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Novelty is not enough: laser-evoked potentials are determined by stimulus saliency, not absolute novelty

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Ronga I, Valentini E, Mouraux A, Iannetti GD. Novelty is not enough: laser-evoked potentials are determined by stimulus saliency, not absolute novelty. *J Neurophysiol* 109: 692–701, 2013. First published November 7, 2012; doi:10.1152/jn.00464.2012.—Event-related potentials (ERPs) elicited by transient nociceptive stimuli in humans are largely sensitive to bottom-up novelty induced, for example, by changes in stimulus attributes (e.g., modality or spatial location) within a stream of repeated stimuli. Here we aimed *1*) to test the contribution of a selective change of the intensity of a repeated stimulus in determining the magnitude of nociceptive ERPs, and *2*) to dissect the effect of this change of intensity in terms of "novelty" and "saliency" (an increase of stimulus intensity is more salient than a decrease of stimulus intensity). Nociceptive ERPs were elicited by trains of three consecutive laser stimuli (S1-S2-S3) delivered to the hand dorsum at a constant 1-s interstimulus interval. Three, equally spaced intensities were used: low (L), medium (M), and high (H). While the intensities of S1 and S2 were always identical (L, M, or H), the intensity of S3 was either identical (e.g., HHH) or different (e.g., MMH) from the intensity of S1 and S2. Introducing a selective change in stimulus intensity elicited significantly larger N1 and N2 waves of the S3-ERP but only when the change consisted in an increase in stimulus intensity. This observation indicates that nociceptive ERPs do not simply reflect processes involved in the detection of novelty but, instead, are mainly determined by stimulus saliency.

habituation; pain; electroencephalography; stimulus intensity

BRIEF INFRARED LASER HEAT pulses selectively activate Aδ and C fiber skin nociceptors in the superficial skin layers (Bromm and Treede 1984). Such stimuli elicit transient event-related potentials [laser-evoked potentials (LEPs)], related to the activation of Aδ nociceptors. The largest LEP wave is a negative-positive complex maximal at the scalp vertex (N2-P2). This complex is preceded by a smaller negative wave (N1) maximal over the central-temporal region contralateral to the stimulated hand (Bromm and Treede 1984; Hu et al. 2010; Valentini et al. 2012). These waves reflect a combination of cortical activities originating from primary and secondary somatosensory cortices, the insula, and the anterior cingulate cortex (Garcia-Larrea et al. 2003; Valentini et al. 2012).

Although widely used to investigate the function of nociceptive pathways in health and disease (Haanpaa et al. 2011), the physiological meaning of LEPs is still debated. Indeed, recent experimental evidence indicates that LEPs may reflect stimulus-triggered brain processes largely unspecific for nociception. Indeed, similar brain responses can be elicited by non-nociceptive sensory stimuli that are never perceived as painful, provided that they are salient (Legrain et al. 2011; Mouraux and Iannetti 2009). Furthermore, the well-known positive correlation between the intensity of perceived pain and the magnitude of LEPs can be disrupted in several experimental conditions, such as stimulus repetition at a short and constant interval (Iannetti et al. 2008; Treede et al. 2003; Wang et al. 2010).

Finally, there is a large amount of evidence that the main LEP waves can be modulated by contextual factors such as temporal predictability (Brown et al. 2008; Wang et al. 2010). However, by exploring the LEP dishabituation triggered by introducing selective changes of stimulus location or modality (i.e., two of the four basic attributes defining a sensory stimulus) we showed that LEPs are highly sensitive to bottom-up, stimulus-driven factors such as novelty (Torta et al. 2012; Valentini et al. 2011). Importantly, the design of these previous experiments did not allow differentiating between the respective effects of novelty and saliency in determining LEP dishabituation.[1]

Here, we examined the respective contributions of novelty and saliency on the magnitude of LEPs by comparing, in two different experiments, the effect of introducing a change consisting of a reduction vs. an increase of stimulus intensity. In *experiment 1* we explored whether the dishabituation of LEPs is strictly determined by absolute novelty (the occurrence of an increase or a decrease of stimulus intensity are similarly novel) or by stimulus saliency (the occurrence of an increase of stimulus intensity is more salient than the occurrence of a decrease of stimulus intensity). In *experiment 2*, we tested whether the dishabituation of LEPs was dependent on the size of the increase or decrease of stimulus intensity.

MATERIALS AND METHODS

Subjects

Ten healthy subjects (4 women) aged 22–36 yr (29.7 ± 4.6 yr; means ± SD) participated in *experiment 1*. Six healthy subjects (5 women) aged 22–37 yr (29.3 ± 5.0 yr; means ± SD) participated in *experiment 2*. All participants gave their written informed consent. The study conformed to the standards required by the Declaration of Helsinki and was approved by the local ethics committee.

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[1] We define novelty as the occurrence of any change in a stream of sensory stimuli (Chen et al. 2010; Grimm and Escera 2011) and saliency as the ability of a stimulus to stand out relative to the sensory background or in respect to preceding stimuli (Itti and Koch 2001; Yantis 2008).

Nociceptive Stimulation

Noxious radiant heat stimuli were generated by an infrared neodymium yttrium aluminum perovskite (Nd:YAP) laser with a wavelength of 1.34 μm (Electronical Engineering, Florence, Italy). The laser beam was transmitted through an optic fiber, and its diameter was set at ~7 mm (38 mm^2) by focusing lenses. All laser pulses were directed to a square area of ~5 × 5 cm on the dorsum of the right hand. A He-Ne laser indicated the area to be stimulated. The duration of the laser pulses was 4 ms. Three different and equally spaced stimulus intensities were used [*experiment 1*: low (L) 3.5 ± 0.7 J; medium (M) 4 ± 0.8 J; high (H) 4.5 ± 0.7 J; means ± SD; *experiment 2*: low (L) 3.25 ± 0.3 J; medium (M) 3.75 ± 0.3 J; high (H) 4.25 ± 0.3 J; means ± SD]. Stimulus intensities were individually adjusted to elicit the three following pain ratings targets: 25/100 for intensity low, 45/100 for intensity medium, and 65/100 for intensity high. In a preliminary experiment, we found that stimuli with these characteristics always produce painful pinprick sensations.

