Neural markers of nociceptive input and pain intensity coding: a signal detection approach

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Abstract

The N2 and P2 vertex waves are the largest electroencephalographic (EEG) responses evoked by transient nociceptive stimuli. N2 and P2 amplitudes often correlate with both stimulus energy and pain intensity. However, studies using paradigms that dissociated afferent input from stimulus salience suggested the N2 and P2 waves primarily reflect supramodal salience, rather than being selective for nociception or pain. Here we investigated how well EEG responses to nociceptive laser stimuli discriminate stimulus intensities and subjective pain in healthy human volunteers. Specifically, we applied signal detection theory (SDT) to calculate how well single-trial amplitudes of stimulus-related EEG responses (contralateral N1 wave, vertex N2 and P2 waves, and gamma-band oscillations) discriminate between two laser energies ($\text{neuro-d}'_{\text{obj}}$), and between participants' reports of "high" or "low" pain intensity ($\text{neuro-d}'_{\text{subj}}$). As a control, we applied the same procedure to similar brain potentials evoked by non-painful electrical stimulation. Participants' perceptual sensitivity to stimulus intensity (d') was similar between modalities, indicating that laser and electrical stimulus discriminations were similarly difficult. Laser-evoked N1, N2 and P2 waves were sensitive to stimulus intensity, whereas the same waves evoked by electrical stimulation showed minimal sensitivity. Inter-individual differences in laser-evoked N1, N2 and P2 $\text{neuro-d}'_{\text{obj}}$ also predicted inter-individual variability in nociceptive sensitivity (d'). Together, these findings show that the laser-evoked potential—elicited by stimuli at long, variable intervals—reflects nociceptive intensity discrimination. Further, the laser-evoked N2 wave captured trial-by-trial variability in pain perception that was unrelated to stimulus intensity. This indicates that the N2 carries particular information about subjective pain intensity.

Keywords: gamma, human, laser, pain, signal detection theory, vertex potential

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Significance Statement

56 Nociceptive laser stimuli evoke a sequence of electroencephalographic (EEG) responses in
57 the time domain: a contralateral N1 followed by N2 and P2 waves centered on the vertex.
58 Here, we used signal detection theory (SDT) to quantify the sensitivity of these waves to
59 nociceptive input and subjective pain intensity while participants discriminated between two
60 nociceptive laser stimulus intensities. The N1, N2 and P2 waves were sensitive to laser
61 stimulus intensity, and reflected the participants' ability to discriminate between stimulus
62 intensities (d'). Together, these findings show that the laser-evoked potential reflects
63 nociceptive intensity discrimination. Additionally, the N2 wave captured trial-by-trial variability
64 in perceived pain intensity that was unrelated to stimulus intensity, indicating that the N2
65 carries additional information about subjective pain level.

Introduction

Pain is a ubiquitous sensory and affective experience, yet it has proven difficult to quantify. Pain measurement is especially problematic in those who cannot report their sensations, such as infants or adults lacking verbal capacity. The search for an objective physiological marker of pain has thus attracted considerable interest (Brodersen et al., 2012; Brown et al., 2011; Davis et al., 2017; Huang et al., 2013; Kucyi and Davis, 2015, 2016; Mouraux and Iannetti, 2018; Smith et al., 2017; Wager et al., 2013). In this study, we used signal detection theory (SDT; Green and Swets, 1966) to identify electrophysiological responses sensitive to the intensity of transient nociceptive stimuli causing pain. Such markers would inform our understanding of sensory aspects of acute pain, including how discriminative capacities of nociceptive pathways change under conditions of disease, pharmacological manipulation, expectation, and cognitive reappraisal. We also looked for neural markers sensitive to idiosyncratic determinants of pain intensity that are independent of sensory processing, and thus fundamental to subjective aspects of pain.

The N2 and P2 vertex waves of the laser-evoked potential (LEP; Beydoun et al., 1993; Bromm and Lorenz, 1998; Chapman et al., 1981; Garcia-Larrea et al., 1997; Iannetti et al., 2005; Kakigi et al., 1989) and gamma-band oscillations (GBOs; Gross et al., 2007; Hu and Iannetti, 2019; Li et al., 2016; Peng et al., 2014; Schulz et al., 2011, 2015; Zhang et al., 2012) have all been proposed as physiological markers of pain intensity. However, N2 and P2 amplitudes are largely determined by supramodal stimulus salience (Iannetti et al., 2008; Mouraux and Iannetti, 2009; Ronga et al., 2013), which, in most experimental circumstances, is tightly coupled with afferent input (Mouraux and Iannetti, 2018). By contrast, laser-induced GBOs over contralateral somatosensory cortex seem to be a more faithful correlate of perceived pain, both within (Zhang et al., 2012) and across subjects (Hu and Iannetti, 2019).

A precise assessment of nociceptive stimulus or subjective pain intensity coding in these electrophysiological signals requires an approach that can distinguish sensory

processing—notably, the capacity to discriminate between higher and lower levels of noxious stimulation—from other factors that contribute to pain reports. We used a well-established SDT model for distinguishing between perceptual sensitivity and bias (Green and Swets, 1966). SDT has been used to characterize the sensitivity of single neurons to visual stimulus properties (Bradley et al., 1987; Britten et al., 1992; Celebrini and Newsome; 1994, Tolhurst et al., 1983; Vogels and Orban, 1990; Zohary et al., 1990). Here, we used SDT to characterize the sensitivity of electrophysiological signals to both nociceptive stimulus energy (neuro- d'_{obj}) and subjective pain intensity (neuro- d'_{subj}). In one session, participants discriminated between two levels of laser stimulation above the threshold for activating A δ -nociceptive afferents. In another session, the same participants discriminated between two levels of non-painful transcutaneous electrical stimulation, which primarily activates A β -mechanoreceptive afferents (Mouraux et al., 2010; Sang et al., 2003). We recorded their electroencephalogram (EEG) during both sessions to measure single-trial N1, N2 and P2 amplitudes and GBO magnitudes. Then we calculated neurometric sensitivity to stimulus energy (neuro- d'_{obj}) and to participants' intensity reports (neuro- d'_{subj}) for each electrophysiological component. We compared these neurometric sensitivities to participants' perceptual sensitivity to stimulus intensity (d').

