

Temporal summation of trigeminal pain in human anterior cingulate cortex

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ABSTRACT

Temporal summation of nociceptive inputs in trigeminal networks can induce central sensitization and maintain chronic pain. We combined functional magnetic resonance imaging and electrically evoked pain-related potentials (PREP) in healthy human subjects to identify brain regions involved in temporal summation of nociceptive inputs. We stimulated the skin innervated by the ophthalmic division of the trigeminal nerve with trains of three, seven and eleven similar nociceptive pulses, while recording evoked hemodynamic or electrical brain responses. We found that PREP amplitudes and pain ratings increased in parallel with increasing train length. Strikingly, only hemodynamic responses in the posterior part of the anterior cingulate cortex (pACC) scaled with individual pain ratings on a verbal rating scale (VRS) and electrically evoked responses (i.e., PREP amplitudes for the three train lengths). These findings indicate that pACC codes temporal summation of trigeminal nociception and in this regard may be important for the development of central sensitization observed in chronic head and facial pain.

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Introduction

Central sensitization of trigeminal nociceptive systems has been suggested a key mechanism for the development of chronic pain (Gracely et al., 1992; Staud et al., 2001). Increased excitability of spinal and supraspinal neurons develops within minutes to hours after sustained nociceptive afferent input. This parallels enhanced responsiveness to nociceptive and non-nociceptive stimuli and enlargement of associated receptive fields (Coderre et al., 1993; Dougherty et al., 1992; Rainville et al., 1997). Repetitive nociceptive stimulation leads to increased firing rates of wide-dynamic range (WDR) neurons that reaches a plateau after several stimuli (Price et al., 1994; Rainville et al., 1997). This mechanism is commonly referred to as temporal summation. Central sensitization is thought to be induced by temporal summation that occurs in early phases of chronic pain which over time induces intracellular signalling cascades and supports long-term neuroplastic changes (Ji and Woolf, 2001). The minimal tonic nociceptive input that is required to sustain these early stages of central sensitization has been regarded as key mechanism in the development and sustenance of chronic pain (Li et al., 1999; Staud et al., 2004).

We utilized an electrical stimulation of cutaneous nociceptive fibers in healthy human subjects as described elsewhere (Kaube et al., 2000). In brief, pain-related evoked potentials (PREP) elicited by a concentric electrode allow quantitative measurements of trigeminal nociceptive transmission which provide a highly sensitive indicator for changes in trigeminal nociception caused by disease or pharmacological interventions (Ayzenberg et al., 2006; Katsarava et al., 2002; Kaube et al., 2002; Obermann et al., 2007). Using this technique, we established a repetitive stimulation protocol to induce temporal summation in cranial and extra-cranial nociceptive systems (Giffin et al., 2004; Katsarava et al., 2006). Here, we recorded electrical and hemodynamic responses evoked by repetitive stimulation to identify sources of temporal summation within the trigeminal nociceptive system. This technique is very different from the classical heat pain paradigm that exploits the manipulation of the inter-stimulus interval (above vs. below 3 Hz) and is mediated by C-fiber induced spinal activation.

The anterior cingulate cortex (ACC) represents a key candidate for coding different aspects of nociceptive information. This has been demonstrated in lesion studies (Bouckoms, 1989; Devinsky et al., 1995; Foltz and White, 1968; Talbot et al., 1995) and by positron-emission-tomography (PET) and functional magnetic resonance imaging (fMRI) (Casey, 2000; Casey et al., 1994; Coghill et al., 1999; Craig et al., 1996; Davis et al., 1997; Jones et al., 1991; Kwan et al., 2000; Ploghaus et al., 1999; Porro et al., 1998; Rainville et al., 1997;

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Sawamoto et al., 2000; Talbot et al., 1991). Besides the well described encoding of affective components (Craig et al., 1996; Rainville et al., 1997; Sawamoto et al., 2000), attentional processing (Gitelman et al., 1999; Peyron et al., 1999) and working memory (Petit et al., 1998), associated with painful stimuli, recent imaging experiments identified different subregions within ACC responsible for coding non-painful and painful stimulation (Buchel et al., 2002).

Combining verbal rating scale (VRS), blood-oxygen-level-dependent (BOLD) fMRI, and electrically evoked pain-related potentials (PREP) we expected to identify possible subregions within ACC reflecting a supraspinal aspect of temporal summation.

Materials and methods

Subjects

Eleven healthy volunteers (8 men) with a mean age of 23.3 ± 2.0 years (range: 19–26 years) were recruited from the University of Duisburg-Essen. All participants gave their written informed consent according to the Declaration of Helsinki. The study protocol was approved by the local ethics committee. Exclusion criteria were headache or other paroxysmal or chronic pain condition, other somatic or psychiatric disease, family history of headache or other pain condition in first relatives. All subjects were investigated a second time in a control experiment as described below, serving as their own controls.

Study design

In all subjects we applied three, seven and eleven train pulses of constant current intensity to the skin innervated by the ophthalmic division of the trigeminal nerve, which corresponded to mild (verbal rating scale, \approx VRS 3), moderate (\approx VRS 5) and severe (\approx VRS 7) pain respectively, to investigate the effect of temporal summation in trigeminal nociceptive processing.

