
363 between PAF and the radius of the area of secondary mechanical hyperalgesia (R). For each
364 estimated PAF, the analyses were performed using Spearman's correlations because normality was
365 not strictly respected for all variables. Yet, as it was respected for our primary aim (relationship
366 between sensorimotor PAF and secondary hyperalgesia), we displayed Pearson's correlation for
367 these analyses.

368 To examine a possible relationship between PAF and pain sensitivity, we also computed Spearman's
369 correlation between PAF and the detection threshold to single electrical stimuli, as well as the
370 average ratings of the intensity of the sensation elicited by the HFS trains. Finally, we also assessed
371 the correlation between inter-individual variations in vision- and sensorimotor-related PAF, and
372 between the estimates of PAF obtained using the different approaches (ICA vs subtraction-based
373 method to isolate vision- and sensorimotor-related alpha-band activity, CoG vs FOOOF method to
374 estimate PAF).

375 Because the primary aim of our study was to test a possible association between PAF of
376 sensorimotor alpha-band oscillations and the susceptibility to develop central sensitization, and
377 because previous studies showed that the extent of the area of secondary hyperalgesia may be a

more reliable readout as compared to changes in pinprick ratings (47), our primary outcome was the correlation between the sensorimotor PAF and the radius of the HFS-induced area of increased pinprick sensitivity. All other correlations were considered as exploratory, thus we did not apply corrections for multiple comparisons.

RESULTS

Pinprick sensitivity before and after HFS

After HFS, all but one participant reported an increased sensitivity to pinprick stimuli at the sensitized forearm as compared to the control arm (Figure 3A). The repeated-measures ANOVA showed a significant TIME × ARM interaction ($F(1, 30) = 48.414$, $p = <.001$, $\eta^2 = 0.617$).

Post hoc comparisons confirmed a significant increase in pinprick ratings at the HFS-treated arm, with a mean increase of 11.854 ($t(31) = 5.424$, $p < 0.001$, Cohen's $d = 0.959$ [0.533 to 1.374], 95% CI for mean difference: 7.397 to 16.311). Conversely, there was a significant decrease in ratings at the control arm, with a mean reduction of 3.312 ($t(31) = -4.177$, $p < 0.001$, Cohen's $d = -0.738$ [−1.126 to −0.342], 95% CI for mean difference: −4.930 to −1.695).

Figure 3. **A**. Individual intensity ratings for pinprick stimuli delivered to the HFS-sensitized forearm and the contralateral non-sensitized forearm, before and after HFS (T-20 and T+40). Note the clear increase in pinprick ratings at the sensitized forearm due to HFS-induced sensitization, and the decrease in pinprick ratings at the non-sensitized forearm possibly due to perceptual habituation. **B**. Estimated area of HFS-induced secondary hyperalgesia (grey contours: individual participants; black contour: group-level average).

HFS-induced area of secondary hyperalgesia

401 At the HFS-treated forearm, an area of increased pinprick sensitivity was found in all but one
402 participant (Figure 3B). Across participants showing an area of increased sensitivity, the extent was
403 101 ± 32 mm along the proximal-distal axis, 53 ± 20 mm along the lateral-medial axis, 77 ± 29 mm
404 and 66 ± 27 mm along the two diagonal axes. The grand average estimated area radius was 68 ± 22
405 mm.

406 **Vision- and sensorimotor-related alpha-band activity isolated using the ICA-based method**

407 After blind source separation using ICA, 19.4 ± 5.5 ICs capturing alpha-band activity were identified in
408 31 out of 32 participants, of which 11.9 ± 5.4 ICs were categorized as vision-related (VISUAL-ALPHA)
409 in 31 out of 32 participants, 5.5 ± 5 ICs were categorized as sensorimotor-related (SM-ALPHA) in 24
410 out of 32 participants and 4.5 ± 4.3 ICs were categorized as mixed (MIXED-ALPHA) in 22 out of 32
411 participants.

412 The reconstructed VISUAL-ALPHA signals clearly captured vision-related alpha-band activity that
413 increased during closure of the eyes, was only minimally affected by finger movements and, most
414 importantly, displayed an occipital scalp topography compatible with activity originating
415 predominantly from visual areas (Figure 4A). Similarly, the SM-ALPHA signals captured sensorimotor-
416 related alpha-band activity that decreased during movement of the fingers, was minimally influenced
417 by closure of the eyes, and exhibited a bilateral centroparietal scalp topography compatible with
418 activity originating from the representation of the left and right hands within the sensorimotor
419 cortex (Figure 4B). The reconstructed MIXED-ALPHA signal captured alpha-band activity that
420 predominated over posterior regions and tended to both increase during closure of the eyes and
421 decrease during finger movements (Figure S1).

422

423 **Figure 4**. **A.** Group-level average frequency spectra of the ICA-separated EEG signals capturing vision-
424 related alpha-band activity (VISUAL-ALPHA signals) at occipital electrodes in the eyes closed at rest
425 (blue), eyes open at rest (red) and eyes open during finger movements (green) conditions. Note the

426 clear increase in alpha-band amplitude during closure of the eyes. Also note the occipital topography
427 of the peak. **B.** Group-level average frequency spectra of the ICA-separated EEG signals capturing
428 sensorimotor-related alpha-band activity (SM-ALPHA signals) at central electrodes. Note the selective
429 decrease of amplitude during finger movements, and the bilateral central-parietal topography
430 compatible with activity originating from the left and right sensorimotor cortices.

