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ORIGINAL ARTICLE

Chemo-somatosensory evoked potentials: A sensitive tool to assess conditioned pain modulation?

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Abstract

Background: Chemo-somatosensory evoked potentials (CSSEPs) elicited by chemical stimulation (CO_2 gas) of the nasal mucosa have been shown to be sensitive enough to pick up even weak analgesic effects. With the present study we wanted to investigate whether CSSEPs are also a sensitive tool to capture endogenous pain inhibitory mechanisms elicited by conditioned pain modulation (CPM; where a first conditioning stimulus reduces the sensitivity for a second test stimulus) with a conditioning stimulus of rather low noxious load.

Methods: Seventeen healthy participants were tested for CPM effects (conditioning stimulus: tonic heat pain with intensities around the pain threshold induced via a thermode; test stimulus: chemonasal stimulation (73% and 78% CO_2)) on CSSEPs and on self-report ratings.

Results: We found significant CPM effects in the CSSEPS, with reduced amplitudes and prolonged latencies at several electroencephalogram (EEG) recording positions when using the lower CO_2 concentration (73% CO_2). In contrast to the visible inhibitory effects on the CSSEPs, subjective ratings of the test stimulus did not reflect CPM action.

Discussion: The experimental pain model using CO_2 stimuli to elicit CSSEPs proved to be sensitive enough to capture weak CPM effects elicited by a conditioning stimulus of rather low noxious load. The usage of such mild noxious conditioning stimuli—in contrast to stimuli of higher noxious load (e.g., cold pressor test)—has the advantage that the activation of other types of pain inhibitory mechanisms in parallel (like attentional distraction, stress-induced analgesia) can be avoided.

Keywords

Chemo-somatosensory evoked potentials, conditioned pain modulation, experimental pain, weak noxious load

History

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Introduction

Conditioned pain modulation (CPM) describes the phenomenon that a first pain stimulus (called the *conditioning stimulus*) reduces the sensitivity for a second pain stimulus (called the *test stimulus*) (Yarnitsky et al. 2010). This ''pain inhibits pain'' phenomenon is supposed to be physiologically mainly based on the diffuse noxious inhibitory controls (DNIC), which were first described by Le Bars et al. (1979, 1981, 1992). Using animal studies, these authors demonstrated that pain occurring in one part of the body reduces pain in the rest of the body by activating these DNIC, which are spinal and supra-spinal (i.e., subnucleus reticularis dorsalis in the brainstem) neural mechanisms that modulate the transmission of nociceptive signals via multireceptive neurons (Bouhassira et al. 1992). DNIC-like mechanisms have also repeatedly been observed in humans, where the application of a tonic pain stimulus has been shown to reduce the sensitivity for a concurrently applied phasic pain stimulus (going along with an increase in pain inhibitory frontal areas before decreasing in primary somatosensory areas (Piche et al. 2009; Moont et al. 2012)). A consensus group decided to use a different terminology for such tests in humans, by calling them CPM and thus, separating them from DNIC, which had been defined in pure physiological terms (Yarnitsky et al. 2010). The growing interest in CPM has been inspired by observations that a deficiency of CPM might be a risk factor for the development of chronic pain (Edwards 2005; Julien et al. 2005; de Souza et al. 2009; King et al. 2009; Yarnitsky et al. 2010; Lewis et al. 2012).

Enthusiasm is dampened, however, by the fact that there is still no agreement on the ''ideal CPM protocol'', which leads to various combinations of different noxious stressors (e.g., electrical current, cold pressor pain, pressure pain) as conditioning and test stimuli. While the effect size of CPM inhibition is less dependent on the subjective pain ratings of the conditioning stimulus, the objective noxious load of the

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conditioning stimulus seems to be more critical (Lautenbacher et al. 2002; Granot et al. 2008). Accordingly, intense and summating conditioning stimuli such as hand immersion in hot or cold water have appeared to be very effective (Lewis et al. 2012). This methodological solution might be promising for some purposes but definitely not for all because such strong and summating conditioning stimuli activate also other inhibitory mechanisms like attentional distraction and stress-induced analgesia. Thus, the intensification of the conditioning stimulus is not necessarily always the best solution, and therefore, it is important to also search for test stimulus probes that might be more sensitive in detecting/discerning CPM effects even when using conditioning stimuli of weak to moderate noxious intensity. This search can be guided by earlier findings on the suitability of experimental pain tests to detect weak analgesic effects. In other words, experimental pain protocols, which have been shown to be sensitive enough to capture analgesic effects of weak opioids or even non-steroidal anti-inflammatory drugs (NSAIDs), might also qualify to assess weak CPM effects. Such an experimental pain model might be supplied by the assessment of chemo-somatosensory evoked potentials (CSSEPs) in response to chemical stimulation (CO_2 gas puffs) of the nasal mucosa. CSSEPs in response to chemonasal stimulation have been repeatedly shown to be sensitive enough to pick up the effects of weak analgesics (Kobal et al. 1990, 1994; Lötsch et al. 1995a, 1995b). Moreover, this experimental pain model has the advantage that it allows for specific stimulation of the nociceptive system without simultaneous excitation of other sensory systems.

Our aim in the present study was to further investigate the CPM methodology; more precisely, to investigate which CPM protocol allows for detecting even weak CPM effects. Given that the chemonasal experimental pain model has been shown to be sensitive to even small changes in nociception, we aimed at investigating whether this model is also sensitive enough to capture the effects of a frequently used conditioning stimulus, which is known to be effective but produces only weak to moderate inhibitory effects, namely, tonic heat pain induced via thermodes attached to the skin with an intensity around the pain threshold (Lautenbacher et al. 2002; Pielsticker et al. 2005; Kunz et al. 2006). Given our interest in CPM methodology, we included various variations with regard to (i) the conditioning stimulus (thermal stimuli with $-1\,^{\circ}C$ below and $+1\,^{\circ}C$ above pain threshold were used), (ii) the test stimulus (chemonasal stimulation with 73% and 78% CO_2 concentration were used), and (iii) stimulation sites (homotopic and heterotopic applications of the conditioning stimulus were compared). These variations should allow us to investigate which combinations are most sensitive to pick up even weak CPM effects.

Materials and methods

Participants

Seventeen healthy participants (9 women and 8 men) between the ages of 23 and 33 years (mean age $= 27.3 \pm 2.7$ years) took part in the study. Exclusion criteria were all kinds of acute and chronic diseases. The diagnostic interview, which was designed for this purpose, aimed especially at the exclusion of nasal diseases, cardiovascular diseases, allergies, hyper-responsiveness to stress, mental disorders, neuropathies, disc diseases, endocrine disorders and nerve injuries as well as dermatosis at the upper extremities. All participants studied were drug free. The protocol was approved by the local ethics committee; all participants gave written informed consent and were paid for participation.

