Abstract

Integration of nociceptive information is essential to produce adapted responses, in order to promote body integrity and survival. However, how the brain integrates nociceptive inputs from different body areas remains unknown. The aim of the present study was to examine the cortical integration of bilateral nociceptive inputs evoked by laser heat stimuli. Sixteen healthy volunteers (8 F, 8 M; age: 25.5 ± 4.3) were recruited to participate in one session during which painful laser stimuli were applied to their hands with two Nd:YAP laser systems. Electroencephalographic activity was recorded to measure laser-evoked potentials and event-related spectral perturbations. Twenty nociceptive stimuli were applied in each of the four counterbalanced conditions: 1) right hand 2) left hand, and both hands with 3) attention to the right or 4) attention to the left. Compared with unilateral conditions, N2 and P2 peak amplitude as well as gamma oscillation power were decreased in bilateral conditions ($p<0.05$), but these effects were not affected by the direction of attention ($p>0.1$). In contrast, pain was not significantly different in any condition ($p>0.05$). These findings show that although more nociceptive inputs reach the brain with multiple nociceptive stimuli, their sensory representation is decreased while pain perception remains unchanged. These interactions between cerebral processing of nociceptive information from different body regions could support coordinated behavioral responses when pain origins from multiple sources.

Cortical integration of bilateral nociceptive signals: when more is less.

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Number of text pages: 25
Number of figures: 4
Number of tables: 1

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Running title: Cortical integration of nociceptive signals

Keywords: Pain, nociception, concurrent stimulation, laser-evoked potentials, time-frequency, cortical integration

1. Introduction

Cerebral integration of sensory information is critical for perception and behavior. This was shown for the visual [41], auditory [40; 41] and somatosensory [44] systems. Although integration of multiple nociceptive inputs is essential to produce adapted responses and promote body integrity and survival, this has been largely overlooked.

Laser heat stimulation is an established method to investigate the nociceptive system [49]. Nd:YAP lasers produce laser-evoked potentials (LEPs), including the N1, N2, and P2 [15; 33; 45; 50; 51]. They also produce event-related spectral perturbations (ERSPs), including increased power below 10 Hz and in the gamma range between 150 and 400 ms, as well as suppression of alpha and beta power between 300 and 1000 ms [36]. While LEP may not reflect pain-related activity per se, but rather stimulus saliency [16; 31; 39], they are a useful tool to examine the cerebral integration of nociceptive inputs. As for ERSPs evoked by painful stimuli, it was suggested that gamma synchronization is related to pain intensity [36], attentional capture by pain [23; 47] and may modulate the impact of spiking neurons on their target [10].

Both LEPs and ERSPs can be modulated by bottom-up (e.g. stimulus saliency) and top-down processes (e.g. selective attention). For instance, increasing stimulus intensity leads to increases in both the N2 and P2 peak amplitude [22]. In contrast, distraction away from the painful stimulus leads to reduced N2 [2; 9; 21] and P2 [2; 21] peak amplitude. Besides, stimulus intensity and selective attention influence responses in alpha and gamma bands, while responses in delta and beta bands are affected by stimulus intensity only [11]. However, how the integration of nociceptive information induced by concurrent bilateral stimulation would be reflected in these brain responses remains unknown.

Studies on somatosensory integration indicate that responses to non-painful somatosensory stimulation in the somatosensory cortex are modulated when multiple stimuli are applied concurrently [3; 13; 32; 43; 44]. For instance, suppressive interference was reported in SI when tactile, electrical, or vibrotactile stimuli were applied bilaterally on both upper limbs compared with unilateral stimulation [44]. From a behavioral perspective, studies have shown that concurrent stimuli to homologous body parts increase the detection threshold of tactile stimuli [5]. These findings may also apply to pain perception and pain-related behaviors, but this remains to be investigated.

The aim of the present study was to examine the cortical mechanisms of nociceptive integration when nociceptive stimuli are applied concurrently. Based on the literature on somatosensory processing of non-painful somatosensory stimuli, we hypothesized that LEPs and ERSPs would be attenuated in bilateral compared with unilateral conditions, consistent with a decrease in their relative sensory representation. In accordance with this idea and based on results from a previous study showing that decreased LEP amplitude by saliency manipulation was not associated with a significant change in pain perception [16], we anticipated that pain intensity would remain unaffected, in spite of the increase in nociceptive inputs arising from the periphery.

2. Materials and methods

2.1 Participants

Nineteen healthy volunteers were recruited by an advertisement on the campus of the Université du Québec à Trois-Rivières. All participants gave written informed consent and acknowledged their right to withdraw from the experiment at any time without prejudice. The procedures were approved by the institutional ethical committees and were in accordance with the

declaration of the revised version of Helsinki. Participants were recruited if they were right-handed and between 18 and 50 years old. They were excluded if they reported chronic pain, had a diagnosed psychiatric or neurologic disorder, or took any medication during the 2 weeks prior to their participation. From the 19 participants recruited, only those who felt a clear pricking pain at or before the maximal laser fluence were retained (n=16; 8 women; range 18–35 years; mean: 25.3, SD: 4.4).

2.2 Experimental procedures

Room temperature was kept constant at 23 °C. Participants sat in a chair with both arms on an armrest (inter-limb distance of 70 cm) with hands in a comfortable and stable pronation position. Participants and experimenters wore safety glasses designed for a 1340 nm wavelength laser during the entire duration of the experiment. Participants were instructed to avoid excessive head and body movement.

The experimental paradigm is illustrated in Figure 1. All participants were submitted to four experimental conditions in a counterbalanced order. For two conditions, stimuli were applied unilaterally to the right or left hand while participants were asked to direct their attention towards the stimulation side (unilateral with attention to right hand: UR, unilateral with attention to left hand: UL). For the other two conditions, both hands were stimulated concurrently while participants were asked to direct their attention to the right hand (bilateral stimulation with attention to the right hand: BR) or left hand (bilateral stimulation with attention to the left hand: BL). Each condition included a series of 20 laser stimuli delivered with an inter-stimulus interval of six seconds. To ensure that selective attention was directed to the right or left hand as instructed, participants provided verbal pain ratings after each stimulus, for the attended hand only.