431

432 **Vision- and sensorimotor-related alpha-band activity isolated using the subtraction-based method**

433 The group-level averages of the subtracted frequency spectra are shown in Figure 5. As expected, the
434 subtraction spectrum EYES-CLOSED-REST minus EYES-OPEN-REST exhibited a clear peak of alpha-
435 band activity that was maximal over occipital electrodes, compatible with alpha oscillations
436 originating predominantly from the visual cortex (Figure 5A).

437 The subtraction spectrum EYES-OPEN-REST minus EYES-OPEN-MVT also exhibited a peak of alpha-
438 band activity, with a topography maximal at central and bilateral parietal electrodes, compatible with
439 activity originating from the hand representation within the left and right sensorimotor cortices
440 (Figure 5B). As compared to the signals obtained using the ICA-based method, the contribution of 1/f
441 noise was minimal in these subtracted spectra.

442

443 **Figure 5**. **A.** Group-level average of the subtracted EEG frequency spectra EYES-CLOSED-REST minus
444 EYES-OPEN-REST conditions at occipital electrodes. Note the clear occipital topography of the peak
445 indicating that this approach effectively isolated vision-related alpha-band activity. **B.** Group-level
446 average of the subtracted frequency spectra EYES-OPEN-REST minus EYES-OPEN-MVT conditions at
447 central electrodes. Note the bilateral central topography of the peak indicating that this approach
448 succeeded in isolating sensorimotor-related alpha-band activity. Also note the lack of an 1/f decay
449 (i.e. the aperiodic component of the EEG).

450

Baseline peak alpha frequency (PAF) estimated using the CoG method

Using the CoG approach and a 9-11 Hz window applied to the VISUAL-ALPHA, SM-ALPHA and MIXED-ALPHA spectra obtained with the ICA-based method, the PAF of vision-related alpha-band activity estimated at occipital electrodes in the VISUAL-ALPHA signals during closure of the eyes was 10.0 ± 0.2 Hz (visual PAF). The PAF of sensorimotor-related alpha-band activity estimated at central electrodes in the eyes open condition at rest was 10.0 ± 0.1 Hz (sensorimotor PAF). The PAF estimated in the MIXED-ALPHA spectra was 10.0 ± 0.1 Hz at occipital electrodes during closure of the eyes, and 10.0 ± 0.1 Hz at central electrodes in the eyes open at rest condition. Using the same CoG approach applied to the spectra obtained with the subtraction-based approach, data from one participant was rejected for estimation of the visual PAF and data from three participants were rejected for estimation of the sensorimotor PAF as their spectra did not exhibit a peak within the frequency window. The visual PAF was 10.0 ± 0.3 Hz while the sensorimotor-related PAF was 9.9 ± 0.5 Hz. Average PAF estimates obtained using the larger 7-13 Hz frequency window are reported in Table 1.

				Visual PAF					Sensorimotor PAF				
				ICA spectra			Subtracted spectra		ICA spectra			Subtracted spectra	
				CoG (9-11 Hz)	CoG (7-13 Hz)	FOOOF	CoG (9-11 Hz)	CoG (7-13 Hz)	CoG (9-11 Hz)	CoG (7-13 Hz)	FOOOF	CoG (9-11 Hz)	CoG (7-13 Hz)
Visual PAF	ICA spectra	CoG (9-11 Hz)	10.0 ±0.2 Hz		0,93	0,87	0,93	0,80	0,27	0,34	0,14	0,15	0,27
		CoG (7-13 Hz)	10.0±0.3 Hz			0,84	0,90	0,92	0,18	0,26	-0,02	0,07	0,16
		FOOOF	10.1 ±0.7 Hz				0,89	0,79	0,13	0,22	0,01	0,08	0,22
	Subtracted spectra	CoG (9-11 Hz)	10.0 ±0.3 Hz					0,89	0,16	0,29	0,25	0,10	0,38
		CoG (7-13 Hz)	10.1 ±0.7 Hz						0,08	0,18	0,15	0,08	0,32
Sensorimotor PAF	ICA spectra	CoG (9-11 Hz)	10.0 ±0.1 Hz							0,90	0,42	0,79	0,70
		CoG (7-13 Hz)	9.9 ±0.3 Hz								0,40	0,77	0,72
		FOOOF	9.7 ±1.4 Hz									0,56	0,73
	Subtracted spectra	CoG (9-11 Hz)	9.9 ±0.5 Hz										0,75
		CoG (7-13 Hz)	10.0 ±0.9 Hz										

Table 1. Spearman's correlation coefficients between visual and sensorimotor PAF estimates obtained using the ICA-based or subtraction-based approaches to isolate visual- and sensorimotor-related alpha-band activity, and the CoG (frequency windows: 9-11 Hz and 7-13 Hz) or FOOOF

methods to estimate peak frequency. Positive and negative correlations are coded in green and red, respectively. Significant correlations ($p<0.05$) are highlighted with red borders

Estimation of Peak Alpha Frequency (PAF) using the FOOOF method

Using the FOOOF approach applied to the VISUAL-ALPHA, SM-ALPHA and MIXED-ALPHA spectra obtained with the ICA-based method, the PAF of vision-related alpha-band activity was 10.1 ± 0.7 Hz, while the PAF for sensorimotor-related alpha-band activity was 9.7 ± 1.4 Hz. The PAF estimated in the MIXED-ALPHA spectra was 10.3 ± 1.0 Hz at occipital electrodes during closure of the eyes, and 10.4 ± 1.2 Hz at central electrodes in the eyes open at rest condition. Average PAF estimates obtained using the larger 7-13 Hz frequency window are reported in Table 1.