Apparatus and procedures

General procedure

All participants took part in two sessions within a period of 3 days. The two sessions differed only in terms of stimulation site of the conditioning stimulus (volar forearm vs. cheek). The order of these stimulation sites was balanced across participants (half of the participants started with stimulation of the volar forearm, whereas the other half started with stimulation of the cheek). The time of day was equivalent for the two sessions with each subject. Each session lasted for approximately 3 h.

Each session included assessment of pain thresholds (right and left body side) being followed by four experimental blocks for the assessment of CPM (a tonic stimulus is applied simultaneously to the phasic pain stimuli). Various practice trials were conducted at the beginning to familiarize participants with stimuli, stimulation, and rating procedures. Figure 1 provides the experimental protocol for each session. During each session, participants were comfortably seated in a sound-attenuated room. In order to help maintain participants' vigilance the participants performed a simple task on a video screen: they had to keep a small square, which could be controlled by a joystick, inside a larger one, which unpredictably moved around. This task was presented during interstimulus intervals.

Apparatus

Heat stimuli (pain threshold, conditioning (tonic) stimulus) were delivered by use of a Peltier stimulator (PATH-Tester; Galfe et al. 1990) with a contact thermode of $1.6 \times 3.6\,cm^2$. Stimulation sites were in one session the volar forearm (right and left body side) and in one session the cheek (right and left body side).

The CO_2 stimuli (test stimulus) were applied to the right and left nostril via the nosepiece of an olfactometer (for a detailed description of the chemical stimulator, see Kobal 1985) which delivered the chemical stimulants without the simultaneous activation of mechano- and thermal receptors of the nasal mucosa. This monomodal chemical stimulation was achieved by mixing the pulse of the stimulant with a constantly flowing air stream with controlled temperature and humidity ($36.5\,^{\circ}C$, 80% relative humidity). The total flow rate was 140 ml/s. Stimulus duration was 200 ms with a rise time below 20 ms. To avoid the respiratory flow of air in the nasal cavity, all participants were trained to practice velopharyngeal closure, while breathing through the mouth. Heat and CO_2 stimuli were applied to the ipsilateral body side

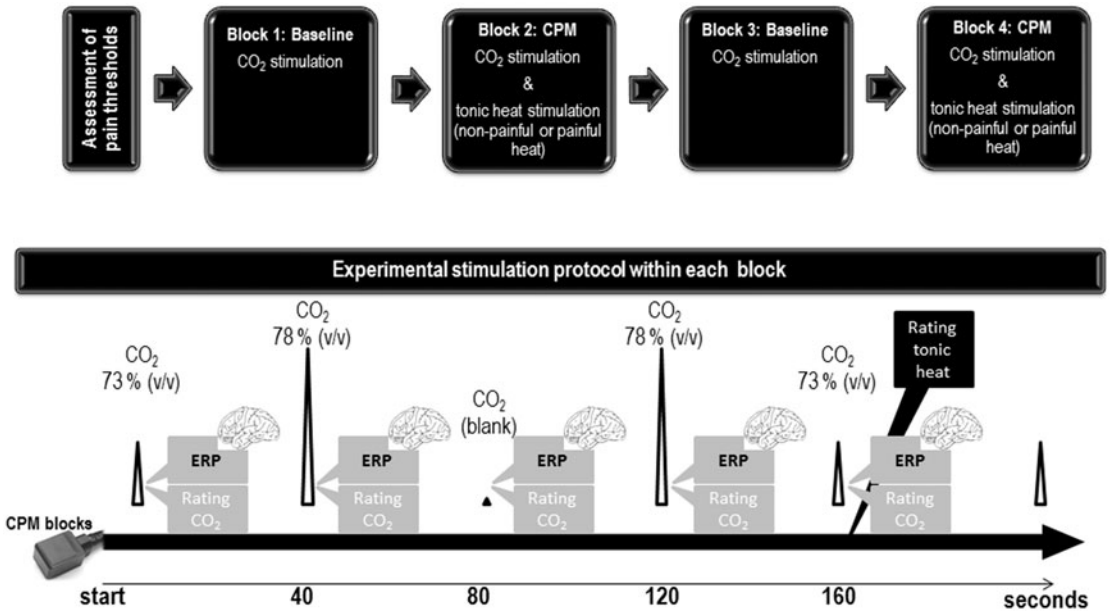

Figure 1. Schematic representation of the experimental protocol and the assessment of CPM effects.

(left nostril: left volar forearm or left cheek; right nostril: right volar forearm or right cheek).

Assessment of pain thresholds

At the beginning of each session, we assessed heat pain thresholds on the right and left volar forearm and on the right and left cheek, respectively (always starting with the right body side). Heat pain thresholds were assessed using the method of adjustment. Participants were asked to adjust a temperature starting from 38 °C, using heating and cooling buttons, until they obtained a level which was barely painful. There were seven such trials; averages of the last six were used as the measure of pain threshold.

Determination of conditioned pain modulation

The inhibitory effects of two types of conditioning stimuli on the perception of the CO_2 stimuli (test stimuli) were tested: non-painful heat (just below pain threshold) and painful heat (slightly above pain threshold). Each treatment by a conditioning stimulus (either ''non-painful heat'' or ''painful heat'') was preceded by a baseline block (CO_2 stimulation without tonic heat) resulting in four experimental blocks (see Figure 1). The interval between blocks 1 and 2 as well as between blocks 3 and 4 was 2 min, whereas the interval between blocks 2 and 3 was 10 min (due to the necessary change of body side). The 10-min interval between blocks 2 and 3 should also ensure that CPM effects elicited during block 2 (see Figure 1) have abated before the start of block 3. Each block lasted for approximately 28 min. We always started with the left body side (blocks 1 and 2) and continued with the right body side (blocks 3 and 4). Participants received both intensities of conditioning stimuli (non-painful heat/painful heat) in each session. The order of intensities was, however, balanced across participants (half of the participants started with non-painful heat (block 2) and received later painful stimulation (block 4), whereas the other half received the opposite order).

Conditioning stimulus. The conditioning stimulus was applied in blocks 2 and 4 (CPM blocks) using a tonic heat pain model (Lautenbacher et al. 1995, 2002; Pielsticker et al. 2005; Kunz et al. 2006). Small heat pulses with an amplitude of 1.3 °C were administered at a constant frequency of 30 pulses per min either at the volar forearm or at the cheek, respectively. In the condition with painful heat, the pulses were tailored to have a base of 0.3 °C below the individual pain threshold and a peak temperature of 1 °C above it. In the condition with non-painful heat, the procedure was the same with the exception that the peak was 0.3 °C below and the base 1.6 °C below pain threshold.[1] This approach allowed a comparison of the effects of tolerable tonic heat pain with the effects of very strong but still non-painful tonic heat.