Subjects were studied twice: In the first session we measured pain-related evoked potentials (PREP), in the second session subjects underwent functional magnetic resonance imaging (fMRI) during nociceptive stimulation (i.e., same stimulation parameters as for PREP). The stimulation setup was identical in both sessions: First a train of 11 pulses was applied with increase of current intensity in steps of 0.2 mA to achieve pain perception of VRS 7. The current intensity remained unchanged thereafter. Individual pain ratings on a verbal rating scale (VRS; 0 = no pain to 10 = worst possible pain)

were obtained for stimulation trains of 7 and 3 pulses, applying the previously determined fixed stimulus intensity in mA. The stimuli, regardless of the number of train-pulses applied, were experienced by the participant as one sharp, stinging pain with a decrease in pain intensity as the number of pulses of the stimulation train decreased. The applied stimuli matched the individual mean pain ratings (VRS 3 \approx mild pain; VRS 5 \approx mild to moderate pain; VRS 7 \approx moderate to severe pain) obtained from each subject. Seven blocks of train-pulse-stimulation, consisting of 3 (i.e., VRS 3), 7 (i.e., VRS 5), or 11 pulses (i.e., VRS 7) per train with identical stimulus intensity in mA (monopolar square wave, duration: 0.5 ms, pulse interval: 5 ms, interstimulus interval: 3.16 s including a jitter of ± 0.02 s) were applied in pseudo-randomized order starting with any of the three pulse trains, followed by any other. Duration of one stimulation block was 22.4 s (= 7 scans) followed by a non-stimulation rest interval of 41.6 s (= 13 scans) (Fig. 1). The stimulation sequence was the same for PREP and fMRI sessions. Sensory and pain thresholds were determined before each session with single pulse stimulation in ascending stimulus intensity until the person reported to feel the stimulation and then little further until it turned to a sharp, pin-prick like pain.

All eleven subjects participated in a control experiment. Here we applied single nociceptive pulses to the same location of the trigeminal nerve, but now with increasing stimulation intensities that again correspond to the three pain levels (VRS 3, VRS 5, and VRS 7). In other words, in this experiment, subjects experienced the same pain intensity as for the pulse trains but now receiving only one stimulus with varying stimulation intensities in mA instead of varying pulse trains with constant stimulation intensities.

Stimulation

For electrical stimulation we used a custom built planar concentric electrode (CE), consisting of a central metal cathode (D: 0.5 mm), an isolation insert (D: 5 mm) and an external anode ring (D: 6 mm) providing a stimulation area of 19.6 mm² (Fig. 1). By virtue of its concentric design and small anode-cathode distance, this surface stimulating electrode produces high current density at low current intensities leading to predominant stimulation of nociceptive A δ -fibers with only little (approx. 10%) contamination of A β -fiber transmission (Kaube et al., 2000, 1999). Skin stimulation was performed over the ophthalmic nerve division of the trigeminal nerve with three parallel connected electrodes placed 10 mm above the right supraorbital nerve with a 10 mm distance between each electrode (Fig. 1). The method of applying multiple pulse trains in

Stimulation paradigm for BOLD-Signal and pain-related evoked potentials

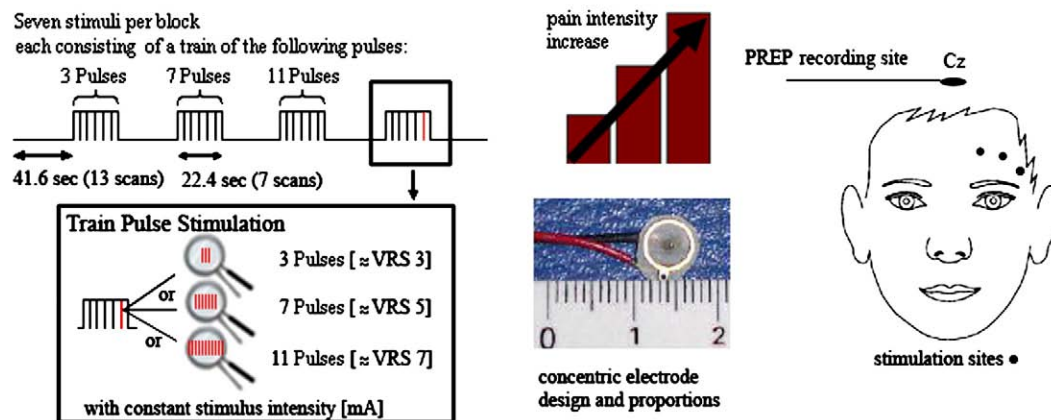


Fig. 1. Experimental setup and stimulation paradigm for blood-oxygen-level-dependent (BOLD) signal and pain-related evoked potential (PREP) conduction inducing an increase in perceived pain intensity by train pulse stimulation using an asymmetric block design. Concentric electrode design and proportion.

neural stimulation has been used to optimize evoked recordings from peripheral and central motor and sensory nerves by temporal summation (Giffin et al., 2004; Inghilleri et al., 1990; Reitter and Johannsen, 1982; Taylor et al., 1993). Multiple pulse stimulation increases the magnitude and persistence of evoked potential responses without losing nociceptive specificity.

Recording of pain related evoked potentials (PREP)

Seven blocks, each consisting of seven sweeps with train pulses of either 3 (i.e., VRS 3), 7 (i.e., VRS 5), or 11 pulses (i.e., VRS 7), were applied to elicit PREP. Recording electrodes were placed at C₂ referenced to linked earlobes according to the international 10–20 system (Fig. 1). Recording: bandwidth 1 Hz to 1 kHz, sampling rate 2.5 kHz, sweep length 300 ms (1401plus, Signal, Cambridge Electronic Design, UK).

Signal analysis was performed by an investigator blinded to the study design. The first sweep was rejected to avoid contamination by startle responses. The remaining 6 sweeps were averaged. NP peak-to-peak amplitudes were analyzed on the averaged file (Fig. 2). Mean values of stimulation blocks for each subject and subsequent mean values for each group were calculated. PC-based offline analysis was performed with custom written software (Matlab 6.5, The MathWorks, Natick, MA, USA).