2.3 Pain ratings

A fixation cross was displayed on a computer monitor in front of participants to minimize eye movements. It remained visible for the duration of the experiment, except when pain ratings were prompted by a visual analogue scale with left (0) and right (100) anchors indicating "no pain" and "worse pain imaginable", respectively. Participants were instructed to rate the attended painful stimulus verbally from 0 to 100 when prompted by this scale. The scale always appeared outside the time window of interest for brain activity analyses.

2.4 Painful Laser Stimulation

Painful stimuli were produced by laser heat pulses using two infrared neodymium yttrium aluminum perovskite lasers (Nd:YAP, DEKA 1380, Electronical Engineering, Florence, Italy), one for each hand. This type of stimulation has been shown to activate nociceptors selectively [17; 35]. The laser beam was transmitted through a 10-meter fiber-optic cable. Laser pulse duration was set at 4 ms and the diameter at 4 mm (≈12.5 mm^2 area). Based on safety recommendations for repeated laser stimuli [25], a maximum fluence limit was set at 20 J/cm^2 (i.e. a 2.25 J upper limit for a 4 mm diameter). The lasers were triggered externally using a stimulus presentation software (E-Prime2, Psychology Software Tools, Sharpsburg, PA, USA). To avoid stimulating the same area more than once per condition, ink markers were drawn on the hand dorsum in the superficial radial nerve territory. The in-built helium-neon laser was used for aiming purpose and stimulation distance was kept constant using the mounted guides on the laser probe.

The pain threshold was determined using a staircase method for each hand separately. Before the beginning of the staircase assessment, participants were told to focus on the pinprick (bee sting) sensation and to report pain intensity verbally. Energy output started at the lowest

possible level (0.5 J) and increased sequentially by 0.25 J increments until pain was reported, or up to the 2.25 J upper limit. The energy at which pain was first reported was repeated three times to obtain a reliable pain threshold. To induce a clear painful pinprick sensation, the energy was then adjusted to two increments (0.5 J) over threshold, or to 2.25 J if this upper limit was reached. For each hand, the participant was then familiarized with the selected stimulus intensity using a sequence of five consecutive stimuli with an inter-stimulus interval of six seconds. Pain intensity was reported after each stimulus and averaged for comparison between hands. Pain intensity discrepancies between hands were corrected by adjusting laser intensity to have comparable ratings (increasing or reducing energy output if already at the security threshold). Another series of three consecutive stimuli were then delivered for each hand at the adjusted stimulus intensity to confirm that pain ratings were comparable between hands.

2.5 Electroencephalographic recordings

Electroencephalographic activity (EEG) was measured using a 64-channel BrainVision system with active electrodes mounted on an actiCAP (Brain Products, Gilching, Germany). Electrodes were nose-referenced and the ground was set at FPz. Signals were digitized at 500 Hz with a hardware band-pass filter of 0.01–100 Hz. Eye movements and blinks were recorded using right eye electrooculography (EOG) with electrodes placed at the suborbital ridge and just lateral to the outer canthus.

2.6 Laser-evoked potentials

EEG data were analyzed offline using EEGLAB v.13.5.4b [7]. Data were filtered using a finite impulse response (FIR) band pass filter (0.1–30 Hz), down sampled to 250 Hz, and re-

referenced to the common average. Data were segmented into stimulus-locked epochs from -100 ms to 800 ms, with time 0 corresponding to the onset of laser stimuli. Baseline correction was made using the -100 to 0 ms window. An Infomax independent component analysis (ICA) was applied using the inbuilt EEGLAB function *Runica* to identify and remove components associated with noise (e.g. eye movement, eye blinks, cardiac and muscle artifacts). Baseline corrected epochs were then averaged for each condition separately to extract LEP components of interest, including the N2 and P2 [9; 15; 23; 33; 39]. The N1 component could not be clearly identified in all subjects after re-referencing to electrode Fz and looking at central electrodes. It is therefore not reported. The N2 was defined as the first major negative deflection occurring between 140 and 220 ms with a maximum amplitude at the vertex (Cz) and the P2 was defined as the first major positive deflection occurring between 230 and 350 ms with a maximum amplitude at the vertex (Cz). From the 16 participants that reported pricking pain, three did not have clear N2 and P2 peaks from their average waveforms. The N2 and P2 calculations were thus performed on data from the remaining 13 subjects.

2.7 Time-frequency analysis

Event-related spectral perturbations (ERSPs) [26; 34] were analyzed for each condition. Data were filtered using a FIR band pass filter (1–100 Hz). Data were segmented into stimulus-locked epochs from -2000 to 2600 ms, with time 0 corresponding to the onset of laser stimuli. As for LEPs, an ICA was applied to remove artifacts as described above. A Morlet wavelet convolution [30] was computed using the channel time-frequency options available in EEGLAB v.13.5.4b [7]. Two hundred time points were generated, and 100 linearly spaced frequencies were computed from 1 to 100 Hz. Variable cycles were used for low and high

frequencies, with 3 cycles for lowest frequencies and up to 15 cycles for highest frequencies. This variable number of cycles allows the wavelet convolution method to provide a better frequency resolution at lower frequencies and a better temporal resolution at higher frequencies [7]. ERSP data were computed in decibels relative to the -400 to -100 ms baseline.

ERSPs were computed for all electrodes separately. For each participant, the time-frequency data of all trials were averaged for each condition separately, resulting in four average time-frequency maps for each electrode. From these maps, two types of analyses were conducted. In the first analysis, the mean power in four time-frequency maps were extracted from the Cz electrode in regions of interest (*time* X *frequency*) based on previous studies [36]: from 2 to 10 Hz between 150 and 400 ms, from 8 to 29 Hz between 300 and 1000 ms, from 30 to 60 Hz between 100 and 350 ms, and from 61 to 100 Hz between 150 and 350 ms. As some previous work has identified components in lower gamma frequencies [1; 4], the gamma band was separated as low and high gamma. The ERSP values for each time-frequency point included in the regions of interest were extracted from each subject. A mean ERSP value was then obtained for each subject and regions of interest by selecting and averaging the values with the 20% highest amplitude (for power increases relative to the baseline) or 20% lowest amplitude (for power decreases relative to the baseline in the case of suppression). This procedure has been used in previous studies for EEG data processing [14; 16; 30; 51] and its main advantage is to allow the selection of wide regions of interests to account for variability across subjects, while reducing the regression to the mean problem with near-zero values.