This design allowed us to evaluate several potential neural markers of nociceptive stimulus and pain intensity coding according to pre-specified criteria (Table 1). First, a viable electrophysiological marker of sensory intensity coding should be sensitive to stimulus energy, and that sensitivity should correlate with participants' perceptual sensitivity. Second, an electrophysiological component preferentially indexing nociceptive intensity coding should exhibit higher neurometric sensitivity to nociceptive laser stimulus intensities than to non-nociceptive electrical stimulus intensities, and a stronger correlation with perceptual sensitivity to laser stimulus intensity than with perceptual sensitivity to electrical stimulus intensity. Third, a marker of subjective pain intensity coding should show sensitivity to

participants' pain intensity reports, beyond any sensitivity to actual laser stimulus energy (i.e., $\text{neuro-d}'_{\text{subj}} - \text{neuro-d}'_{\text{obj}} > 0$).

Table 1. Criteria for evaluating potential electrophysiological markers of sensory intensity coding, nociceptive intensity coding, and subjective pain intensity coding.

Construct	Criteria that a putative marker should fulfill
Sensory stimulus intensity coding	<ol style="list-style-type: none"> 1. Sensitive to (nociceptive or non-nociceptive) stimulus intensity ($\text{neuro-d}'_{\text{obj}} > 0$) 2. Neurometric sensitivity correlated with perceptual sensitivity across participants ($\text{neuro-d}'_{\text{obj}} \propto d'$)
Nociceptive stimulus intensity coding	<ol style="list-style-type: none"> 1. More sensitive to nociceptive stimulus intensity than to non-nociceptive intensities ($\text{laser neuro-d}'_{\text{obj}} > \text{electrical neuro-d}'_{\text{obj}}$) 2. Stronger correlation across participants between neurometric sensitivity and perceptual sensitivity to nociceptive stimuli, compared to correlation for non-nociceptive stimuli ($\text{laser neuro-d}'_{\text{obj}} - d' \text{ corr} > \text{electrical neuro-d}'_{\text{obj}} - d' \text{ corr}$)
Subjective pain intensity coding	<ol style="list-style-type: none"> 1. More sensitive to participants' pain intensity reports than to nociceptive stimulus intensity ($\text{laser neuro-d}'_{\text{subj}} > \text{laser neuro-d}'_{\text{obj}}$)

Materials and Methods

Participants

Seventy-seven participants were recruited to participate in the study through the participant database of the Institute of Cognitive Neuroscience at University College London (UCL). Eligibility criteria included being 18-40 years of age, not having sensitive skin, dark skin (for laser safety reasons) or a dermatological condition, and not having taken any analgesic medications within 24 hours prior to the experiment. Five participants did not complete the study because their A δ -nociceptor stimulation thresholds (described below

under Procedure) were too high to safely set two discriminable levels of radiant heat stimulation within the A δ -nociceptive range. Another two participants voluntarily chose to withdraw from the study before completing the second session. A further eleven experimental sessions had to be aborted because of failure of either the laser, electrical stimulator, or EEG equipment. Additionally, the EEG data of two participants could not be analyzed because of excessive muscle artifacts and high frequency noise. Thus, our study is based on 57 participants' data. Those participants were between 19 and 37 years old ($M = 24.42$ years, $SD = 4.77$ years). All were right-handed, 37 were female and 20 were male. Participants gave written informed consent to participate in the study, which was approved by the UCL Research Ethics Committee (0847/004) and carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki).

Materials

Radiant heat stimulation was delivered to the left hand dorsum with a skin temperature feedback-controlled CO₂ laser stimulation device (wavelength: 10.6 μm , spot size: 6 mm diameter; LSD, SIFEC, Ferrières, Belgium), which selectively stimulates epidermal free nerve endings of A δ - and C-nociceptive afferents. Each laser stimulus lasted 100 ms, including temperature ramp-up (~80 ms) and plateau. Transcutaneous electrical stimulation of the superficial radial nerve was delivered by a DS5 isolated bipolar current stimulator (Digitimer, Welwyn Garden City, UK) connected by clip leads to two disposable self-adhesive electrodes placed at the inner left wrist, with the cathode approximately 2.5 cm proximal to the anode. Each electrical stimulus was a square-wave pulse lasting 10 ms. Stimulus duration was determined in pilot-testing to evoke vertex potentials similar in mean amplitude to those evoked by the laser, but without causing a sensation of pain.

Radiant heat and electrical stimulation were controlled by analog voltage signals to the laser device and electrical stimulator, respectively. A computer running Labview 2012 (National Instruments, Austin, TX, USA) was used to control stimulus delivery and record participant responses from a standard numeric keypad.

EEG was recorded with an ActiveTwo 64-scalp channel system (Biosemi, Amsterdam, Netherlands) connected to a second computer. Five additional Ag/AgCl electrodes were positioned around the eyes to record electrooculogram (EOG) for artifact removal (at the outer canthi of the eyes for horizontal EOG, and above and below the right eye for vertical EOG). Another electrode was placed on the nose for offline re-referencing. DC offset on all channels was kept within ± 40 μ V. The signals were digitized with an online sampling rate of 1024 Hz. We also recorded skin temperature during laser stimulation and current output of the stimulator during electrical stimulation.

Procedure

The experiment took place over two sessions. These were run on consecutive days at the same time of day, to minimize any effects of diurnal variation in perception (Glynn and Lloyd, 1976; Strian et al., 1989). In one session, participants discriminated between two intensities of radiant heat stimulation that caused a sensation of “pinprick” on the left hand dorsum. Both radiant heat intensities were above the threshold for stimulating A δ -nociceptive afferents (see below). In the other session, participants discriminated between two intensities of transcutaneous electrical stimulation of the superficial radial nerve, resulting in a tapping or tingling sensation on the left hand dorsum. All participants reported that neither electrical stimulus intensity felt painful. Session order was counterbalanced between participants.