Experimental Design and Psychophysics

Experiment 1. Before starting the recording we delivered a small number of low-energy laser pulses to the dorsum of the right hand to familiarize the subjects with the stimuli. The experiment consisted in a single session divided into five recording blocks. In each block, we delivered 20 trains of three laser stimuli (S1, S2, and S3: a triplet) at a constant interstimulus interval (ISI) of 1 s. The time interval between each triplet ranged between 20 and 25 s (rectangular distribution). Between each pulses of a given triplet, the laser beam was manually displaced by ~1 cm along a proximal-distal line on the hand dorsum (Iannetti et al. 2008). The direction of this displacement was balanced in each block (10 triplets in the proximal direction and 10 triplets in the distal direction). A proximal-distal spatial displacement was used to minimize the variations in thickness and innervation of the irradiated skin (Schlereth et al. 2001) and, consequently, in the strength of the nociceptive afferent volley not directly related to the change of stimulus intensity. The order of recording blocks was balanced across subjects.

In two blocks, triplets were composed of stimuli of the same intensity. In one of these two blocks, stimuli were all of low intensity (LLL). In the other block, stimuli were all of high intensity (HHH). Before the beginning of each of these two blocks subjects were verbally informed of the stimulus intensity (either L or H). In the remaining three blocks, S1 and S2 were always of medium intensity (M), while S3 was either of the same intensity as S1 and S2 (MMM) or of a different intensity (MML or MMH). The occurrence of each type of triplet (MMM, MML, and MMH) was balanced and pseudorandomized within each block. The maximum number of consecutive triplets of the same type was three. Approximately 5 s before the onset of the triplet, participants were verbally informed of the intensity of the stimuli composing the triplet.

Between 3 and 6 s after the end of each triplet, subjects were asked to rate verbally the intensity of the Aδ-related pricking sensation elicited by each of the three laser stimuli composing each triplet, using a numerical rating scale ranging from 0 to 100, where 0 was defined as "no pain," and 100 as "pain as bad as it could be." This procedure provided ratings for each individual percept elicited by each individual laser pulse of the triplet. Previous experiments have demonstrated that subjects are able to remember and rate reliably and independently the intensity of the sensation elicited by each of the three stimuli constituting a triplet (Iannetti et al. 2008).

Since variations in baseline skin temperature may modulate pain perception (Tjolsen et al. 1988), an infrared thermometer was employed to ensure that hand temperature remained constant across blocks.

Experiment 2. The experimental design of *experiment 2* was identical to *experiment 1* except for what follows. In each of the five blocks we delivered 24 triplets. In two blocks, triplets were composed of stimuli of the same intensity. In one of these two blocks, stimuli were all of low intensity (LLL). In the other block, stimuli were all of high intensity (HHH). The remaining three blocks were composed of four different types of triplet: MMH, LLH, MML, and HHL. The occurrence of each type of triplet was balanced and pseudorandomized within each block.

Electroencephalogram Recording

Participants were seated in a comfortable chair in a silent, temperature-controlled room. They were asked to focus on the stimuli, keep their eyes open, and look at a fixation point. A screen blocked the view of the hand.

The electroencephalogram (EEG) was recorded using 32 Ag-AgCl electrodes placed on the scalp according to the International 10–20 system and referenced to the nose. Electrode impedances were kept <5 kΩ. In addition, the electro-oculogram (EOG) was recorded from two surface electrodes, one placed over the right lower eyelid and the other placed lateral to the outer canthus of the right eye. Signals were amplified and digitized at a sampling rate of 1,024 Hz (SD32; Micromed, Treviso, Italy).

EEG Analysis

EEG data were preprocessed and analyzed using Letswave (http://nocions.webnode.com/letswave; Mouraux and Iannetti 2008) and EEGLAB (Delorme and Makeig 2004). Continuous EEG data were segmented into epochs using a time window ranging from 0.5 s before S1 to 1.5 s after S3 of each triplet (total epoch duration: 4 s) and band-pass filtered (1–30 Hz) using a fast Fourier transform filter. Each epoch was baseline corrected using the interval from −0.2 to 0 s as reference. Artifacts due to eye blinks or eye movements were subtracted using a validated method based on an Independent Component Analysis (Jung et al. 2000). In all data sets, independent components related to eye movements had a large EOG channel contribution and a frontal scalp distribution. Finally, epochs with amplitude values exceeding ±100 μV (i.e., epochs likely contaminated by artifacts) were excluded.

Epochs belonging to the same experimental condition were averaged time locked to the onset of S1. Thus five average waveforms (LLL, HHH, MMM, MMH, and MML) were obtained for each subject in *experiment 1*, and six average waveforms (LLL, HHH, MMH, LLH, MML, and HHL) for each subject in *experiment 2*. To explore experimental effects on the N1 wave, average waveforms were re-referenced to Fz (Hu et al. 2010; Treede et al. 2003).

Statistical Analysis of Intensity of Perception

Pain ratings were rescaled in each subject between 0 and 100, defining 0 as the smallest pain rating (across S1, S2, and S3) and 100 as the largest pain rating (across S1, S2, and S3) of that subject. A two-way, repeated-measures ANOVA was performed to explore the effect of the factors "stimulus intensity" (two levels: low, high) and "intensity change" (two levels: change, no change), as well as their possible interaction, on the pain ratings elicited by S3.

Statistical Analysis of LEPs

In *experiment 1*, a point-by-point, two-way, repeated-measures ANOVA was performed to explore the effect of the factors stimulus intensity (two levels: low, high) and intensity change (two levels: change, no change) on the S3-LEP. This analysis allowed testing the contribution of the direction of the change in intensity (increase vs. decrease) in dishabituating the LEP response. Specifically, the finding of a significant interaction between the factors stimulus intensity and intensity change would indicate that the direction of the change in intensity is important to determine the response dishabituation induced by the novel stimulus. The point-by-point analysis yielded three distinct waveforms, representing the significance of the main effect of each of the two factors across time, as well as their interaction (Mouraux and Iannetti 2008). A point-by-point, paired t-test was used as post hoc analysis to explore the difference between the S3-LEP in triplets MMH and HHH and between the S3-LEP in triplets MML and LLL. The threshold for statistical significance was set at $P = 0.05$.

In *experiment 2*, a point-by-point, paired t-test was used to explore the difference between the S3-LEPs in triplets MMH vs. LLH, MMH vs. HHH, and LLH vs. HHH, as well as between the S3-LEPs in triplets MML vs. HHL, MML vs. LLL, and HHL vs. LLL. The threshold for statistical significance ($P = 0.05$) was corrected for the number of t-tests, giving a final statistical threshold of $P = 0.0083$.