For the second analysis, a data-driven approach was used to test for differences across all time-frequency points between 0 and 1000 ms for the Cz electrode. As no attention effect was observed in previous analyses, this analysis compared unilateral (UR and UL) and bilateral (BR

and BL) conditions. Specific spectral bands were defined as follows: delta (2 to 3 Hz), theta (4 to 7 Hz), alpha (8 to 12 Hz), beta (13 to 29 Hz), low gamma (30 to 60 Hz) and high gamma (61 to 100 Hz). To that end, the cluster correction method was used [27]. Cluster correction is a non-parametric method that limits the multiple comparison problems without being overly conservative. Firstly, for each specific spectral band, the differences between unilateral and bilateral conditions were computed in *t*-values at each time-frequency point. A Monte Carlo permutation analysis with 2000 permutations was used to create a permutation distribution, with the null hypothesis being that the data from both conditions are drawn from similar probability distributions. For a two-tailed *t*-test at alpha-level 0.05, all *t*-values lower or greater than the 2.5^{th} and 97.5^{th} percentile on the permutation distribution were selected. From this selection, temporally and spectrally adjacent *t*-values with similar magnitude and sign were clustered. All the *t*-values comprised in a cluster were summed, and the largest cluster-level statistic was taken as test statistics. Its *p*-value was then calculated under a permutation distribution obtained using the procedure just described. Since the *p*-values for smaller clusters are calculated under the same distribution, this approach reduces the false alarm rate at the expense of reduced sensitivity for smaller clusters. Time-frequency clusters were then explored on a time-frequency-electrode level. Within a given time-frequency cluster, the time and frequency pair with the highest *t*-value was selected for time and frequency plotting across all electrodes. Permutation analysis was performed again, by clustering adjacent electrodes with similar magnitude and sign. The grand average time-frequency map for the group was also obtained for each condition by averaging data across subjects, for illustration purposes.

2.8 Statistical Analysis

Data analysis was conducted using Statistica v13.1 (Dell Inc., Tulsa, OK, USA). All results are expressed as mean ± SEM and statistical threshold was set at $p \leq 0.05$ (two-tailed). Data distribution was assessed for normality with the Kolmogorov-Smirnov test. Sphericity was assessed with Mauchly's test and corrected with the Greenhouse-Geisser correction when appropriate. Pain intensity, N2 and P2 peak amplitude as well as ERSP values were compared between conditions using repeated-measures ANOVA with two within-subject factors, including *Stimulation* (unilateral vs. bilateral) and *Attention* (left vs. right). Effect sizes are reported based on partial eta-squared (η^2_p).

3. Results

3.1 Laser-evoked potentials

Laser heat stimuli produced the expected LEPs in all conditions, including the N2 and P2 components, with a central scalp distribution and a maximum at Cz (see Figure 2). The latencies of N2 and P2 peaks were also as expected and are reported in Table 1.

N2 peak amplitude was strongly decreased in the bilateral compared with unilateral conditions (*main effect:* $F_{1,12} = 14.0$, $p = 0.003$, $\eta^2_p = 0.54$). However, selective attention did not modulate N2 peak amplitude for unilateral and bilateral conditions combined (*main effect:* $F_{1,12} = 0.2$, $p = 0.7$, $\eta^2_p = 0.02$) or between unilateral and bilateral conditions (*interaction:* $F_{1,12} = 2.5$, $p = 0.14$, $\eta^2_p = 0.17$). As for N2 peak latency, it was not significantly different between unilateral and bilateral conditions (*main effect:* $F_{1,12} = 1.3$, $p = 0.3$, $\eta^2_p = 0.10$) and not significantly affected by selective attention for unilateral and bilateral conditions combined (*main effect:* $F_{1,12} = 1.6$, p =

0.3, η^2_p = 0.11) or between unilateral and bilateral conditions (*interaction:* $F_{1,12}$ < 0.01, p = 0.9, η^2_p < 0.01).

Similar effects were observed for P2 peak amplitude that was strongly decreased in the bilateral compared with unilateral conditions (*main effect:* $F_{1,12}$ = 19.5, p < 0.001, η^2_p = 0.62). Also, selective attention did not modulate P2 peak amplitude for unilateral and bilateral conditions combined (*main effect:* $F_{1,12}$ = 2.1, p = 0.17, η^2_p = 0.15) or between unilateral and bilateral conditions (*interaction:* $F_{1,12}$ = 0.1, p = 0.8, η^2_p < 0.01). As for P2 peak latency, it was not significantly different between unilateral and bilateral conditions (*main effect:* $F_{1,12}$ = 0.5, p = 0.5, η^2_p = 0.04), not significantly affected by selective attention for unilateral and bilateral conditions combined (*main effect:* $F_{1,12}$ = 0.2, p = 0.9, η^2_p < 0.01) or between unilateral and bilateral conditions (*interaction:* $F_{1,12}$ = 0.1, p = 0.7, η^2_p = 0.01).

Together these results indicate that cortical integration of concurrent bilateral laser heat stimuli is reflected in decreased N2 and P2 peak amplitude while latency is unaffected.

3.2 Event-related spectral perturbations

Laser heat stimuli evoked robust event-related spectral perturbations in all conditions (see Figure 3a). The mean power increase in the regions of interest of 2–10 Hz or 8–29 Hz was not significantly different between the unilateral and bilateral conditions (*main effect:* $F_{1,15}$ = 0.5, p = 0.5, η^2_p = 0.03 and $F_{1,15}$ = 2.5, p = 0.13, η^2_p = 0.14, respectively). Similarly, selective attention did not modulate power for unilateral and bilateral conditions combined (*main effect:* $F_{1,15}$ = 0.05, p = 0.82, η^2_p = 0.004 and $F_{1,15}$ = 1.14, p = 0.3, η^2_p = 0.07, respectively) or between unilateral and bilateral conditions (*interaction:* $F_{1,15}$ = 0.24, p = 0.63, η^2_p = 0.02 and $F_{1,15}$ = 0.22, p = 0.64, η^2_p = 0.01, respectively). In contrast, low-gamma (30–60 Hz) power was decreased in the bilateral