Radiant heat stimulation session

Participants were first exposed to a single radiant heat stimulus at 45 °C to familiarize them with the sensation. Next, they completed a 3-down/1-up staircase procedure to determine their individual threshold for A δ -nociceptor activation. On each staircase trial, a single laser stimulus was delivered after a random delay between 10 and 15 s, and the participant was instructed to react to the stimulus as soon as they felt it by pressing a key. Starting at 38 °C, the stimulus temperature increased after every reaction time (RT) slower

than 650 ms, and decreased after three consecutive RTs faster than 650 ms at the same stimulus temperature. This 650-ms criterion provides a valid, objective measure of A δ -nociceptor activation, based on the difference in conduction velocity between A δ - and C-nociceptive afferents (Churyukanov et al., 2012; Mouraux et al., 2003). The staircase step size started at 4 °C, then reduced to 2 °C after the first reversal, and then to 1 °C after the second reversal. The staircase was terminated after 8 reversals, and the stimulus temperature at the final 6 reversals were averaged to obtain the A δ -nociceptor threshold ($M = 48.76$ °C, 95% CI = [47.98, 49.55]).

Next, the experimenter delivered one radiant heat stimulus 2 °C above the individual's A δ -nociceptor threshold, and another stimulus 6 °C above the threshold. The participant was told that these were the low and high intensities of laser stimulation, respectively. They then completed a practice block of 20 trials. In each trial, a single laser stimulus at either the low or the high intensity was delivered, followed by a beep after approximately 2 s signaling the participant to make a response. The participant reported the intensity of each stimulus by pressing one of two response keys. Each stimulus intensity was delivered 10 times in a randomized order. There was a random inter-trial interval between 10 and 15 s. If the participant made fewer than 65% correct responses, the high laser stimulus intensity was increased by 1 °C, the two intensities were re-demonstrated, and the practice block was run again. If the participant made more than 85% correct responses, the low laser stimulus intensity was increased by 1 °C, the two intensities were re-demonstrated, and the practice block was run again. This procedure continued until two intensities that yielded 65-85% correct responses ($M = 3.85$ °C, 95% CI = [3.70, 4.00]) were identified.

After setting up the EEG recording, participants completed the main experiment, which consisted of 6 blocks (20 trials each) of the same intensity discrimination task they performed in the practice block. Low and high stimulus intensities were not adjusted further. However, if participants made fewer than 65% correct responses in any block, the two

intensities were re-demonstrated to them before they started the next block. Participants were given breaks of 2-3 min between blocks.

Transcutaneous electrical nerve stimulation session

Participants were first exposed to a single electrical stimulation of the superficial radial nerve at 0.8 mA to familiarize them with the sensation. Next, they completed a 3-down/1-up staircase procedure to determine their individual detection threshold for the electrical stimulation. On each staircase trial, a single electrical stimulus was delivered after a random delay between 10 and 15 s, followed immediately by a beep. After the beep, the participant pressed one key if they felt the stimulus, or another key if they did not feel anything. Starting at 0.4 mA, the stimulus current increased after every missed stimulus, and decreased after three stimuli at the same intensity were detected consecutively. The staircase step size started at 0.4 mA, then reduced to 0.2 mA after the first reversal, and then to 0.1 mA after the second reversal. The staircase was terminated after 8 reversals, and the stimulus current intensities at the final 6 reversals were averaged to obtain the detection threshold ($M = 0.56$ mA, 95% CI = [0.49, 0.63]).

Next, the experimenter delivered one electrical stimulus at 3 times the individual's detection threshold, and another stimulus at 4 times the detection threshold. The participant was told that these were the low and high intensities of electrical stimulation, respectively. The participant was asked whether either stimulus was painful. If they responded that a stimulus was painful, then it was lowered in steps of 0.1 mA until it was no longer painful. Participants then completed a practice block of 20 trials. In each trial, a single electrical stimulus at either the low or the high intensity was delivered, followed by a beep after approximately 2 s signaling the participant to make a response. The participant pressed one key if they thought it was the low intensity electrical stimulus, or another key if they thought it was the high intensity electrical stimulus. Each stimulus intensity was delivered 10 times in a randomized order. There was a random inter-trial interval between 10 and 15 s. If the participant made fewer than 65% correct responses, the high electrical stimulus intensity

was increased by 0.1-0.2 mA, the two intensities were re-demonstrated, and the practice block was run again. If the participant made more than 85% correct responses, the low laser stimulus intensity was increased by 0.1-0.2 mA, the two intensities were re-demonstrated, and the practice block was run again. This procedure continued until it identified a difference between the two intensities that yielded 65-85% correct responses ($M = 0.56$ mA, 95% CI = [0.49, 0.64]).

After setting up the EEG recording, participants completed the main experiment, which consisted of 6 blocks (20 trials each) of the same intensity discrimination task they performed in the practice block. Low and high stimulus intensities were not adjusted during the main experiment. If participants made fewer than 65% correct responses in any block, the two intensities were re-demonstrated to them before they started the next block. Participants were given breaks of 2-3 min between blocks.

EEG preprocessing and single-trial amplitude/power estimation

EEG preprocessing was done in MATLAB 2015a (Mathworks, Natick, MA, USA) using the EEGLAB toolbox, version 13.6.5 (Delorme and Makeig, 2004). EEG signals were re-referenced offline to the nose channel, high pass-filtered at 0.5 Hz, segmented into epochs from -500 to 1000 ms relative to stimulus onset, and baseline-corrected by subtracting the pre-stimulus period from -500 to 0 ms. For radiant heat stimulation, stimulus onset was considered the time at which skin temperature reached the individual's A δ -nociceptor stimulation threshold on each trial. For transcutaneous electrical stimulation, stimulus onset was the rising edge of the square-wave pulse. Electrical stimulation produced an artifact in the EEG signal that was removed by linearly interpolating the epoched signal from -5 to 20 ms.