To account for multiple comparisons in the point-by-point statistical analysis of the ERP waveform, intervals were considered as significant only when lasting >50 ms. F, T, and P values are given at the maximum peak of each significant interval.

RESULTS

Experiment 1

Quality and intensity of perception. At each of the three intensities used (L, M, and H), laser stimuli elicited a clear sensation of pinprick pain in all subjects, related to the activation of Aδ fibers (Bromm and Treede 1984). All subjects were able to clearly distinguish between the three stimulus intensities. The pain ratings elicited by stimuli of each intensity in each type of triplet are shown in Fig. 1. When considering the sensations elicited by S3, the absolute difference between the intensity ratings of L and M (22.6 ± 6.9) was similar to the absolute difference between the intensity ratings of H and M (19.2 ± 10.5). Notwithstanding the possibly nonlinear nature of the rating scale, this suggests that the absolute change introduced by decreasing stimulus intensity (MML) was similar to the absolute change introduced by increasing stimulus intensity (MMH).

As expected, there was a significant main effect of the factor stimulus intensity on the ratings of the painful sensation elicited by S3 [$F(1,9) = 224.94$; $P < 0.0001$] with a positive correlation between the energy of the laser stimulus and the intensity of pain perception. There was no main effect of the factor intensity change [$F(1,9) = 1.20$; $P = 0.301$], and no interaction between the two factors [$F(1,9) = 0.30$; $P = 0.866$]. Hence, intensity of perception was determined by the energy of the stimulus, independently of its absolute novelty.

Laser-evoked brain potentials. Grand average waveforms of the LEPs obtained in each of the five different experimental conditions are shown in Figs. 2 and 3. In all conditions, the scalp topographies of the N2 and P2 waves were remarkably similar: the N2 wave was maximal at the scalp vertex (electrode Cz) and extended bilaterally toward temporal regions, whereas the P2 wave was more centrally distributed (Fig. 2).

The effects of stimulus intensity and intensity change on the magnitude of LEPs are reported below.

MAIN EFFECT OF STIMULUS INTENSITY. There was a significant main effect of the factor stimulus intensity on the amplitude of the LEP elicited by S3, with larger amplitudes at higher intensity levels (Fig. 4). At Cz, this factor was a significant source of variance within three different time intervals: 141–247 ms [coinciding with the latency of both the N1 and the N2 waves; $F(1,9) = 35.19$; $P = 0.0002$], 274–386 ms [coinciding with the latency of the P2 wave; $F(1,9) = 17.51$; $P = 0.0024$], and 538–730 ms [coinciding with the latency of the negative shoulder following the P2 wave; $F(1,9) = 23.28$; $P = 0.0009$].

MAIN EFFECT OF INTENSITY CHANGE. There was a significant main effect of the factor intensity change on the amplitude of the LEP elicited by S3. At Cz, this factor was a significant source of variance within the time interval 168–227 ms [coinciding with the latency of both the N1 and the N2 waves; $F(1,9) = 8.35$; $P = 0.0179$], with higher amplitudes in triplets entailing a change of stimulus intensity (Fig. 4). The scalp topography of this effect revealed a maximum at C3 (the electrode contralateral to the stimulated side) and extended towards temporal regions especially on the side contralateral to the stimulated hand (Fig. 4).

INTERACTION BETWEEN STIMULUS INTENSITY AND INTENSITY CHANGE. Importantly, there was a significant interaction between the factors stimulus intensity and intensity change (Fig. 4) within the time interval 161–221 ms [coinciding with the latency of both the N1 and N2 waves; $F(1,9) = 22.28$; $P = 0.0011$], thus indicating that the direction of the intensity change is important in determining LEP dishabituation. The post hoc paired t-tests revealed that in the time interval coinciding with the latency of both the N1 and N2 waves the amplitude of the S3-LEP was significantly larger in triplets MMH than in triplets HHH ($T_9 = 19.11$; $P < 0.0001$) but not significantly different in triplets MML and

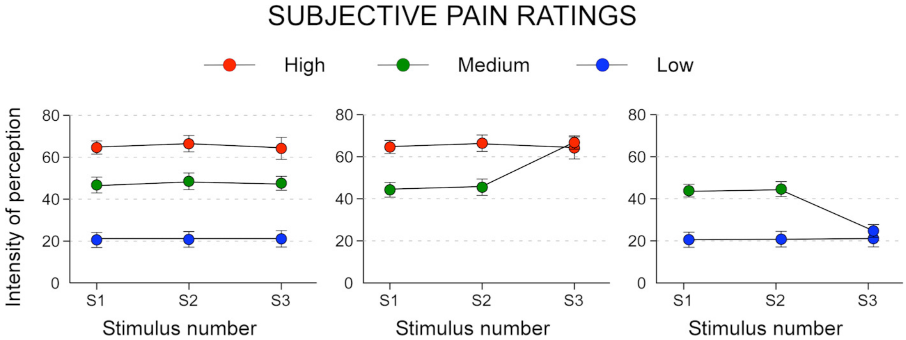


Fig. 1. Relationship between stimulus intensity, stimulus repetition and pain ratings (*experiment 1*). Trains of neodymium yttrium aluminum perovskite (Nd:YAP) laser stimuli were delivered on the hand dorsum. Each train consisted of three stimuli (S1-S2-S3, a triplet) delivered at a constant interstimulus interval (ISI) of 1 s. Three equally spaced stimulus intensities (low, L; medium, M; and high, H) were used. While the intensity of S1 and S2 was always identical, the intensity of S3 was either identical to S1 and S2 (e.g., LLL, MMM, and HHH) or different (e.g., MML and MMH). x-axis, stimulus number; y-axis, rescaled intensity of pain perception. Error bars represent the SE. Note that the intensity of perception was significantly and positively correlated with the intensity of the laser stimulus, with stronger stimuli leading to higher intensities of perception. Note also that stimulus repetition (S1-S3) did not affect the intensity of pain perception.