To avoid sensitization effects, tonic heat stimulation was interrupted for 2 min after half of the CO_2 stimuli had been applied (after the 20th CO_2 stimulus; which was approximately after 12 min of tonic stimulation). During this break the skin of the subject was actively cooled by reducing the temperature of the thermode to 20 °C.

Test stimuli. During each experimental block 32 CO_2 test stimuli (73% and 78% CO_2 (v/v), $n = 16$ for each concentration category) and 8 control stimuli (blanks with no CO_2) were delivered, resulting in 40 chemonasal stimuli. The stimuli were arranged in series of 5 stimuli (2 stimuli with strong concentration (78%), 2 stimuli with medium concentration (73%), and 1 control stimulus) and were administered pseudorandomly. The inter-stimulus interval was 40 s (see Figure 1). The two concentrations used elicit moderate to strong pain intensities without reaching tolerance level (Lötsch et al. 1997).

Rating scale

After the application of each CO_2 stimulus, the participants had to rate the perceived pain intensity of the test stimulus (resulting in 40 ratings per experimental block) by using a

visual analogue scale (VAS) displayed on a computer screen. Participants were instructed to rate the intensity of each stimulus in relation to a standard CO_2 stimulus (73% (v/v)) which was presented at the beginning of experimental blocks 1 and 3. The intensity of this standard stimulus was defined as the center of the VAS being labeled with the number ''100''. Intensities that were perceived as more intense than the standard stimulus had to be rated above 100 and intensities being perceived as less intense had to be rated below 100.

Additionally, in blocks 2 and 4 (CPM blocks) the participants had to rate the perceived intensity of the conditioning heat stimulus in regular intervals after each 5th CO_2 stimulus (see Figure 1). More precisely, after the 5th, 10th, 15th, 20th, 25th, 30th, 35th, and 40th presentation of the CO_2 stimulus the participants were asked to give pain ratings for the conditioning stimulus, resulting in eight ratings per CPM block. For this purpose another VAS was displayed on the computer screen. The number ''100'' in the middle of the scale indicated the threshold between painful and non-painful heat intensities, so that all non-painful sensations were asked to be rated below 100 and all painful ones above 100.

Chemo-somatosensory event-related potentials (CSSEPs)

The electroencephalogram (EEG) was recorded from five positions (Cz, C3, C4, Fz, Pz) of the 10/20 system referenced to linked earlobes (A1 + A2). Blink and eye movement artifacts were monitored from an additional site (Fp2/A1 + A2). EEG records of 2048 ms duration were digitized (sampling frequency was 250 Hz). Data were evaluated by OFFLAP (Kobal 1981) and DATAN programs (Brandl, unpublished). The analyses were performed manually with the operator of the program being blind to the condition. Baseline was defined as the average level during the period 100 ms before stimulus onset. Pain-related chemo-somatosensory event-related potentials were obtained in response to all test stimuli in blocks 1, 2, 3, and 4. For further analyses we used the base-to-peak amplitude and latency of the P2 response (a measure of analgesic effect; Lötsch 1995a).

Statistics

Data were statistically analyzed using SPSS version 11.0 for Windows. All data are given as means ± SD.

Assessment of pain thresholds

To evaluate the effect of stimulation site (volar forearm vs. cheek) on the pain threshold, a paired *t*-test was conducted.

Assessment of CPM effects

Conditioning stimulation. VAS ratings of the conditioning stimulation were analyzed using analyses of variance with repeated measurement with three within-subject factors, ''condition'' with two levels non-painful heat, painful heat, ''stimulation site'' with two levels volar forearm, cheek, and ''time of assessment'' with eight levels eight points of time.

CPM effects on pain ratings. To evaluate possible CPM effects on the perception of the CO_2 stimuli, analyses of variance with repeated measurements were conducted with two within-subject factors, ''CPM'' (with two levels baseline, tonic heat) and ''stimulation site'' (with two levels volar forearm, cheek). These analyses were conducted separately for the two intensities of the test stimulus (CO_2 concentrations 73% and 78% (v/v)) and the two intensities of the conditioning stimulus (non-painful heat and painful heat), resulting into four analyses by combination.

CPM effects on evoked potentials (P2 component). To evaluate possible CPM effects on pain-related chemo-somatosensory event-related potentials (CSSEPs) obtained in response to CO_2 stimuli, multivariate analyses of variance with repeated measurements were conducted with three within-subject factors, ''CPM'' (with two levels baseline, tonic heat), ''stimulation site'' (with two levels volar forearm, cheek), and ''cortical area'' (with five levels Cz, C3, C4, Fz, Pz); the dependent variables were amplitude and latency. These analyses were conducted separately for the two intensities of the test stimulus (CO_2 concentrations 73% and 78% (v/v)) and the two intensities of the conditioning stimulus (non-painful heat and painful heat), resulting in four analyses by combination.

Correlation between CPM effects on pain ratings and on CSSEPs

We further wanted to investigate whether CPM effects on CSSEPs are correlated with CPM effects on pain ratings. To do this we computed difference scores for CPM-related changes in (i) self-report and in (ii) CSSEP responses. Since CSSEP responses were composed of amplitude and latency values and were recorded from many sites, we focused on the more common value ''amplitude'' and selected the most pertinent electrode site (which proved to be Fz; see Results section) and the most pertinent CO_2 intensity (which proved to be 73% (v/v); see Results section) for the calculation of difference scores; in order to reduce the number of analyses. These difference scores were then entered into correlation analyses. We conducted correlation analyses separately for stimulation site (arm, cheek) and separately for tonic heat intensities (warm, pain).

In case of significant effect, *post hoc* analyses (analyses of variances and *t*-tests) were conducted. The value of α was set to 0.05 throughout.

Results

Assessment of pain thresholds

The assessment of pain thresholds was undertaken in order to tailor the tonic heat stimulation (conditioning stimulus) to the individual pain sensitivity in the experimental blocks 2 and 4 (see Figure 1 where the sequence of the experimental protocol is depicted). The stimulation site had a significant effect on heat pain thresholds ($T(16) = 2.36$; $p = 0.031$), with lower pain thresholds at the cheek (mean: 43.8 °C, SD: 0.85) compared to the volar forearm (mean: 44.4 °C, SD: 0.87).