compared with unilateral conditions (*main effect:* $F_{1,15} = 4.4$, p = 0.05, $\eta^2_p = 0.23$; see Figure 3b). This low-gamma suppression in the bilateral condition affected several scalp regions with a marked difference at central electrodes (see Figure 3c, top row). Besides, selective attention did not produce significant effects for unilateral and bilateral conditions combined (*main effect:* $F_{1,15} = 1.81$, p = 0.2, $\eta^2_p = 0.11$) or between unilateral and bilateral conditions (*interaction:* $F_{1,15} = 1.77$, p = 0.2, $\eta^2_p = 0.11$). Lastly, high-gamma (61–100 Hz) power was strongly suppressed in bilateral compared with unilateral conditions (*main effect:* $F_{1,15} = 14.13$, p = 0.002, $\eta^2_p = 0.48$; see Figure 3b). This suppression also affected several scalp regions with a marked difference at central electrodes (see Figure 3c, bottom row). Again, selective attention did not produce significant effects for unilateral and bilateral conditions combined (*main effect:* $F_{1,15} = 0.94$, p = 0.34, $\eta^2_p = 0.06$) or between unilateral and bilateral conditions (*interaction:* $F_{1,15} = 0.6$, p = 0.45, $\eta^2_p = 0.04$).

Considering these significant effects, it may be expected that the 8-29 Hz oscillations also be modulated. Since the 8-29 Hz suppression usually originates, in part, from electrodes over the sensorimotor cortices, further analyses were performed by clustering electrodes in hemispheres ipsilateral (CP2, CP4, C2, and C4) and contralateral (CP1, CP3, C1, and C3) to stimulation, in order to confirm the results. No main effects of *Hemisphere* (ipsi- vs. contralateral), *Stimulation* or *Attention* and no interactions were observed (all p>0.3, all $\eta^2_p<0.07$).

The permutation analysis with cluster-correction revealed differences between unilateral and bilateral conditions in the alpha, beta and high-gamma frequency bands (see Figure 4a). While alpha power was tonically suppressed from 300 to 1000 ms during unilateral stimulation, an opposite and significantly different pattern emerged from 575 to 825 ms for bilateral stimulation (p = 0.01). For beta power, bilateral stimulation produced a stronger and tonic power suppression at frequencies neighbouring 20 Hz from 270 to 570 ms compared with unilateral stimulation (p =

0.006). For high-gamma power, two widespread clusters corresponding to decreased power for bilateral compared with unilateral conditions were observed between 75 and 85 Hz: one centred at 190 ms ($p = 0.002$), and the other extending between 340 and 575 ms ($p = 0.007$). Permutations with cluster corrections at the electrode level revealed that the differences were mostly distributed at Cz and adjacent electrodes over the sensorimotor cortex (see Figure 4b).

3.3 Pain intensity ratings and stimulus intensity

Participants reported light pain during unilateral (right hand: 8.1 ± 2.0; left hand: 15.5 ± 4.3) and bilateral (attention to right: 8.2 ± 1.6; attention to left: 10.3 ± 2.5) laser stimulation. Mean pain ratings were not significantly different between bilateral compared with unilateral conditions (*main effect:* $F_{1,12} = 3.7$, $p = 0.08$, $\eta^2_p = 0.24$). Moreover, pain ratings were not significantly affected by selective attention (main effect: $F_{1,12} = 1.8$, $p = 0.2$, $\eta^2_p = 0.13$). In addition, selective attention did not significantly modulate pain ratings for bilateral compared with unilateral conditions (interaction: $F_{1,12} = 3.0$, $p = 0.11$, $\eta^2_p = 0.20$). These results are consistent with the individual adjustment of stimulus intensity to produce comparable pain perception on both hands and they indicate that selective attention did not modulate pain. To confirm that stimulus intensity was comparable for each hand although individual adjustment was made using pain ratings, laser power was compared using a paired T-test. Stimulus intensity was not significantly different between the left and the right hands (1.72 ± 0.12 and 1.78 ± 0.11 J, respectively; $T(15) = 1.0$, $p = 0.33$), consistent with the lack of pain rating difference between unilateral conditions and ruling out the possibility that the effects reported above may be due to different stimulus intensity.

4. Discussion

The novel finding of the present study is that cortical integration of bilateral nociceptive inputs is reflected in decreased nociceptive brain activity. To explain this reduction, several mechanisms will be considered below. Nonetheless, these findings suggest that although more nociceptive inputs reached the brain, the sensory representation of stimuli was decreased. These interactions between cerebral processing of nociceptive information from different body regions could support coordinated behavioral responses when pain origins from multiple sources.

4.1 Changes in saliency

The specificity of LEPs to pain perception or to activation of the "pain neuromatrix" was revised in recent years [20; 22]. It was proposed that LEPs most likely reflect a saliency detection system [22]. In the present study, the two nociceptive stimuli were temporally aligned and matched in terms of pain perception. Stimulus intensity, another determinant of stimulus saliency, was also comparable. Considering that LEP amplitude was proposed to represent stimulus saliency [16; 22], we suggest that both stimuli were of comparable saliency, based on their comparable LEP amplitude. Accordingly, we propose that the relative saliency of one stimulus was lower when applied concurrently with the second stimulus. Indeed, saliency depends on how much a stimulus stands out from the sensory background. Thus, the unilateral hand stimulus stood out from a sensory background that was controlled to be minimal. When presented concurrently to the other stimulus, we propose that the saliency of this stimulus was reduced since its sensory background comprised a competing stimulus. To test this hypothesis, future studies could manipulate the saliency of the competing stimulus and thus, the sensory background.

Thus, we propose that concurrent nociceptive information arising from both hands is integrated in the brain, resulting in a weaker sensory representation of each nociceptive stimulus. We also propose that this is critical to generate coordinated behavioral responses to nociceptive inputs from both hands without giving priority to one of the two pain sources. In accordance with this interpretation, repeated application of a nociceptive stimulus with a short inter-stimulus interval leads to decreased LEP amplitude [16]. In this study, series of three laser stimuli were applied on the hand with a 1 s inter-stimulus interval. LEP reduction was observed from the 2nd stimulus, with no further reduction for the 3rd stimulus. This is consistent with the idea that the stimulus was less salient when preceded by the same sensory input. As in the present study, decreased LEP amplitude was not associated with significant changes in pain perception.

