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Somatosensory-evoked potentials indicate increased unpleasantness of noxious stimuli in response to increasing stimulus intensities in the rat

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Abstract

Recently, it has been shown in rats that specific characteristics of somatosensory-evoked potentials (SEPs) recorded from different sites on the scalp correlate differently to the amount of unpleasantness experienced by the animal following noxious stimulation. It was shown that the SEP recorded from vertex (Vx-SEP) did correlate with the unpleasantness, whereas the SEP recorded from the primary somatosensory cortex (SI-SEP) did not. In the present study, we further investigated the relationship between the Vx-SEP, SI-SEP and the unpleasantness of noxious stimuli. Therefore, different groups of rats were subjected to a SEP fear-conditioning paradigm in which the unconditioned stimulus (US), represented by noxious stimuli applied to evoke SEPs, was paired to a conditioned stimulus (CS) represented by a tone. Different stimulus intensities of the US were applied in the different groups. After CS–US presentation, CS-induced fear-conditioned behaviour was analysed in relation to the characteristics of the Vx- and SI-SEP during CS–US presentation. Results showed that increasing stimulus intensities led to increased SEP amplitudes, which were paralleled by an increased amount of CS-induced fear-conditioned behaviour. No differences between Vx-SEP and SI-SEP were found. The increase in the SEPs in parallel with the increased amount of fear-induced behaviour further supports the SEP to be a potentially valuable tool for studying acute pain and analgesia in animals.
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1. Introduction

Evoked potentials recorded from the scalp after noxious stimulation (SEPs) represent neuronal processing of the noxious stimulus in the brain [6,17,38]. SEPs recorded from vertex (Vx-SEP) in man show interesting characteristics making them useful to study acute pain and analgesia. Vx-SEPs (1) are sensitive to analgesic intervention [2,4,24]; (2) show a positive correlation with the unpleasantness of the noxious stimuli applied to evoke the SEP [2,5,14,17,19]; (3) are absent or altered in patients suffering from deficits in pain sensation [8,17,23,26,27,37].

In animals, SEPs are also of interest to study acute pain and analgesia. SEPs recorded in animals (1) are sensitive to analgesic intervention [4,18,25,35,40,41,42] and (2) a recent study in rats showed a correlation between the Vx-SEP and the unpleasantness of the noxious stimuli applied to evoke the SEP [42] using a model based on Pavlovian fear-conditioning

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[43]. This Pavlovian fear-conditioning model consists of two sessions executed on consecutive days. On day 1 (training session) rats are subjected to a paradigm in which an innocuous tone, the conditioned stimulus (CS), is presented paired to the unconditioned stimulus (US), which is represented by noxious stimuli applied to evoke a SEP (SEP stimulation paradigm). On day 2 (testing session) the strength of the rat's CS–US association is investigated by determining the duration of freezing behaviour after presenting the CS without the US. The literature provides ample evidence that the duration of freezing behaviour is to be considered to reflect the unpleasantness of the noxious stimuli experienced by the animal [1,12,43]. Thus, correlating the SEPs recorded on day 1 with the duration of freezing behaviour recorded on day 2 shows whether the SEP indicates the unpleasantness of the noxious stimuli. In a previous study, using this model in combination with the μ-opioid analgesic fentanyl applied to modulate the unpleasantness of the US, it was shown that SEPs recorded from vertex (Vx-SEP) did signal the unpleasantness of noxious stimuli whereas SEPs recorded from the primary somatosensory cortex (SI) (SI-SEP) did not [42]. However, analgesic drugs can influence the outcome of Pavlovian fear-conditioning tasks by other mechanisms than analgesic actions alone [10,31,45]. Therefore, the relation between the Vx-SEP and the unpleasantness of the noxious stimuli, found in the previous study [42], might be confounded by the use of fentanyl. Although the possible co-influences of fentanyl were argued to be of little importance in the previous study [42], the present study further investigated the relation between both the Vx-SEP and the SI-SEP and the unpleasantness of the noxious stimuli. To this aim, we used the SEP fear-conditioning model while modulating the US unpleasantness by using different stimulus intensities instead of a pharmacological intervention.

2. Materials and methods

2.1. Animals and surgery

Animal care and experimentation were performed in accordance with protocols approved by the Science Committee and the local Animal Experimentation Committee (Utrecht University, Utrecht, The Netherlands).