Image acquisition, processing and statistical analysis

We used a Siemens Sonata 1.5 Tesla scanner (Siemens, Erlangen, Germany) with a standard circularly polarized volume head coil. For

functional imaging two sessions with 420 T₂* weighted echo planar imaging (EPI) scans per session were acquired (TE=55 ms, TR=3200 ms, flip angle=90°, acquisition time for the entire test period was 22.4 min for each session). Each scan consisted of 34 transaxial slices (slice thickness=3 mm, interslice gap=0.3 mm, matrix 64×64, field of view 384 mm, voxel size 3.4×3.4×3.4 mm). Twenty-one blocks consisting of 20 scans each (13 rest scans, 7 activation scans) were presented in pseudo random order in each session. Preparation and pain-intensity adjustment were performed outside the scanner as described above. Subjects were asked to rate the experienced pain intensity after the end of each block on a verbal rating scale (VRS). An HF filter was interconnected between the stimulator outside the scanner and stimulation electrodes inside the scanner to reduce electrode artefacts. An additional anatomical T₁-weighted 3D scan was obtained from each subject using a magnetization prepared rapid acquisition gradient echo (MP-RAGE) sequence (TR/TE/TI=2400 ms/4.38 ms/1200 ms, flip angle=8°, field of view=256 mm, 160 slices, voxel size 1×1×1 mm³).

Functional sequences were analyzed using statistical parametric mapping software (SPM5; <http://www.fil.ion.ucl.ac.uk/>) based on the general linear model. Pre-processing of data included realignment of scans to the first scan of the series (neglecting three scans to account for relaxation effects) and stereotactic normalization (Friston et al., 1995) into the standard EPI template from the Montreal Neurological Institute (MNI) (Evans et al., 1994). For correct assignment of anatomical location within the Talairach space (Talairach and Tournoux, 1988) we used a transformation algorithm provided by Brett et al. (2002). To account for interindividual variability, images were spatially smoothed with an isotropic Gaussian kernel of 8 mm full width at half maximum (FWHM).

For individual subject analysis (first-level), a general linear model was specified using multiple regression analysis (Friston et al., 1995). As described above, we applied a simple block design with alternating scans of rest and activation (13 rest scans, 7 activation scans). Thus, for each subject, the statistical first-level model consisted of 4 regressors: VRS 3, VRS 5, VRS 7 and rest. We assessed pain-related hemodynamic responses by contrasting each pain condition with rest condition. The resulting *t*-statistics (i.e., comparison between stimulation vs. rest blocks) constitute of activation maps for each pain condition (i.e., VRS 3, VRS 5, VRS 7).

At group level (second level), random-effects model was used to test for within group effects using a full factorial design. Activation maps from first-level analysis (i.e., VRS 3, VRS 5, VRS 7) were entered into a second-level analysis. Pain related responses were whole brain corrected with family-wise error (FWE) correction. Level of significance was set at *p*<0.05. First, we tested for the main effect of stimulation (see Fig. 3A). Then we computed the 1st Eigenvariate across all voxels within a sphere of 8 mm radius, centered on the peak coordinate of brain regions that survived *p*<0.05 (FWE-corrected). The 1st Eigenvariate is the principal component or, in other words, the typical subject response for each pain level over voxels within the chosen sphere (Abler et al., 2007; Cardoner et al., 2007). Note that this data is unbiased or free from any a-priori assumptions. We used this data to test for a linear parametric increase response function that reflects the provided stimuli and the experienced pain-intensity using SPSS 14.0 (SPSS Inc. Chicago, IL, USA). For this we performed a simple regression analysis using Pearson parametric correlation of VRS, PREP and fMRI data. To reconfirm the established regions of activation we performed a time by condition interaction on the second-level with an ANOVA using the factor train pulse stimulation and the factor single pulse stimulation with the three different pain intensities (VRS 3, VRS 5, VRS 7) as regressors. The following contrasts were used to define the actual stimulation level by condition interaction: 1 2 3 – 1 – 2 – 3 and – 1 – 2 – 3 1 2 3 respectively. Due to our strong a priori hypothesis we were able to perform a small-volume correction (SVC) using an eight mm sphere corresponding to the volume used to

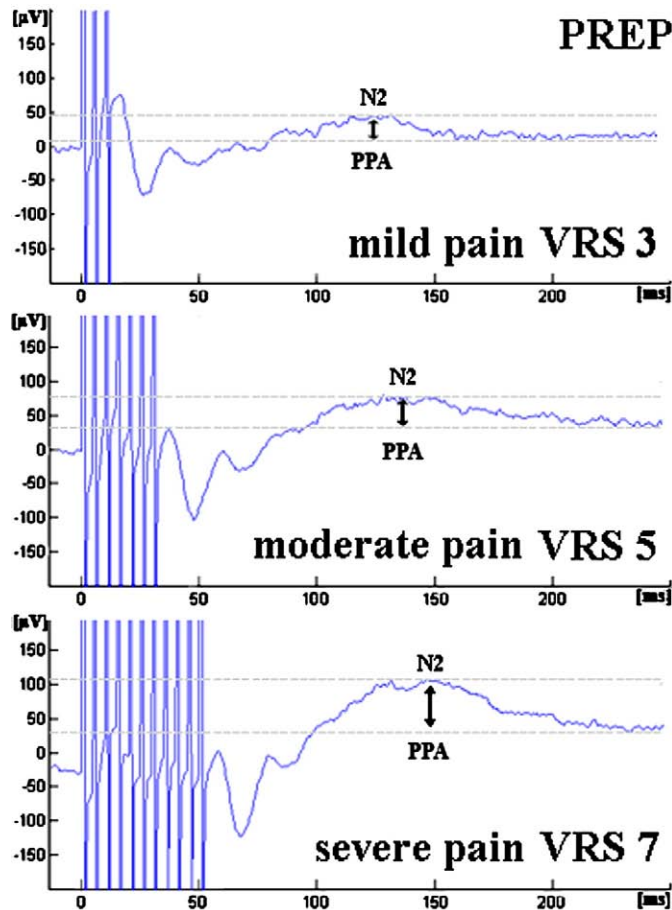


Fig. 2. Example recordings of typical pain-related evoked potentials (PREP). There is a pronounced increase in amplitudes (μV) with increasing pain-intensity experienced by the subject due to increasing temporal summation effect.