Another factor that should be considered is the stimulated body region. This was examined in previous studies using repeated laser stimuli to test whether saliency is spatially specific. In the repeated laser stimulus paradigm mentioned above, when the location of the third stimulus was changed from one hand to the other, no dishabituation of LEPs was observed [48], suggesting that saliency is not spatially specific. Conversely, when the third stimulus changed from the foot to the hand, dishabituation was observed, arguing for spatial specificity [29]. However, this dishabituation was not observed when changing the third stimulus from the hand to the foot, which argues against spatial specificity. To reconcile these discrepancies, we suggest that there is a saliency gradient, where a stimulus with the same characteristics has a different saliency depending on the body region on which it is applied.

4.2 Response suppression

Previous findings indicate that bilateral somatosensory stimuli applied to homologous body parts produce response suppression [13; 32], increase tactile detection threshold [5; 6] and decrease tactile discrimination abilities (reviewed in [44]). Accordingly, some brain areas may integrate information from a body part, regardless of the body side [5]. If so, a suppression of redundant information is expected during bilateral stimulation. This is consistent with the reduction of LEPs during bilateral stimulation in the present study. Coherent with this interpretation, dishabituation does not occur in the repeated stimulus paradigm, if the third stimulus of the series is applied on the contralateral hand [48], as if it was the same body region. In contrast, when two foot stimuli are followed by a hand stimulus, dishabituation occurs [29]. Response suppression alone is unlikely to explain LEP reduction observed in this study. Other mechanisms such as those presented above likely contribute to this effect as well.

4.3 Top-down inhibition

Nociceptive inputs could be modulated in the spinal cord by segmental processes. While this cannot be ruled out, to the best of our knowledge, no mechanism was shown to produce such inhibition with concurrent bilateral A-δ fibre inputs. Nociceptive activity could also be modulated by descending pathways from the cortex or the brainstem. Diffuse noxious inhibitory controls (DNIC) [18] or conditioned pain modulation (CPM) [53] and cortical projections to brainstem regions involved in these mechanisms [8] produce such inhibition. These mechanisms are unlikely to explain the present findings since DNIC and CPM are triggered by tonic stimuli that activate a spino-bulbo-spinal loop [19; 52]. Indeed, this inhibitory system cannot be effective when short concurrent stimuli are applied, since nociceptive inputs are already ascending when inhibitory

feedback reaches their spinal origin. Other top-down mechanisms from cortical to subcortical regions involved in expectations and cognitive control, such as the dorsolateral prefrontal cortex [42], may contribute to the present reduction of LEPs. For example, spatial integration of pain can be dynamically altered by top-down attentional control as shown by the reduction in pain when dividing attention between two painful stimuli delivered 10 cm apart, and by the increase in pain when directing attention to only one of the two stimuli [37]. However, this possibility also seems unlikely considering that pain ratings were unaffected in the present study.

4.4 Brain oscillations

Two approaches were used to compare brain oscillations in bilateral and unilateral conditions. The first one, using predetermined time-frequency regions of interest, revealed a suppression of low- and high-gamma oscillations during bilateral stimulation. The second one, a permutation analysis applied on the whole time-frequency range, revealed suppression of beta oscillations from 270 to 570 ms and of high-gamma oscillations between 180 and 200 ms and between 340 and 575 ms during bilateral stimulation. Considering that oscillations below 10 Hz correspond to the laser-evoked potentials [36], a decrease of 2-10 Hz oscillations was expected in the time window of LEPs. However, it is likely that the hypothesis-driven method, based on a broader time window (150-400 ms), was less sensitive than peak assessment. Also, while gamma and theta oscillations often act as coupled oscillators, previous studies show that gamma oscillations are unaffected by repeated laser stimuli while theta power decreases [54]. Moreover, when comparing attended and unattended painful laser stimuli, gamma power increases while delta/theta power remains similar [11]. The present findings are consistent with these results, indicating a gamma-theta dissociation. The gamma power decrease may reflect changes in

processes related to selective attention and sensory processing as suggested previously [10]. As for the beta suppression, it most likely reflects changes in the motor cortex [38], possibly to prevent movement during the task, as instructed to participants. In contrast to these suppressions, a late alpha power increase between 575 and 825 ms was observed during bilateral stimulation. This may reflect alerting and task-related processing [14].

4.5 Special considerations for gamma oscillations

Recent studies have explored the significance of pain-related gamma oscillations evoked by phasic pain (reviewed in [36]). Using three consecutive laser stimuli, a study reported that gamma oscillations, as opposed to LEPs, are insensitive to habituation. Based on these findings, it was proposed that gamma oscillations reflect the encoding of pain intensity [54]. However, pain reduction by placebo is not associated with changes in gamma oscillations [46], suggesting that gamma oscillations do not simply encode pain intensity, but also possibly context-dependent sensory processing [46]. Furthermore, gamma oscillations recorded intracranially in the insula, an important region of the saliency network [28], strongly habituate following three consecutive laser stimuli [24], suggesting in this case that gamma oscillations reflect saliency. It is likely that the multidimensional pain experience is associated with gamma oscillations from multiple subsystems that possibly overlap in time and space, leading to conflicting results. Future studies are needed to reconciliate these diverging views and provide a better understanding of what represent gamma oscillations and their modulation in specific regions.

Besides, pain-induced gamma oscillations over central areas were reported to be negatively associated with visually induced gamma oscillations over occipital areas [47]. Because gamma oscillations are associated with attentional processes, this effect suggests a transient and

involuntary attentional capture of attention by pain [12]. In the present study, attenuation of gamma power in the bilateral condition may be due to a transient shift in the attended body location. This could reflect a way of reducing the impact of neurons activated by nociceptive inputs on their target [10] to take into account the bilateral origin of nociceptive inputs and thus promote an adapted behavior.

5. Conclusion

In summary, concurrent bilateral nociceptive stimulation leads to reduced laser-evoked potentials and high-gamma oscillation power compared with unilateral stimulation. We propose that this cerebral integration may be essential for coordinated behavioral responses.

Acknowledgements

This work was supported by a grant from the Natural Sciences and Engineering Research Council of Canada (grant #06659) and the Canadian Foundation for Innovation (grant #33731) to MP. The contribution of MP was supported by the research Chair in Pain Neurophysiology from the Université du Québec à Trois-Rivières (UQTR) and the Fonds de Recherche du Québec en Santé (FRQS). The contribution of Nabi Rustamov was supported by the Normand Danis postdoctoral fellowship from the Fondation de Recherche en Chiropratique du Québec and by a postdoctoral fellowship from Fonds de Recherche du Québec en Nature et Technologie (FRQNT). The authors have no financial or other relationship that might lead to a conflict of interest. The authors would also like to thank Simon Schreiber for his help with data collection.