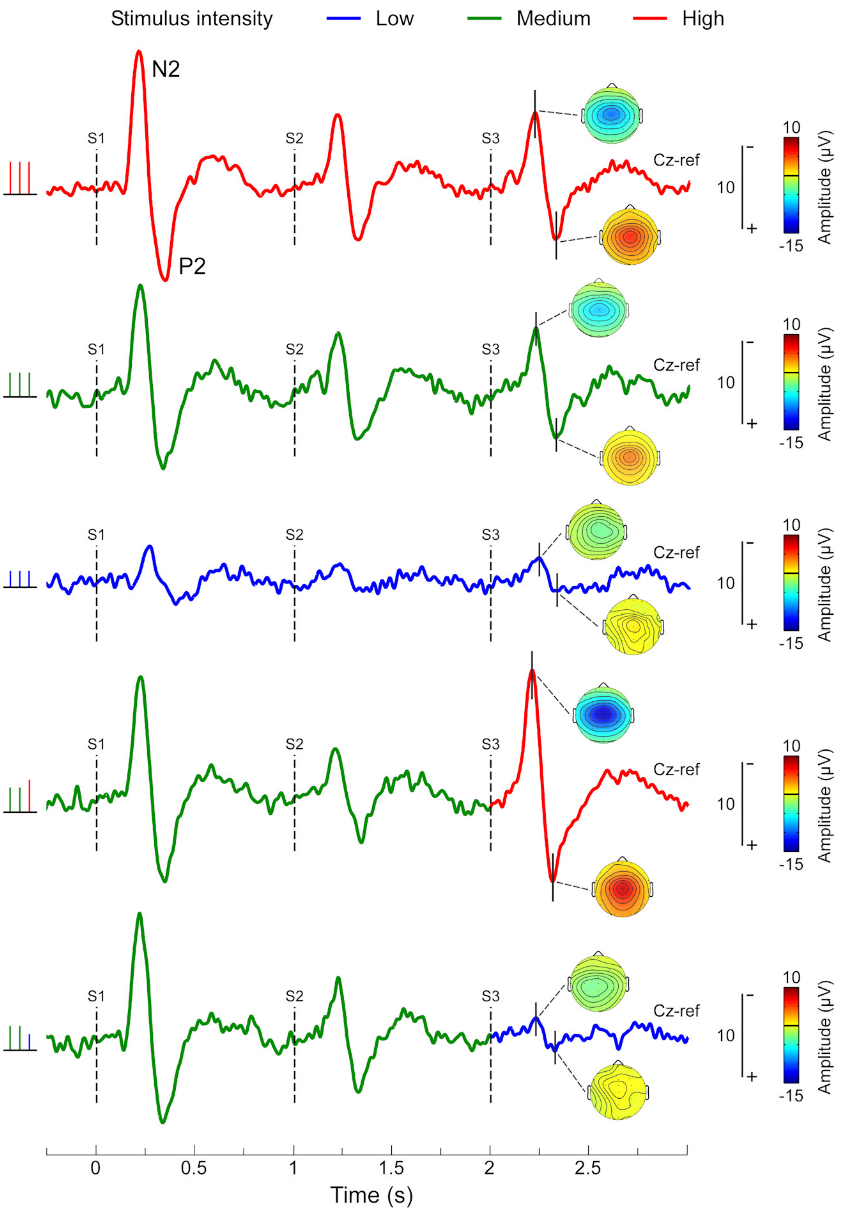


Fig. 2. Effect of stimulus intensity and intensity change on laser-evoked brain potentials (LEPs; *experiment 1*). Group-level average LEPs elicited by S1, S2 and S3. Displayed signals are recorded from the vertex (Cz). Three equally spaced stimulus intensities (L, M, and H) were used. While the intensity of S1 and S2 was always identical, the intensity of S3 was either identical to S1 and S2 (e.g., LLL, MMM, and HHH) or different (e.g., MML and MMH). *x*-axis, time (seconds); *y*-axis, amplitude (μV). Scalp maps obtained at peak latency of the N2 and P2 waves of the S3-LEP. Note the significant amplitude reduction between S1 and S2, and the larger amplitude of the S3-LEP in triplets MMH compared with triplets HHH.

LLL ($T_9 < 0.4$; $P > 0.1$). This indicates that only increases in stimulus intensity are effective in determining response dishabituation. The scalp topography of the stimulus intensity × intensity change interaction was similar to that of the factor intensity change, maximal at C3 and extending bilaterally towards the temporal regions.

Importantly, when comparing the LEP waveforms using the montage to display the N1 wave (i.e., C3 referenced to Fz; Hu et al. 2010), there was also a significant interaction between the factors stimulus intensity and intensity change [$F(1,9) = 14.2$; $P = 0.0044$], thus confirming that, such as the N2 wave, the amplitude enhancement of the N1 wave was present when the change consisted in an increase of stimulus intensity but not when it consisted in a decrease of stimulus intensity (Figs. 3 and 5).

Experiment 2

Laser-evoked brain potentials. Grand average waveforms of the LEPs obtained in each of the six different experimental conditions are shown in Fig. 6.

Experiment 2 yielded three main results (Fig. 7).

First, the amplitude of the LEP elicited by S3 was significantly larger in triplets MMH than in triplets HHH ($T_5 = 31.18$; $P = 0.0001$). This effect was observed in the 154- to 214-ms time interval (coinciding with the latency of both the N1 and the N2 waves). In contrast, the amplitude of the LEP elicited by S3 was similar in triplets LLL and MML ($T_5 < 0.2$; $P > 0.8$). These observations confirm the findings of *experiment 1*.

Second, the amplitude of the LEP elicited by S3 in triplets LLH and MMH was not different ($T_5 < 0.7$; $P > 0.5$), thus indicating that the magnitude of LEP dishabituation was not dependent on the size of the intensity increase.

Third, the amplitude of the LEP elicited by S3 in triplets HHL and MML was not significantly different ($T_5 < 2.5$; $P > 0.05$), thus indicating that the lack of dishabituation observed in *experiment 1* between the S3-LEP of triplets MML and LLL was not due to the fact that the change in stimulus intensity was too small.