For analysis in the time domain, the data were low pass-filtered at 30 Hz. An independent components analysis (ICA) was used to identify and remove components associated with eye blinks and movement artifacts. In a further artifact rejection stage, we

rejected any epochs with a signal exceeding ± 80 μ V from EOG channels, or a signal exceeding ± 100 μ V from any scalp channel. To extract single-trial peak N2 and P2 amplitudes, we first identified the latencies of the maximal negative and positive peaks in the grand average evoked potential recorded from Cz between 85 and 650 ms after stimulus onset, with the restriction that the positive peak should always follow the negative peak. Then, for each individual trial, we searched a window of ± 75 ms around the grand average N2 latency for the most negative amplitude, and a window of the same length around the grand average P2 latency for the most positive amplitude. These values were recorded as the N2 and P2 amplitudes evoked on that trial.

The lateralized N1 component can be difficult to separate from the larger N2 component of the vertex potential. Thus, before analyzing the N1 component, we removed ICA components associated with the N2 and P2 vertex waves (i.e., components showing a centrally distributed scalp topography maximal at the vertex, with time courses of negative/positive activity consistent with N2/P2 latencies; see Hu et al., 2010). We also re-referenced channel T8—contralateral to the stimulated hand—to Fz, a standard montage for detecting the N1 in LEP studies (Cruccu et al., 2008). Note that a similar component maximal from electrodes overlying the contralateral parieto-temporal cortex has been found in brain potentials evoked by electrical stimulation of the fingers or the wrist (Allison et al., 1992; Garcia-Larrea et al., 1995; Kida et al., 2004; Kunde and Treede, 1993; Treede et al., 1988). Next, we identified the latency of the maximal negative peak in the grand average evoked potential recorded from T8 between 85 and 200 ms after stimulus onset. For each individual trial, we searched a window of ± 75 ms around that latency for the most negative amplitude, which was recorded as the single-trial N1.

For analysis in the time-frequency domain, the data were low pass-filtered at 100 Hz. Artifact rejection proceeded as described above, using ICA and automated epoch rejection based on amplitude thresholds. A time-frequency decomposition from 4-100 Hz in 4-Hz steps was run in the FieldTrip toolbox (Oostenveld et al., 2011) We used a Fourier transform

with a fixed 250-ms Hanning window that slid from -500 ms to 1000 ms in steps of 20 ms. The resulting single-trial power spectrograms were expressed as the absolute change in power from baseline (-400 to -100 ms relative to stimulus onset). We ran a cluster-based permutation test (1000 randomizations; test $\alpha = .05$, cluster $\alpha = .001$) on the time-frequency gamma-band data (30-100 Hz) from the laser stimulation condition to identify time and frequency windows of laser-induced GBOs over contralateral somatosensory cortex. That test found no positive clusters of GBO enhancement relative to the pre-stimulus baseline. However, since we had a strong a priori hypothesis about a relation between pain intensity and GBOs, we selected frequency (60-92 Hz) and time (100-300 ms) windows of interest based on previous studies that found a correlation between perceived pain intensity and GBOs over contralateral somatosensory cortex (Gross et al., 2007; Schulz et al., 2011; Zhang et al., 2012). We calculated the average power within these windows of interest from electrode C6 to obtain single-trial values of GBOs.

Calculation of SDT measures

For behavioral responses, trials in which the participant responded “high” to the higher stimulus energy were counted as “hits” and trials in which the participant responded “high” to the lower stimulus energy were counted as “false alarms”. Perceptual sensitivity (d') and bias (C) were calculated using the following standard formulas (Green and Swets, 1966):

$$d' = Z(\text{hit rate}) - Z(\text{false alarm rate})$$

$$C = -0.5 * [Z(\text{hit rate}) + Z(\text{false alarm rate})]$$

We then calculated neurometric sensitivities to physical stimulus energy (neuro- d'_{obj})—analogous to perceptual d' , but based on splitting the continuous distribution of component amplitudes into a “high” and a “low” category. Each participant’s single-trial N1, N2, P2, and GBO amplitudes were first sorted by magnitude. N1 and N2 amplitudes were sign-reversed so that a more positive value indicated a larger amplitude. We then set a range of plausible amplitude cutoffs for each participant, session, and component to convert the amplitudes

from graded to categorical (“high”/“low”) responses (Fig. 1A). A single-trial amplitude was considered a “high” response if above the cutoff, or a “low” response if below the cutoff. Cutoffs were chosen based on the percentage of trials labeled a “low” response versus a “high” response in each session (5-95%, in steps of 1%). Trials in which a “high” component amplitude was evoked by the higher stimulus energy were counted as “hits”, whereas trials in which a “high” component amplitude was evoked by the lower stimulus energy were counted as “false alarms”. We then calculated d' at each cutoff according to the standard formula. We considered the mean d' across all cutoffs to be the $\text{neuro-}d'_{\text{obj}}$ of that component (Fig. 1B).

Figure 1. Illustration of the procedure used to estimate the neurometric sensitivity ($\text{neuro-}d'$) of stimulus-related EEG components, showing the P2 component as an example. A) For each participant, stimulus modality (laser or electrical stimulation), and EEG component (N1, N2, P2, and GBOs), multiple cutoffs were chosen to subdivide the distribution of single-trial component amplitudes into “high” and “low” response categories. B) Measures of neurometric sensitivity to physical stimulus energy ($\text{neuro-}d'_{\text{obj}}$) and to the participant’s intensity reports ($\text{neuro-}d'_{\text{subj}}$) were then calculated at each amplitude cutoff. For the actual analysis, we set a range of cutoffs based on the percentage of trials categorized as a “low” versus a “high” response (5-95%, in 1% steps). We then took the average $\text{neuro-}d'$ as our

measure of neurometric sensitivity for that component. Note that these images are for illustrative purposes only, and do not show the full range of amplitude cutoffs used, nor the final results of the complete dataset.

