Single Pulse and Pulse Train Modulation of Cutaneous Electrical Stimulation: A Comparison of Methods

Esther M. van der Heide, Jan R. Buitenweg, Enrico Marani, and Wim L. C. Rutten

Summary: Changing the amplitude of single rectangular pulse stimuli (SP) has the disadvantage of recruiting tactile and nociceptive fibers in a changing, unknown proportion. Keeping the amplitude constant, but applying a varying number of pulses in a train is another way of stimulus variation, keeping the proportion constant. So, pulse trains (PT) with a variable number of pulses but fixed amplitude might be more suitable to study nonperipheral aspects of processing of stimuli. In this study, we compared the effects of PT and SP stimulation on subjective Numeric Rating Scale scores of perceived stimulus strength and evoked potentials (EP). A total of 41 healthy subjects were electrically stimulated at the left forearm or left middle fingertip using SP and PT stimuli. Numeric Rating Scale scores and EPs were averaged from 105 randomized stimuli at 5 stimulus amplitudes or number of pulses for each subject. The relationships between stimulus amplitudes or number of pulses, EP components and Numeric Rating Scale scores differed depending on the stimulation method and stimulus location. Although the repeatedly reported Numeric Rating Scale-EP (N150-P200) correlation was reproduced for SP at the fingertip, no significant correlation was found for SP stimulation at the forearm. For PT this correlation was found for both stimulus locations. These findings demonstrate that SP and PT involve different ways of processing. The two methods result in different Numeric Rating Scale scores and EP components. Furthermore, PT stimulation is less dependent on stimulus location.

Key Words: Cutaneous electrical stimulation, Pulse train, Evoked potential.

(*J Clin Neurophysiol* 2009;26: 54–60)

In both clinical and fundamental pain research, peripheral and central changes in neural functions are widely acknowledged to play a key role in chronifying pain (Apkarian et al., 2005; Coderre et al., 1993; Woolf and Salter 2000). However, observation of the underlying neurophysiological mechanisms remains difficult.

Perceived pain strength, for example reported by the Numeric Rating Scale (NRS), is frequently used for measuring the subjective pain experience. Yet, for understanding the mechanisms of pain the subjective pain experience is not sufficient and neurophysiological measures are required. Therefore, in several studies evoked potentials (EPs) are used to measure cortical activations that reflect central processing of noxious stimuli, applied using thermal energy (laser or contact heat) or electrical current [see for reviews: (Bromm and Lorenz, 1998; Kakigi et al., 2005; Treede et al., 2003)].

The measured peak-to-peak EP amplitudes seem to correlate with subjectively reported pain intensities: subjective ratings of identical stimuli are correlated to EP components and peak-to-peak EP amplitudes (Iannetti et al., 2005; Lousberg et al., 2005). Other studies have used different stimulation strengths e.g., to evaluate if generated EPs could be related to subjective ratings (Chen et al., 1979; Kanda et al., 2002) or to explore the differences in activation of cortical areas by changing stimulus amplitudes (Torquati et al., 2002). Naturally, well defined stimuli are essential for such studies with varying strength.

Laser stimulation permits selective stimulation of cutaneous nociceptive fibers at different locations on the body (Bromm and Lorenz, 1998; Kakigi et al., 2003; Kakigi et al., 2005). In some studies the stimulus strength is modulated by varying the power of a laser pulse (Nahra and Plaghki, 2003; Ohara et al., 2004; Timmermann et al., 2001). In other studies, increasing the duration of laser stimuli resulted in a linear increase of subjective ratings and EP components and besides a relationship between peak-to-peak amplitudes and subjective ratings was reported (Kanda et al., 2002). However, Chen et al., (2002) showed that subjective ratings of contact heat are not only changed by increasing energy levels but also by increasing the area of stimulation. In spite of these merits of heat stimulation, a disadvantage of laser stimulation is receptor fatigue and peripheral sensitization, both disturbing the transduction of stimulus power into neural activity (Arendt-Nielsen and Chen, 2003).

Intracutaneous electrical stimulation (IES) (Bromm and Meier, 1984) was also commonly used to evoke pain sensations. An advantage of electrical stimulation over heat stimulation is a good control of timing of neural activation. In most studies using different electrical stimulation strengths, the amplitude of a single pulse is varied, [e.g., (Chapman et al., 1999; Stancak et al., 2003)]. Often a linear increase of subjective ratings and modulated EP amplitudes or peak-to-peak amplitudes by a changing stimulus amplitude was reported (Chen et al., 1979; Chapman et al., 1999; Flor et al., 2002). Conversely, multiple pulses of equal amplitude are also perceived stronger and are applied even in combination with changing stimulus amplitudes (Bromm and Scharein, 1982; Dowman, 1994; Inui et al., 2006). Additionally, a train of increasing numbers of pulses resulted in increased subjective ratings, and tend to saturate for higher levels (Giffin et al., 2004). However, since both nociceptive and tactile afferents are activated, electrical stimulation became less popular after introduction of laser stimulation. To improve electrical selective stimulation, an alternative method of electrical stimulation using a pushpin-like needle electrode (epidermal stimulation, ES) has been introduced recently, which preferentially stimulates Aδ-fibers (Inui et al., 2002).

From the above it follows that changing the stimulus strength of both thermal and electrical stimulation results in modulation of the neural activity. However, this activity can be modulated in two different manners: spatially and temporally. By increasing the area of a thermal stimulus more receptors are activated and results in a changing number of activated fibers. Increasing the stimulus amplitude of a single electrical pulse enlarges the area of recruitment resulting in a similar change in activation. However, due to unknown local distribution of tactile and nociceptive fibers, these fiber

From the Institute for Biomedical Technology (BMTI), Biomedical Signals and Systems Group, EEMCS, University of Twente, Enschede, The Netherlands.
Address correspondence and reprint requests to Esther M. van der Heide, M.Sc., Biomedical signals and systems group, Department EEMCS, University of Twente, Zuidhorst 210, P.O. Box 217, 7500AE Enschede, The Netherlands; e-mail: e.m.vanderheide@ewi.utwente.nl.