Assessment of potential CPM effects

Conditioning stimuli

The VAS ratings for the conditioning stimuli are displayed in Figure 2. As expected, the conditioning stimuli designed to

reflect non-painful heat (−1 °C below the pain threshold) differed significantly from the stimuli designed to reflect painful heat (+1 °C above the pain threshold) ($F(1,16) = 27.92$; $p < 0.001$), with painful heat leading to higher VAS ratings. The factors ''stimulation site'' (volar forearm, cheek) ($F(1,16) = 3.24$; $p = 0.091$) and ''time of assessment'' (the conditioning stimulus was rated in intervals of approximately 160 s, see Figure 1) ($F(7,112) = 0.88$; $p = 0.523$) did not have any significant effects on the VAS ratings of the conditioning stimuli. However, we found a significant interaction effect between ''stimulation site'' and ''condition'' ($F(1,16) = 10.06$; $p = 0.006$). The difference in VAS ratings between non-painful and painful heat seems to be more pronounced when heat was applied to the volar forearm compared to the cheek (see Figure 2). We conducted *post hoc* analyses to clarify whether at both stimulation sites (volar forearm and cheek) painful heat led indeed to higher VAS ratings compared to non-painful heat (one-sided test). At the volar forearm, VAS ratings for painful heat were significantly increased compared to non-painful heat ($F(1,16) = 31.044$; $p < 0.001$). At the cheek, the difference between ratings for painful heat and non-painful heat was also significant ($F(1,16) = 4.277$; $p = 0.028$). There was also a significant interaction between ''condition'' and ''time of assessment'' ($F(7,112) = 2.26$; $p = 0.035$), with ratings for painful heat increasing over time while VAS ratings for non-painful heat remained or even slightly decreased over time (see Figure 2).

CPM effects on pain ratings

The effects of the non-painful and painful conditioning stimuli on the VAS ratings of the CO_2 stimuli are displayed in Figure 3. As can be seen, pain ratings of CO_2 stimuli with medium concentration (73% (v/v)) were neither affected by tonic non-painful heat ($F(1,16) = 2.83$; $p = 0.602$), nor

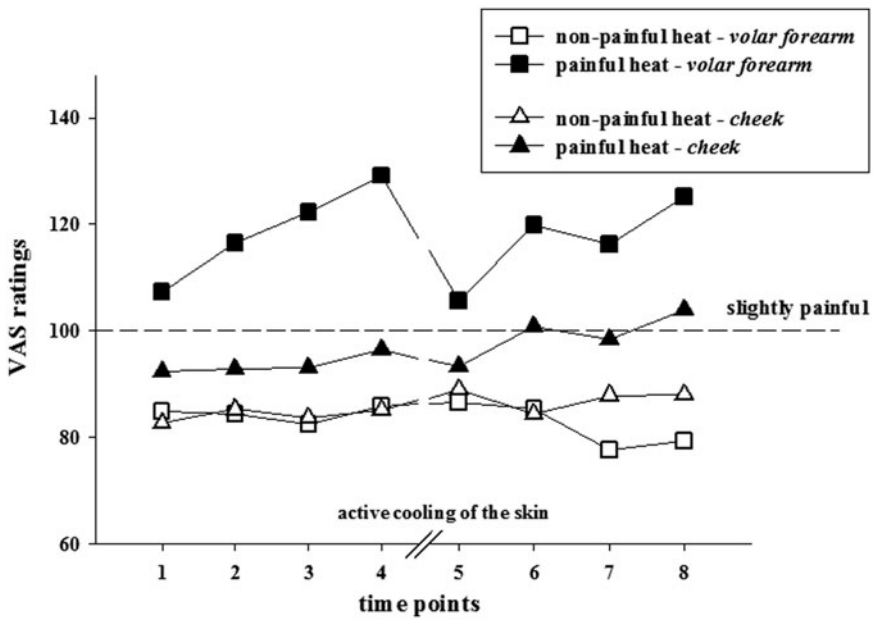

Figure 2. VAS ratings (mean ± SD) of perceived intensity of the conditioning stimuli (non-painful heat; painful heat) during stimulation at the volar forearm and at the cheek.

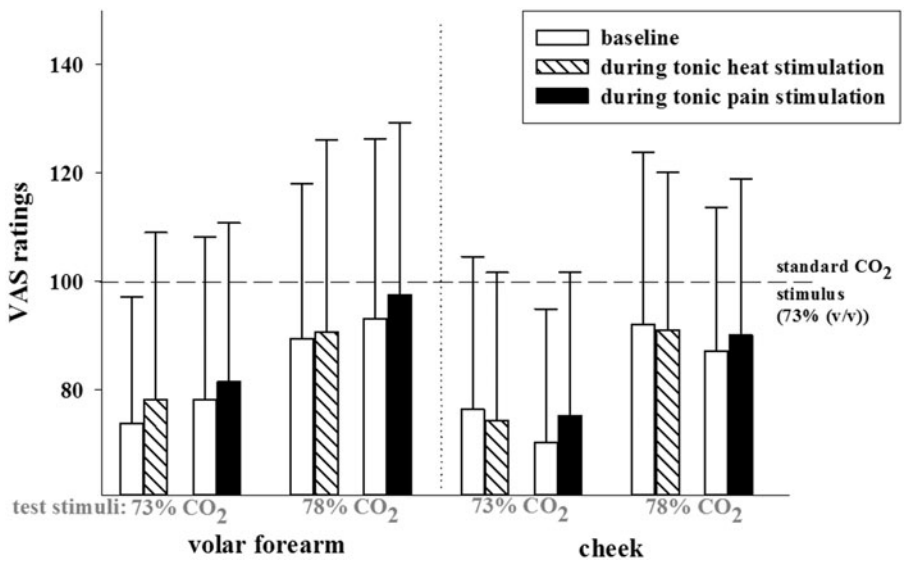

Figure 3. VAS ratings (mean ± SD) for the CO_2 test stimuli (73% and 78% (v/v)) during baseline (no concurrent stimulation) and during the concurrent application of conditioning stimuli (non-painful heat or painful heat at two stimulation sites: volar forearm and cheek).

Table I. Univariate findings for the effects of the conditioning stimuli (non-painful and painful heat) on pain-related chemo-somatosensory event-related potentials (amplitude and latency values) obtained in response to CO_2 stimuli (73% and 78% (v/v)).

	Amplitude			Latency		
Factors (main effects and interaction effects)	*F*	df	*p*	*F*	df	*p*
Conditioning stimulus: **tonic heat**; test stimuli: **73% (v/v)**						
CPM (baseline, tonic heat)	**14.64**	**(1,16)**	**0.001**	2.32	(1,16)	0.148
Stimulation site (volar forearm, cheek)	**5.52**	**(1,16)**	**0.041**	0.54	(1,16)	0.471
Cortical area (Cz, C3, C4, Fz, Pz)	**13.86**	**(4,64)**	**<0.001**	2.36	(4,64)	0.062
Conditioning stimulus: **tonic pain**; test stimuli: **73% (v/v)**						
CPM (baseline, tonic pain)	**5.89**	**(1,16)**	**0.027**	**8.22**	**(1,16)**	**0.011**
Stimulation site (volar forearm, cheek)	2.09	(1,16)	0.168	2.01	(1,16)	0.176
Cortical area (Cz, C3, C4, Fz, Pz)	**9.45**	**(4,64)**	**<0.001**	1.10	(4,64)	0.363
Conditioning stimulus: **tonic heat**; test stimuli: **78% (v/v)**						
CPM (baseline, tonic heat)	2.62	(1,16)	0.125	0.01	(1,16)	0.965
Stimulation site (volar forearm, cheek)	**7.61**	**(1,16)**	**0.014**	0.99	(1,16)	0.336
Cortical area (Cz, C3, C4, Fz, Pz)	**10.51**	**(4,64)**	**<0.001**	**436.3**	**(4,64)**	**<0.001**
Conditioning stimulus: **tonic pain**; test stimuli: **78% (v/v)**						
CPM (baseline, tonic pain)	0.74	(1,16)	0.403	2.20	(1,16)	0.157
Stimulation site (volar forearm, cheek)	1.33	(1,16)	0.265	0.06	(1,16)	0.808
Cortical area (Cz, C3, C4, Fz, Pz)	**9.38**	**(4,64)**	**<0.001**	1.74	(4,64)	0.153