To quantify information about subjective intensity perception, we also calculated neurometric sensitivity to participants' intensity reports ($\text{neuro-d}'_{\text{subj}}$) for N1, N2, P2, and GBOs. This analysis used the same procedure as described above, except that a trial was counted as a "hit" if the categorical label of the amplitude/power value and the participant's own response were both "high". A trial was considered a "false alarm" if the amplitude/power value was labeled "high" but the participant responded "low".

Experimental design and statistical analysis

Statistical tests were performed in JASP 0.8.6.0 (JASP Team, 2018). We used two-tailed one-sample t -tests against 0 ($\alpha = .05$) to test whether stimulus-evoked N1, N2, P2, and GBO components were sensitive to the physical energy of the stimulus ($\text{neuro-d}'_{\text{obj}}$). Two-tailed paired-samples t -tests assessed whether the same components were more sensitive to participants' intensity reports ($\text{neuro-d}'_{\text{subj}}$) than to physical stimulus energy ($\text{neuro-d}'_{\text{obj}}$). We also used paired samples t -tests to compare perceptual sensitivity (d'), perceptual bias (C), neurometric sensitivities ($\text{neuro-d}'_{\text{obj}}$), and mean component amplitudes between the nociceptive laser stimulation session and the innocuous electrical stimulation session. We report means and 95% confidence intervals (CIs) in the figures and the text.

Pearson correlation tests examined relations between inter-individual differences in perceptual sensitivity, neurometric sensitivity ($\text{neuro-d}'_{\text{obj}}$), and bias. Correlations were corroborated using Spearman's rank order method, which is more resistant to outliers. Two-tailed Steiger's Z tests implemented in R package "cocor" (Diedenhofen and Musch, 2015) were used to compare Pearson correlation coefficients between laser and electrical stimulus modalities.

Results

Perceptual sensitivity (d') and bias (C) of human judgements

We used paired samples t -tests to compare the perceptual sensitivity (d') and bias (C) of human volunteers between stimulation sessions. There was no difference between perceptual sensitivity (d') to nociceptive laser stimulus intensity ($M = 1.54$, 95% CI = [1.40, 1.67]) and non-painful electrical stimulus intensity ($M = 1.59$, 95% CI = [1.46, 1.72]), $t(56) = -0.51$, $p = .613$, Cohen's $d_z = -0.07$. This indicates that our stimulus intensity calibration procedure successfully matched high/low stimulus discriminability between the laser stimulation and electrical stimulation sessions. There was also no difference in bias (C) between the laser stimulation session ($M = 0.30$, 95% CI = [0.21, 0.39]) and the electrical stimulation session ($M = 0.22$, 95% CI = [0.12, 0.31]), $t(56) = 1.58$, $p = .119$, Cohen's $d_z = 0.21$. This implies that participants were not more inclined to respond "high" to one stimulus modality than the other.

We also used Pearson correlation tests to investigate associations between individual differences in perceptual sensitivity (d') and bias (C) across sessions. There was no correlation between sensitivity to laser stimulus intensity and sensitivity to electrical stimulus intensity, $r = -0.15$, $p = .268$ (Fig. 2A). This reinforces the idea that perception of laser and electrical stimuli relied on separate afferent pathways. There was, however, a positive correlation between biases in intensity reports across sessions, $r = 0.33$, $p = .013$ (Fig. 2B). This suggests that our bias measure reflects stable inter-individual, supramodal differences in the tendency to report higher or lower intensities of sensation, regardless of the actual intensity or modality of somatosensory stimulation. Spearman's rank order correlation tests showed the same pattern of results (d' : $\rho = -0.10$, $p = .451$; C: $\rho = 0.38$, $p = .003$).

Figure 2. Correlations across participants between signal detection theory measures obtained from the laser stimulation and electrical stimulation sessions. A) Correlations in perceptual sensitivity (d'). B) Correlations in bias (C). Note that a positive bias indicates a higher criterion for reporting “high” intensity, and thus a lower probability of “high” responses.

Comparison of mean EEG responses evoked by laser and electrical stimulation

Figure 3A shows the average nociceptive laser-evoked potentials (LEPs) and non-nociceptive electrical somatosensory-evoked potentials (SEPs), separately for low intensity and high intensity stimuli. We used paired-samples t -tests to compare the single-subject mean N1, N2, and P2 amplitudes between stimulus modalities. There was no difference between the laser-evoked ($M = 14.38 \mu V$, 95% CI = [12.97, 15.78]) and electrically evoked N1 amplitudes ($M = 15.41 \mu V$, 95% CI = [13.97, 16.84]), $t(56) = -1.25$, $p = .216$, Cohen's $d_z = -0.17$. The laser-evoked N2 ($M = 18.44 \mu V$, 95% CI = [16.96, 19.90]) was larger than the electrically evoked N2 ($M = 14.89 \mu V$, 95% CI = [13.28, 16.50]), $t(56) = 5.40$, $p < .00001$, Cohen's $d_z = 0.72$. On the other hand, the laser-evoked P2 ($M = 24.73 \mu V$, 95% CI = [22.86, 26.60]) was *smaller* than the electrically evoked P2 ($M = 30.56 \mu V$, 95% CI = [28.14, 32.99]), $t(56) = -7.36$, $p < .00001$, Cohen's $d_z = -0.98$.

Figure 3. Stimulus-related EEG signals. A) Grand-average nociceptive laser-evoked potential (left) and electrical somatosensory-evoked potential (right), including N1, N2, and P2 components. Evoked potentials are shown separately for low and high stimulation levels. See text for explanation of epoching. B) Scalp maps showing changes in gamma-band power induced by nociceptive laser stimulation (left) and innocuous electrical stimulation (right) within the time (100-300 ms) and frequency (60-92 Hz) regions of interest. The red box indicates electrode C6 over the contralateral somatosensory cortex.