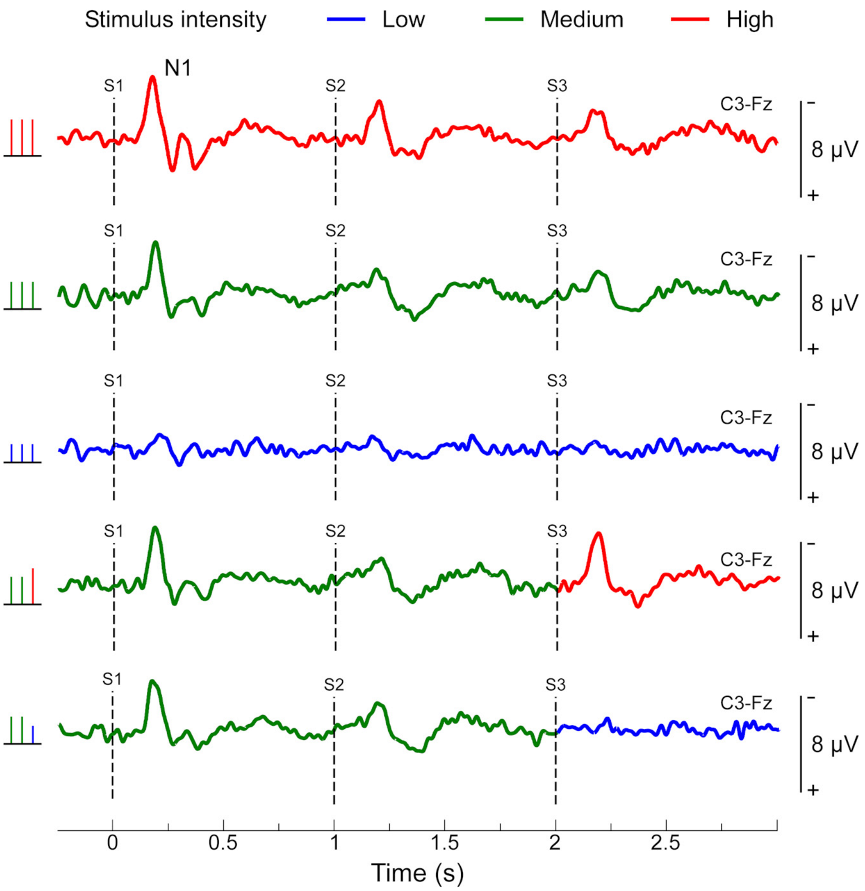


Fig. 3. Effect of stimulus intensity and intensity change on the N1 waveform of LEPs (*experiment 1*). Group-level average LEPs elicited by S1, S2, and S3. Displayed signals are recorded from the central electrode contralateral to the stimulated hand (C3), referenced to Fz (Hu et al. 2010). *x*-axis, time (seconds); *y*-axis, amplitude (μV). Note the larger amplitude of the S3-LEP in triplets MMH compared with triplets HHH.

DISCUSSION

Our results show that introducing a selective change in the intensity of a monotonously repeated laser stimulus produces a significant dishabituation of both the N1 and the N2 waves of the LEP elicited by S3 but only when the change entails an increase of the stimulus intensity (*experiment 1*: significant interaction between the factors stimulus intensity × intensity change; Figs. 4 and 5). The lack of dishabituation when there is a decrease of stimulus intensity is not explained by the size of such intensity reduction (*experiment 2*; Figs. 6 and 7). The finding that the dishabituation of LEPs is sensitive to the direction of the change in intensity indicates that stimulus saliency, and not merely novelty, plays an important role in determining the magnitude of LEPs.

Importantly, the amount of dishabituation did not appear to be dependent on the size of the increase of stimulus intensity (*experiment 2*, Figs. 6 and 7). Indeed, a similar dishabituation was observed when the intensity was increased from low to high (triplets LLH) and from medium to high (triplets MMH). This finding indicates that the dishabituation of LEPs reflects the detection of an increase of saliency above a certain threshold, in an "all or nothing" fashion.

Dissecting Novelty from Probability of Occurrence

In the present study, we define novelty as the occurrence of a change of intensity in a stream of otherwise identical laser

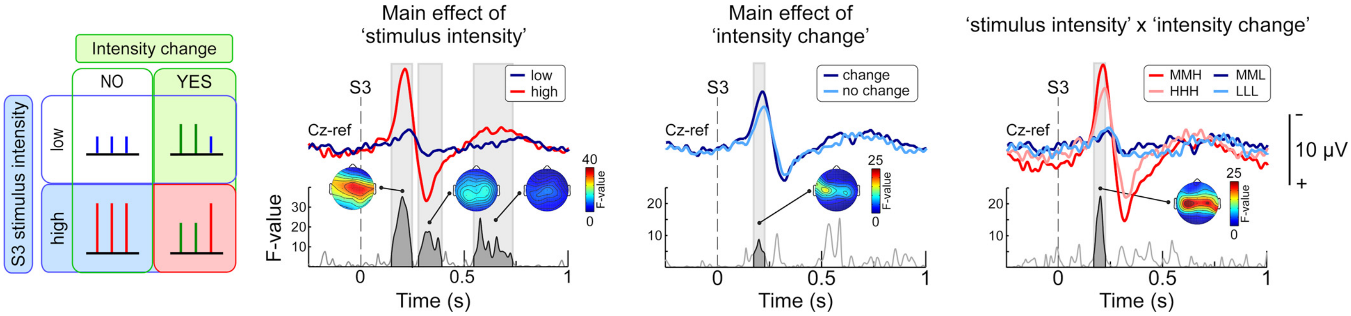
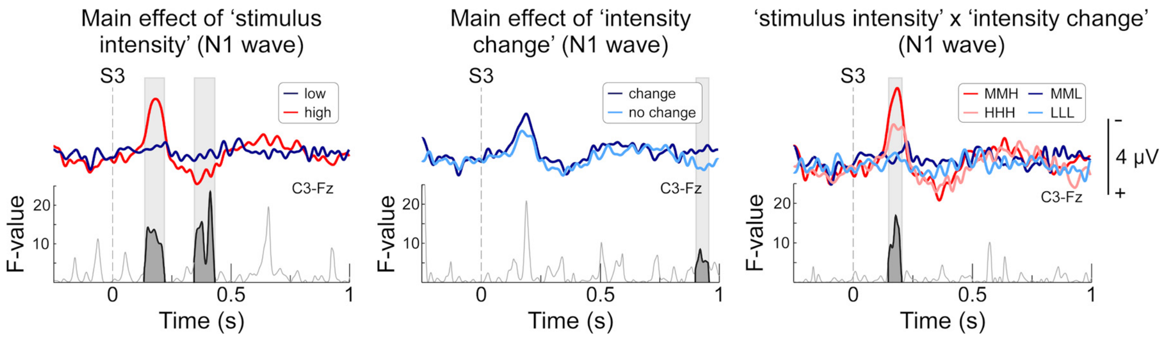


Fig. 4. Effect of the direction of a change in stimulus intensity on LEPs (*experiment 1*). Time course of the effect of the factors stimulus intensity (two levels: low and high) and intensity change (two levels: change and no change), as well as their possible interaction on the S3-LEP was assessed using a repeated-measures ANOVA performed on each time point of each subject's averaged waveforms at Cz (nose reference). *F* values at each time point are represented below the LEP waveforms. Time intervals where the difference between conditions was significant are highlighted in gray (consecutivity threshold = 50 ms), and the corresponding scalp topographies are shown. Note the significant interaction in the time interval corresponding to both the N1 and the N2 waves (161–221 ms; *right*), indicating that the effect of intensity change is significant only when the intensity of S3 is increased.