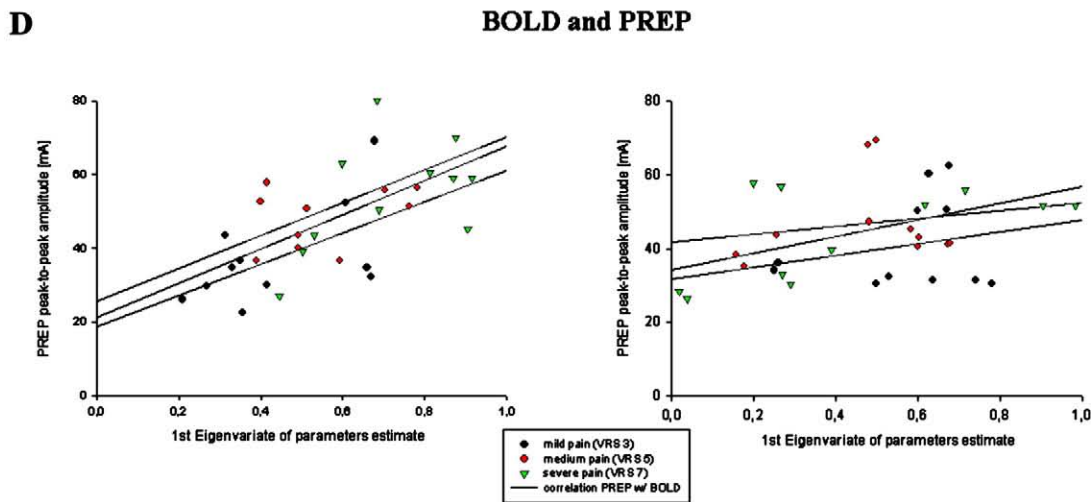
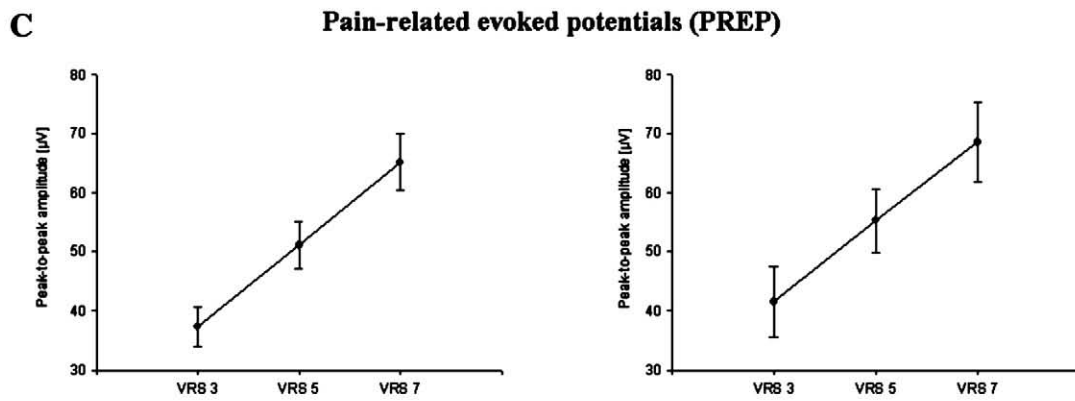
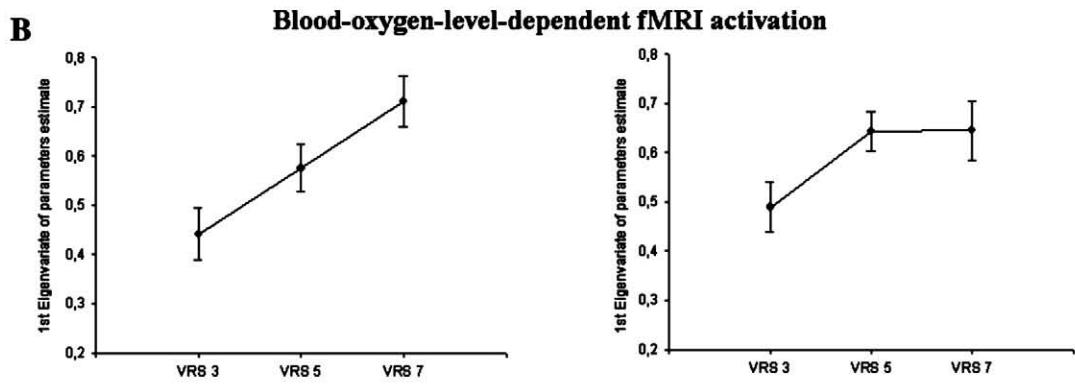
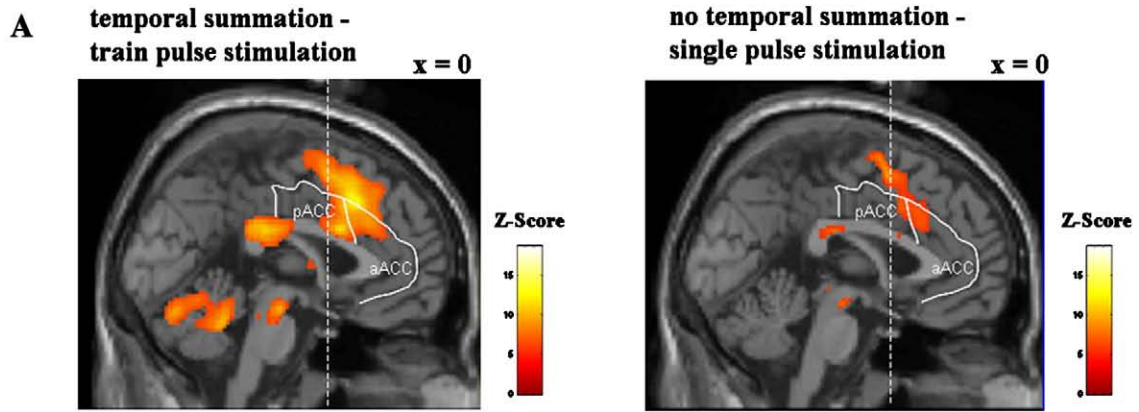