Adult male Wistar rats (HsdCpb:WU, Harlan Netherlands BV, Horst, The Netherlands, body weight 300–350 g, $n = 48$) were permanently instrumented with epidurally placed EEG recording electrodes. After induction of anaesthesia with 0.3 mg/kg fentanyl (i.p., Fentanyl Janssen®, Janssen-Cilag BV, Tilburg, The Netherlands) and 0.3 mg/kg medetomidine (i.p., Domitor®, Pfizer Animal Health BV, Capelle a/d IJssel, The Netherlands), the animals were fixed in a stereotaxic apparatus (Model 963, Ultra Precise Small Animal Stereotaxic, David Kopf Instruments, Tujunga, CA, USA). Epidural electrodes (wired stainless steel screws, tip diameter 0.6 mm, impedance 300–350 Ω, Fabory DIN 84A-A2, Fabory, Nieuwegein, The Netherlands) were implanted at the vertex (4.5 mm caudal to bregma, 1.0 mm right from midline), the primary somatosensory cortex (2.5 mm caudal to bregma, 2.5 mm right from midline), and bilateral in the frontal sinus (10.0 mm rostral to bregma, 1.0 mm left and right from midline, respectively) [40]. All electrodes were wired to an eight-pin receptacle (Mecap Preci-Dip 917-93-108-41-005, Preci-Dip Durtal SA, Delémont, Switzerland) and fixed to the skull with dental cement (Simplex Rapid, Associated Dental Products Ltd., Swindon, UK). At the end of surgery, anaesthesia was antagonized with 1 mg/kg atipamezole (s.c., Antisedan®, Pfizer Animal Health BV, Capelle a/d IJssel, The Netherlands) and 0.15 mg/kg buprenorphine (s.c., Temgesic®, Schering-Plough, Amstelveen, The Netherlands). Postoperative analgesia was provided by 0.15 mg/kg buprenorphine administered s.c. at 8 h intervals for 3 days after surgery. One animal did not survive surgery.

2.2. Fear-conditioning (training session)

The fear-conditioning sessions on day 1 (training session) were performed under the same conditions and using the same procedures as described previously [42,43]. In brief, after 30 min of acclimatization in the experimental room the animals were fitted in a tight fitting jacket and an electrical stimulation device (two brass electrodes of 3 mm in diameter tapered towards the end, fixed in a silicon tube, which enclosed the tail base [40]) was fixed at the left tail base and tightened by Velcro tape for maximal fixation. Subsequently, the animals were placed in the fear-conditioning box, and the receptacle at the animal's head was wired, via a swivel-connector, to the SEP recording device. Through the open middle of this swivel-connector, the stimulation device was wired to a Grass-stimulator via a separate swivel-connector. This approach allowed free movement of the animal during the session. The sessions consisted of 10 CS–US pairings and started after 15 min of acclimatization in the box. The US (square wave pulses, $n = 72$ of 2 ms duration each, with a stimulus frequency of 0.5 Hz; total time 144 s) was presented 10 s after onset of the CS (a 40 s 1500 Hz tone, 85 dB sound pressure level) creating a 30 s overlap. Time between subsequent CS onsets varied between 358 and 502 s. The US intensity was 0.0, 0.5, 1.0, 2.0, 3.0 or 5.0 mA for group 0.0, 0.5, 1.0, 2.0, 3.0 and 5.0, respectively ($n = 8$ for every group except for group 0.0, $n = 7$). During the fear-conditioning sessions SEPs were recorded in the freely moving animal for every US presentation, as described previously [39]. In brief, SEPs were recorded from the vertex and the primary somatosensory cortex electrodes with the ipsi- and contra-lateral frontal sinus electrodes serving as reference and ground, respectively. Signals were amplified 2000 times, band-pass filtered between 15 and 300 Hz and digitized online at 2000 Hz. For each SEP trial, 72 subsequent data segments of 256 data points (25 ms pre-stimulus, 102.5 ms post stimulus) were recorded and averaged online.

2.3. Assessment of CS–US association (testing session)

The sessions to assess the CS–US association on day 2 (testing session) were performed under the same conditions and using the same procedures as described previously [42,43]. In brief, after 30 min of acclimatization in the experimental room the animals were placed in the testing box. After 15 min of acclimatization in the box the testing session, consisting of 10 CS presentations, started. Time between subsequent CS onsets varied between 196 and 370 s. During these sessions the behaviour of the animals was videotaped continuously.

2.4. Data and statistical analysis

Calculations were performed with the aid of Microsoft Excel 2000. Statistical analysis was performed with SPSS 11.0 for Windows. Differences were considered to be significant when $P < 0.05$.