Main effect of ‘stimulus intensity’ (N1 wave)

Main effect of ‘intensity change’ (N1 wave)

‘stimulus intensity’ x ‘intensity change’ (N1 wave)

Fig. 5. Effect of the direction of a change in stimulus intensity on the N1 waveform of LEPs (*experiment 1*). Time course of the effect of the factors stimulus intensity (two levels: low and high) and intensity change (two levels: change, no change), as well as their possible interaction on the S3-LEP was assessed using a repeated-measures ANOVA performed on each time point of each subject’s averaged waveforms at C3 referenced to Fz. Time intervals where the difference between conditions was significant are highlighted in gray (consecutivity threshold = 50 ms).

stimuli. This definition is important, because, when referred to a sensory stimulus, the term novel is used with different meanings. Some researchers label as novelty related the effects observed in response to stimuli that have not been previously experienced before (Stoppel et al. 2009). In contrast, several studies (e.g., Grimm and Escera 2011; Legrain et al. 2009) use the term novel to describe stimuli having a low probability of occurrence compared with standard repeated stimuli having a high probability of occurrence. Importantly, stimuli with low probability of occurrence are also novel in the sense that they are different relative to the preceding stimuli (i.e., novelty is also determined by changes in their attributes). Electrophysiological correlates of the neural activity involved in the processing of novelty due to low probability of occurrence consist in a characteristic ERP pattern comprised sequentially of the mismatch negativity (MMN; Naatanen and Picton 1987) and the “novelty P3” or P3a wave (Friedman et al. 2001). The MMN is a negative wave obtained by subtracting the ERPs elicited by standard stimuli from the ERPs elicited by novel stimuli with low probability of occurrence. Importantly, the neural processes underlying the MMN do not appear to be strictly related to the occurrence of a physical change in the attributes of the stimulus relative to the preceding stimuli. Instead, they appear to be mainly determined by the detection of high-order, infrequent changes in a stimulation pattern. Indeed, it has been shown that when presenting few trials composed of five identical auditory tones (e.g., xxxxx) within a block of many trials composed of four identical auditory tones followed by a different tone (e.g., xxxxy), an MMN is elicited by the fifth identical “x” stimulus (Wacongne et al. 2011: note that this particular kind of MMN is also referred to as “repetition negativity,” e.g., Nordby et al. 1988; Horváth and Winkler 2004). In other words, although all stimuli in trials xxxxx are identical, the fifth “x” stimulus elicits an MMN since it represents an infrequent violation to the standard pattern xxxxy.

In the present study, we explored the novelty effect due to the occurrence of a change in a specific stimulus attribute (i.e., intensity), without the confound represented by the probability of stimulus occurrence. To isolate such novelty effects we matched the probability of occurrence of the increases and decreases of stimulus intensity. Importantly, only intensity increases produced a significant dishabituation of the corresponding LEP, whereas intensity decreases did not (Fig. 4).

LEPs: Saliency vs. Novelty

When we described the habituation of the ERPs elicited by three repeated laser pulses, each identical in terms of the four main attributes defining a sensory stimulus (modality, location, intensity, and timing; Iannetti et al. 2008), we showed that these responses habituate strongly to stimulus repetition. However, we were unable to determine which of these stimulus attributes are determinant in causing response habituation. In two subsequent experiments we showed that introducing a selective change in the sensory modality of the stimulus produced a significant dishabituation of the ERPs (Valentini et al. 2011), whereas introducing a selective change in its spatial location did not (Torta et al. 2012). These findings suggest that different attributes defining a stimulus are not equally effective in inducing a dishabituation of the ERP response. Importantly, the observation of similar dishabituation patterns in the ERPs elicited by auditory stimuli (Valentini et al. 2011) indicates that such dishabituation mechanisms, triggered by bottom-up stimulus changes, are not modality specific (Fruhstorfer 1971). This gives further support to the notion that the largest part of the ERPs elicited by transient sensory stimuli presented at long inter-stimulus intervals (e.g., >4 s) reflect nonmodality-specific neural activities (Mouraux and Iannetti 2009). Therefore, it is likely that the response dishabituation observed in the current study in response to increases of stimulus intensity (Figs. 2 and 4) is not pain specific but reflects a general mechanism related to the detection of salient sensory events regardless of the sensory modality through which these events are conveyed.

Importantly, the design of our previous experiments exploring the effects of introducing a change in stimulus modality or spatial location (Torta et al. 2012; Valentini et al. 2011) did not allow differentiating between the respective effects of novelty and saliency in determining response dishabituation. In contrast, the design of the present experiment, entailing changes in the intensity of the applied stimuli, allowed us to explore the effect of the direction of change. This is important, as it permits dissecting the effects of absolute novelty compared with increased saliency in determining the response dishabituation. Indeed, the occurrence of a change within a stream of identical stimuli necessarily introduces novelty but not all changes are equally salient. In triplets MML and MMH (Fig. 2), the introduction of a low intensity stimulus was as novel as the introduction of a high intensity stimulus.