Figure 3B shows scalp maps of baseline-corrected GBOs within the time (100-300 ms) and frequency (60-92 Hz) windows of interest (WOI). The signal from electrode C6, over the

somatosensory cortex contralateral to the stimulus side, was used for GBO analyses (see red box in Fig. 3B). A Shapiro-Wilk test indicated that GBO power differences between laser and electrical stimulation conditions were not normally distributed ($p < .001$), so we used a non-parametric Wilcoxon signed rank test to compare GBO power between laser and electrical stimulation. This test showed that laser-induced GBO power was higher than electrically induced GBO power, $W(56) = 1204$, $p = .003$, $r = 0.46$. In fact, electrical stimulation induced a reduction in GBO power ($M = -0.05 \mu V^2$, 95% CI = $[-0.09, -0.02]$), whereas laser stimulation left GBO power unchanged ($M = 0.00 \mu V^2$, 95% CI = $[-0.04, 0.04]$).

Neurometric sensitivity to stimulus intensity (neuro- d'_{obj})

We used one-sample t -tests to test whether stimulus-evoked N1, N2, P2, and WOI-defined GBO components were sensitive to the physical energy of the stimulus (neuro- d'_{obj}). N1, N2, and P2 LEP components were all sensitive to laser stimulus intensity [N1: $t(56) = 3.46$, $p = .001$, Cohen's $d_z = 0.46$; N2: $t(56) = 6.66$, $p < .00001$, Cohen's $d_z = 0.88$; P2: $t(56) = 14.69$, $p < .00001$, Cohen's $d_z = 1.95$], whereas laser-induced GBOs showed no such sensitivity, $t(56) = 0.19$, $p = .851$, Cohen's $d_z = 0.02$. Only the P2 SEP component was sensitive to electrical stimulus intensity, $t(56) = 7.50$, $p < .00001$, Cohen's $d_z = 0.99$. N1, N2, and GBO components showed no sensitivity to the physical energy of the electrical stimulus [N1: $t(56) = 1.36$, $p = .180$, Cohen's $d_z = 0.18$; N2: $t(56) = 0.60$, $p = .554$, Cohen's $d_z = 0.08$; GBOs: $t(56) = -0.88$, $p = .385$, Cohen's $d_z = -0.12$].

Next, we used paired samples t -tests to compare neurometric sensitivities between laser stimulation and electrical stimulation sessions. (Fig. 4A). Both the laser-evoked N2 and P2 components were more sensitive to laser stimulus energy than the corresponding electrically evoked N2 and P2 components were to electrical stimulus energy [N2: $t(56) = 4.79$, $p = .00001$, Cohen's $d_z = 0.63$; P2: $t(56) = 8.13$, $p < .00001$, Cohen's $d_z = 1.08$]. There was no difference between the neuro- d'_{obj} of the laser-evoked N1 and the neuro- d'_{obj} of the electrically evoked N1, $t(56) = 1.69$, $p = .097$, Cohen's $d_z = 0.22$, nor between the neuro- d'_{obj} values obtained from laser-induced and electrically induced GBOs, $t(56) = 0.77$, $p = .446$,

Cohen's $d_z = 0.10$. These results demonstrate that laser-evoked N2 and P2 vertex waves were more sensitive to laser stimulus intensity than the electrically evoked N2 and P2 waves were to electrical stimulus intensity. On the other hand, the N1 and GBOs showed little or no sensitivity to the intensity of either type of stimulation.

Figure 4. Neurometric sensitivities of pre-defined EEG components of interest: N1, N2, P2, and GBOs. A) Neurometric sensitivities to actual stimulus intensity (neuro- d'_{obj}), showing comparisons between laser and electrical stimulation. B) Correlations between perceptual sensitivity (d') and the neurometric sensitivity of each component to stimulus energy (neuro- d'_{obj}). C) Neurometric sensitivities to subjective perception of intensity, i.e., the difference between each component's sensitivity to participants' intensity reports (neuro- d'_{subj}) and its sensitivity to physical stimulus energy (neuro- d'_{obj}). Laser stimulation data are shown in light gray, and electrical stimulation data are shown in dark gray. Error bars show 95% confidence intervals.

Correlations between perceptual sensitivity (d') and neurometric sensitivity (neuro- d'_{obj})

We used Pearson correlations to investigate the relation between perceptual sensitivity and neurometric sensitivity to the energy of laser and electrical stimulation (Fig. 4B). Participants' perceptual sensitivity to laser stimulus intensity (d') correlated with the corresponding neurometric sensitivities (neuro- d'_{obj}) of the laser-evoked N1 ($r = 0.37$, $p = .005$), N2 ($r = 0.28$, $p = .036$), and P2 ($r = 0.66$, $p < .00001$). There was no correlation between perceptual sensitivity to laser stimulus intensity and the corresponding neurometric sensitivity of laser-induced GBOs ($r = 0.14$, $p = .304$). By contrast, there were no correlations between participants' perceptual sensitivity to electrical stimulus intensity and the corresponding neurometric sensitivities of any of the EEG responses to electrical stimulation (N1: $r = 0.01$, $p = .948$; N2: $r = 0.18$, $p = .167$; P2: $r = 0.18$, $p = .192$; GBOs: $r = 0.00$, $p = .996$). This suggests that LEPs track participants' ability to discriminate stimulus intensity levels, whereas GBOs and electrically evoked SEPs do not.