ISSN: 0736-0258/09/2601-0054

types are activated in variable and unknown proportion with changing electrical stimulus amplitude. This proportion is largely unknown in most skin areas. Interaction between activated fibers, e.g., in the dorsal horn (gate control theory) (Melzack and Wall, 1965), may result in differences in perceived stimulus strength. Conversely, the activity can be changed by temporal modulation of the amount of neural activity in a relative constant proportion of fibers. Due to the coding mechanisms of the receptor, varying the thermal energy results in fibers firing more action potentials with a higher frequency (Kandel et al., 2000). It is widely acknowledged that electrical stimuli directly activate neural fibers instead of skin receptors (Bromm and Lorenz, 1998; Kajimoto et al., 2002). Hence, by increasing the number of pulses (NoP) in a train of pulses with fixed amplitude more action potentials are generated in a constant number of activated fibers (with an interpulse-interval larger then the refractory period). Although with ES Aδ-fibers are stimulated more preferentially, an increase of stimulus amplitude would result in a changing distribution of activated fibers. Yet, by changing the NoP in a train this could be improved, leading to well defined varying electrical stimulus strength.

Both spatial and temporal modulations of neural activity change subjective ratings and EPs. The question arises if both modulations are equivalent and cause similar effects. A linear relationship between subjective ratings and stimulus strength was shown for increasing stimulus amplitude (Chen et al., 1979) but in contrast a nonlinear relationship was reported for change of number of electrical pulses in a train (Giffin et al., 2004). Besides, an EP component showed nonlinear modulation for an increasing thermal pulse length (Kanda et al., 2002) whereas increasing electrical stimulus amplitudes changed EP components linearly (Chen et al., 1979). A systematic comparison between the two modulations has not been reported before.

Therefore, the objective of this study was to investigate differences in subjective ratings and EP components by spatial and temporal modulations using electrical stimuli. Using electrical stimuli, the neural activity will be modulated in two manners; by changing the stimulus amplitude of a single pulse (SP) or by changing the NoP in a pulse train (PT). In the present study a similar electrode was used as in IES stimulation. The differential effect of both SP and PT on stimulus processing was evaluated using two response scores: subjective pain rating scale (NRS) scores and both contralateral and vertex EP component amplitudes.

METHODS

Subjects

A total of 36 right-handed, healthy female subjects (age 22.51 ± 2.81) participated. All subjects gave their written informed consent according to the Declaration of Helsinki. The study was approved by the ethical committee of the Academical Hospital Maastricht.

Electrical Stimulation

The subjects were electrically stimulated at the left anterior lateral forearm or left middle fingertip. Stimulation at the fingertip corresponds to the IES method (Bromm and Meier, 1984). We expected that PT is less sensitive to the local fiber distribution compared with the SP method. Hence, stimuli were also applied at the forearm where the local fiber density is lower (Chien et al., 2001; Nolano et al., 2003) and the distribution different.

An electrode with a 1 mm diameter tip of gold in an insulating material was used. A small opening was drilled in the upper layer of the skin of the fingertip using a dental gimlet with the same diameter as the tip of the stimulation electrode (Bromm and Meier, 1984). If the sensation threshold (I_S) was higher than 1 mA the preparation was regarded insufficiently and tried again. As no thick horn layer is present at the forearm, no special preparation was required there. A rectangular surface electrode (a 4 × 9 cm Klinerva Blue Electrode) was placed with a distance of at least 10 cm at the upper part of the left forearm as an anode. The stimuli were generated by a battery-driven computer controlled current stimulator. The stimulus was a current bipolar rectangular pulse with a stimulus duration of 0.2 milliseconds. Such a stimulus produces a clear pinprick sensation. The electrode was placed in a way that all subjects reported a mild pricking sensation at I_S.

Sensation and Pain Threshold

For each subject, the stimulus amplitudes corresponding to the subjective I_S and pain threshold (I_P) were determined before a protocol. Thresholds were obtained by the ascending method of limits by increasing the stimulus amplitude with steps of 0.1 mA starting at a level of zero. Mean I_S and I_P for both electrode locations are shown in Table 1.

Single Pulse Method

For SP, the stimulus amplitude of a SP was varied depending on the obtained I_S and I_P (see Eqs. below).

$$I_{-50\%} = I_P - 0.50 \cdot (I_P - I_S) \quad (1)$$

$$I_{-25\%} = I_P - 0.25 \cdot (I_P - I_S) \quad (2)$$

$$I_{0\%} = I_P \quad (3)$$

$$I_{+25\%} = I_P + 0.25 \cdot (I_P - I_S) \quad (4)$$

$$I_{-50\%} = I_P + 0.50 \cdot (I_P - I_S) \quad (5)$$

In anticipation of habituation effects (Milne et al., 1991), the minimum stimulus amplitude was set in between I_S and I_P. Decreasing the amplitude further below this minimum stimulus amplitude would probably result in large numbers of unperceived stimuli.

Pulse Train Method

The fixed stimulation current for PT was chosen similar to the minimum stimulus amplitude $I_{-50\%}$ of SP (Eq. 1). Since we used an IES electrode, selective stimulation of nociceptive afferents (Aδ-fibers) alone is probably not possible. To activate Aδ-fibers as selective as possible we therefore chose the minimum stimulus amplitude of SP as stimulus amplitude of PT.

The NoP for PT varied from 1, 3, 5, 7, to 9 pulses. The interpulse interval (IPI) between 2 subsequent pulses in the pulse train was 5 milliseconds. With 5 milliseconds IPI, i.e., well outside the refractory period, fibers have enough time to regenerate. To make sure that stimulation by PT was tolerable, the five NoP were applied in increasing order before the protocol. Although the stimulus amplitude of PT was below the subjective I_P, subjects described stimulation by a train of five pulses as a clear pricking painful sensation.