Primary analysis: correlation between baseline sensorimotor PAF and the area radius of HFS-induced secondary hyperalgesia

Using the data from the 24/32 participants in which the ICA-based approach succeeded in isolating sensorimotor-related alpha-band activity, no significant correlation between the sensorimotor PAF and the radius of the HFS-induced area of secondary hyperalgesia was found, neither when using the CoG estimates of PAF (CoG window 9-11 Hz: Spearman's rho = 0.167, p = 0.434, Pearson's r = 0.267, p = 0.207; CoG window 7 – 13 Hz: Spearman's rho = 0.207, p = 0.330, Pearson's r = 0.132, p = 0.539), nor when using the FOOOF estimates of PAF (Spearman's rho = -0.203, p = 0.339, Pearson's r = -0.252, p = 0.235) (Table 2 and Figure 6).

Similarly, when using the PAF estimates obtained from the subtraction-based spectra, there was no significant correlation between the sensorimotor PAF and the radius of the HFS-induced area of secondary hyperalgesia (CoG window 9-11 Hz: Spearman's rho=-0.068, p=0.729, Pearson's r = -0.092, p = 0.641; CoG window 7-13 Hz: Spearman's rho=-0.095, p=0.624, Pearson's r = -0.107, p = 0.581) (Table 2 and Figure 6).

	Visual PAF					Sensorimotor PAF				
	ICA spectra			Subtracted spectra		ICA spectra			Subtracted spectra	
	CoG (9-11 Hz)	CoG (7-13 Hz)	FOOOF	CoG (9-11 Hz)	CoG (7-13 Hz)	CoG (9-11 Hz)	CoG (7-13 Hz)	FOOOF	CoG (9-11 Hz)	CoG (7-13 Hz)
Secondary hyperalgesia radius	-0,23	-0,33	-0,23	-0,12	-0,28	0,17	0,21	0,20	-0,07	-0,10
Change in pinprick sensitivity at HFS forearm (ΔNRS_{HFS})	-0,07	-0,16	0,02	-0,25	-0,29	-0,03	-0,11	-0,02	0,00	-0,11
Change in pinprick sensitivity at non-sensitized forearm (ΔNRS_{NS})	-0,49	-0,42	-0,44	-0,48	-0,36	-0,17	-0,25	0,00	-0,01	-0,08
Change in pinprick sensitivity at HFS minus non-sensitized forearm ($\Delta\Delta NRS$)	0,09	-0,02	0,20	-0,08	-0,16	0,12	0,06	-0,02	0,07	-0,07
Baseline pinprick ratings	0,31	0,29	0,40	0,25	0,20	0,22	0,25	-0,34	-0,03	0,07
HFS pain ratings	0,20	0,09	0,31	0,15	0,01	0,36	0,39	-0,03	0,15	0,17
Electrical detection threshold	-0,29	-0,11	-0,17	-0,12	0,03	-0,13	-0,12	0,05	0,11	0,05

Table 2. Spearman's correlation coefficients between visual and sensorimotor PAF estimates and the following measures: the area radius of HFS-induced secondary hyperalgesia area, the changes in pinprick sensitivity at the HFS-sensitized and non-sensitized forearms, the detection threshold to a single electrical pulse averaged across the two forearms, the baseline pinprick ratings averaged across the two forearms, and the pain ratings reported during HFS. Positive and negative correlations are coded in green and red, respectively. Significant correlations ($p<0.05$) are highlighted with red borders.

Figure 6. Scatter plots showing the absence of a significant correlation between the radius of the HFS-induced area of secondary hyperalgesia and the sensorimotor peak alpha frequency (PAF), regardless of the method used to isolate sensorimotor-related activity (A: ICA-based approach; B: subtraction-based approach) or the method used to estimate the PAF (center of gravity using a narrow 9-11 Hz or wide 7-13 Hz frequency window, FOOOF algorithm).

Exploratory analyses

Correlation between baseline sensorimotor PAF and changes in pinprick sensitivity after HFS. Such as for the area of HFS-induced secondary hyperalgesia, there was no significant correlation between the sensorimotor PAF and (1) the magnitude of the HFS-induced increase in pinprick sensitivity at the sensitized forearm compared to the contralateral forearm ($\Delta\Delta$NRS), (2) the change in pinprick

513 sensitivity at the HFS-sensitized forearm (ΔNRS_{HFS}) and (3) the change in pinprick sensitivity at the
514 contralateral non-sensitized forearm (ΔNRS_{NS}) (Table 2).

515 *Correlation between baseline visual PAF and the area of HFS-induced secondary hyperalgesia.* Such as
516 for the sensorimotor PAF, there was no significant correlation between the visual PAF and the radius
517 of the area of HFS-induced secondary hyperalgesia (Table 2).

518 *Correlation between baseline visual PAF and the changes in pinprick sensitivity after HFS.* There was
519 also no significant correlation between the visual PAF and the increase in pinprick sensitivity at the
520 sensitized forearm compared to the contralateral forearm ($\Delta\Delta NRS$) or the change in pinprick
521 sensitivity at the HFS-sensitized forearm (ΔNRS_{HFS}). However, and regardless of the method used to
522 estimate the visual PAF, there was a significant negative correlation between the visual PAF and the
523 change in pinprick sensitivity at the non-sensitized forearm (ΔNRS_{NS}): participants with a higher visual
524 PAF at baseline exhibited a stronger perceptual habituation to pinprick stimulation at the non-
525 sensitized forearm (Table 2 and Figure 7).

526 *Correlation between baseline PAF and baseline pain ratings*. There was no significant correlation
527 between either the visual PAF nor the sensorimotor PAF and (1) the detection threshold to single
528 electrical pulses averaged across the two forearms, (2) the baseline pinprick ratings averaged across
529 the two forearms, or (3) the intensity of the pain during HFS (Table 2).