The factor ''CPM'' indicates whether evoked potentials in response to the CO_2 stimuli changed during the simultaneously applied tonic heat stimulation.
The factor ''stimulation site'' refers to the body site the tonic heat was applied to (conditioning stimulus).
The factor ''cortical area'' refers to the electrode sites used to assess evoked potentials.
Significant effects are marked in bold.

by painful heat stimulation ($F(1,16) = 2.65$; $p = 0.123$). The same was found for ratings of CO_2 stimuli with strong concentration (78% (v/v)), where we again found no significant CPM effects (non-painful heat: $F(1,16) = 0.07$; $p = 0.801$; painful heat: $F(1,16) = 1.77$; $p = 0.202$). Furthermore, the stimulation site of the conditioning stimuli (volar forearm vs. cheek) did also have no significant effects on the VAS ratings of the CO_2 stimuli (all p values > 0.05). Neither did we find significant interaction effects between ''CPM'' and ''stimulation site'' (all p values > 0.05).

CPM effects on CSSEPs (P2 component)

The effects of the conditioning stimuli on pain-related CSSEPs obtained in response to CO_2 stimuli are presented in Table I (univariate effects) and Figures 4 and 5. We found significant CPM effects for the P2 component of the evoked potential in response to CO_2 stimuli with medium concentration (73% (v/v)). Non-painful heat stimulation ($F(2,15) = 7.07$; $p = 0.007$) as well as painful heat stimulation ($F(2,15) = 15.07$; $p < 0.001$) led to significant changes in the CO_2 evoked potentials compared to baseline. As can be seen in Table I (univariate effects on amplitude and latency) and Figures 4(a) and 5(a), tonic non-painful and painful heat led to significant reductions of P2 amplitudes. Moreover, the latencies of the evoked potential were prolonged under tonic pain stimulation (see Table I and Figure 5b).

In contrast to these findings, tonic heat stimulation had no effects on CSSEPs obtained in response to CO_2 stimuli with the stronger concentration (78% (v/v)). We found neither during non-painful heat ($F(2,15) = 1.24$; $p = 0.318$) nor during painful heat stimulation ($F(2,15) = 2.08$; $p = 0.160$) significant CPM effects (univariate findings for amplitude and latency are presented in Table I). Given the non-significant CPM effects, we refrained from depicting the descriptive values for the stronger test stimulus in the figures.

The stimulation site of the conditioning stimuli (volar forearm vs. cheek) yielded mostly non-significant effects in the multivariate analyses of variance conducted. Only evoked potentials (CSSEPs) obtained in response to CO_2 stimuli with strong concentration (78% (v/v)) during baseline/non-painful heat stimulation differed between stimulation sites ($F(2,15) = 3.96$; $p = 0.042$). As can be seen in Table I, this effect was due to differences in the amplitude magnitudes; with reduced amplitudes in the session where heat was applied to the cheek.

We found significant effects for the location of electrode placement (''cortical area''). This was true for both CO_2 concentrations (73% and 78% (v/v)) and occurred regardless of the concurrently applied tonic heat intensities (all p values < 0.001). As can be seen in Table I, these effects were mostly due to differences in amplitude magnitudes, with highest amplitudes recorded at Cz and C4 and lowest amplitudes recorded at C3 (see also Figures 4a and 5a).

None of the interactions between ''CPM'', ''stimulation site'', and ''cortical area'' yielded any significant effects in the multivariate analyses of variance conducted (all p values > 0.05) and thus, no univariate findings are presented in Table I.

Correlation between CPM-related changes in CSSEPs and in pain ratings

As can be seen in Figure 6, we found a significant correlation between CPM-related changes in pain ratings and CSSEPs (Fz was selected out of the different recording sites, given that CPM effects (based on the effect sizes) were most pronounced

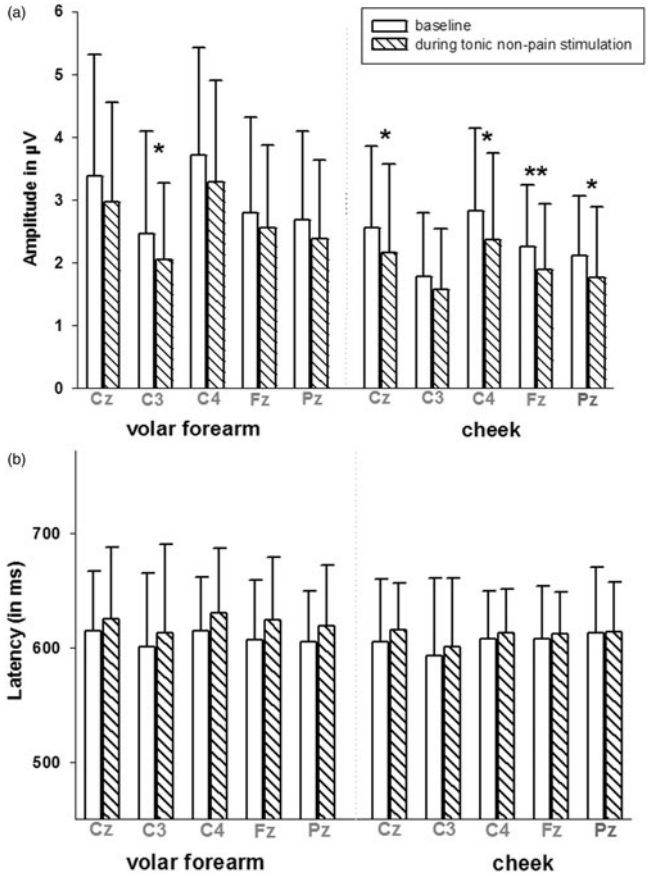

Figure 4. Pain-related chemo-somatosensory evoked potentials (mean ± SD for amplitude P2) obtained in response to CO_2 stimuli of moderate concentration (73% (v/v)) during the concurrent application of **non-painful** heat: (a) amplitude values; (b) latency values.