We repeated our correlation tests using Spearman's rank order method, which is more resistant to outliers. Those tests showed the same pattern of results. Participants' perceptual sensitivity to laser stimulus intensity (d') correlated with the corresponding neurometric sensitivities (neuro- d'_{obj}) of the laser-evoked N1 ($\rho = 0.36$, $p = .006$), N2 ($\rho = 0.28$, $p = .032$), and P2 ($\rho = 0.63$, $p < .00001$). There was no correlation between perceptual sensitivity to laser stimulus intensity and the corresponding neurometric sensitivity of laser-induced GBOs ($\rho = 0.08$, $p = .576$). There were no correlations between participants' perceptual sensitivity to electrical stimulus intensity and the corresponding neurometric sensitivities of any of the EEG responses to electrical stimulation (N1: $\rho = 0.01$, $p = .920$; N2: $\rho = 0.11$, $p = .426$; P2: $\rho = 0.15$, $p = .265$; GBOs: $\rho = -0.06$, $p = .648$).

We then used Steiger's Z tests to formally ascertain whether the neurometric sensitivities of EEG responses to laser stimulation (neuro- d'_{obj}) correlated more strongly with participants' perceptual sensitivity (d') than the corresponding neurometric sensitivities of

EEG responses to electrical stimulation did. The neurometric sensitivities of the laser-evoked N1 and P2 waves tracked participants' ability to discriminate laser stimulus intensities better than the corresponding electrically evoked components tracked their ability to discriminate electrical stimulus intensities [N1: $Z = 1.98$, $p = .048$; P2: $Z = 3.24$, $p = .001$]. On the other hand, the neurometric sensitivities of the N2 wave and GBOs did not track perceptual sensitivity to laser intensity better than perceptual sensitivity to electrical stimulus intensity [N2: $Z = 0.52$, $p = .604$; GBOs: $Z = 0.71$, $p = .476$].

Neurometric sensitivity to subjective perception of intensity (neuro- d'_{subj})

To quantify the amount of information each EEG component carried about subjective pain level, over and above the component's sensitivity to physical stimulus energy, we used paired samples t -tests to compare each component's sensitivity to participants' intensity reports (neuro- d'_{subj}) with its sensitivity to the physical energy of the stimulus (neuro- d'_{obj} ; Fig. 4C). The laser-evoked N2 was more sensitive to participants' intensity reports than to physical stimulus energy, $t(56) = 2.46$, $p = .017$, Cohen's $d_z = 0.33$. None of the other EEG responses to laser stimulation were differentially sensitive to subjective and objective intensity [N1: $t(56) = 0.24$, $p = .807$, Cohen's $d_z = 0.03$; P2: $t(56) = 0.15$, $p = .885$, Cohen's $d_z = 0.02$; GBOs: $t(56) = 0.92$, $p = .363$, Cohen's $d_z = 0.12$]. This suggests that the N2 LEP component of LEPs carries some unique information about subjective pain level, beyond that which is attributable to the stimulus itself. Surprisingly, electrically induced GBOs were *negatively* sensitive to participants' intensity reports (i.e., GBOs were lower on trials where participants reported feeling a higher intensity stimulus), $t(56) = -3.35$, $p = .001$, Cohen's $d_z = -0.44$. None of the electrically evoked SEP components were differentially sensitive to subjective and objective intensity [N1: $t(56) = 0.15$, $p = .879$, Cohen's $d_z = 0.02$; N2: $t(56) = 1.68$, $p = .100$, Cohen's $d_z = 0.22$; P2: $t(56) = -1.23$, $p = .223$, Cohen's $d_z = -0.16$].

Discussion

We developed a neurometric SDT approach to identify electrophysiological markers of nociceptive stimulus intensity and pain intensity coding (Table 1). We adapted the standard SDT measure of perceptual sensitivity (d') to estimate measures of neurometric sensitivity to stimulus energy (neuro- d'_{obj})—i.e., how well each EEG response discriminated between high vs low levels of nociceptive radiant heat stimulation, or between levels of non-painful transcutaneous electrical nerve stimulation in a control condition. Importantly, we never required participants to discriminate between a nociceptive and a non-nociceptive level of stimulation, or to detect “pain”. Instead, we used a signal detection approach to investigate intensity coding *within* identified nociceptive and non-nociceptive sensory pathways. Although previous studies have searched for neural markers of pain (Brodersen et al., 2012; Brown et al., 2011; Davis et al., 2017; Gross et al., 2007; Hu and Iannetti, 2019; Huang et al., 2013; Kucyi and Davis, 2015, 2016; Li et al., 2016; Mouraux and Iannetti, 2018; Peng et al., 2014; Schulz et al., 2011, 2015; Smith et al., 2017; Wager et al., 2013; Zhang et al., 2012), ours is the first to combine three important methodological features. First, we defined nociceptive laser stimulus energies relative to an objective measure of A δ -nociceptor activation, (Churyukanov et al., 2012; Mouraux et al., 2003), rather than a subjective report of pain. Second, we studied the percept resulting from laser stimulation using an SDT method that separates sensitivity from bias. Third, we studied the percept with respect to its intensity dimension, rather than with respect to the presence/absence of any specific sensory label of “pain”. This last point means that our task does not depend on a criterion or threshold for an individual to apply the label “pain”.

We found that LEPs show discriminative coding of nociceptive stimulus intensities. Interestingly, the N2 and P2 LEP waves were more sensitive to laser stimulus intensity than the N2 and P2 SEP waves were to innocuous electrical stimulus intensity (Fig. 4A). Moreover, participants who were better able to distinguish between lower and higher levels of laser stimulation also tended to have N1, N2, and P2 LEP components with higher

neurometric sensitivities to laser stimulus energy (Fig. 4B). By contrast, we found no such relation between perceptual sensitivity to electrical stimulation and the neurometric sensitivities of the electrically evoked N1, N2, and P2 components. Direct comparisons between stimulus modalities showed that the laser-evoked N1 and P2 components tracked participants' perceptual sensitivity to nociceptive intensities better than the electrically evoked N1 and P2 tracked perceptual sensitivity to non-painful stimulus intensities. Together, these results suggest that LEPs index nociceptive stimulation levels within the spinothalamic A δ afferent pathway better than SEPs index somatosensory stimulation levels within the lemniscal A β pathway. The laser-evoked P2 showed the strongest evidence for nociceptive intensity coding, according to our pre-determined criteria (Table 1).