530 *Correlation between vision- and sensorimotor-related PAF*. Both visual and sensorimotor PAF
531 estimates derived using ICA-based and subtraction-based approaches to isolate visual- and
532 sensorimotor-related alpha-band activity and computed using the CoG and FOOOF methods were
533 highly correlated, indicating good consistency across the estimation techniques (Table 1). In contrast,
534 there was no significant correlation between the visual PAF and the sensorimotor PAF, indicating that
535 they likely reflect distinct oscillatory processes (Table 1). This highlights the importance of
536 considering these rhythms as functionally separate when assessing their relevance to perception.

537 *Mixed alpha-band activity*. Using the ICA-based approach, independent components (ICs) capturing
538 alpha-band activity that increased during closure of the eyes and decreased during finger movements
539 were identified in 22/32 participants. The PAF estimates of these MIXED-ALPHA signals were strongly
540 correlated with the visual PAF, but also showed significant correlation with the sensorimotor PAF
541 using the largest window, indicating that these signals may reflect the fact ICA did not fully dissociate
542 the two types of alpha-band activity (Table S1). There was no significant correlation between the PAF
543 of MIXED-ALPHA signals and the secondary hyperalgesia radius, the changes in pinprick sensitivity
544 after HFS, the electrical detection threshold and the HFS pain ratings. A weak correlation was
545 reported for the baseline pinprick ratings and mixed PAF at occipital electrodes (Table S2).

546

547 **Figure 7**. Scatter plots showing the correlation between the post-HFS decrease in pinprick sensitivity
548 at the non-sensitized forearm and the visual peak alpha frequency (PAF), present across methods
549 used to isolate visual-related activity (A: ICA-based approach; B: subtraction-based approach) and
550 methods to estimate the PAF (center of gravity using a narrow 9-11 Hz or wide 7-13 Hz frequency
551 window, FOOOF algorithm).

552

553 **DISCUSSION**

554 The results of the present study do not support our hypothesis that interindividual variations in peak-
555 alpha frequency are associated with the susceptibility to develop HFS-induced secondary
556 hyperalgesia. Indeed, in our sample of 32 healthy participants and using different approaches to
557 isolate sensorimotor- and vision-related alpha-band activity and estimate peak frequency in the
558 resting EEG, there was no significant correlation between sensorimotor or visual PAF estimates and
559 neither the spatial extent of the HFS-induced secondary hyperalgesia area, nor the magnitude of the
560 HFS-induced increase in pinprick sensitivity at the sensitized forearm.

561 **Functional dissociation between vision- and sensorimotor-related alpha-band activity**

562 Previous reports of a relationship between PAF and sensitivity to pain have often interpreted the
563 measured interindividual differences in PAF as reflecting differences in sensorimotor-related alpha-
564 band activity. However, the topographical distribution of the alpha-band in resting EEG predominates
565 over posterior areas, and its most prominent feature is its sensitivity to closure of the eyes. Hence,
566 measures of PAF in resting EEG may be expected to mainly reflect vision-related alpha-band
567 oscillations, even when the PAF is estimated at central electrodes overlying the sensorimotor cortex
568 (17,19,55). For this reason, several authors have attempted to isolate sensorimotor- from vision-
569 related alpha oscillations using an Independent Component Analysis and selecting components
570 displaying a central topography as compared to an occipital topography (18,52,54,65).

571 In the present study, we propose two functional approaches to isolate vision- and sensorimotor-
572 related alpha oscillations, based on the sensitivity of vision-related alpha oscillations to closure of the
573 eyes and the sensitivity of sensorimotor-related alpha oscillations to movement execution rather
574 than their topography. In the ICA-based approach, the functional properties of vision- and
575 sensorimotor-related alpha-band activity allowed separating alpha-band activity into visual-related
576 components that were sensitive to closure of the eyes and sensorimotor-related components that
577 were sensitive to the performance of bilateral finger movements. In the subtraction-based approach,
578 we subtracted the EEG spectra obtained during eyes open and eyes closed recordings at rest to
579 isolate the alpha-band increase occurring during eye closure, while we subtracted the EEG spectra
580 obtained during eyes open at rest and eyes open during bilateral finger movements to capture the
581 change in alpha-band activity occurring during movement. Both the ICA-based approach and the
582 subtraction-based approach showed that the alpha-band activity sensitive to closure of the eyes had
583 a clear occipital topography compatible with activity originating from visual areas, whereas the
584 alpha-band activity sensitive to moving of the fingers exhibited a bilateral central topography,
585 compatible with activity originating from left and right sensorimotor areas.

586 It should be noted that in the ICA-based approach, visual-related alpha-band activity was isolated in
587 31/32 participants, whereas sensorimotor-related alpha-band activity was isolated in 24/32

588 participants. This may be related to the fact that the sensorimotor mu rhythm is not always observed
589 (5).

590 **Visual and sensorimotor PAF estimates**

591 Previous studies using the center-of-gravity (CoG) method to estimate PAF have shown that the
592 choice of frequency window can markedly influence results (55,58,65). Some studies have employed
593 relatively narrow windows (9-11 Hz) to minimize the influence of the aperiodic 1/f component of the
594 EEG frequency spectrum (17–19,52), while other studies have used larger windows from 7 to 11 Hz
595 (57), 7.4 to 12 Hz (56) or 7.5 to 13 Hz (58). In the present study, the CoG was computed using both
596 narrow (9-11 Hz) and wide (7-13 Hz) windows to assess the robustness of the estimates. Additionally,
597 CoG estimates computed on the subtracted spectra are expected to be minimally influenced by the
598 1/f aperiodic component, as this component should be largely cancelled by the subtraction
599 procedure. Finally, we also used the FOOOF method to remove the aperiodic component and
600 estimate PAF using a Gaussian fit rather than computing the center of gravity (62).