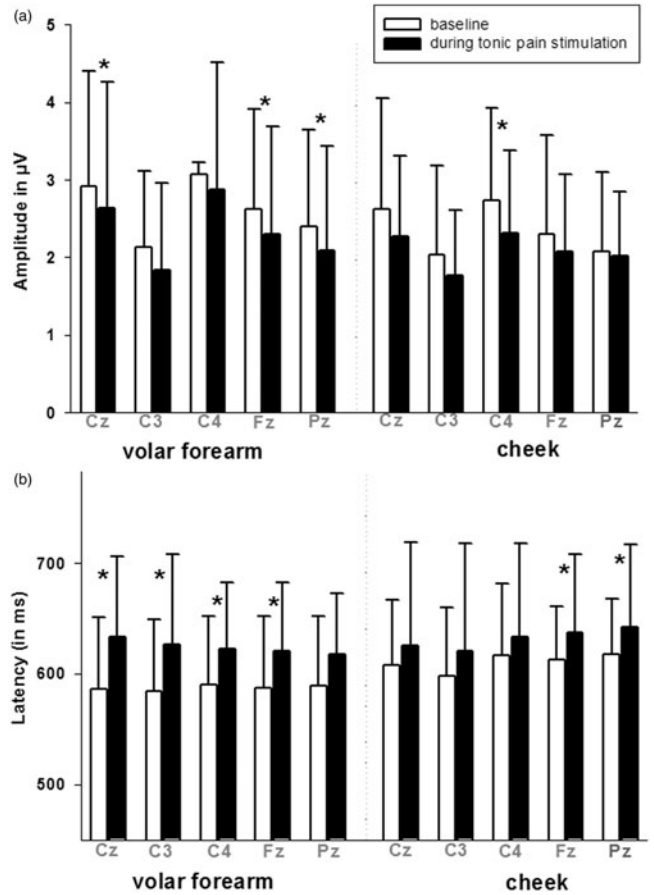

Figure 5. Pain-related chemo-somatosensory event-related potentials (mean ± SD for amplitude P2) obtained in response to CO_2 stimuli of moderate concentration (73% (v/v)) during the concurrent application of **painful** heat: (a) amplitude values; (b) latency values.

here) when the conditioning stimulus was applied to a remote body site (the forearm) from the test stimuli. In contrast, when the tonic stimulus was applied to a proximate body site (the cheek) no significant correlations between evoked potentials and subjective pain ratings could be observed.

In summary, tonic non-painful (−1 °C below the pain threshold) and painful heat (+1 °C above the pain threshold) as conditioning stimuli reduced the P2 amplitude and increased latency for the weaker of the two test CO_2 stimuli. This CPM effect occurred irrespectively of the site of application of the conditioning stimulation (forearm, cheek) and site of recording of the potentials evoked by the test stimuli (Cz, C3, C4, Fz, Pz). In contrast, no CPM effects could be verified by considering the VAS ratings of the test stimuli. Nevertheless, we found significant positive correlations between CPM-related changes in VAS ratings and in CSSEPs when the stimulation sites of the test and the conditioning stimuli were remote enough or in other words, when heterotopic stimulation sites were used.

Discussion

We used a chemonasal pain model (CO_2 gas) to investigate whether this pain model is sensitive enough to capture CPM effects triggered by tonic heat pain of weak noxious load as conditioning stimulus. In the current study, we obtained the following findings: (i) the experimental pain model detected CPM effects in the CSSEPs, with reduced amplitudes and prolonged latencies at several EEG recording positions; (ii) the CPM effect was only visible when using the lower intensity of the test stimulus (73% CO_2); (iii) the CPM effect was more effective using the heterotopic conditioning stimulation (tonic painful heat) at the volar forearm as compared to the homotopic stimulation at the cheek; (iv) in contrast to the visible inhibitory effects on the CSSEPs, subjective ratings of the test stimulus did not reflect CPM action. We will discuss these findings in detail below.

Reduction of the amplitude P2 of the CSSEPs can be regarded as a sensitive indicator of analgesic effects, which has been demonstrated in various studies of non-opioid and opioid analgesics with this experimental pain model (Kobal et al. 1994; Lötsch et al. 1995a; Kraetsch et al. 1996; Thürauf et al. 1996; Renner et al. 2007). It is commonly accepted that earlier components of the evoked potential reflect more the sensory input while later components like the P2 rather reflect the cerebral signal processing. This has also been demonstrated for evoked potentials in response to painful stimulation of the tooth-pulp, given that earlier components of evoked potentials correlated with the physical stimulus intensity, while later components were related to the estimates of pain intensity (Chen et al. 1979). Therefore, the P2 amplitude reduction as well as the P2 latency increase that we found

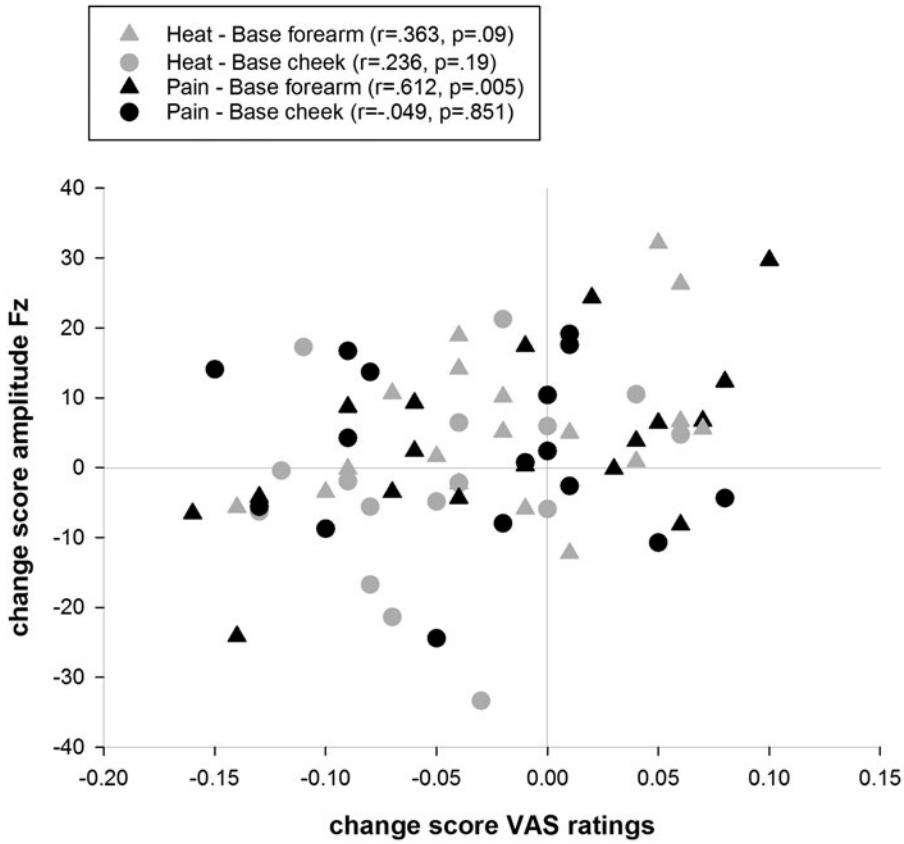

Figure 6. Correlations between CPM-related changes in self-report ratings and pain-related chemo-somatosensory event-related potentials (Fz amplitude) obtained in response to CO_2 stimuli of moderate concentration (73% (v/v)).

during tonic heat stimulation of low noxious load, suggest analgesic effects of CPM at a cerebral level.