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Figure legends

Figure 1. Experimental paradigm

The four conditions are presented at the bottom of the figure, along with the stabilization procedure. Condition order was counterbalanced. Each condition included 20 stimuli and each trial lasted 6 seconds, during which laser-heat stimuli were applied and pain was rated. The painful laser stimulus is represented by the lightning symbol. Pain ratings were prompted immediately after each stimulus using a visual analogue scale with left (0) and right (100) anchors indicating “no pain” and “worse pain imaginable”, respectively. ISI: inter-stimulus interval.

Figure 2. Laser-evoked potentials.

a: time course of the average laser-evoked potentials for the four conditions. Unilateral conditions are depicted as full lines and bilateral conditions as dashed lines. **b**: scalp topography for the average N2 (top) and P2 (bottom) peaks for all four conditions. **c**: average N2 and P2 peak values for all four conditions. Unilateral conditions are depicted as unicolor bars and bilateral conditions as dashed bars. ** $p<0.01$ for the main effect of STIMULATION (unilateral vs. bilateral).

Figure 3. Event-related spectral perturbations in regions of interest.

a: average event-related spectral perturbation analysis for each condition. Units are in decibels relative to baseline (-400 to -100 ms). Dashed areas represent the four regions of interests: 2–10 Hz (150 to 400 ms), 8–29 Hz (300 to 1000 ms), 30–60 Hz (100–350 ms), and 61–100 Hz (150–350 ms). **b**: average results from the top 20% values within the region of interest for event-related synchronization and lowest 20% values for event-related desynchronization for the four

conditions. * p<0.05; ** p<0.01 for the main effect of STIMULATION (unilateral vs. bilateral). **c**: scalp topography for the low—and high gamma (30–60 Hz and 61–100 Hz) at the time X frequency peak.

Figure 4. Event-related spectral perturbations across all time points.

a: average event-related spectral perturbation analysis shown from 0 to 1000 ms and for frequency bands with significant differences at p<0.05 after 1000 permutations with cluster correction between unilateral (left and right merged together) and bilateral conditions (attention to left hand and right hand merged together): high-gamma (61–100 Hz), beta (13–29 Hz) and alpha (8–12 Hz). Units are in decibels relative to baseline (-400 to -100 ms). The white cross on each plot depicts the time-frequency point with the highest *t* value. Data from this point is used for scalp topography, for illustration purposes. **b**: scalp topography of electrodes with significant differences between unilateral and bilateral conditions at the time-frequency point represented by a white cross in (a) for high-gamma, beta and alpha frequency bands.

Summary

When concurrent nociceptive stimuli are applied, the amplitude of laser-evoked N2 and P2 as well as gamma oscillation power are decreased, while pain remains unchanged.

Figure 1

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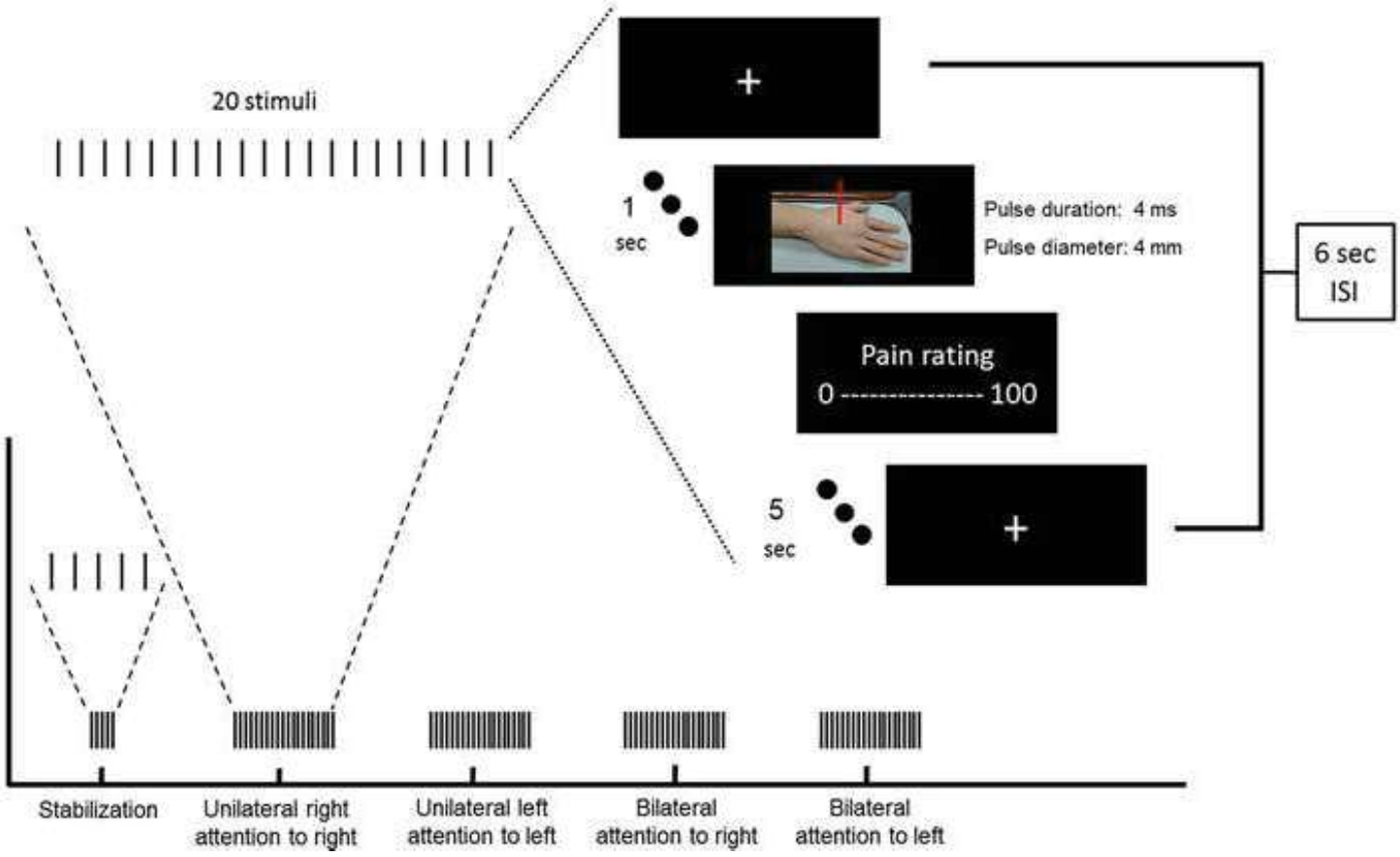