TABLE 1. Ranges and Means (±SD) of I_S and I_P for the Forearm (n = 26) and Fingertip (n = 30)

	I_S		I_P	
Location	Range (mA)	Mean ± SD (mA)	Range (mA)	Mean ± SD (mA)
Fingertip	0.1–1.0	0.46 ± 0.26	0.5–3.3	1.76 ± 0.72
Forearm	0.1–1.3	0.47 ± 0.26	0.7–4.0	2.06 ± 0.74

Means obtained of all subjects of the four groups.
I_S, sensation threshold; I_P, pain threshold.

EEG Recordings

Electrical brain activity was continuously recorded using a 64-channel EEG Refa-72 system (ANT, the Netherlands). Ag/AgCl electrodes were placed according to the international 10-5 system (Waveguard EEG cap). The scalp electrode impedance was less than 5 k[Omega]. The ground electrode was placed at the forehead. An electrode was placed above and under the left eye for electrooculogram recording. Furthermore, subjects were instructed to fix their eye on a point in front of them. Data recorded at C_Z referred to linked earlobes (A_1A_2) and data recorded at C_4 referred to F_Z were analyzed. EEG was recorded at a sample frequency of 1 kHz. The signals were filtered offline at band-pass 0.3 to 120 Hz. Data from -10 up to -100 milliseconds prestimulus was used for baseline correction. The time window of analysis was 100 milliseconds to 400 milliseconds poststimulus. EEG data was recorded using ASA software (ANT software BV, the Netherlands) and data analysis was performed in Matlab (The Mathworks Inc.).

Numeric Rating Scale

Subjects were asked to rate orally the perceived strength of each stimulus on an 11 point NRS. Zero corresponded to "no sensation" whereas 10 corresponded to "strongest imaginable pain." The first stimulus corresponded for SP with the I_P $I_{0\%}$ (Eq. 3) and for PT with a train of 5 pulses at $I_{-50\%}$ (Eq. 1). The subjects were instructed to rate the first stimulus with a six.

Procedure

Four experiments consisting of two protocols were performed. In Table 2, the four experiments and sample sizes can be found. In each protocol, one combination of stimulus location and stimulation method was tested. The order of protocols was randomized in each experiment.

A protocol consisted of a total of 105 stimuli with 21 stimuli for each of the 5 stimulus amplitudes (SP) or 5 NoP in a pulse trains (PT). The stimuli were applied semi-randomly. The inter stimulus interval between 2 successive stimuli was varied randomly between 10 and 12 seconds.

Data Analysis

Grand average EPs (C_Z-A_1A_2, C_4-F_z,) were obtained of each of the five stimulus amplitudes or NoP of all protocols. First, trials with an electrooculogram artifact exceeding $\pm 100\mu$V in a time window of -10 to -100 milliseconds pre stimulus and 60 to 400 milliseconds post stimulus were rejected. Subsequently, the accepted data was visually inspected for missed electrooculogram artifacts and muscular artifacts. At least 11 trials should be accepted for each of the 5 subject EPs obtained in a protocol. If one of the 5 subject EPs had fewer than 10 accepted trials, the subject was excluded from analysis of the concerning protocol.

Furthermore, mean NRS scores were obtained at all five stimulus amplitudes (SP) or at all five NoP (in PT).

TABLE 2. Composition of Four Experiments Each Consisting of Two Protocols

	SP	PT
Forearm	exp1 (n = 6)	exp1 (n = 6)
	exp4 (n = 11)	exp2 (n = 9)
Fingertip	exp4 (n = 11)	exp2 (n = 9)
	exp3 (n = 10)	exp3 (n = 8)

A protocol is a combination of stimulus location and modulation method.
The number of subjects of each protocol in an experiment is shown.
SP, single pulse; PT, pulse train; exp, experiment.

To allow pooling of the data (NRS scores and EPs) of subjects participating in identical protocols in different groups, the data were statistically tested for difference using a one-way Analysis of Variance (ANOVA). Furthermore, the one-way ANOVA was used to analyze the difference between the I_S and I_P of stimulation of the forearm and finger.

For each protocol both NRS scores and prominent EP component amplitudes were analyzed against stimulus amplitudes or NoP, using one-way ANOVA. We analyzed the following EP components, recorded at C_Z-A_1A_2: P90 at 90 milliseconds, P300 at 290 milliseconds and N150-P200 peak-to-peak EP amplitude with N150 at 140 milliseconds and P200 at 190 milliseconds. Furthermore, we analyzed EP components recorded at C_4-F_Z and C_3-F_Z: P50 at 50 milliseconds, N90 at 90 milliseconds. A linear regression analysis was performed to determine the correlation between NRS scores and EP components and N150-P200 peak-to-peak amplitude.

The effect of stimulus location was analyzed by using recorded EPs at the minimum stimulus amplitude (Eq. 1) of experiment 2 and 4. In these experiments subjects were stimulated at both fingertip and forearm with SP or PT. Since the minimum stimulus amplitude was equal for both, the EPs (C_4-F_Z and C_3-F_Z) were statistically tested using a one-way ANOVA for the effect experiment. Subsequently, the data of the experiments was pooled and the effect of stimulus location on the early contralateral P50 and N90 was statistically tested with a repeated measured ANOVA. All statistical tests were performed at a level of significance $P < 0.05$.

RESULTS

Numeric Rating Scale Scores of Single Pulse

Mean NRS scores were obtained for each of the five stimulus amplitudes by SP. The mean NRS scores for stimulation at the fingertip and the forearm are shown in Fig. 1A. A linear relationship was found between stimulus amplitude and NRS score for both locations. The effects were significant [fingertip: $F(4,100) = 26.45$; $P < 0.0001$ and forearm: $F(4,80) = 26.76$; $P < 0.0001$].

Numeric Rating Scale Scores of Pulse Train

Mean NRS scores were obtained for each of five NoP. The scores are shown in Fig. 1B. The relationship between NRS scores and NoP was comparable for both stimulus locations. The effect was significant [fingertip: $F(4,80) = 28.13$; $P < 0.0001$ and forearm: $F(4,70) = 13.44$; $P < 0.0001$].