The CPM effects observed in the CSSEP amplitudes and latencies were, however, not significantly reflected by the participants' ratings of the test stimulus. This might seem surprising, however, previous experiments using the chemo-nasal pain model have also reported a great amount of variance in pain ratings when assessing the analgesic effects of NSAIDs by use of CO_2 stimuli that clearly exceeded the variance found in CSSEPs (Kobal et al. 1990; Renner et al. 2007). Therefore, it is possible that the subjective responses to CO_2 stimulation of the nasal mucosa might be less sensitive to change compared to the P2 component of the CSSEPs. Moreover, CPM paradigms have been assumed to assess the net effect of complex facilitatory and inhibitory mechanisms of pain processing (Yarnitsky et al. 2010). Thus, finding no CPM effect on self-report ratings does not mean that no inhibitory or excitatory processes have been induced by the concurrent tonic stimulation but it might simply be the case that the ratings represent an aggregation of many influences. In line with this argument, we did find significant correlations between CPM-related changes in evoked potentials and in subjective ratings (when heterotopic stimulation sites were used (this being the more ''classical'' CPM design)); with those participants showing the greatest CPM effects in CSSEPs also showing the greatest inhibition in their self-report ratings. Accordingly, even though we found no overall CPM effect on pain ratings we are still confident that CPM action was elicited in our paradigm given the significant correlations between changes in evoked potentials and changes in self-report ratings.

In this study, two concentrations of CO_2 were used for painful stimulation of the nasal mucosa. One reason for using two concentrations is to reduce response bias giving the participants a choice to discriminate between different painful intensities. We observed a significant CPM effect (reduction in amplitude and increase in latency for P2) in response to the weaker stimuli but not any significant changes in response to the stronger stimuli (78% v/v). This is not a novel finding since the ''weaker'' stimuli have appeared to be more sensitive to changes in CSSEPs induced by different types of analgesics (Kobal et al. 1990; Lötsch et al. 1995b; Renner et al. 2007). It is possible that a conditioning stimulus of higher noxious load would have been necessary to elicit CPM effects on the stronger CO_2 stimulus.

The observed CPM effects during homotopic and heterotopic stimulation differed: (i) In case of heterotopic stimulation (volar forearm and nasal mucosa), a marginal CPM effect was evident during non-painful conditioning stimulation (only at recording position C3); the CPM effect became prominent during the stronger and more painful conditioning stimulation (decreased P2 amplitudes at Cz, Fz, and Pz and prolonged latencies at several recording positions; see Figures 4 and 5). Finding the CPM effect to be stronger when using conditioning stimulation of higher nociceptive load is well in line with previous findings (e.g., Granot et al. 2008). (ii) In case of homotopic stimulation, the effect pattern differed from the heterotopic stimulation. The weaker conditioning

stimulus at the cheek with non-painful intensities decreased P2 amplitudes at several recording positions, but this effect was not enhanced by the stronger conditioning stimulus with more painful intensities (see Figures 4 and 5). In contrast, during the stronger conditioning stimulation we observed a significant decrease in P2 amplitude only at one recording position (C4) in combination with prolonged latencies at Fz and Pz. Thus, increasing the intensity of the conditioning heat stimulus had opposing effects on the processing of the test stimulus, depending on the site of stimulation. In summary, we observed stronger CPM effects during stronger heterotopic conditioning stimulation and, in contrast, weaker CPM effects during stronger homotopic conditioning stimulation, suggesting that the latter does not reflect the activation of the DNIC-like mechanisms but a segmental interaction with additive effects of the conditioning and test stimuli.

Limitations

We also have to acknowledge several limitations of the present study. The sample size might appear rather small. However, we used a within-subject design and based our power calculation (SAMPLE POWER; SPSS) on previous findings using the chemonasal pain model (CO_2 gas) to investigate the effect of weak analgesics (e.g., Lötsch et al. 1995a) (we based our power calculation on CSSEPs as the outcome measure of main interest). Based on previous findings, a sample of 17 proved to be sufficient to provide strong power at the significance level at 0.05 for within-subject analyses. Moreover, the tonic heat pain applied to the cheek was not always rated as being painful by the participants. However, given that an intensity of 44.8 °C (average temperature applied to the cheek) surely lies within the nociceptive range and given that the painful intensity was rated as significantly stronger than the non-painful intensity, we are confident that we succeeded in applying a conditioning stimulus of a low noxious intensity both to the volar forearm as well as to the cheek. It might also seem surprising that VAS ratings of the CO_2 test stimuli were rated lower than ''100'', although 100 was defined as a control stimulus of the lower of the two CO_2 concentrations (73%). However, this phenomenon can be observed in almost all studies using the same protocol as we did (e.g., Lötsch et al. 1997). The reason for this is most likely adaptation or habituation processes taking place over time (Hummel and Livermore 2002). Although the experimental pain model using CO_2 stimuli to elicit CSSEPs has several advantages (e.g., specific stimulation of the nociceptive system without simultaneous excitation of other sensory systems), the induction and assessment methods are laborious as well as rather unpleasant for the participant. Moreover, this experimental pain model is unsuitable to study regional pain conditions but is more useful for generalized pain conditions.

Conclusions

Altogether, heterotopic tonic heat applied via thermodes as conditioning stimulus led—as expected—to CPM effects, which could be detected by CSSEPs induced by chemonasal stimuli (CO_2 gas with 73% (v/v)). This stimulus intensity has repeatedly been shown to be very sensitive to weak analgesic effects. The CPM effects increased with stronger conditioning stimuli (higher noxious load). In contrast, subjective ratings of the test stimulus did not reflect any inhibitory action. Nevertheless, CPM changes in evoked potentials were correlated with changes in subjective ratings (when heterotopic stimulation sites were used). This finding indicates that CPM actions were also present in subjective ratings, even though to a much lesser degree.

The experimental pain model assessing CSSEPs in response to CO_2 stimuli proved to be a sensitive test for capturing weak CPM effects elicited by a conditioning stimulus of rather low noxious load. The usage of such mild noxious conditioning stimuli—in contrast to stimuli of higher noxious load (e.g., cold pressor test)—has the advantage that the activation of other types of pain inhibitory mechanisms in parallel (like attentional distraction, stress-induced analgesia) can be more effectively avoided. This advantage might not only be useful in experimental studies on CPM effects, but might also prove to be of clinical usefulness. Indeed, the usage of mildly painful conditioning stimuli might even be mandatory in clinical settings because facilitatory effects have been observed in pain patients which might lead to intolerable pain levels when using strongly painful conditioning stimuli during CPM diagnostics.