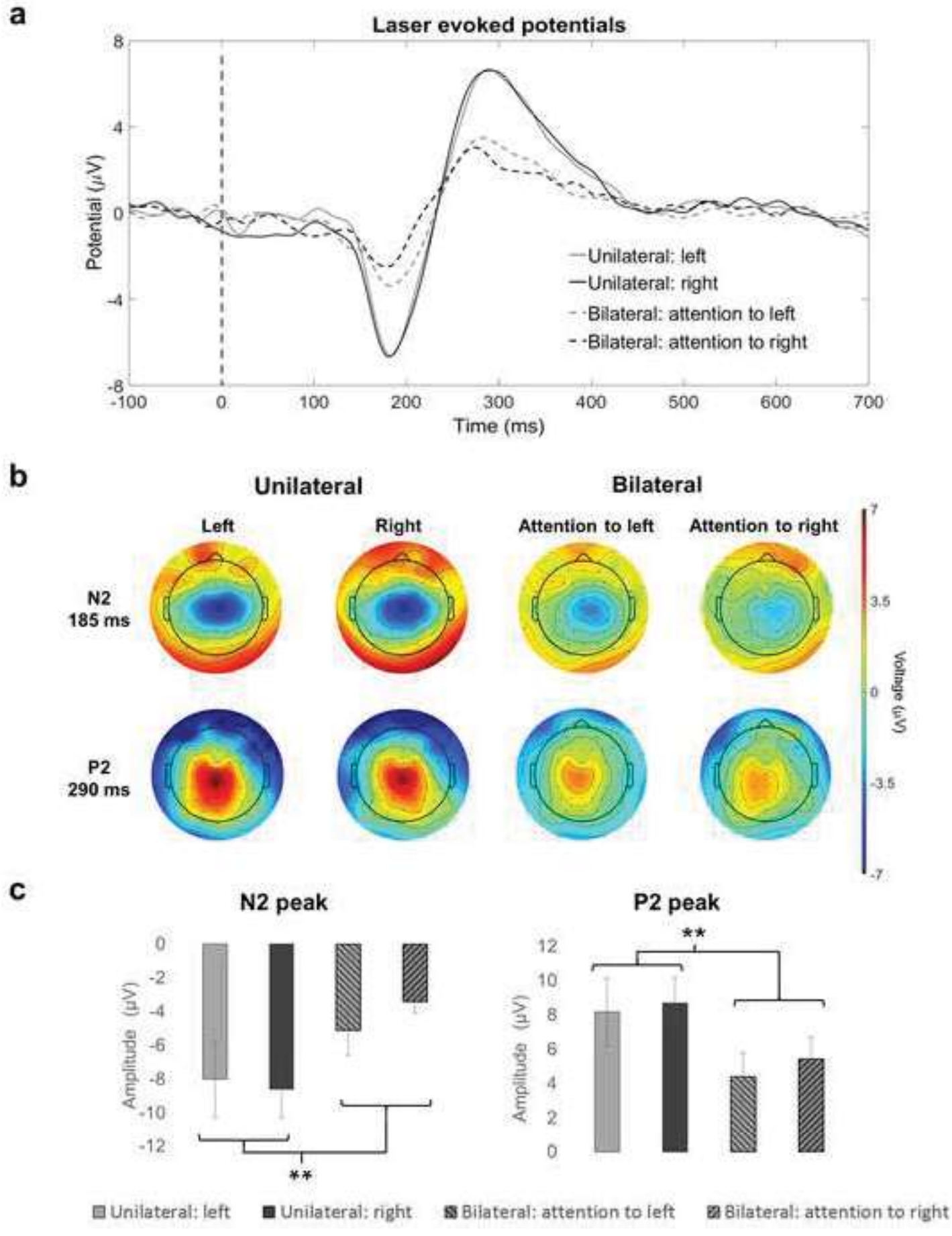

Figure 3

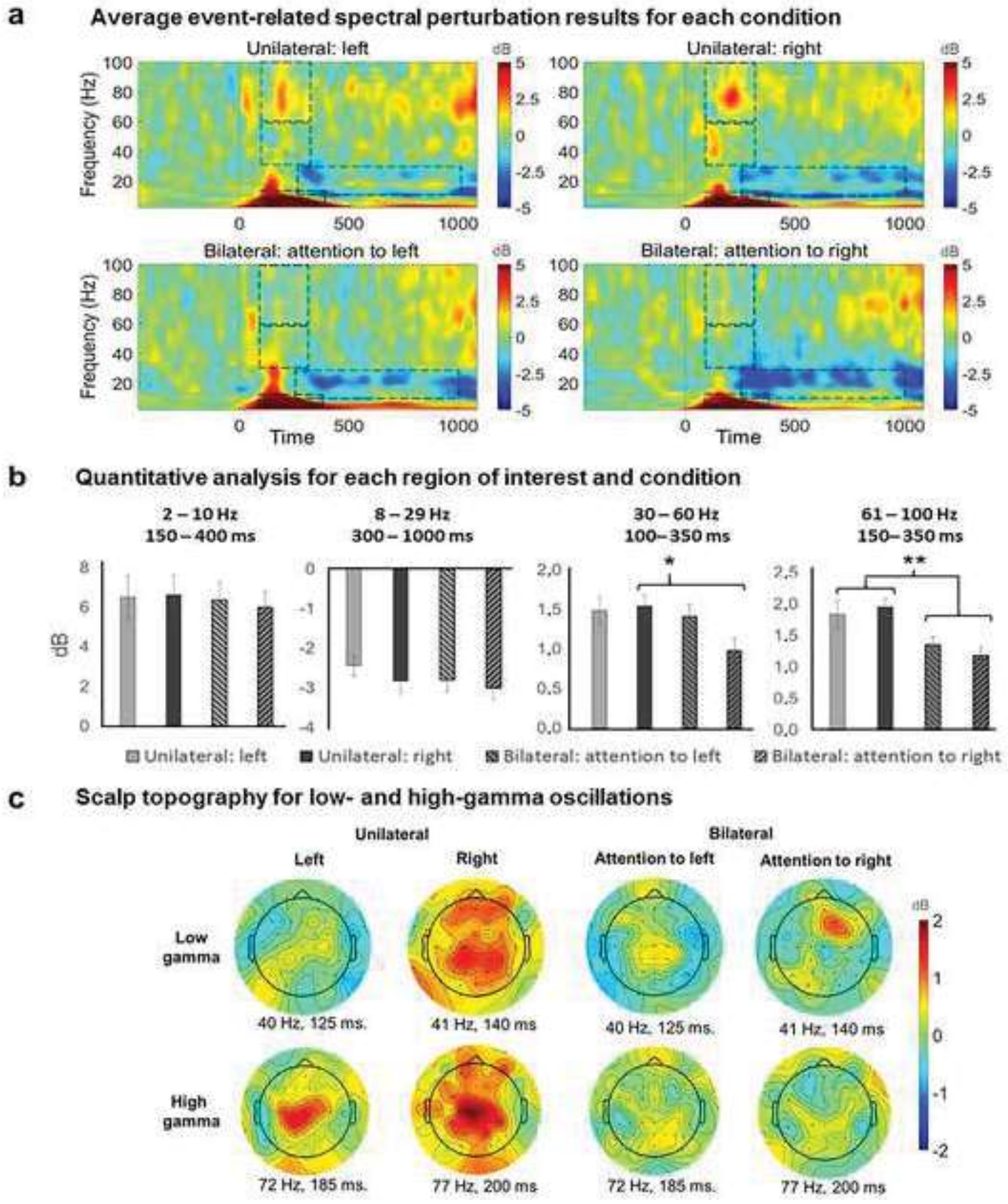

Figure4

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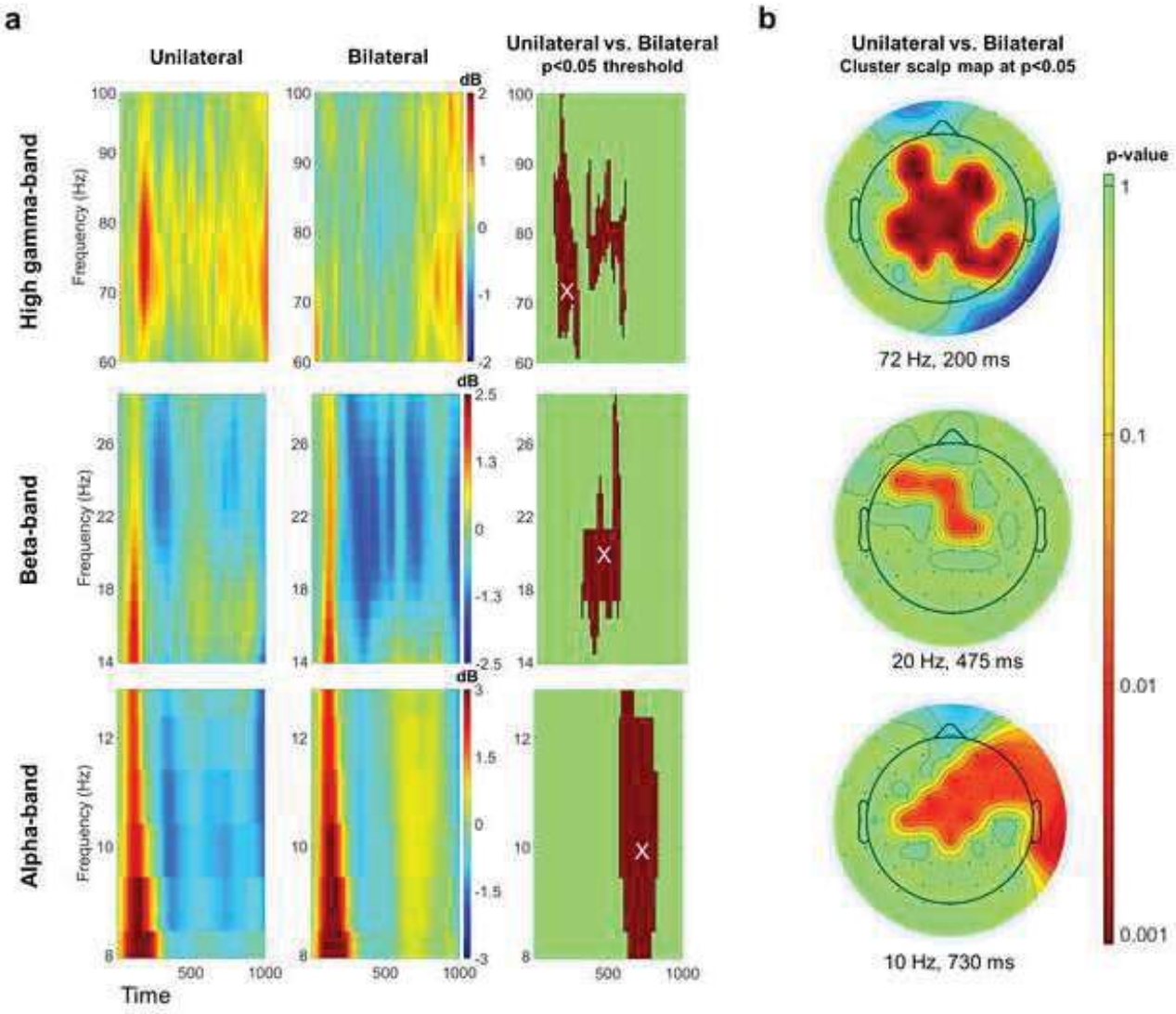

Table 1. N2 and P2 peak latencies

	Unilateral right stimulation	Unilateral left stimulation	Bilateral stimulation attention to the right	Bilateral stimulation attention to the left
N2 latency (mean ± SEM)	185.2 ± 6.7	180.9 ± 5.7	179.4 ± 7.1	174.5 ± 7.1
P2 latency (mean ± SEM)	292.3 ± 9.3	295.4 ± 9.5	289.8 ± 10.6	288.3 ± 12.1