Short trains of intra-epidermal electrical stimulation to elicit reliable behavioral and electrophysiological responses to the selective activation of nociceptors in humans.

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Abstract

Currently, the study of nociception in humans relies mainly on thermal stimulation of heat-sensitive nociceptive afferents. To circumvent some limitations of thermal stimulation, it was proposed that intra-epidermal electrical stimulation (IES) could be used as an alternative method to activate nociceptors selectively. The selectivity of IES relies on the fact that it can generate a very focal electrical current and, thereby, activate nociceptive free nerve endings located in the epidermis without concomitantly activating non-nociceptive mechanoreceptors located more deeply in the dermis. However, an important limitation of IES is that it is selective for nociceptors only when very low current intensities are used. At these intensities, the stimulus generates a very weak percept, and the signal-to-noise ratio of the elicited evoked potentials (EPs) is very low. To circumvent this limitation, it was proposed that the strength of the nociceptive afferent volley could be increased through temporal summation, using short trains of repeated IES. Here, we characterized the intensity of perception and EPs elicited by trains of 2, 3 and 4 IES delivered using a 5-ms inter-stimulus interval. We found that both the intensity of perception and the magnitude of EPs significantly increased with the number of pulses. In contrast, the latency of the elicited EPs was not affected by the number of pulses, indicating that temporal summation did not affect the type of activated fibers and, therefore, that trains of IES can be used to increase the reliability of stimulus-evoked responses while still preserving its selectivity for nociceptors.

1. Introduction

During the last decades, investigation of the neurophysiological mechanisms underlying nociceptive processing and pain perception has relied mainly on the thermal stimulation of cutaneous Aδ- and C-fiber free nerve endings [17]. For example, thermal stimuli generated by laser stimulators have been used extensively because of their indisputable selectivity for heat-sensitive nociceptors [1]. In addition, due to their high power, lasers can generate very steep heating ramps, and thus elicit synchronous afferent discharges enabling the recording of time-locked responses such as event-related brain potentials (ERPs) or reaction times [2]. More recently, intra-epidermal electrical stimulation (IES) [10] and electrical stimulation using a small surface concentric electrode [11] have been proposed as alternative methods to activate nociceptors selectively and, thereby, explore nociception [10]. The rationale for these stimulation techniques relies on the fact that nociceptive free nerve endings are preferentially located in the epidermis, while non-nociceptive mechanoreceptors are mainly located more deeply in the dermis. Therefore, pulses of electric current spatially restricted to the epidermis could activate nociceptors selectively. These alternative methods could circumvent some limitations of laser stimulation, such as skin overheating and lesion due to stimulus repetition, and delay or relative desynchronization of the nociceptive afferent volley due to transduction of thermal energy into a neural impulse. However, these stimulation techniques suffer from their own limitations, in particular, the need to use low stimulation current intensities to guarantee its selectivity for nociceptors. Indeed, it has been shown that if IES is delivered using a strong intensity (e.g. an intensity corresponding to the pain threshold), the stimulus is not selective for nociceptors because it also activates more deeply located low-threshold mechanoreceptors [5,18]. In particular, it was shown that selective denervation of nociceptive free nerve endings by prolonged topical application of capsaicin abolishes the behavioral and electrophysiological responses to laser stimuli and IES delivered at low current intensities

(corresponding to twice the absolute detection threshold; 0.18±0.25mA) but does not affect the responses to conventional transcutaneaous electrical stimulation and IES delivered at a stronger intensity of current (2.5 mA) [15]. Thus, there is converging evidence that IES can activate nociceptors selectively, if and only if low intensities of current are used [14]. The important drawback is that at such low intensities, a single pulse of IES elicits a very weak sensation and the signal-to-noise ratio of the elicited ERPs is low, possibly because of the very small number of recruited afferents. This drawback has probably limited the use of this technique for pain research, and as a consequence, its availability. To circumvent the lack of spatial summation, some authors have proposed to deliver short trains of electrical pulses (e.g. three pulses delivered at a 5-ms inter-stimulus interval) [7,11,13,16,20,21], with the aim of increasing the strength of the nociceptive afferent volley through temporal summation. However, in these studies, the latency of the elicited ERPs was not systematically analyzed. As the latency of ERP components depends on the conduction velocity of the sensory fibers, and, therefore, on the type of fiber activated by the eliciting stimulus, it is important to ensure that temporal summation does not affect the type of fibers activated by IES. The aim of the present study was to compare the magnitude and latency of the perception and ERPs elicited by trains of 2, 3 or 4 pulses of IES delivered using a 5-ms inter-stimulus interval.