601 Both for the visual PAF and the sensorimotor PAF, there was a strong correlation between the
602 different PAF estimates, indicating good consistency between estimation techniques.

603 Interestingly, and regardless of the method used to estimate PAF, the interindividual variations in
604 visual PAF were not significantly correlated with the interindividual variations in sensorimotor PAF.
605 This indicates that the two reflect at least partially distinct functional processes. Supporting this
606 distinction, previous studies have shown that the mu-rhythm and alpha rhythm can differ in
607 frequency and amplitude despite occupying the same frequency band (66). In light of these
608 observations, future research should investigate whether the previously reported association
609 between PAF and pain sensitivity (18,19,21,57,67) are predominantly driven by visual- versus
610 sensorimotor-related alpha-band activity.

611 **Lack of association between PAF and the susceptibility to develop secondary hyperalgesia**

612 Our study found no significant association between either visual or sensorimotor PAF and the
613 susceptibility to develop HFS-induced secondary hyperalgesia. This suggests that the frequency
614 characteristics of alpha-band activity do not play a modulatory role in the induction of central
615 sensitization. Notably, recent studies examining whether cognitive factors that are known to
616 modulate pain perception – such as attention and expectations – may also influence the
617 susceptibility to develop experimentally-induced central sensitization have reported mostly negative
618 results (68,69). Collectively, this suggests that the propensity to develop central sensitization may be
619 minimally influenced by cognitive factors and the functional state of the brain.

620 Whether this absence of association between PAF and secondary hyperalgesia is specific for HFS-
621 induced hyperalgesia in healthy volunteers, or whether it generalizes to other experimental methods
622 to induce hyperalgesia, as well as to hyperalgesia in patients remains an open question requiring
623 further studies.

624 Finally, it is important to acknowledge that the sample size in our study was based on prior work
625 reporting relatively large correlation coefficients. As such, our study was underpowered to detect
626 smaller effect sizes.

627 **Association between visual PAF and habituation at the non-sensitized forearm**

628 An incidental finding of the present study was a negative association between visual PAF and
629 perceptual habituation to pinprick stimulation. Specifically, participants with a higher visual PAF
630 showed a more pronounced decrease in pinprick ratings at the non-sensitized forearm. This raises an
631 intriguing possibility: could previous findings linking a lower PAF with greater pain sensitivity be
632 partially explained by this relationship between PAF and habituation? If individuals with a lower PAF
633 habituate less to repeated or sustained nociceptive stimuli as compared to individuals with a higher
634 PAF, they would also be expected to report higher average pain ratings when exposed to such
635 stimuli. Such an interpretation would also be compatible with the fact that the studies having shown
636 a relationship between PAF and sensitivity to pain have relied on methods inducing sustained pain

637 such as capsaicin-heat pain, NGF injection, and tonic heat pain (18–20,67), while studies employing
638 more phasic pain stimuli have reported no or only marginally-significant associations between PAF
639 and pain ratings (70).

640

641 **CONCLUSION**

642 In this study conducted in healthy volunteers, interindividual variations in visual or sensorimotor PAF
643 were not significantly associated with differences in the susceptibility to develop HFS-induced
644 secondary hyperalgesia. This suggests that PAF does not modulate the propensity to develop central
645 sensitization. However, exploratory analyses revealed that individuals with a higher visual PAF
646 tended to exhibit stronger perceptual habituation to pinprick stimulation. Further studies are needed
647 to confirm this incidental observation, and examine whether it might contribute to the previously
648 reported association between PAF and pain sensitivity.

649

650

651 **DATA AVAILABILITY:**

652 Source data are available at https://doi.org/10.6084/m9.figshare.29145725

653

654 **SUPPLEMENTAL MATERIAL:**

655 Supplemental Fig. S1 & Table S1-S2 DOI: https://doi.org/10.6084/m9.figshare.29145695

656

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665

666 **DISCLAIMERS**

667 The statements and opinions presented here reflect the author's view and neither IMI nor the
668 European Union, EFPIA, or any Associated Partners are responsible for any use that may be made of
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670

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674

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676 interpreted results of experiment, prepared figures, drafted manuscript, approved final version of
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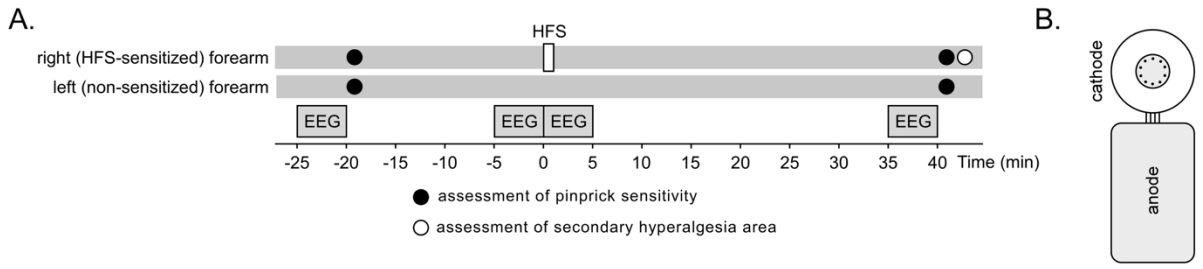

Figure 1. A. Timeline of the experiment. HFS was applied to the right volar forearm. EEG segments lasting 5 minutes and including three conditions (eyes open at rest, eyes closed at rest, eyes open while performing bilateral finger movements) were recorded 25 and 5 minutes before HFS (T-25 and T-5), immediately after HFS, and approximately 35 minutes after HFS (T+35). Pinprick sensitivity of the left and right volar forearms was assessed before HFS (T-20) and approximately 40 minutes after HFS (T+40). The spatial extent of the HFS-induced area of secondary hyperalgesia was assessed approximately 40 minutes after HFS (T+40). **B.** HFS was delivered to the forearm skin using a cathode consisting of 10 blunt tungsten pins arranged on a circle with a diameter of 5 mm and a 24x20 mm anode conductive gel pad placed next to the cathode.