Since we only compared noxious radiant heat with innocuous electrical stimulation, we cannot determine whether this effect is exclusive to nociceptive processing, or whether it might be present in other stimulus modalities. Visual or auditory stimulation, for example, might evoke vertex potentials with neurometric sensitivity similar to, or even better than, the neurometric sensitivity of the laser-evoked potential. Likewise, we cannot rule out the possibility that the relatively long electrical stimulus duration we used may have precluded detection of electrophysiological components that index processing within the A β lemniscal pathway. For instance, we could not measure the neurometric sensitivities of earlier electrical stimulus-evoked components such as the N20 or the P30 (Treede and Kunde, 1995) because of the duration of our stimuli and the difficulty in resolving these smaller components on single trials. Despite these limitations, our results indicate that, when laser stimuli are delivered at long and variable intervals, the laser-evoked potential carries information about intensity coding within the nociceptive system. This point is pertinent given evidence that the vertex potential contains non-specific components related to stimulus salience (Iannetti et al., 2008; Mouraux and Iannetti, 2009). Of course, salience and intensity are often related, with stronger stimuli typically being more salient. Both properties could be proxies for a common survival-relevant dimension of threat.

Of all the electrophysiological components we evaluated, only the laser-evoked N2 wave fulfilled the criteria for a neural marker of subjective pain intensity coding (Table 1). The N2 component contained information about participants' pain intensity reports, over and above that which was attributable to the physical energy of the laser stimulus itself (Fig. 4C). Using partial correlations, Carmon and colleagues (1980) similarly found that LEP amplitude correlated better with perceived pain intensity than with laser stimulus energy. However, they took a measure of the area under the whole waveform, rather than separately testing the amplitudes of individual LEP components, which may reflect different underlying processes. Our study refines this previous result by demonstrating that the N2 LEP component, specifically, discriminates between subjective pain intensities better than laser stimulus energies, whereas the N1 and P2 components are similarly sensitive to both subjective and objective intensity.

Some have proposed that the laser-evoked N2 arises from activity in bilateral operculo-insular areas (including secondary somatosensory cortex, SII) (Frot et al., 1999; Garcia-Larrea et al., 2003; Peyron et al., 2002; Tarkka and Treede, 1993; Valeriani et al., 1996), and the contralateral primary somatosensory cortex (Ohara et al., 2004b; Schlereth et al., 2003). We found that a possible marker of activity in these brain regions carries information about idiosyncratic determinants of subjective pain intensity. Importantly, our finding cannot establish a causal role for the N2 or its neural generators in pain perception. Although our result is consistent with the idea that the SII and posterior insula are involved in sensory pain discrimination (Bornhøvd et al., 2002; Frot et al., 2007; Garcia-Larrea, 2012; Hofbauer et al., 2001; Iannetti et al., 2005; Lockwood et al., 2013; Mazzola et al., 2006; Ohara et al., 2004a; Ostrowsky et al., 2002; Timmermann et al., 2001), there is plenty of evidence indicating that these brain areas do not play a specific or exclusive role in pain perception (e.g., Davis et al., 2015; Iannetti and Mouraux, 2010; Mouraux and Iannetti, 2018).

Numerous prior studies found a correlation between GBOs and perceived pain (e.g., Gross et al., 2007; Hu and Iannetti, 2019; Li et al., 2016; Peng et al., 2014; Schulz et al., 2011, 2015; Zhang et al., 2012). However, we found no evidence of laser-induced GBOs, nor did we find any relation between GBO power and either subjective pain intensity or nociceptive stimulus energy. A recent study that used painful mechanical pinprick stimulation also failed to find any stimulus-induced GBOs attributable to brain activity (van den Broeke et al., 2017). Another study recorded intracerebral laser-induced GBOs from the insula that were specific to nociceptive stimulation, but challenged the idea that those GBOs necessarily correlate with perceived pain intensity (Liberati et al., 2018). Both those studies and the present findings cast doubt on the characterization of GBOs as an *obligatory* measure of graded pain intensity. The exact relation between pain intensity perception and GBOs may depend upon particular properties of the nociceptive stimulus, such as stimulus modality and duration, rather than tracking pain perception in the same way across all contexts. The neural source of GBOs and their method of measurement may be other important determinants of their relation to pain intensity (Liberati et al., 2018).

On the other hand, we found that an electrically induced GBO desynchronization was more sensitive to perceived stimulus intensity than to actual stimulus energy. That is, a larger reduction in GBO power was associated with a report of “high” non-painful stimulus intensity from the participant. This shows that the electrically induced reduction in GBO power contained information about the subjective perception of innocuous somatosensory stimulation. This finding was not predicted, and should be taken with caution. Future studies may determine whether a relation between electrically induced GBO desynchronization and innocuous somatosensory intensity perception is reliable and functionally significant.

In sum, we have developed a measure of neurometric sensitivity (neuro-d') which estimates the sensitivity of EEG signal features to properties of sensory stimuli. Using this method, we have shown that LEP amplitudes—especially the N2 and P2 components—discriminate intensities of nociceptive stimulation transmitted by the A δ nociceptive pathway,

and they do so better than electrically evoked SEPs discriminate intensities of innocuous somatosensory stimulation. Further, the ability of the laser-evoked N1, N2, and P2 waves to discriminate between higher and lower nociceptive laser stimulus energies correlated well with the corresponding discriminative abilities calculated from participants' behavioral responses. This indicates that LEPs contain more information about nociceptive processing than SEPs contain about somatosensory processing, at least with the kinds of stimuli we used here (delivered at long and variable intervals). Interestingly, only the N2 LEP component contained information about subjective perception of pain intensity, over and above information attributable to the objective difference between lower and higher laser stimulus energies. This suggests that the N2—uniquely amongst the EEG components evoked by nociceptive laser stimulation that we investigated here—codes a subjective aspect of pain perception.

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