2. Methods

Eleven volunteers took part in the study (4 women, aged from 21 to 45 years) with no prior history of neurological, psychiatric or chronic pain disorder. Written informed consent was obtained and all experimental procedures were approved by the local ethics committee and conformed to the latest revision of the Declaration of Helsinki.

IES was delivered to the right hand dorsum using a stainless steel concentric bipolar electrode developed by Inui et al. [10] (Nihon Kohden, Japan). The electrode consists of a needle cathode (length: 0.1 mm, Ø: 0.2 mm) surrounded by a cylindrical anode (Ø: 1.4 mm). By gently pressing the device against the skin, the needle electrode was inserted in the epidermis of the hand dorsum, within the sensory territory of the superficial radial nerve. In order to guarantee the selectivity of the nociceptive stimulation, the intensity of the stimulus was individually adjusted to twice the absolute detection threshold to a single 0.5 ms constant-current square-wave pulse (DS7 Stimulator, Digitimer Ltd., UK). The detection threshold was estimated using an adaptive algorithm [3]. After positioning the electrode, single-pulse stimuli were applied using a staircase procedure, with detection vs. non detection as criterion, by increasing or decreasing the intensity of the electrical current in steps of 0.01 mA. The procedure was interrupted after the occurrence of four staircase reversals. The staircase converged towards the intensity at which the probability of detecting the stimulus was 50% [3]. The intensity was then set to twice the detection threshold, defined as the average of the intensity delivered at the four staircase reversals, with an intensity of $\leq$0.50 mA as restrictive criterion [4,6]. If this criterion was not met, the electrode was displaced and the adaptive staircase procedure was restarted.

During a first session, stimuli were applied using a single pulse or a train of 2, 3 or 4 pulses separated by a 5-ms inter-pulse interval. The different types of stimuli were repeated 5 times in random order. After each stimulus, the participants were asked to rate the perceived intensity of the stimulus using a numerical rating scale (NRS) extending from 0 to 100 (0 = not perceived; 100 = maximum pain; 50 = limit between non-painful and painful domains of sensation).

During a second session, the electroencephalogram (EEG) was recorded using 19 Ag-AgCl electrodes placed on the scalp according to the International 10-20 system and referenced to

linked earlobes (A1-A2). Ocular movements and eye-blinks were recorded using two additional bipolar electrodes placed at the upper-left and lower-right sides of the left eye. The signals were amplified, digitized at a 167 Hz sampling rate (PL-EEG, Walter Graphtek, Germany). Stimuli were applied using a train of 2, 3 or 4 pulses separated by a 5-ms inter-pulse interval, delivered in a random order in three consecutive blocks of 30 trials each (one block = 10 trials x 3 stimulus types). Within a block, the inter-train interval varied randomly from 5 to 10 s (rectangular distribution). Each block was separated by a 2-5 min pause. Participants were asked to press a button held in the left hand as soon as they perceived the stimulus. The mean reaction time (RT) recorded relative to stimulus onset was used as a measure of response speed. RTs greater than 1000 ms were considered as undetected. We also examined the frequency distribution of RTs according to stimulus type. For this purpose, RTs were grouped in 100-ms bins extending from 0 to 1000 ms.

Offline analyses of the EEG data were carried out using Brain Vision Analyzer 1.05 (Brain Products GmbH, Germany) and Letswave 5 (Université catholique de Louvain, Belgium). The continuous EEG recordings were band-pass filtered (0.5-45 Hz) and segmented into 2000 ms epochs extending from -500 to +1500 ms relative to stimulus onset. Artifacts produced by eye blinks and eye movements were corrected using an Independent Component Analysis [9]. Signals were re-referenced according to a common average reference, and baseline-corrected from -500 to 0 ms. Epochs containing artefacts were identified by visual inspection and excluded from further analyses (rejected epochs constituted less than 15% of the total number of epochs). The epochs were then averaged according to the number of pulses (2, 3 or 4). Furthermore, an additional set of average waveforms were computed to test the effect of repetition. For each subject, the full set of epochs were split into four blocks according to trial order (blocks 1 to 4) and number of pulses (2, 3 or 4), yielding 6 average waveforms for each subject. Within each average waveform, the latency and amplitude of three distinct peaks

were measured as follows. First, a negative peak (N2) was identified as the most negative peak obtained at Cz within 200-300 ms after stimulus onset. Second, a positive peak (P2) was defined as the most positive peak obtained at Cz within 300-400 ms after stimulus onset. The peak-to-peak amplitude of the N2-P2 complex was obtained by subtracting the N2 peak amplitude from the P2 peak amplitude. Third, a negative peak (N1) was identified at the contralateral electrode T3 re-referenced to Fz, within 120-170 ms after stimulus onset.