Effect of Stimulus Location

Figure 2A shows the pooled grand average EPs (C_4-Fz) for stimulation at the fingertip and forearm. These grand averages were obtained by pooling the data of both SP and PT at the minimum stimulus amplitude.

Stimulation at the fingertip resulted in a clear positive peak around 50 milliseconds (C_4-F_Z), significantly different from the potential for stimulation at the forearm [$F(1,19) = 13.55$; $P < 0.002$]. Furthermore, the N90 (C_4-F_Z) was significant different for stimulus location [$F(1,19) = 9.41$; $P < 0.006$]. Besides C_4-F_Z we also analyzed pooled grand average EPs measured at C_3-F_Z (Fig. 2C). For potentials measured at the ipsilateral electrode C_3 versus F_Z no significant difference for stimulus location was found.

Evoked Potentials of Single Pulse

Grand average EPs (C_Z-A_1A_2) of five stimulus amplitudes for stimulation at the fingertip and forearm are shown in Figs. 3A and 3C respectively. For both stimulus locations, the relationship between the P300 EP component amplitude and stimulus amplitude (Fig. 3E) was comparable to the relationship between NRS and stimulus amplitude (Fig. 1). Increasing stimulus amplitude resulted

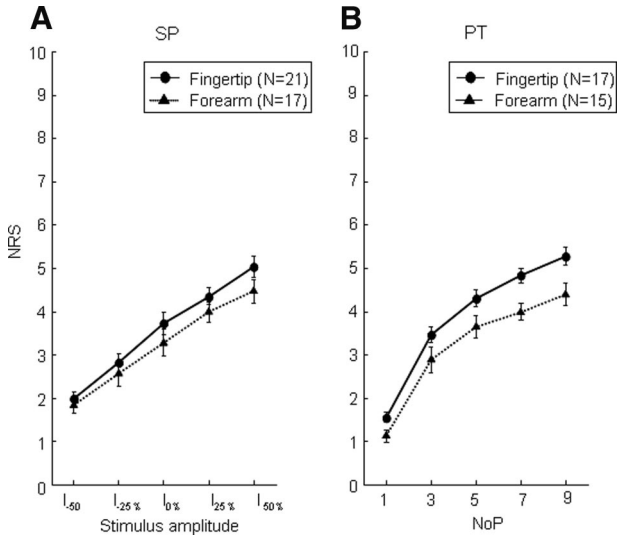

FIGURE 1. Mean Numeric Rating Scale (NRS) scores (± SEM) of all five stimulus amplitudes for single pulse (SP) (**A**) and all five NoP for PT (**B**) for stimulation at the forearm and fingertip. Each symbol represents the mean NRS score of all accepted sweeps of all included subjects at the stimulus amplitude or NoP under test.

in increasing EP component amplitude. The effect of stimulus amplitudes on the P300 EP component was only significant for stimulation at the fingertip [fingertip: F (4,100) = 5.31, $P < 0.0001$ and forearm F (4,80) = 2.15, $P = 0.082$]. Furthermore, stimulus amplitude had no effect on N150-P200 for both fingertip [F(4,100) = 1.25, $P = 0.30$) and forearm (F(4.80) = 0.16, $P = 0.96$].

Evoked Potentials of Pulse Train

Figures 3B and 3D show grand average EPs (C_Z-A_1A_2) of all five NoP for stimulation at the fingertip and the forearm. A stimulation

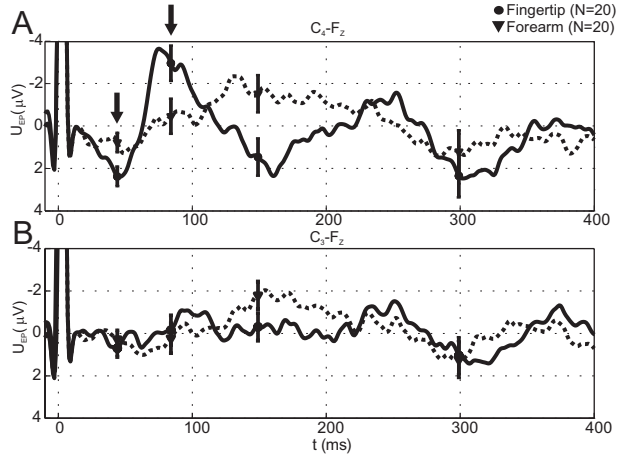

FIGURE 2. Pooled grand average evoked potentials (EPs) (±SEM) measured at C_4-F_Z (contralateral to stimulus location) (A) and at C_3-F_Z (ipsilateral to stimulus location) (**B**) for both stimulus locations. Data was pooled of experiment two and experiment four of both the SP and PT method for stimulation with a single pulse at minimum stimulus amplitude $I_{-50\%}$. Significant effect ($P < 0.05$) indicated by an arrow.