Table 1
Pain-related evoked potential (PREP) results using train pulse stimulation

PREP	VRS 3 PPA [μ V]	VRS 5 PPA [μ V]	VRS 7 PPA [μ V]
Subject 1	30.1	50.7	60.4
Subject 2	29.1	41.7	45.2
Subject 3	62.6	76.5	99.1
Subject 4	34.8	36.5	59.1
Subject 5	52.2	55.7	59.1
Subject 6	36.5	43.5	50.4
Subject 7	29.6	40.0	43.5
Subject 8	26.1	62.6	87.0
Subject 9	32.2	51.4	70.1
Subject 10	43.5	67.8	80.0
Subject 11	34.8	43.5	73.0
Mean	37.4	51.8	66.1

PPA = peak-to-peak amplitude; VRS = verbal rating scale.

calculate the 1st Eigenvariate. The T_1 -weighted structural volume was co-registered to the functional scans by normalizing it to a T_1 -weighted template in the same space as the T_2^* EPI template used to normalize the functional data set. Behavioural and electrophysiological data were analyzed using a multifactorial analysis of variance (MANOVA) for repeated measures and post-hoc test Bonferoni correction.

Results

Pain ratings and perception data

Mean sensory threshold was $0.7 \pm \text{SEM } 0.3$ mA (range: 0.4–1.4 mA), mean pain threshold was $1.2 \pm \text{SEM } 0.3$ mA (range: 0.6–1.8 mA) and mean moderate to severe pain (VRS 7) was reached at $3.6 \pm \text{SEM } 1.1$ mA (range: 2.1–5.8 mA) using 11 train pulse stimulation. We found a significant difference between individual pain ratings for the three pulse train lengths (3 pulse train = mean VRS 3 $3.2 \pm \text{SEM } 0.2$ (range: 2–4); 7 pulse train = mean VRS 5 5.1 ± 0.1 (range: 4.5–6); 11 pulse train = VRS 7 $7.0 \pm \text{SEM } 0.0$; MANOVA for repeated measures: $F = 103.15$, $df = 2$, $p < 0.001$). Post-hoc pair-wise comparisons showed differences comparing VRS 3 vs. VRS 5 ($p = 0.003$); VRS 5 vs. VRS 7 ($p = 0.001$); VRS 3 vs. VRS 7 ($p < 0.001$). The average pain ratings over each block increased corresponding to increasing train pulses and correlated with train length for all 11 subjects (mean $r = 0.75 \pm \text{SEM } 0.2$) in the first experiment (i.e., PREP). Fig. 2 plots the relationship between individual pain ratings and pulse train length in regard to PREP amplitudes (i.e. 3, 7 and 11 nociceptive stimuli; see also Fig. 3C).

In the control experiment, where we applied single pulses with increasing stimulation intensities (i.e. instead of changing train length, see Methods for further details) to increase perceived pain, mean sensory threshold was $0.7 \pm \text{SEM } 0.6$ mA (range: 0.4–1.6 mA), mean pain threshold was $1.4 \pm \text{SEM } 0.6$ mA (range: 0.8–2.6 mA). Mean mild pain (VRS 3) was reached at $3.2 \pm \text{SEM } 0.7$ mA (range: 2.4–4.0 mA), moderate pain (VRS 5) was reached at $4.75.1 \pm \text{SEM } 0.9$ mA (range: 4.0–6.0), while mean severe pain (VRS 7) was reached at $6.8 \pm \text{SEM } 1.4$ mA (range: 4.4–8.4 mA). We found a significant difference between individual pain ratings for the three stimulation intensities (MANOVA for repeated measures: $F = 19.5$, $df = 2$, $p = 0.008$). Post-hoc pair-wise comparisons showed differences comparing VRS 3 vs. VRS 5 ($p = 0.006$); VRS 5 vs. VRS 7 ($p = 0.004$); VRS 3 vs. VRS 7 ($p = 0.002$). Pain ratings correlated with stimulus intensities for all subjects (mean $r = 0.79 \pm \text{SEM } 0.6$).

We found no difference in sensory threshold ($p = 0.398$) and pain threshold ($p = 0.291$) between the two experiments.

Pain-related evoked potential data

Individual PREP results are summarized in Table 1. Peak-to-peak amplitudes (PPA) increased systematically with increasing train length of nociceptive pulses induced by the temporal summation effect (Fig. 2). PPA for the pulse train of three ($37.4 \pm \text{SD } 11.2$ μ V, range: 26.1–62.6 μ V), seven ($51.8 \pm \text{SD } 13.3$ μ V, range: 36.5–76.5 μ V), and eleven ($66.1 \pm \text{SD } 17.6$ μ V, range: 43.5–99.1 μ V) differed significantly (MANOVA for repeated measures $F = 17.5$, $df = 9.0$, $p = 0.001$, Fig. 2). Post-hoc pair-wise comparisons showed differences comparing three train pulse vs. seven train pulse ($p = 0.001$); seven train pulse vs. eleven train pulse ($p = 0.002$); three train pulse vs. eleven train pulse ($p = 0.001$). The PREP PPA positively correlated with pain ratings; for VRS 7/PPA 11 pulse train ($r = 0.81$; $p < 0.001$), VRS 5/PPA 7 pulse train ($r = 0.72$; $p < 0.001$), VRS 3/PPA 3 pulse train ($r = 0.72$; $p = 0.001$).