The effect of the number of stimuli was assessed using an ANOVA for repeated measures (GraphPad 5, GraphPad Software Inc., CA) with *stimulus type* as within-subject factor with four levels (1 vs. 2 vs. 3 vs. 4 pulses) for the intensity of perception, and three levels (2 vs. 3 vs. 4 pulses) for RTs and ERP amplitudes and latencies. For the N2-P2 difference, the ANOVA also included *time* as second within-subject factor (blocks 1 vs. 2 vs. 3 vs. 4), in order to assess the effect of stimulus repetition. Analyses included data from unperceived stimuli (NRS = 0 in the first session, trials with no button press in the second session), except for RTs. Post-hoc analyses using paired *t*-tests were performed when necessary. Significance level was set at $p < 0.05$.

3. Results

The group-level mean ±SD of the absolute detection threshold was 0.09 ±0.07 mA. With an intensity set at twice the detection threshold, the mean ±SD intensity of perception (NRS) was 25 ±15 for a single pulse, 40 ±18 for 2 pulses; 51 ±14 for 3 pulses and 59 ±13 for 4 pulses, as shown in Figure 1A. The ANOVA revealed a significant effect of *stimulus type* on the intensity of perception ($p<0.001$). All post-hoc t-tests were significant: 1 vs. 2 pulses, 2 vs. 3 pulses, and 3 vs. 4 pulses (all $p<0.013$).

The group-level mean ±SD of RTs was 397 ±47ms, 341 ±49ms and 330 ±51ms for trains of 2, 3 and 4 pulses, respectively. The ANOVA showed that RTs were significantly different according to stimulus type (p<0.001). Post-hoc comparisons showed that RTs to 2 pulses were significantly greater than RTs to 3 pulses (p<0.001), and that RTs to 3 pulses were significantly greater than RTs to 4 pulses (p<0.010). As shown in Figure 1B, most stimuli were detected with RTs between 300 and 500 ms, independently of stimulus type. Such RTs are compatible with the conduction velocity of myelinated A-fibers [3].

Clear ERPs were identified in 9 of the 11 participants. Figure 2 illustrates the group-level average ERP waveforms elicited by 2, 3 and 4 pulses as well as the group-level scalp topographies of the N1, N2 and P2 waves. The mean peak latencies and amplitudes of these components are detailed in Table 1.The peak-to-peak amplitude of the N2-P2 complex was significantly affected by *stimulus type* (p = 0.021) and *time* (p = 0.015). The interaction between the two factors was not significant (p = 0.348). This indicates a significant effect of the numbers of IES pulses and a significant effect of stimulus repetition on the magnitude of the elicited ERPs. In addition, it suggests that the effect of stimulus repetition was not different for the different types of stimuli. Separate analyses for N1, N2 and P2 amplitudes showed a significant effect of *stimulus type* for N1 (p = 0.030) and P2 (p = 0.039), but not for N2 (p = 0.302). Post-hoc comparisons revealed a significant difference between 2 and 4 pulses for both N1 (p = 0.028) and P2 (p = 0.044). There was no significant difference between 2 and 3 pulses (N1: p = 0.203; P2: p = 0.202), as well as between 3 and 4 pulses (N1: p = 0.285; P2: p = 0.519). Comparison of the N1, N2 and P2 latencies did not reveal any significant effect of *stimulus type* (N1: p = 0.257; N2: p = 0.641; P2: p = 0.816).

4. Discussion

The present study confirms that when care is taken to position the electrode, the absolute detection threshold of IES lies at very low intensities. Notably, these absolute detection threshold values are far below the values at which a single pulse of IES elicits a percept qualified as painful (e.g., 1.6 ±0.5 mA; [5]). Importantly, the temporal summation resulting from increasing the number of IES pulses significantly increased the intensity of the elicited percept. Therefore, manipulation of the numbers of pulses represents a viable approach to increase the strength of the nociceptive stimulus without changing the intensity of the electrical current and, thus, ensuring that the stimulus remains selective for nociceptors. Similarly, the amplitude of the ERPs elicited by IES was also increased by the numbers of pulses. However, the relationship between number of pulses and ERP magnitude was not exactly similar to the relationship between number of pulses and intensity of perception. Indeed, whereas increasing the number of pulses appeared to be related to a gradual increase of perceived intensity, the increase of ERP magnitude with the number of pulses appeared to reach a plateau between 3 and 4 pulses.