The SEP signals were evaluated using the rate dispersion factor (RDF). The RDF, an expression of the overall shape of the SEP waveform in the latency range studied, is obtained by averaging the absolute differences between all pairs of subsequent sampled data points y_k in a specified latency range from x to m (see Eq. (1)) [13,29,40]. Both decreases in amplitude and increases in latency of the SEP components, decrease the value of the RDF, whereas both increases in amplitude and decreases in latency of the SEP components increase the value of the RDF. When choosing the RDF latency range (x to m) in the group with the strongest signals (5.0 mA group), individual signals at all stimulus intensities can be analyzed using this fixed latency range. Therefore, the RDF is a highly objective method to evaluate SEP signals without the need of choosing peak amplitudes in individual signals, which is prone to confounding errors, especially when signals are weak.

For the Vx-SEP, the RDFs were calculated for the latency range (x to m) of the positive-to-negative complex previously described as the P15–N20 complex [42], recorded in the 5.0 group. For the SI-SEP the RDFs were calculated for the latency range (x to m) of the positive-to-negative complex previously described as the P12–N20 complex [42], recorded in the 5.0 group. For both the Vx-SEP and the SI-SEP, the RDF was calculated as the percentage change over groups, using the mean RDF value of both Vx-SEP and SI-SEP of the 5.0 group as

100%, since in this group the signals had the highest amplitudes and the shortest latencies, resulting in the highest RDFs.

$$\mathrm{RDF} = \frac{1}{m-x}\sum_{k=x+1}^{m} |y_k - y_{k-1}| \qquad (1)$$

The duration of freezing behaviour during the testing sessions was analyzed using the video recordings. Freezing behaviour was defined as the absence of all visible movements with the exception of breathing movements and pendulum motion of the head, while the animal sits in a tensed posture [21]. Freezing was scored only during presentation of the CS. The behavioural data were scored blindly with respect to the stimulus intensity used and the scorings of the principal investigator [HvO] demonstrated a high correlation with the scorings of a second observer who was unaware of the aims and procedures of this experiment (Pearson's correlation coefficient 0.959, $P < 0.001$, $n = 36$).

For each animal, the SEP data of the 10 trials were averaged for both recording sites and subsequently analysed using a repeated measurement analysis of variance with fixed factor "group" and repeated factor "recording site". The freezing data of the 10 trials were averaged and subsequently analysed using a one-way analysis of variance with factor "group".