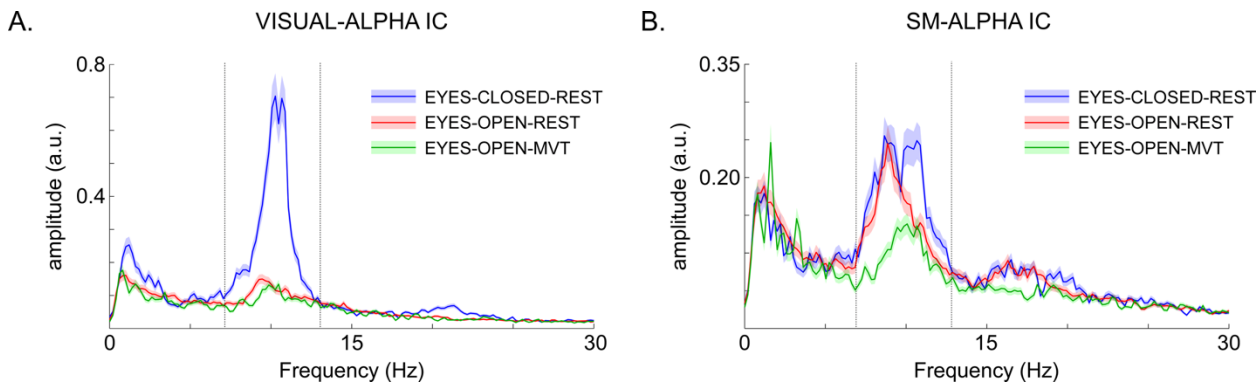

Figure 2. **A.** EEG frequency spectrum of a representative independent component (IC) categorized as capturing vision-related alpha-band activity (VIS-ALPHA) that was increased during closure of the eyes (EYES-CLOSED-REST condition) as compared to the eyes open conditions (EYES-OPEN-REST and EYES-OPEN-MOVEMENT). **B.** EEG frequency spectrum of a representative IC categorized as capturing sensorimotor-related alpha-band activity (SM-ALPHA) that was decreased during finger movements (EYES-OPEN-MVT condition) as compared to rest (EYES-OPEN-REST and EYES-CLOSED-REST conditions). Y-axis: arbitrary units. X-axis: frequency in Hz. The vertical grey lines represent the 6 Hz frequency window used to assess the alpha-band activity.

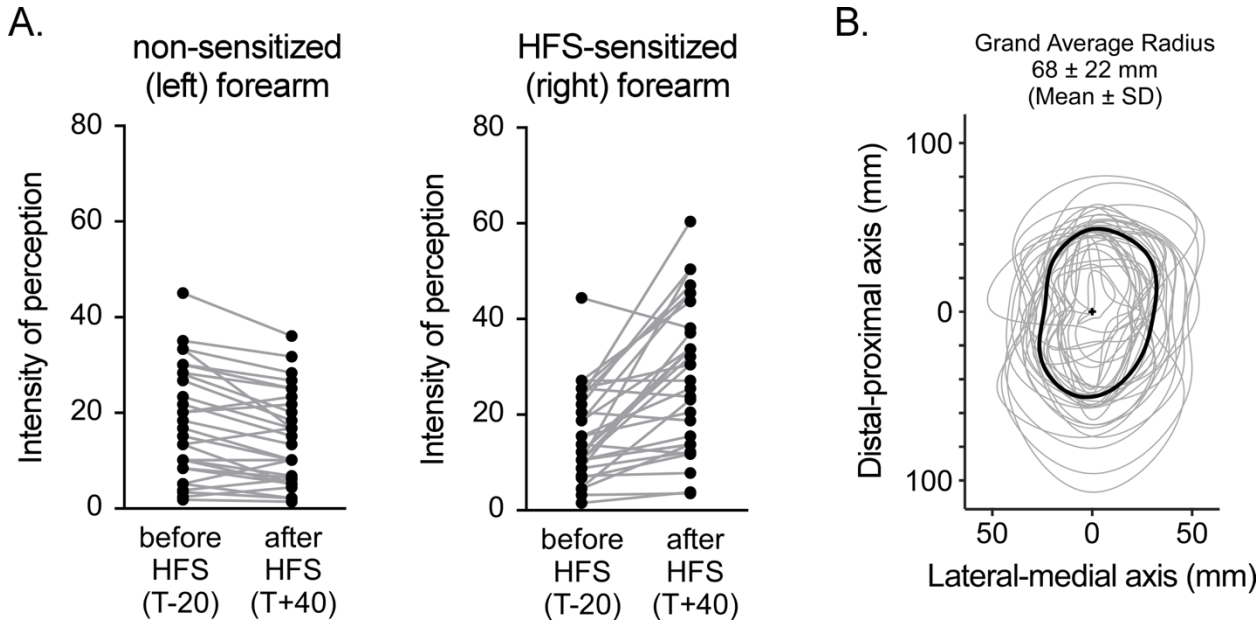

Figure 3. **A**. Individual intensity ratings for pinprick stimuli delivered to the HFS-sensitized forearm and the contralateral non-sensitized forearm, before and after HFS (T-20 and T+40). Note the clear increase in pinprick ratings at the sensitized forearm due to HFS-induced sensitization, and the decrease in pinprick ratings at the non-sensitized forearm possibly due to perceptual habituation. **B**. Estimated area of HFS-induced secondary hyperalgesia (grey contours: individual participants; black contour: group-level average). The origin (+) corresponds to the center of the cathode of the HFS electrode.