There was a significant effect of the number of pulses on reaction time latencies. This could be explained by either a difference in peripheral conduction time or a difference in the central processing time required to detect and respond to the incoming sensory input [19]. Increasing the number of pulses could have led to the activation of additional fibers brought to a subthreshold potential by the preceding pulses and, hence, could have led to the activation of faster, non-nociceptive A-fibers. However, this interpretation seems unlikely. Indeed, there was no effect of the number of pulses on the latency of the elicited ERP components, suggesting that the ERPs elicited by the different numbers of pulses were related to the activation of the same type of afferents, i.e. predominantly Aδ-fiber afferents [15]. The effect of the number of pulses on reaction time latencies was thus probably related to the fact that

increasing the number of pulses increases the strength of the nociceptive input and, hence, facilitates and speeds its detection (e.g. [8]).

Taken together, our results indicate that increasing the number of pulses constitutes an appropriate procedure to increase the signal-to-noise ratio of the brain responses elicited by IES without jeopardizing its selectivity for nociceptive afferents. In contrast, previous studies showed that increasing the intensity of the electrical current decreases significantly the latencies of the elicited ERPs, suggesting that in this case, the elicited ERPs are no longer related to the activation of Aδ-fibers but to the concomitant activation of non-nociceptive Aβ-fibers [5,18]. As compared to laser stimulation, IES could be particularly useful in experimental contexts requiring short inter-stimulus intervals, stimulation at various locations or combination with other types of stimuli. Of course, the fact that the selectivity of IES depends crucially on current intensity constitutes an important limitation of the method, especially to explore nociceptive processing in patients with sensory disorders. However, this limitation could be partially circumvented by the use of a very strict procedure to define current intensity, as well as the use of short trains to increase the strength of the nociceptive afferent volley through temporal summation.

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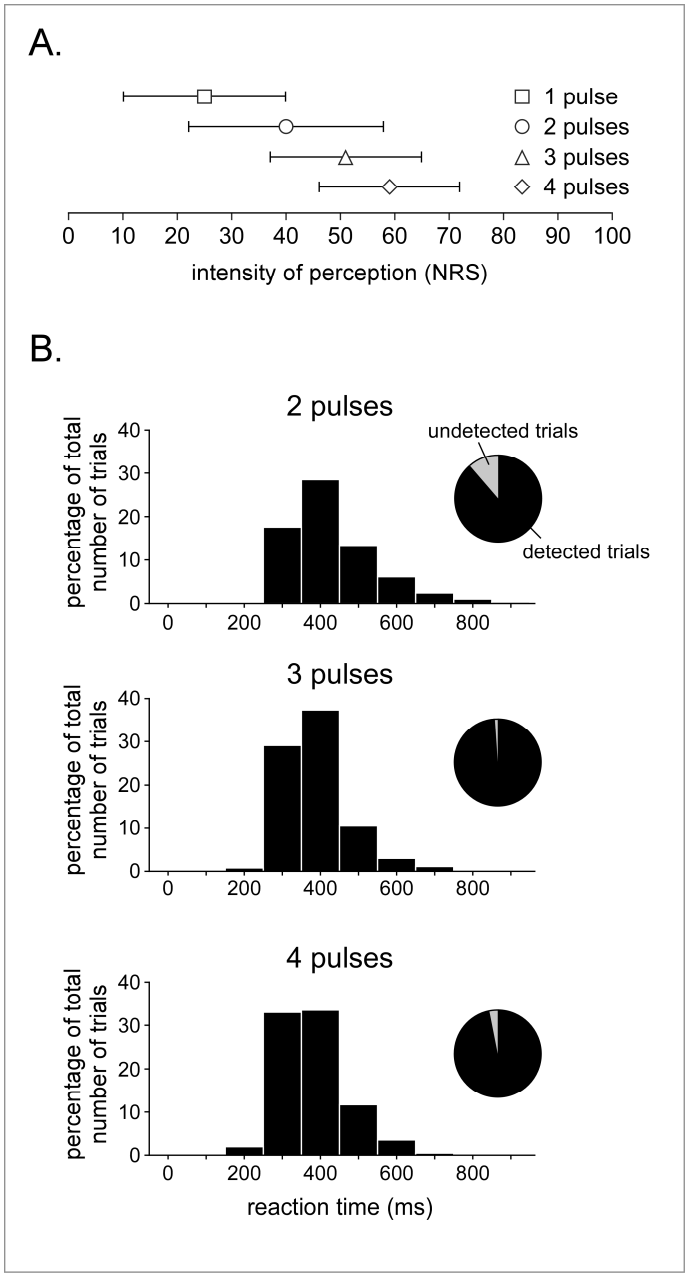
292 Figure legends



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294 Figure 1. A. Group-level average intensity of the perception elicited by 1, 2, 3 and 4 pulses of
295 IES delivered to the right hand dorsum (the whisker plots represent the standard deviation
296 across participants). B. The histograms represent the frequency distribution of the reaction-
297 times to IES using 2, 3 and 4 pulses (bins of 100 ms). The pie charts represent the proportion
298 of detected (black) and undetected (grey) trials.