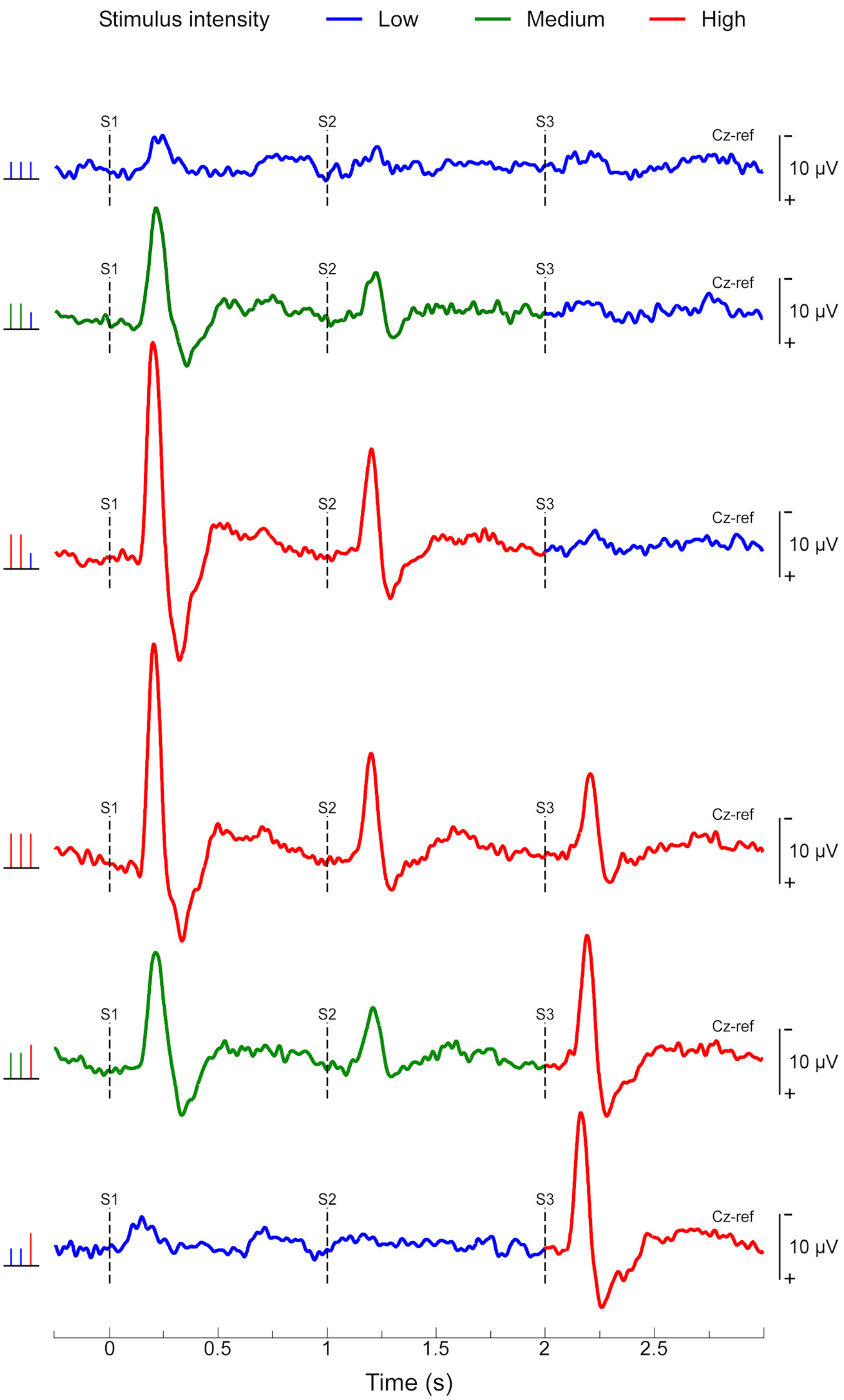


Fig. 6. Effect of the size of intensity change on LEPs (*experiment 2*). Group-level average LEPs elicited by S1, S2, and S3. Displayed signals are recorded from the vertex (Cz). Three equally spaced stimulus intensities (L, M, and H) were used. *x*-axis, time (seconds); *y*-axis, amplitude (μV). Note the similarity between the amplitudes of the S3-LEP in triplets MMH and LLH. Note also the similarity between the amplitudes of the S3-LEP in triplets MML and HHL, indicating that the lack of dishabituation observed in *experiment 1* in response to intensity decreases was not due to the fact that the decrease in stimulus intensity was too small.

Furthermore, the absolute physical change in stimulus intensity, as well as the absolute change in intensity of perception between S2 and S3, was identical in triplets MML and MMH. However, the introduction of a high intensity stimulus (representing a change characterized by an increase of energy compared with the preceding stimuli) is more salient than the introduction of a low intensity stimulus (representing a change characterized by a decrease of energy compared with the preceding stimuli). In the context of the current paradigm, in which we uniquely employed transient nociceptive stimuli embedded in a silent background, low-intensity stimuli were unavoidably less salient than the preceding medium-intensity stimuli. This is in accordance with the definition of saliency as "the ability of a stimulus to stand out in respect to preceding stimuli" (Itti and Koch 2001).

Thus, if LEP dishabituation is solely determined by novelty caused by absolute changes in stimulus intensity in a stream of otherwise identical stimuli, a similar enhancement of LEP magnitude should be observed when comparing MMH and HHH triplets and when comparing MML and LLL triplets (i.e., following an increase or a decrease of stimulus intensity). Conversely, if LEP dishabituation is more generally deter-