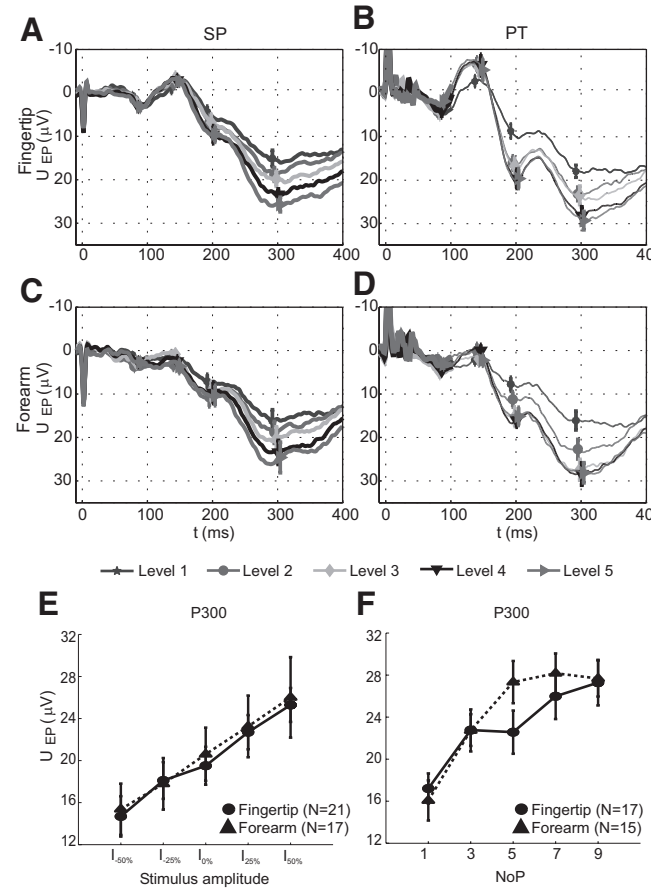

FIGURE 3. Grand average evoked potentials (EPs) (±SEM) measured at C_Z-A_1A_2 of five stimulus amplitudes for stimulation at the fingertip (**A**) and forearm (**C**) by SP. Grand average EPs (±SEM) measured at C_Z-A_1A_2 of five NoP for stimulation at the fingertip (**B**) and forearm (**D**) by PT. Amplitude (±SEM) of P300 EP component measured at C_Z-A_1A_2 for SP (**E**) and PT (**F**) for stimulation at the fingertip and forearm. The levels mentioned in the figure correspond to stimulus amplitudes (SP) or NoP (PT).

artifact can be distinguished during the first milliseconds of the EPs, lasting up to 45 milliseconds for stimulation with 9 pulses. Although the stimulus duration increases with the NoP, latency shifts of the EP components did not follow accordingly (significance not tested).

A significant modulation of the amplitudes the P300 EP component by the PT method was observed for both stimulus locations [fingertip: F (4,80) = 4.11, $P < 0.0044$ and forearm F (4,70) = 7.14, $P < 0.0001$]. Figure 3F illustrates the relationship between P300 EP component amplitudes and the NoP in a pulse train. Again it was comparable with the relationship between NRS and the NoP. The effect of stimulus NoP on EP components was also significant for N150-P200 peak-to-peak EP amplitude for both fingertip [F (4,80) = 3.73, $P = 0.0078$] and forearm [F (4.70) = 2.69, $P = 0.038$].

Evoked Potentials C_4-F_Z for Stimulation at the Fingertip

In Fig. 4 the grand average EPs recorded contralaterally at C_4-F_Z for both SP and PT are shown, for stimulation at the fingertip. For the PT method, a significant effect of increasing NoP in a pulse

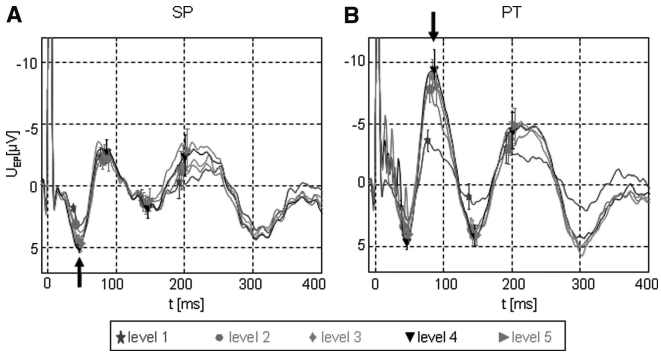

FIGURE 4. Grand average evoked potential (EP) (±SEM) measured at the contralateral electrode (C_4-F_Z) of all five stimulus amplitudes by SP (**A**: n = 21) and all five NoP by PT (**B**: n = 17) for stimulation at the fingertip. Significant effect ($P < 0.05$) indicated by an arrow. The levels mentioned in the figure correspond to stimulus amplitudes (SP) or NoP (PT).

train on the EP amplitude appears for the N90 [$F(4,80) = 3.60$, $P = 0.009$]. For SP no significant effect on N90 was obtained [$F(4,100) = 0.16$, $P = 0.96$]. For the SP method the effect of stimulus amplitude on only the P50 EP amplitude (C_4-F_Z) was significant [$F(4,100) = 3.47$, $P = 0.01$].

Correlation Numeric Rating Scale–Evoked Potential

For both SP and PT method and for both stimulus locations, the relationship between the measured NRS scores and the EP components (measured at C_Z-A_1A_2) was tested by a linear regression analysis. The P300 EP component and the N150-P200 peak-to-peak amplitude were tested. Figures 5A and 5B show the correlations. For both SP and PT at both stimulus locations a significant relationship between P300 EP amplitude and NRS can be seen. It is notable that for the N150-P200 peak-to-peak amplitude no relationship was found for SP at the forearm whereas this relationship was found for SP at the fingertip and PT at both stimulus locations.

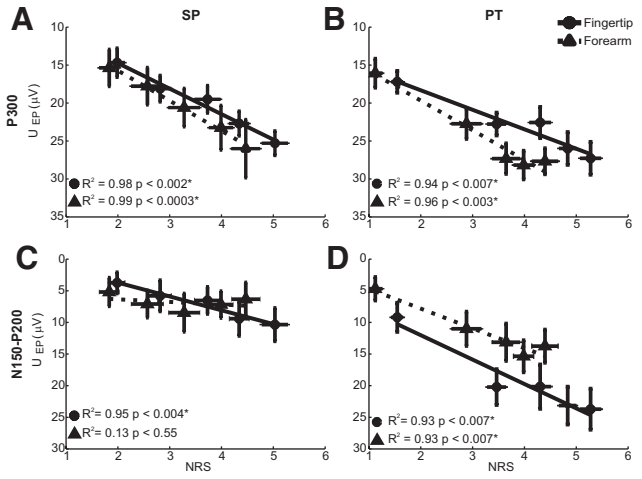

FIGURE 5. Linear regression analysis for correlation between Numeric Rating Scale (NRS) scores (± SEM) and amplitudes evoked potential (EP) components (± SEM) measured at the vertex (C_Z-A_1A_2) for both SP (**A**: n = 21, **C**: n = 17) and PT (**B**: n = 17, **D**: n = 15) at the fingertip and forearm. Correlations are shown for both P300 amplitude (A, B) and peak-to-peak amplitude N150–P200 (C, D).