In the control experiment (i.e., single pulses with varying stimulation intensities) PREP PPA increased with increasing pain-intensities (Table 2): PPA amplitude for mild pain (VRS 3) was $37.1 \pm \text{SD } 10.3$ μ V (range: 28.5–60.4 μ V), for moderate pain (VRS 5) $52.1 \pm \text{SD } 13.6$ μ V (range: 32.1–80.3 μ V), and for severe pain (VRS 7) $60.6 \pm \text{SD } 14.9$ μ V (range: 40.9–90.3 μ V). Stimulation intensities for the three pain levels differed (MANOVA for repeated measures: $F = 22.6$, $df = 1.0$, $p = 0.007$). Post-hoc pair-wise comparisons showed differences comparing stimulation intensity VRS 3 vs. VRS 5 ($p = 0.003$); stimulation intensity VRS 5 vs. VRS 7 ($p = 0.005$); stimulation intensity VRS 3 vs. VRS 7 ($p = 0.006$). PREP PPA amplitudes correlated to reported VRS ratings; for VRS 7/PPA 11 pulse train ($r = 0.76$; $p = 0.003$), VRS 5/PPA 7 pulse train ($r = 0.73$; $p = 0.008$), VRS 3/PPA 3 pulse train ($r = 0.78$; $p = 0.002$). We found no difference in PREP PPA ($p = 0.254$) between the two experiments.

Imaging data

The painful nature of both stimulation paradigms was confirmed by the response of brain regions previously associated with trigeminal pain processing (Becerra et al., 1999; Borsook et al., 2004) (Table 3). We however aimed to identify the brain region coding temporal summation of trigeminal nociceptive stimuli. Using the same stimulation protocol for electrophysiological and fMRI measures, we searched for hemodynamic responses that correlated with electrophysiological responses (i.e., PREP) and pain ratings (i.e., VRS; Fig. 3).

When looking at the main effect of stimulation (Fig. 3A) we found a significant increase of hemodynamic responses with increasing pain (i.e., 1st Eigenvariate; MANOVA for repeated measures: $F = 56.5$, $df = 2$, $p < 0.001$) from the posterior part of the ACC (pACC) ($x = 0$, $y = 10$, $z = 30$) only. Post-hoc pair-wise comparisons showed differences comparing 11 trains vs. 7 trains ($p = 0.001$); 7 trains vs. 3 trains ($p = 0.002$); 3 trains vs. 11 trains ($p < 0.001$). The linear increase of hemodynamic changes in pACC correlated significantly with linear increases of individual pain ratings (VRS 7: $r = 0.72$; $p < 0.001$; VRS 5: $r = 0.76$; $p < 0.001$; VRS 3: $r = 0.79$; $p < 0.001$) and peak-to-peak amplitudes (PPA 11 pulses: $r = 0.56$; $p = 0.011$; PPA 7 pulses: $r = 0.62$; $p = 0.009$; PPA 3 pulses: $r = 0.68$; $p = 0.008$; Fig. 3D). Hemodynamic changes in the posterior cingulate cortex (PCC) ($x = -2$, $y = -30$, $z = 31$), the insula ($x = 42$, $y = 0$, $z = -7$) and the somatosensory cortex (SII) ($x = 46$, $y = -58$, $z = 57$) also increased with pain, but did not

Fig. 3. (A) Main effects of painful trigeminal stimulation (effects of interest; $p < 0.05$; family wise error (FEW) whole brain corrected) for varying pulse trains (with constant stimulation intensities, see left panel) and single pulses (with varying stimulation intensities, see right panel). Eight millimeter radius volumes of interest (VOI) were defined and the 1st Eigenvariate was analyzed in relation to increasing pain-intensity within the posterior part of the anterior cingulate cortex (pACC) with local maximum of activation at $x = 0$, $y = 10$, $z = 30$. Dotted line indicates a vertical through the anterior commissure (VAC). (B) Increase in pain was achieved by increasing train pulse stimulation inducing a temporal summation effect (left panel) and genuine increase in stimulus intensity in mA using single pulse stimulation as control experiment (right panel). (C) Pain-related evoked potentials (PREP) showed a similar, linear increase with increasing pain intensity in both experiments on a verbal rating scale (VRS; 0 = no pain, 10 = worst possible pain). (D) Pain related evoked potentials (PREP) peak-to-peak amplitude (PPA) correlated with hemodynamic (BOLD) response only for train pulse stimulation but not for single pulses.

Table 2

Pain-related evoked potential (PREP) results with single pulse stimulation and increasing current intensity mA (control experiment)

PREP	VRS 3 PPA [μ V]	VRS 5 PPA [μ V]	VRS 7 PPA [μ V]
Subject 1	29.2	52.1	61.1
Subject 2	28.5	39.4	42.9
Subject 3	60.4	80.3	90.3
Subject 4	30.4	49.6	57.8
Subject 5	41.1	50.5	52.1
Subject 6	50.5	68.3	78.4
Subject 7	30.8	32.1	40.9
Subject 8	30.4	43.1	50.9
Subject 9	30.3	48.1	65.2
Subject 10	40.5	62.1	71.0
Subject 11	36.1	47.3	55.7
Mean	37.1	52.1	60.6

PPA = peak-to-peak amplitude; VRS = verbal rating scale; mA = milliampere.

reach the level of significance. We used the nomenclature by Kwan et al. (2000) to describe subregions in the cingulate cortex.

In the control experiment, where we applied single nociceptive pulses, pACC did not show this linear increase as for pulse-trains (MANOVA for repeated measures: $F = 0.534$, $df = 2$, $p = 0.457$) and did not correlate to VRS ratings (VRS 7: $r = 0.28$; $p = 0.287$; VRS 5: $r = 0.15$; $p = 0.612$; VRS 3: $r = 0.24$; $p = 0.382$) or PREP (PPA 11 pulses: $r = 0.32$; $p = 0.598$; PPA 7 pulses: $r = 0.32$; $p = 0.267$; PPA 3 pulses: $r = 0.10$; $p = 0.774$; Fig. 3B). The peak activation within the pACC was located slightly more dorsal and caudal compared to that seen for pulse-trains ($x = 4$, $y = 6$, $z = 29$). The control experiment revealed similar hemodynamic activations in the PCC, insula and SII as observed in the main experiment, but again failed the level of significance.