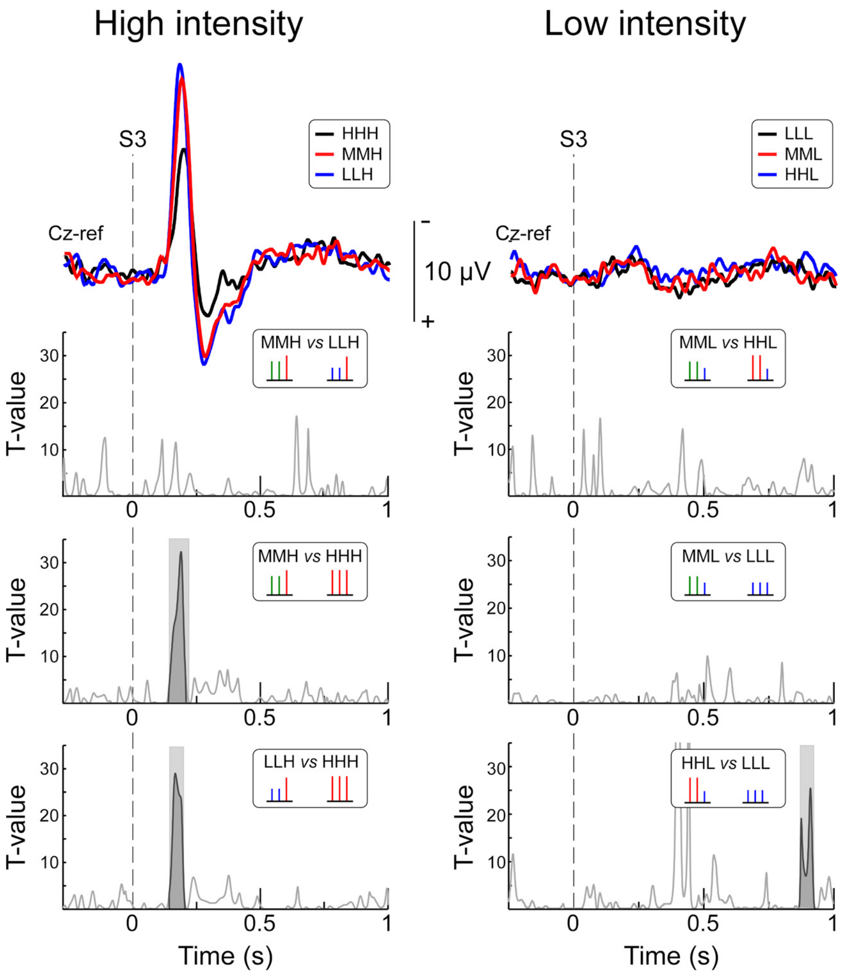


Fig. 7. Effect of the size of intensity change on LEPs (*experiment 2*). Time courses of the effect of size of intensity changes were assessed using paired *t*-tests performed on each time point of each subject's averaged waveforms at Cz (nose reference). Time intervals where the difference between conditions was significant are highlighted in gray (consecutivity threshold = 50 ms).

mined by stimulus saliency, a greater enhancement of LEP magnitude should be observed in MMH than in MML triplets.

Our results, showing that the direction of the change in stimulus intensity is an important determinant of LEP dishabituation, indicate that saliency, and not simply novelty, determines LEP magnitude (Fig. 4). Importantly, the lack of LEP dishabituation following a decrease of stimulus intensity could not be explained by the size of the reduction in stimulus intensity, as no dishabituation was observed in the S3-LEP of both MML and HHL triplets, corresponding to a reduction of stimulus intensity from medium to low and from high to low, respectively (*experiment 2*; Figs. 6 and 7).

In support of the conclusion that the observed LEP dishabituation is determined by the saliency of the stimulus rather than by its novelty, there is the observation that the S3-LEPs in triplets MMH and HHH only show differences in the N1-N2 time window, and not later in the waveforms, specifically when the novelty-related P3a component (sometimes labeled as "novelty P3") occurs (Friedman et al. 2001).

The observation of a direction-specific effect of stimulus intensity on the magnitude of laser-evoked N1 and N2 waves is similar to the finding that only increasing the intensity of rare auditory stimuli enhances the magnitude of the auditory N1 wave (Jacobsen et al. 2003). This observation supports the view that the N1 wave of auditory ERPs and the N1 and N2 waves of laser ERPs reflect functionally similar brain processes (Mouraux and Iannetti 2009). Interestingly, a recent study investigating the EEG responses elicited by transient heating or transient cooling of the skin (heat- and cool-evoked potentials) showed that temperature increases are reflected in a larger amplitude of the N2 wave, whereas temperature decreases of the same magnitude are not (Greffrath et al. 2010). Consistent with these findings, we observed that the intensity change effect was only present when intensity was increased and consisted in an enhancement of the N2 wave (Fig. 4).

Interestingly, the significant interaction between the factors intensity change and stimulus intensity started already 150 ms after the onset of the stimulus, i.e., during the time interval corresponding to the laser-evoked N1 wave, which represents an early cortical response to nociceptive input, largely generated in S1 (Valentini et al. 2012). This indicates that novelty resulting from increasing stimulus intensity modulates the processing of nociceptive input already at its early stages and confirms the notion that these early stages of nociceptive cortical processing are already sensitive to the context in which the stimuli are delivered (Iannetti et al. 2008; Valentini et al. 2011).

LEP Dishabituation to Intensity Increases: an "All or Nothing" Response?

Experiment 2 showed that LEP dishabituation caused by the increase of stimulus intensity was not dependent on the size of the increase (Figs. 6 and 7); that is, the magnitude of LEP dishabituation was similar, regardless of whether it was triggered by a 0.5-J increase in stimulus intensity (i.e., from medium to high), or by a 1.0-J increase in stimulus intensity (i.e., from low to high). This observation suggests that the neural system whose activity is captured by the LEP dishabituation is tuned to detect, above a certain threshold, any increase of stimulus intensity within a stream of repeated stimuli. In other words, the neural system generating the LEPs does not seem to be able to finely discriminate the size of the intensity increase within the stream of repeated stimuli. Instead, it produces a response of the same magnitude following any increase of stimulus intensity. This observation agrees with the notion that the largest part of LEPs and other "vertex potentials" (Mouraux and Iannetti 2009) reflects the activity of a system important for the effective detection of salient and potentially threatening events happening in the sensory environment (Iannetti and Mouraux 2010; Legrain et al. 2011). It has been recently suggested that the detection of salient information is mediated at least in part by direct thalamocortical projections through which salient sensory information reaches multimodal cortical areas responsible for its detection directly from the thalamus, i.e., without being first processed in primary and secondary unisensory areas. Such a saliency-detection system is parallel to the processing of finer stimulus attributes, which are transmitted in a modality-specific fashion from the thalamus to the relevant primary sensory areas (Liang et al. 2012). The lack of fine stimulus discrimination in the observed LEP dishabituation (Fig. 7) might represent additional evidence that the cortical system generating such dishabituation coincides with the "saliency network" (Corbetta and Shulman 2002; Iannetti and Mouraux 2010; Mouraux et al. 2011) that, by receiving direct thalamic input, provides an effective but crude way to rapidly detect salient sensory events and guide appropriate defensive behaviors (Liang et al. 2012).

Conclusion

The present study contributes to characterize the stimulus features determining the magnitude of the different components of the ERP response elicited by nociceptive stimuli. By comparing the effect of a change consisting of a reduction vs. an increase of stimulus intensity, we show that increases but not decreases of stimulus intensity are able to induce LEP dishabituation. This indicates that saliency, rather than novelty per se, is a major determinant of LEP magnitude. Furthermore, the amount of LEP dishabituation was similar for small and large increases of stimulus intensity, suggesting that LEP dishabituation reflects the activity of a system responding to salient sensory input in an all or nothing fashion.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

Author contributions: I.R., E.V., A.M., and G.D.I. conception and design of research; I.R. performed experiments; I.R. analyzed data; I.R., E.V., A.M., and G.D.I. interpreted results of experiments; I.R., E.V., A.M., and G.D.I. prepared figures; I.R., E.V., A.M., and G.D.I. drafted manuscript; I.R., E.V., A.M., and G.D.I. edited and revised manuscript; I.R., E.V., A.M., and G.D.I. approved final version of manuscript.

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