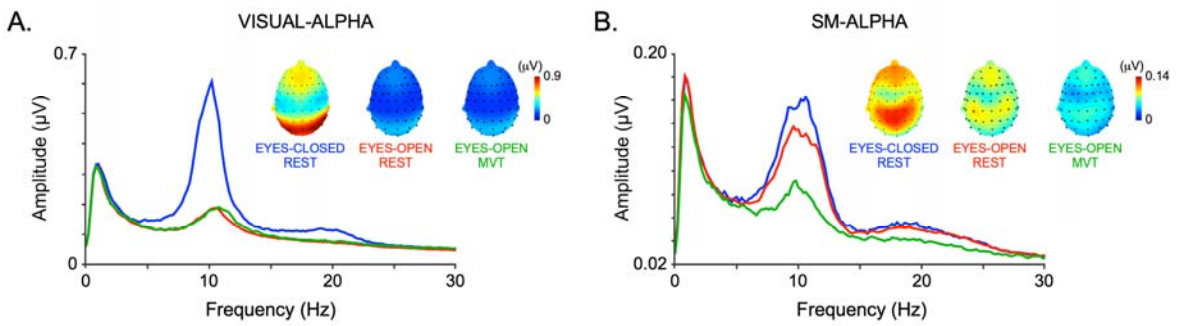

Figure 4. **A.** Group-level average frequency spectra of the ICA-separated EEG signals capturing vision-related alpha-band activity (VISUAL-ALPHA signals) at occipital electrodes in the EYES-CLOSED-REST (blue), EYES-OPEN-REST (red) and EYES-OPEN-MVT (green) conditions. Note the clear increase in alpha-band amplitude during closure of the eyes. Also note the occipital topography of the peak. **B.** Group-level average frequency spectra of the ICA-separated EEG signals capturing sensorimotor-related alpha-band activity (SM-ALPHA signals) at central electrodes. Note the selective decrease of amplitude during finger movements, and the bilateral central-parietal topography compatible with activity originating from the left and right sensorimotor cortices.

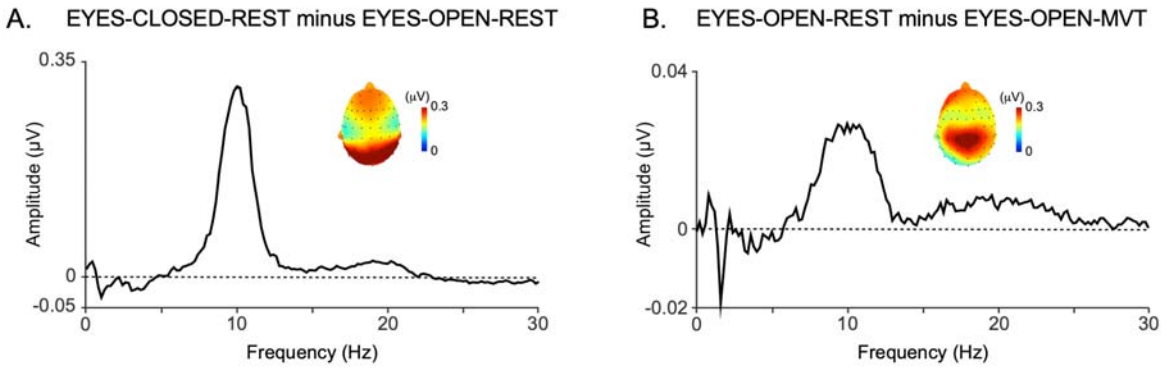

Figure 5. **A.** Group-level average of the subtracted EEG frequency spectra EYES-CLOSED-REST minus EYES-OPEN-REST conditions at occipital electrodes. Note the clear occipital topography of the peak indicating that this approach effectively isolated vision-related alpha-band activity. **B.** Group-level average of the subtracted frequency spectra EYES-OPEN-REST minus EYES-OPEN-MVT conditions at central electrodes. Note the bilateral central topography of the peak indicating that this approach succeeded in isolating sensorimotor-related alpha-band activity. Also note the lack of an 1/f decay (i.e. the aperiodic component of the EEG).