In the stimulation level by condition interaction comparing train pulse and single pulse activation peak activation was located within the pACC ($x = 0$, $y = 10$, $z = 30$) (Table 4), the brainstem ($x = 9$, $y = -22$, $z = -23$), and the hippocampus ($x = 24$, $y = -18$, $z = -7$) contralateral to the site of stimulation (Fig. 4).

Discussion

We investigated trigeminal pain processing following increasing painful stimulation using a) train pulse stimulation (temporal summation) with constant current intensity, and b) single pulse stimulation with increasing stimulus intensity in mA. We measured: a) subjective pain perception using verbal rating scale (VRS), b) pain-

Table 3

Pain-related hemodynamic responses (fMRI activation) using train pulse stimulation (left Z-values) and single pulse stimulation (right Z-values)

Pain-related hemodynamic response					
Anatomical region	x	y	z	Z-value train pulse stimulation	Z-value single pulse stimulation
Posterior ACC ^a	0	10	30	7.12 ^a	6.07
Posterior cingulate cortex	-2	-30	31	7.00	6.50
Insula	42	0	-7	Inf.	8.24
SII	46	-58	57	7.33	5.75
SI	48	-37	53	6.78	6.80
Ventral thalamus	8	-16	13	7.43	5.84
Dorsolateral prefrontal cortex	52	22	33	6.04	5.85
Frontal lobe	4	16	49	7.58	5.93
Putamen	20	4	13	6.79	5.69
Cerebellum	-36	-68	-25	6.62	5.64
Midbrain/Pons	6	-26	-17	6.95	6.13

All $p < 0.05$, whole brain corrected using family-wise error correction (FWE); x , y , z (mm).

^a Related to temporal summation effects.

Table 4

Stimulation level by condition interaction comparing train pulse and single pulse stimulation

Stimulation level by condition interaction	x	y	z	Z-value	p_{SVC}
Posterior ACC	0	10	30	4.93	0.018
Midbrain/pons	9	-22	-23	4.35	0.044
Hippocampus (CA)	24	-18	-7	4.02	0.047

$p < 0.001$ uncorrected; SVC = small-volume correction (8 mm sphere).

related evoked potentials (PREP), and c) hemodynamic response of pain associated structures.

The two experimental setups, with train pulse and single pulse stimulation, both resulted in a similar increase of subjective pain perception, to a parallel augmentation of PREP, and comparable BOLD response within the pain matrix. Main differences between the two experiments were observed in the ACC. When train pulse stimulation was used, the pACC showed a linear increase in BOLD signal following increased pain perception similar to the recorded PREP. When the increase in pain perception was induced by increasing the stimulus intensity in mA, stimulation did not result in a comparable linear BOLD activation pattern within the pACC. Other supraspinal regions such as the PCC, insula, and somatosensory cortex (SII) showed similar responses in both experimental settings and therefore are not likely to be attributed to the central processing of temporal summation. They probably reflect the increase in pain-intensity but do not modulate temporal summation.

Our data is in line with previous reports of ACC encoding of pain and its location within the brain fits current knowledge of similar functioning regions in pain processing. Previous research provided conclusive evidence for a distinction between brain activity involved in the encoding of pain dimensions such as sensation and feeling of pain, and brain activity involved in evaluative and other components that are not directly related to the subjective experience of pain itself such as recognition of stimulus intensity, classification of the type of pain, representing pain dimensions by a word or a number, etc. (Kong et al., 2006; Staud et al., 2007). The ACC was the focus of several studies suggesting that it not only takes part in the affective encoding of pain (Rainville et al., 1997; Sawamoto et al., 2000), but is also involved in primary sensory encoding such as stimulus intensity-related responses (Becerra et al., 1999; Buchel et al., 2002; Davis et al., 1997; Kwan et al., 2000; Sikes et al., 2008). This is further supported by anatomic studies that demonstrated projections to the ACC from thalamic midline and intralaminar nuclei (Marini et al., 1996; Musil and Olson, 1988; Robertson and Kaitz, 1981; Vogt et al., 1987), as well as the ventral part of the ventrobasal complex (Yasui et al., 1988). These regions receive input from the spinothalamic tract and have been shown to contain nociceptive neurons (Albe-Fessard et al., 1985; Craig et al., 1994). These nociceptive neurons have been located in the pACC (Hutchison et al., 1999; Sikes and Vogt, 1992). Thus, the ACC seems to have specific spatial patterns of activation within its substructures and surrounding medial wall regions. Distinct areas within the ACC have been attributed to specific tasks with respect to its complexity and modality (Buchel et al., 2002; Kwan et al., 2000; Paus et al., 1998; Picard and Strick, 1996; Sikes et al., 2008). The posterior part of the ACC which encompasses Brodman area 23 and posterior area 24 seems to be particularly involved in the sensory-integrative aspect of pain processing, while the anterior portion of the ACC is mainly encoding the attentional aspect of pain (Davis et al., 1997; Hutchison et al., 1999; Sikes and Vogt, 1992). Buchel et al. (2002) differentiated stimulus-related and pain-related activation within the ACC. They located pain-related responses mainly to the dorsal ACC slightly anterior to the anterior commissure ($x = 3$, $y = 6$, $z = 48$). Porro et al. (1998) reported pain-related fMRI responses in a similar region ($x = 7$, $y = 6$, $z = 34$). The response of our experiment was located close to this area but was shifted slightly rostral and