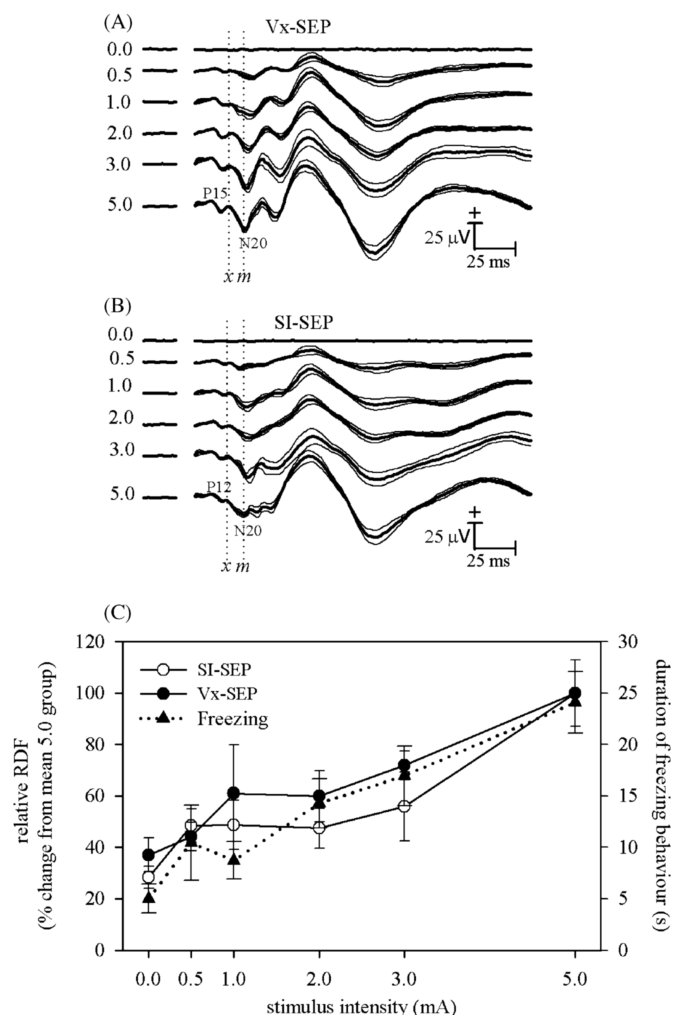

Fig. 1. SEPs in relation to stimulus intensity. (A) and (B) Grand mean (bold lines) ± S.E.M. (plain lines) waveforms of the Vx-SEP and SI-SEP, respectively, at the different stimulus intensities ($n = 8$ for every intensity except for intensity 0.0, $n = 7$). Dashed lines x and m indicate the latency range for which the RDF was calculated (see text). (C) Percentage change in the RDFs calculated over the latency range x to m for both the Vx-SEP and the SI-SEP in relation to the duration of freezing behaviour. Data are presented as mean ± S.E.M. ($n = 8$ for every intensity except for intensity 0.0, $n = 7$). Note the two different y-axes; left for the RDF and right for the duration of freezing behaviour.

3. Results

The grand average waveforms of the Vx-SEP and SI-SEP are shown in Fig. 1A and B, respectively. At the intensity of 5.0 mA the Vx-SEP P15 and SI-SEP P12 occurred at a latency of 14.37 ± 0.09 and 13.95 ± 0.06 ms, respectively (mean ± S.E.M.). The Vx-SEP N20 and SI-SEP N20 occurred at a latency of 18.51 ± 0.37 and 18.21 ± 0.1 ms, respectively (mean ± S.E.M.).

As shown in Fig. 1C, increasing stimulus intensities resulted in increasing RDF values for both Vx-SEP and SI-SEP. Relative RDFs calculated over the specific latency range (x to m) did not show differences for recording site [recording site × group: $F(5,41) = 0.578$, $P = 0.717$; recording site: $F(1,41) = 3.028$, $P = 0.089$] but significantly increased with higher stimulus intensities [group: $F(5,41) = 7.064$, $P < 0.001$].

None of the experimental groups showed freezing behaviour during the 15 min of acclimatization in the testing box. Onset of the CS, however, induced freezing behaviour. The duration of freezing behaviour increased significantly with increasing stimulus intensities [group: $F(5,41) = 6.207$, $P < 0.001$]. As shown in Fig. 1C, the duration of freezing behaviour paralleled the increase in RDFs of the SEPs.

4. Discussion

Data from the present study show that increasing stimulus intensities result in an increase of the RDF of both the Vx-SEP and the SI-SEP, which is paralleled by an increase in the duration of freezing behaviour. From these findings, it is concluded that the SEPs indicate the unpleasantness of the noxious stimuli experienced by the animals. Although previous studies demonstrated that increasing stimulus intensities of noxious stimuli (1) lead to an increase of SEP amplitudes [39,40,41] and (2) lead to an increase in the duration of freezing behaviour [1,12], these data were obtained in separate and unrelated studies. In the present study, however, the data were generated in an integrative approach, providing the possibility to demonstrate a direct relation between the SEPs and the unpleasantness of the noxious stimuli.

The SEP fear-conditioning model in this study was used without pharmacological intervention since (analgesic) drugs might influence the outcome of Pavlovian fear-conditioning tasks by other mechanisms than the principle (analgesic) actions on the US alone. First, administering drugs can impair the formation of a CS–US association at the level of the amygdala, while both CS and US were perceived normally by the animal [10,20,31]. Second, training animals in a drugged state might introduce state-dependent learning. State-dependent learning is described as a situation in which a subject can only recall an association formed under a certain state (e.g. a drugged state) when it is in that state again [28,30,45]. Third, administration of drugs from several classes have been shown to reduce auditory evoked potential amplitudes, as such indicating a reduction in the

perception of auditory input, i.e. the CS [33]. Taken together (1) impairment of a CS–US association at the level of the amygdala; (2) state-dependent learning; (3) a reduced or absent perception of the CS, might result in a reduced or absent CS–US association, leading to short durations of freezing behaviour, while the animal possibly did experience the US as unpleasant. Therefore, one might mistakenly conclude that the reduced SEP amplitude, paralleled by a reduced duration of freezing behaviour, indicates a reduction in the perception of noxious stimuli. Although a previous study that used the SEP fear-conditioning model [42] already argued these drug-induced confounding co-influences to be of limited importance, the present study explicitly demonstrates that SEPs indicate the unpleasantness of noxious stimuli in the rat, without possible confounding drug effects.

In this study, no differences could be demonstrated between the Vx-SEP and SI-SEP in relation to the unpleasantness of noxious stimuli. Although seemingly contradictory to previous work [42], the present findings are in line with (1) the hypothesis previously presented regarding the background of the Vx-SEP and the SI-SEP [40–42] and (2) the differences regarding the experimental set-up between the two studies.