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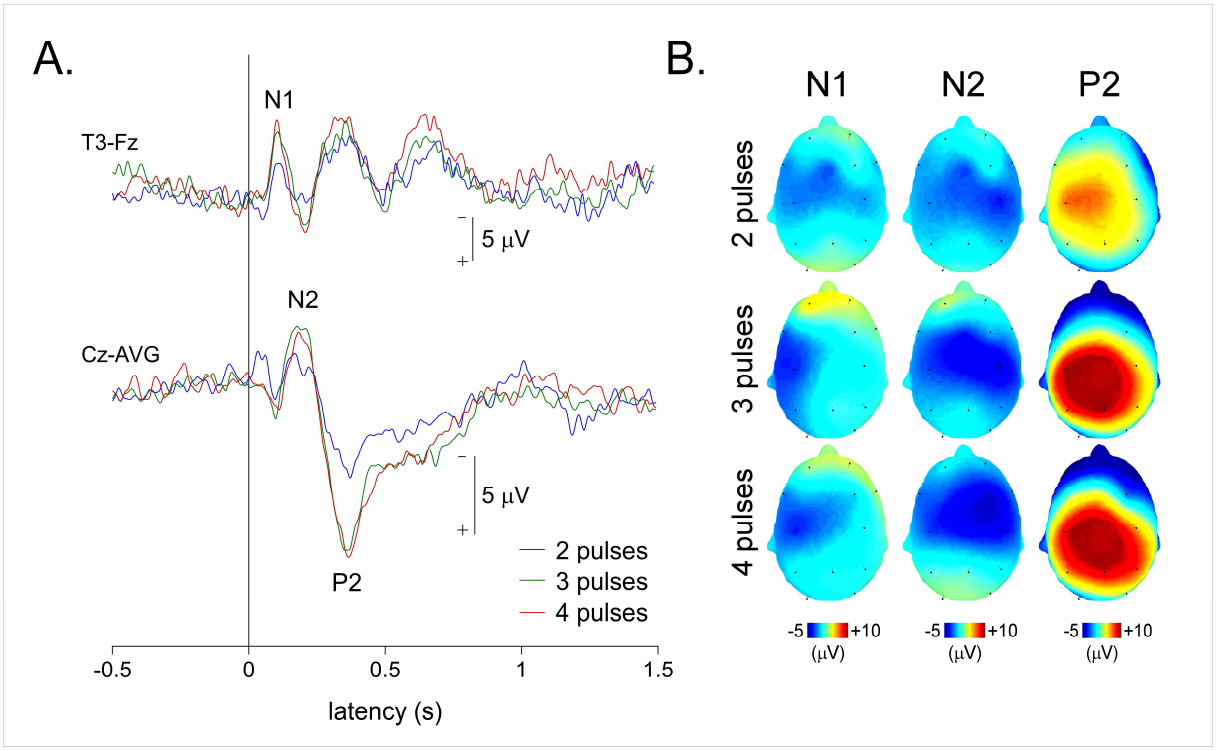

Figure 2. A. Group-level average waveforms of the ERPs elicited by 2, 3 and 4 pulses of IES delivered to the right hand dorsum obtained at electrode Cz (average reference) and T3 (Fz reference). B. Group-level average scalp topographies of the N1, N2 and P2 waves elicited by 2, 3 and 4 pulses of IES.

313 Table 1

		Number of pulses of IES		
		2	3	4
Latencies (ms)				
	N1	149 ±102	151 ±109	144 ±102
	N2	228 ±111	220 ±106	223 ±101
	P2	369 ±113	363 ±118	367 ±105
Amplitudes (μV)				
	N1	-6.7 ±3.3	- 11.6 ±7.3	- 12.0 ±5.8
	N2	-5.4 ±2.3	- 6.5 ±3.7	- 7.3 ±3.4
	P2	9.1 ±5.7	13.4 ±8.5	12.8 ±7.9
N2-P2 difference (μV)		14.5 ±5.6	19.9 ±9.9	20.1 ±9.7

314

315 Table 1.Latencies and amplitudes (mean ±standard deviation) of the ERP components N1, N2
316 and P2, and the N2-P2 amplitude difference, according to the number of pulses of the intra-
317 epidermal stimulation.