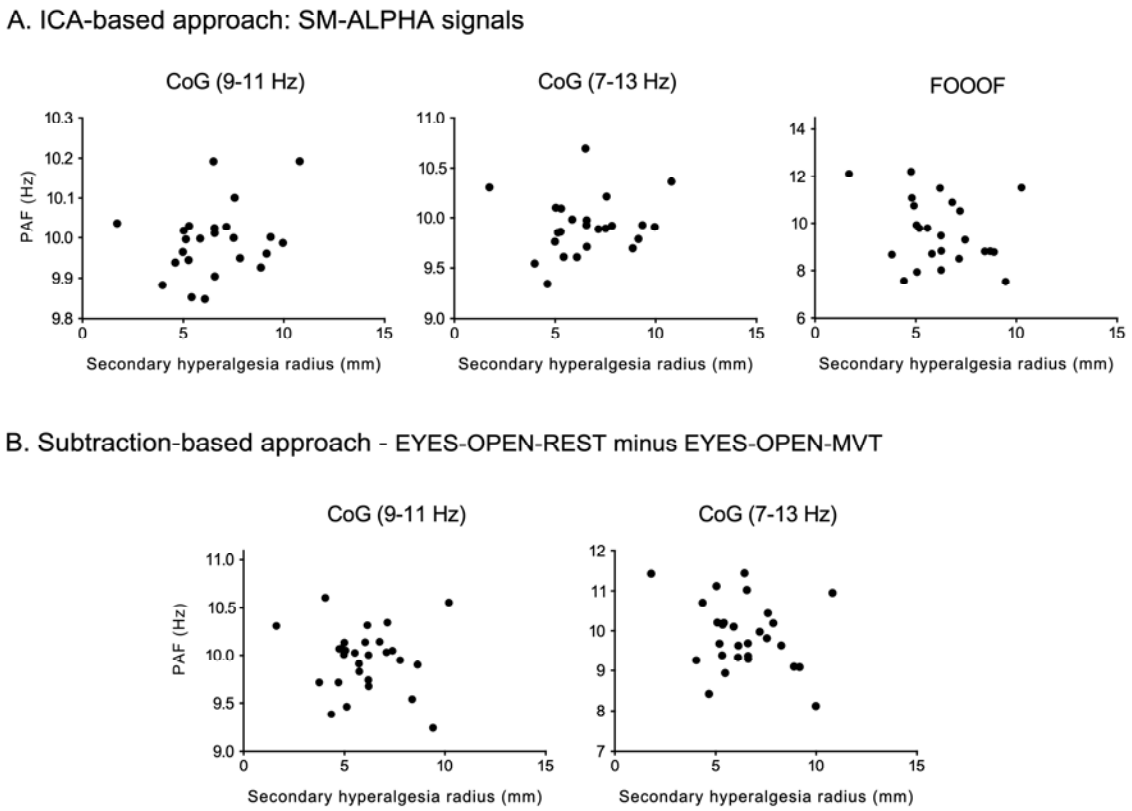

Figure 6. Scatter plots showing the absence of a significant Spearman's correlation between the radius of the HFS-induced area of secondary hyperalgesia and the sensorimotor peak alpha frequency (PAF), regardless of the method used to isolate sensorimotor-related activity (A: ICA-based approach; B: subtraction-based approach) or the method used to estimate the PAF (center of gravity using a narrow 9-11 Hz or wide 7-13 Hz frequency window, FOOOF algorithm).

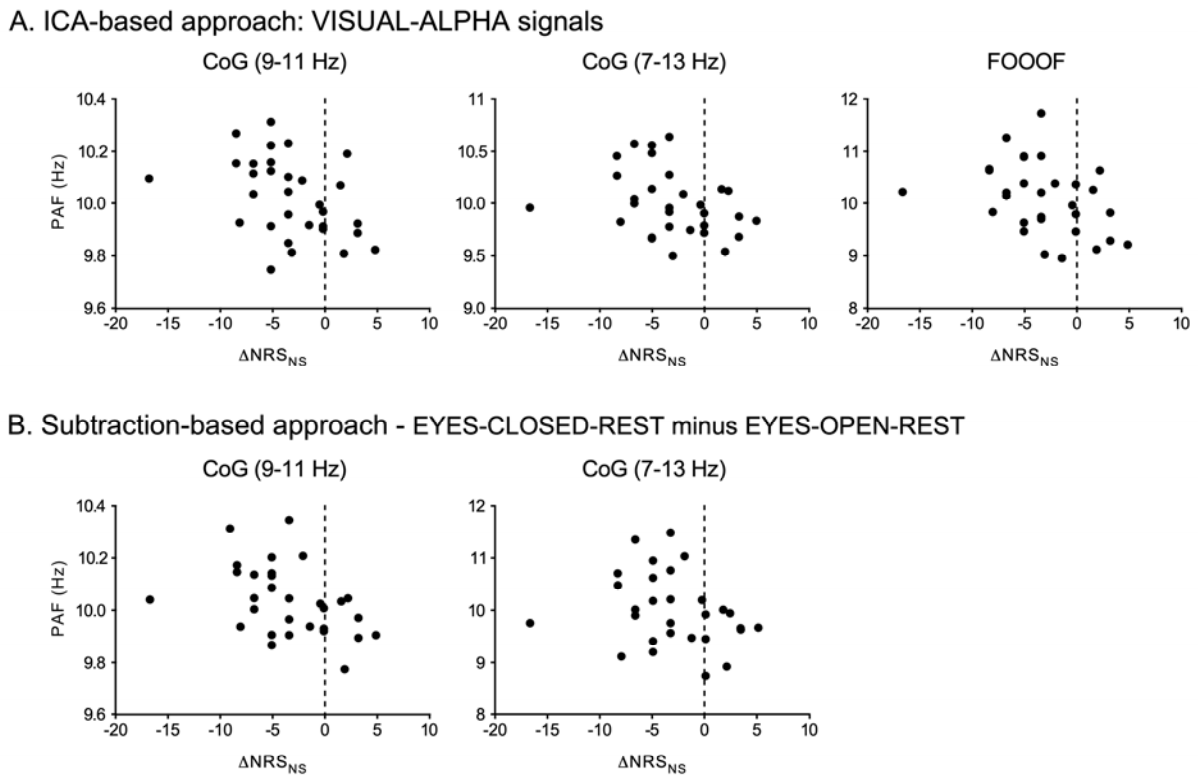

Figure 7. Scatter plots showing the Spearmans's correlation between the post-HFS decrease in pinprick sensitivity at the non-sensitized forearm and the visual peak alpha frequency (PAF), present across methods used to isolate visual-related activity (A: ICA-based approach; B: subtraction-based approach) and methods to estimate the PAF (center of gravity using a narrow 9-11 Hz or wide 7-13 Hz frequency window, FOOOF algorithm).