It was hypothesized that the Vx-SEP and SI-SEP are related to separate functional pain mechanisms, being a mechanism signalling emotional affective aspects (unpleasantness) of noxious stimuli and one signalling sensory discriminative aspects (e.g. place, duration, intensity) of noxious stimuli, respectively [40–42]. In the present study, noxious stimuli of different stimulus intensities were used to modulate the impact of the US. When using different stimulus intensities, the unpleasantness and the sensory discriminative aspects of the noxious stimuli are modulated in parallel [32,34,44]. This explains the absence of dissociation between the Vx-SEP and the SI-SEP with respect to their correlation with the unpleasantness of the noxious stimuli. In contrast, using the SEP fear-conditioning model in combination with a μ-opioid agonist to modulate the impact of the US has been shown to result in dissociation between the Vx-SEP and the SI-SEP with respect to their correlation with the unpleasantness of the noxious stimuli [42]. This is likely caused by the fact that the neuroanatomical pain pathway involved in unpleasantness contains more μ-opiate receptors than the pathway encoding for sensory discriminative aspects [3,9,16,36]. Combined, the present and previous findings [42] show that both the Vx-SEP and the SI-SEP indicate the perception of noxious stimuli but that only the Vx-SEP indicates the unpleasantness of the noxious stimuli.

The SEPs were generated using electrical stimulation, which in principal, can co-activate Aα-fibers involved in motor processing, Aβ-fibers primarily involved in tactile sensation next to Aδ- and C-fibers primarily involved in nociception [6]. However, the SEP components in the latency range studied have been reported to be primarily related to nociception [35,39,40], which is of primary importance when using these components as indicators of pain and analgesia.

First, conduction velocities are considered to be specific for different classes of nerve fibers, e.g. motor (Aα: 60–70 m/s), tactile (Aβ: 30–60 m/s) or nociceptive (Aδ: 4–30 m/s and C: 0.4–1.8 m/s) fibers [6]. Based on these conduction velocities and the distance between the stimulus location and the recording site in this study (approximately 0.15–0.20 m), primarily Aδ-fiber mediated responses will be found within the latency range examined in the present study. According to the international literature Aδ-, compared to Aβ-fibers, primarily mediate nociceptive responses. However, up to 60% of the nociceptive responses in rats (and other species) are reported to be mediated by Aβ-fibers, which is generally neglected [11]. Therefore, conduction velocities alone cannot definitively distinguish between responses representing nociceptive or tactile sensations.

Second, μ-opiate receptor agonists specifically modulate nociceptive processing at both peripheral and (supra-)spinal levels but not tactile processing [18,36]. Since the μ-opioid receptor agonist fentanyl affects both the Vx-SEP and SI-SEP [41,42], these responses are considered to be primarily related to nociception and not tactile sensations.

Finally, the correlation between the duration of freezing behaviour and Vx-SEP and SI-SEP, studied after presentation of the CS, further supports these signals to be primarily related to nociception rather than to tactile sensations.

In conclusion, the SEP components in the latency range studied in the present study are considered to be related to nociception which is in line with other studies using SEPs evoked by electrical stimulation to study nociception [7,15,35,39–43].

Although many animal models exist to study acute pain and analgesia, many of these models are based on nocifensive reflexes (e.g. tail flick, nocifensive withdrawal reflex), which have been shown to be present also in the decerebrate or spinalized animal [22]. Therefore, these models do not necessarily involve higher cerebral structures and functions and consequently, do not provide unequivocal insight in the animal's emotional unpleasant experience. Furthermore, these models have limited suitability for studying the different classes of analgesic drugs and may be easily confounded by learning effects and many other environmental factors [22].

In contrast to the approaches discussed above, we argue that recording of SEPs after noxious stimulation is a powerful tool to study pain and analgesia in animals, rather than nociception and anti-nociception. First, SEPs represent the neural processing of noxious stimuli at the cerebral level [8,17,38]. Second, the Vx-SEP, is sensitive to different classes of analgesic drugs [37]. Third, SEPs can be recorded and analyzed in a highly objective and standardized way, reducing animal and environmental influences to a minimum. Fourth, combining the results of the present and previous study [39] demonstrates that the Vx-SEP potentially is a reliable indicator for unpleasantness whereas the SI-SEP potentially is a reliable indicator for sensory discriminative aspects of noxious stimuli in the rat. Since pain primarily involves the emotional unpleasant component of nociception, the Vx-SEP is considered to be the preferred signal when it comes to studying acute pain and analgesia in animals.

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