DISCUSSION

In the current study, a comparison between SP and PT stimulation methods was performed. Both methods, applied at fingertip as well as forearm, influence NRS scores and EP components in specific ways, as discussed below.

Effect of Single Pulse and Pulse Train on Numeric Rating Scale Scores

A linear relationship was found between SP stimulus amplitudes and NRS scores (Fig. 1A). Linearity was also reported by Chapman et al.,(1999) for electrical SP stimuli at the fingertip and by Chen et al.,(1979) for dental stimuli. Furthermore, linearity showed up for power modulated laser stimuli at the dorsum of the left hand (Chen et al., 2006; Kanda et al., 2002; Nahra and Plaghki, 2003; Ohara et al., 2004).

The PT method yielded a curved relationship (Fig. 1B). In literature, only Giffin et al., (2004) presents comparable results, for electrical PT stimuli at the forehead.

Relationship Between Numeric Rating Scale Scores and Evoked Potential Components

The relationship between NRS scores and (peak-to-peak) EP amplitudes (Fig. 5) is reported for several stimulation methods at different locations at the body. The reports pertain not only to modulated stimuli (Chen et al., 1979; Kakigi et al., 1989; Kanda et al., 2002) but also to stimulation with identical stimuli (Beydoun et al., 1993; Iannetti et al., 2005).

P300

For all four protocols P300 and NRS showed a linear dependency. This suggests that the P300 can be used as a neurophysiological correlate of the subjective perceived stimulus strength. However, P300 not only reflects sensory processing but also cognitive processes like attention/distraction (Becker et al., 2000; Reinvang 1999; Zaslanski et al., 1996). It should be noted that in our experiment attention to the stimulus is controlled by the task to rate each stimulus. Therefore, this cognitive component is similar for all protocols and all stimuli. Thus this cognitive component does not influence the P300.

N150-P200

In this study, NRS scores varied almost linearly with N150-P200 peak-to-peak amplitudes, for PT at both stimulus locations. Furthermore, variation was also found for SP at the fingertip, but not for SP at the forearm. An explanation for the latter can be sought in differences in local fiber density and distribution, at fingertip and forearm. Modulation by SP changes the recruitment and proportion of the activated fiber types (touch and nociceptive) in the skin depending on local fiber density and distribution. The local fiber density is larger at the fingertip than at the forearm (Chien et al., 2001; Kelly et al., 2005; Liang et al., 2006; Nolano et al., 2003; Pan et al., 2001).

Effect of Single Pulse and Pulse Train Method on N150-P200 (C_Z-A_1A_2)

For both stimulus locations, the N150-P200 peak-to-peak amplitude (C_Z-A_1A_2) varied under the influence of PT stimuli, but not by SP stimuli (Figs. 3A–D). No significant change of EP amplitude by SP was found for stimulation at the fingertip, which is contrary to earlier studies using SP with IES at the fingertip (Flor et al., 2002; Miltner et al., 1989). This might be attributed to differences in stimulation charge in (mA · s). The small stimulation charges in our

study might not have resulted in significant effect of stimulus amplitude. However, a clear change in amplitude was found by PT stimulation at the fingertip and forearm. Notably, this change was obtained using PT at minimum stimulus amplitude ($I_{-50\%}$).

The absence of N150-P200 variation with SP is remarkable. For SP a sufficient change in number and proportion of activated fibers should result in changing N150-P200 amplitudes. Possibly, the differences between the five stimulus SP amplitudes in the current study may not have been sufficient.

Effect of Single Pulse and Pulse Train Method on P300 (C_Z-A_1A_2)

The P300 EP amplitude (C_Z-A_1A_2) varied along with SP variation (only fingertip stimulation) as well as with PT variation (at both stimulus locations). Although SP at the forearm did not show significant modulation a linear increase of EP amplitude with stimulus amplitude was found (Fig. 3E). Inui reported a P300 EP component, for preferential Aδ stimulation as well as for non-noxious electrical stimulation, using a larger electrode (Inui et al., 2002). The P300 may also reflect cognitive processes (see paragraph Relationship between Numeric Rating Scale scores and Evoked Potential components).

Contralateral Evoked Potential Components P50 and N90

The P50 and N90 EP components are clearly represented in EPs measured at the contralateral electrode C_4 versus F_Z. The EP amplitude of these two components was sensitive for SP or PT stimulation. The relationship between EP component amplitude and stimulus amplitude or NoP was similar to that between NRS and stimulus amplitude or NoP.

Effect of Stimulus Location

The effect of two stimulus locations on EPs was tested, at minimum stimulus amplitude $I_{-50\%}$. Evoked Potentials measured at the contralateral electrode (C_4-F_Z) showed significant effects of different location for the P50 and N90 component (Fig. 2B). An explanation for this may be that differences in local fiber density, as present between fingertip and forearm, result in different distributions and number of stimulated afferents. Furthermore, both stimulus locations have a different cortical representation (somatotopic organization) which may also lead to different EP shapes and amplitudes.

Effect of Single Pulse and Pulse Train Method on P50

The early P50 EP component (C_4-F_Z) amplitude was significantly sensitive for SP at the fingertip. This was not observed for PT at the fingertip, although a clear peak is present [Fig. 4, due to stimulation artifacts modulation of P50 could only be tested for 1, 3, and 5 pulses (data not shown)]. Incoming Aβ information, which is fast in the periphery (Kandel et al., 2000) and relayed to the fast dorsal column medial lemniscus (Desmedt and Cheron, 1980; Marani and Schoen, 2005; Willes Jr and Coggeshall, 1991), is held responsible for P50. The P50 was also reported following mechanical pulses or vibration (Hamalainen et al., 1990). The changing P50 amplitude for SP at the fingertip is probably a result of an increasing number of activated Aβ-fibers, resulting in increased neural activity.

No significant change in P50 amplitude was found at the forearm, both for SP and PT (data not shown). The effect of stimulus location ascribed above may explain the differences in P50 potentials between fingertip and forearm.