Note

1. It has to be acknowledged that the intended fine-tuned difference of 1.3 °C between stimuli might have varied slightly due to accuracy constriction of the machinery used.

Declaration of interest

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References

Bouhassira D, Villanueva L, Bing Z, le Bars D. 1992. Involvement of the subnucleus reticularis dorsalis in diffuse noxious inhibitory controls in the rat. Brain Res 595:353–357.

Chen AC, Chapman CR, Harkins SW. 1979. Brain evoked potentials are functional correlates of induced pain in man. Pain 6:365–374.

de Souza JB, Potvin S, Goffaux P, Charest J, Marchand S. 2009. The deficit of pain inhibition in fibromyalgia is more pronounced in patients with comorbid depressive symptoms. Clin J Pain 25:123–127.

Edwards RR. 2005. Individual differences in endogenous pain modulation as a risk factor for chronic pain. Neurology 65:437–443.

Galfe G, Lautenbacher S, Hölzl R, Strian F. 1990. Diagnosis of small fibre neuropathy: Computer-assisted methods of combined pain and thermal sensitivity determination. Hospimedica 8:38–48.

Granot M, Weissman-Fogel I, Crispel Y, Pud D, Granovsky Y, Sprecher E, Yarnitsky D. 2008. Determinants of endogenous analgesia magnitude in a diffuse noxious inhibitory control (DNIC) paradigm: Do conditioning stimulus painfulness, gender and personality variables matter? Pain 136:142–149.

Hummel T, Livermore A. 2002. Intranasal chemosensory function of the trigeminal nerve and aspects of its relation to olfaction. Int Arch Occup Environ Health 75:305–313.

Julien N, Goffaux P, Arsenault P, Marchand S. 2005. Widespread pain in fibromyalgia is related to a deficit of endogenous pain inhibition. Pain 114:295–302.

King CD, Wong F, Currie T, Mauderli AP, Fillingim RB, Riley 3rd JL,. 2009. Deficiency in endogenous modulation of prolonged heat pain in patients with irritable bowel syndrome and temporomandibular disorder. Pain 143:172–178.

Kobal G. 1981. Elektrophysiologische Untersuchungen des menschlichen Geruchssinns. Stuttgart: Thieme. pp 171.

Kobal G. 1985. Pain-related electrical potentials of the human nasal mucosa elicited by chemical stimulation. Pain 22:151–163.

Kobal G, Hummel C, Nuernberg B, Brune K. 1990. Effects of pentazocine and acetylsalicylic acid on pain-rating, pain-related evoked potentials and vigilance in relationship to pharmacokinetic parameters. Agents Actions 29:342–359.

Kobal G, Hummel C, Gruber M, Geisslinger G, Hummel T. 1994. Dose-related effects of ibuprofen on pain-related potentials. Br J Clin Pharmacol 37:445–452.

Kraetsch HG, Hummel T, Lotsch J, Kussat R, Kobal G. 1996. Analgesic effects of propyphenazone in comparison to its combination with caffeine. Eur J Clin Pharmacol 49:377–382.

Kunz M, Scholl KE, Schu U, Lautenbacher S. 2006. GABAergic modulation of diffuse noxious inhibitory controls (DNIC): A test by use of lorazepam. Exp Brain Res 175:363–371.

Lautenbacher S, Roscher S, Strian F. 1995. Tonic pain evoked by pulsating heat: Temporal summation mechanisms and perceptual qualities. Somatosens Mot Res 12:59–70.

Lautenbacher S, Roscher S, Strian F. 2002. Inhibitory effects do not depend on the subjective experience of pain during heterotopic noxious conditioning stimulation (HNCS): A contribution to the psychophysics of pain inhibition. Eur J Pain 6:365–374.

Le Bars D, Dickenson AH, Besson JM. 1979. Diffuse noxious inhibitory controls (DNIC). I. Effects on dorsal horn convergent neurones in the rat. Pain 6:283–304.

Le Bars D, Chitour D, Clot AM. 1981. The encoding of thermal stimuli by diffuse noxious inhibitory controls (DNIC). Brain Res 230: 394–399.

Le Bars D, Villanueva L, Bouhassira D, Willer JC. 1992. Diffuse noxious inhibitory controls (DNIC) in animals and in man. Patol Fiziol Eksp Ter 4:55–65.

Lewis GN, Heales L, Rice DA, Rome K, McNair PJ. 2012. Reliability of the conditioned pain modulation paradigm to assess endogenous inhibitory pain pathways. Pain Res Manag 17:98–102.

Lötsch J, Geisslinger G, Mohammadian P, Brune K, Kobal G. 1995a. Effects of flurbiprofen enantiomers on pain-related chemo-somatosensory evoked potentials in human subjects. Br J Clin Pharmacol 40: 339–346.

Lötsch J, Mohammadian P, Hummel T, Florin S, Brune K, Geisslinger G, Kobal G. 1995b. Effects of azapropazone on pain-related brain activity in human subjects. Br J Clin Pharmacol 40: 545–552.

Lötsch J, Marchl R, Kobal G. 1997. The influence of stimulus duration on the reliability of pain ratings after nociceptive stimulation of the nasal mucosa with CO_2. Eur J Pain 1:207–213.

Moont R, Crispel Y, Lev R, Pud D, Yarnitsky D. 2012. Temporal changes in cortical activation during distraction from pain: A comparative LORETA study with conditioned pain modulation. Brain Res 1435:105–117.

Piche M, Arsenault M, Rainville P. 2009. Cerebral and cerebrospinal processes underlying counterirritation analgesia. J Neurosci 29: 14236–14246.

Pielsticker A, Haag G, Zaudig M, Lautenbacher S. 2005. Impairment of pain inhibition in chronic tension-type headache. Pain 118: 215–223.

Renner B, Clarke G, Grattan T, Beisel A, Mueller C, Werner U, Kobal G, Brune K. 2007. Caffeine accelerates absorption and enhances the analgesic effect of acetaminophen. J Clin Pharmacol 47:715–726.

Thürauf N, Fleischer WK, Liefhold J, Schmid O, Kobal G. 1996. Dose dependent time course of the analgesic effect of a sustained-release preparation of tramadol on experimental phasic and tonic pain. Br J Clin Pharmacol 41:115–123.

Yarnitsky D, Arendt-Nielsen L, Bouhassira D, Edwards RR, Fillingim RB, Granot M, Hansson P, Lautenbacher S, Marchand S, Wilder-Smith O. 2010. Recommendations on terminology and practice of psychophysical DNIC testing. Eur J Pain 14:339.