Effect of Single Pulse and Pulse Train Method on N90

Contrary to the P50, the amplitude of the N90 (C_4-F_Z) wave did not significantly change for SP at the fingertip. At the forearm, no significant change in N90 amplitude was found for both SP and PT (data not shown).

However, for PT at the fingertip the amplitude changed distinctly with NoP (Fig. 4). Furthermore, although the total stimulus duration increases with NoP, no latency shift was observed for the N90 latency with NoP.

In several studies, N90 potentials are observed after mechanical stimulation as well as non noxious electrical stimulation (Hamalainen et al., 1990; Inui et al., 2003*a*; Inui et al., 2003*b*). Hence, at least Aβ activity is likely to be involved in N90 generation. Using preferential Aδ activation with epidermal stimulation Inui reported SI activity starting around 93 milliseconds (Inui et al., 2003*a*). Recently Wang (Wang et al., 2007) showed that laser stimulation evokes potentials peaking around 109 to 119 milliseconds but with onset latencies of 88 to 105 milliseconds. In their study a new analysis method was used taking into account latency jittering. The N90 in the current study could be associated with (interactive) processing of Aβ and/or Aδ activation.

CONCLUSIONS

The current results show that SP and PT stimulation act differently on EP components, at different stimulus sites. Some EP components varied only by one of both methods and some by both. SP changed the amplitude of P50, at the fingertip. For SP variation of NRS with N150-P200 was observed only for stimulation at the fingertip. The amplitude of the N90 EP component changed only under PT stimulation at the fingertip. PT results for both stimulus sites in similar relationships between NRS and N150-P200 peak-to-peak EP amplitudes. Stimulation at different locations of the body can be useful to research cortical reorganization in chronic pain patients (Flor et al., 1997).

The used stimulation electrode activates both Aβ and Aδ fibers. Nevertheless, at I_S subjects reported a pricking sensation indicating the activation of nociceptive fibers. So, although the stimulus amplitude of PT was chosen below the subjective I_P, yet Aδ-fibers were activated.

Increasing the stimulus amplitude in the SP method increases the number of activated fibers depending on local fiber density and distribution. The change in proportion of activated Aβ-fibers and Aδ-fibers by SP is unknown, resulting in an unknown change in neural activity. Pulse Train is a more controlled method, keeping the proportion of activated nociceptive and tactile fibers constant and giving better temporal control of neural activity.

It was shown that the PT method results in comparable results as the SP method; they both change EP components and subjective ratings. Yet, the seemingly saturating modulation by PT and difference in modulation of the N90 is remarkable. The question arises if parts of the nociceptive system are involved in the N90. Further research is required to interpret the obtained differences in modulation by SP and PT in terms of neurophysiological mechanisms.

ACKNOWLEDGMENT

The authors thank Lida Dam, Nathalie Schopman, Geert Schrijver and Cecile de Vos for their assistance with the measurements.

REFERENCES

Apkarian AV, Bushnell MC, Treede RD, Zubieta JK. Human brain mechanisms of pain perception and regulation in health and disease. *Eur J Pain.* 2005; 9:463–484.

Arendt-Nielsen L, Chen ACN. Lasers and other thermal stimulators for activation of skin nociceptors in humans. *Clin Neurophysiol.* 2003;33:259–268.

Becker DE, Haley DW, Urena VM, Yingling CD. Pain measurement with evoked potentials: combination of subjective ratings, randomized intensities and long interstimulus intervals produces a P300 confound. *Pain.* 2000;84:37–47.

Beydoun A, Morrow TJ, Shen JF, Casey KL. Variability of laser-evoked poten-

tials: attention, arousal and lateralized differences. *Electroencephalogr Clin Neurophysiol.* 1993;88:173–181.
Bromm B, Lorenz J. Neurophysiological evaluation of pain. *Electroencephalogr Clin Neurophysiol.* 1998;107:227–253.
Bromm B, Meier W. The intracutaneous stimulus: a new pain model for algesimetric studies. *Meth Find Exp Clin Pharmacol.* 1984;6:405–410.
Bromm B, Scharein E. Response plasticity of pain evoked reactions in man. *Physiol Behav.* 1982;28:109–116.
Chapman CR, Oka S, Bradshaw DH, et al. Phasic pupil dilation response to noxious stimulation in normal volunteers: relationship to brain evoked potentials and pain report. *Psychophysiology.* 1999;36:44–52.
Chen AC, Niddam DM, Crawford HJ, et al. Spatial summation of pain processing in the human brain as assessed by cerebral event related potentials. *Neurosci Lett.* 2002;328:190–194.
Chen ACN, Richard Chapman C, Harkins SW. Brain evoked potentials are functional correlates of induced pain in man. *Pain.* 1979;6:365–374.
Chen WT, Yuan RY, Shih Y-H, et al. Neuromagnetic SII responses do not fully reflect pain scale. *Neuroimage.* 2006;31:670–676.
Chien HF, Tseng TJ, Lin WM, et al. Quantitative pathology of cutaneous nerve terminal degeneration in the human skin. *Acta Neuropathol* (*Berl*). 2001; V102:455–461.
Coderre TJ, Katz J, Vaccarino AL, Melzack R. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. *Pain.* 1993;52:259–285.
Desmedt JE, Cheron G. Central somatosensory conduction in man: neural generators and interpeak latencies of the far-field components recorded from neck and right or left scalp and earlobes. *Electroencephalogr Clin Neurophysiol.* 1980;50:382–403.
Dowman R. SEP topographies elicited by innocuous and noxious sural nerve stimulation. II. Effects of stimulus intensity on topographic pattern and amplitude. *Electroencephalogr Clin Neurophysiol.* 1994;92:303–315.
Flor H, Braun C, Elbert T, Birbaumer N. Extensive reorganization of primary somatosensory cortex in chronic back pain patients. *Neurosci Lett.* 1997;224: 5–8.
Flor H, Knost B, Birbaumer N. The role of operant conditioning in chronic pain: an experimental investigation. *Pain.* 2002;95:111–118.
Giffin NJ, Katsarava Z, Pfundstein A, et al. The effect of multiple stimuli on the modulation of the nociceptive blink reflex. *Pain.* 2004;108:124–128.
Hamalainen H, Kekoni J, Sams M, et al. Human somatosensory evoked potentials to mechanical pulses and vibration: contributions of SI and SII somatosensory cortices to P50 and P100 components. *Electroencephalogr Clin Neurophysiol.* 1990;75:13–21.
Iannetti GD, Zambreanu L, Cruccu G, Tracey I. Operculoinsular cortex encodes pain intensity at the earliest stages of cortical processing as indicated by amplitude of laser-evoked potentials in humans. *Neuroscience.* 2005;131: 199–208.
Inui K, Tran TD, Qiu Y, et al. Pain-related magnetic fields evoked by intraepidermal electrical stimulation in humans. *Clin Neurophysiol.* 2002;113: 298–304.
Inui K, Tran TD, Qiu Y, et al. A comparative magnetoencephalographic study of cortical activations evoked by noxious and innocuous somatosensory stimulations. *Neuroscience.* 2003a;120:235–248.
Inui K, Tsuji T, Kakigi R. Temporal analysis of cortical mechanisms for pain relief by tactile stimuli in humans. *Cereb Cortex.* 2006;16:355–365.
Inui K, Wang X, Qiu Y, et al. Pain processing within the primary somatosensory cortex in humans. *Eur J Neurosci.* 2003b;18:2859–2866.
Kajimoto H, Kawakami N, Tachi S. Electrocutaneous display with receptor selective stimulations. *Electron Commun Japan (Part II: Electronics*). 2002; 85:40–49.
Kakigi R, Inui K, Tamura Y. Electrophysiological studies on human pain perception. *Clin Neurophysiol.* 2005;116:743–763.
Kakigi R, Shibasaki H, Ikeda A. Pain-related somatosensory evoked potentials following CO2 laser stimulation in man. *Electroencephalogr Clin Neurophysiol.* 1989;74:139–146.
Kakigi R, Tran TD, Qiu Y, et al. Cerebral responses following stimulation of unmyelinated C-fibers in humans: electro-and magneto-encephalographic study. *Neurosci Res.* 2003;45:255–275.
Kanda M, Matsuhashi M, Sawamoto M, et al. Cortical potentials related to assessment of pain intensity with visual analogue scale (VAS). *Clin Neurophysiol.* 2002;113:1013–1024.
Kandel ER, Schwartz JH, Jessell TM. *Principles of Neural Science*, Vol. 4, New York: McGraw-Hill, 2000.
Kelly EJ, Terenghi G, Hazari A, Wiberg M. Nerve fibre and sensory end organ density in the epidermis and papillary dermis of the human hand. *Br J Plast Surg.* 2005;58:774–779.
Liang HW, Hsieh ST, Cheng TJ, et al. Reduced epidermal nerve density among hand-transmitted vibration- exposed workers. *J Occup Environ Med.* 2006; 48:549–555.
Lousberg R, Vuurman E, Lamers T, et al. Pain report and pain related evoked potentials operantly conditioned. *Clin J Pain.* 2005;21:262–271.
Marani E, Schoen JHR. A Reappraisal of the Ascending Systems in Man, with Emphasis on the Medial Lemniscus, Vol. 179, Berlin: Springer, 2005.
Melzack R, Wall PD. Pain mechanisms: a new theory. *Science.* 1965;50:971–979.
Milne RJ, Kay NE, Irwin RJ. Habituation to repeated painful and non-painful cutaneous stimuli: a quantitative psychophysical study. *Exp Brain Res.* 1991;87:438–444.
Miltner W, Johnson JR, Braun C, Larbig W. Somatosensory event-related potentials to painful and non-painful stimuli: effects of attention. *Pain.* 1989;38: 303–312.
Nahra H, Plaghki L. The effects of A-fiber pressure block on perception and neurophysiological correlates of brief non-painful and painful CO2 laser stimuli in humans. *Eur J Pain.* 2003;7:189–199.
Nolano M, Provitera V, Crisci C, et al. Quantification of myelinated endings and mechanoreceptors in human digital skin. *Ann Neurol.* 2003;54:197–205.
Ohara S, Crone NE, Weiss N, et al. Amplitudes of laser evoked potential recorded from primary somatosensory, parasylvian and medial frontal cortex are graded with stimulus intensity. *Pain.* 2004;110:318–328.
Pan CL, Lin YH, Lin WM, et al. Degeneration of nociceptive nerve terminals in human peripheral neuropathy. *Clin Neurosci.* 2001;12:787–792.
Reinvang I. Cognitive event-related potentials in neuropsychological assessment. *Neuropsychol Rev.* 1999;9:231–248.
Stancak A, Svoboda J, Rachmanova R, et al. Desynchronization of cortical rhythms following cutaneous stimulation: effects of stimulus repetition and intensity and of the size of corpus callosum. *Clin Neurophysiol.* 2003;114: 1936–1947.
Timmermann L, Ploner M, Haucke K, et al. Differential coding of pain intensity in the human primary and secondary somatosensory cortex. *J Neurophysiol.* 2001;86:1499–1503.
Torquati K, Pizzella V, Della Penna S, et al. Comparison between SI and SII responses as a function of stimulus intensity. *Neuroreport.* 2002;13:813–819.
Treede RD, Lorenz J, Baumgartner U. Clinical usefulness of laser-evoked potentials. *Neurophysiol Clin.* 2003;33:303–314.
Wang X, Inui K, Kakigi R. Early cortical activities evoked by noxious stimulation in humans. *Exp Brain Res.* 2007;180:481–489.
Willes WD Jr, Coggeshall RE. *Sensory Mechanisms of the Spinal Cord.* New York: Plenum Press, 1991.
Woolf CJ, Salter MW. Neuronal plasticity: increasing the gain in pain. *Science.* 2000;288:1765–1768.
Zaslanski R, Sprecher E, Katz Y, et al. Pain-evoked potentials: what do they really measure. *Electroencephalogr Clin Neurophysiol.* 1996;100:384–392.