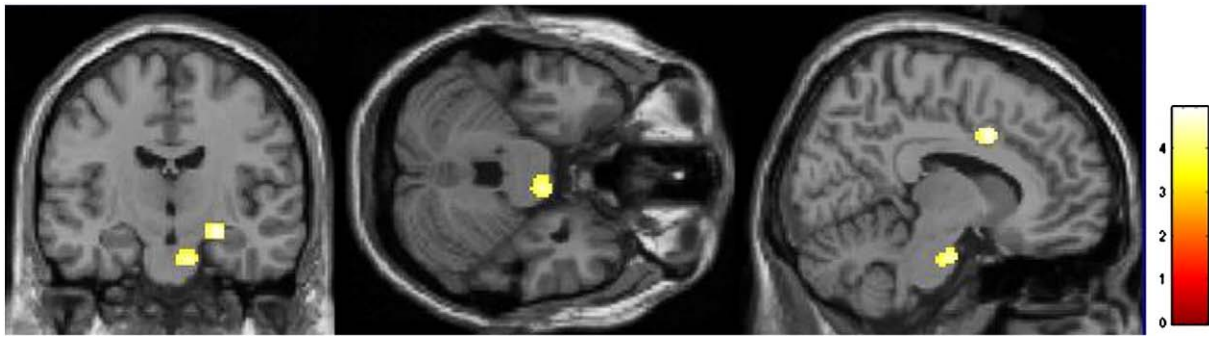


Fig. 4. Time by condition interaction comparing train pulse and single pulse stimulation ($p < 0.001$ uncorrected). A small-volume correction (SVC) with eight mm sphere was used in parallel to the calculation of 1st Eigenvariate. Peak activation was found in the pACC, brainstem and hippocampus.

ventral within the pACC ($x=0, y=10, z=30$). Stimulus intensity related activation was reported to be located more dorsal ($x=-3, y=3, z=51$) within the ACC (Buchel et al., 2002) and close to the activation we recorded in our control experiment ($x=4, y=6, z=29$). The response function we obtained from the control group in this region resembles that reported by Buchel et al. (2002) for stimulus intensity related responses. This locates central representation of temporal summation in between stimulus intensity and pain intensity processing, which makes sense regarding its proposed physiologic function. Feeling pain more severe, when it is applied constantly or in fast series makes this sensory input much more important regarding the integrity of the organism, than one single mild to moderate pain stimulus could. The close spatial relationship to areas encoding for emotional and attentional aspects of pain further support the important role pACC might play in development and sustenance of chronic pain conditions. Staud et al. (2007) confirmed the ACC as pain-modulating region when pain is induced by temporal summation. Similar to our experiment they modulated the perceived pain intensity by increasing the number of applied stimuli, but used heat pulse trains that mainly evoked C-fibers and stimulated the plantar surface of the foot. Their main activation within the ACC was located more rostral compared to ours, but comparison is only visual as they do not provide exact coordinates and do not use the nomenclature introduced by Kwan et al. (2000). The differences in location could have the following explanations: 1. it could be hypothesized that a somatotopic organisation within the ACC exists, which could explain the difference between face and foot stimulation; 2. A δ - and C-fiber related transmission may also have different central representations within the ACC.

Using a stimulation level by condition interaction we were able to reconfirm the role of the pACC in temporal summation, but also identified two different regions that did not show up in our descriptive analysis on electrophysiological basis, i.e. the brainstem (midbrain/pons) and the hippocampus contralateral to the site of stimulation. The brainstem is thought to be extremely relevant in the modulation of pain. Activation of brainstem nuclei within the pain descending pathways were found both during anticipation and during actual painful stimulation (Fairhurst et al., 2007; Keltner et al., 2006). Detailed investigation of the human brainstem is complicated by several technical fMRI limitations including poor spatial resolution, local field inhomogeneity-induced signal loss, image distortion and arterial pulsation (Tracey and Iannetti, 2006). Our results suggest the caudal midbrain and pons nuclei to play a role in central sensitization. Due to the limited statistical power, these results have to be interpreted with caution and will need further validation in future experiments. The same applies for the found activation in the hippocampus. It makes perfect sense for the hippocampus to be included in processing repetitive painful stimulation, but whether this structure plays an active part in pain processing in this regard requires further investigation.

Some limitations to these findings have to be addressed. The comparison with electrophysiological data made it necessary to pool data and to compare it on group level, as PREP show a considerably large inter- and intraindividual variability in amplitude specification. This is also the reason we refrained from using an event-related fMRI design. The fine spatial and temporal resolution of individual subject analysis in fMRI recordings was therefore partially lost. While the location of BOLD activation was consistent with previous research, the undetermined interindividual differences in activation limit our description to a broader area within the pACC and also limit the explanatory power of the indicated stimulus response function. Due to this limitation we cannot exclude the involvement of other, yet undetermined supraspinal regions in the central processing of temporal summation. Moreover, the differences seen in PREP amplitudes and VRS ratings may have been influenced by sleep deprivation, divided attention, coffee, cigarette smoking, etc. which were not controlled.

Our temporal summation paradigm is evoked electrically and activates mainly A δ -fibers within the above mentioned limits. A δ -fibers are depolarized directly by electrical stimulation and skin nociceptors are bypassed. The stimulation leads to perception of a localized, sharp, pin-prick like pain which after several applied pulse trains results in minor hyperalgesia lasting between seconds and few minutes. The conduction velocity measured using PREP was 15–20 m/s corresponding to that of A δ -fibers (Katsarava et al., 2006). As A δ -fibers show rapid attenuation on repetitive pain input (Price et al., 1971) this indicates that the evoked sensitization is probably due to central mechanisms. Electrical stimulation enables us to increase the painful stimulation not only by an increase of current and thus stimulus intensity, but also by applying multiple pulse stimuli inducing temporal summation. The C-fiber related dull, burning second pain that is described by many authors as typical model for 'wind-up' (Staud et al., 2007; Vierck et al., 1997) is not equivalent to our stimulation technique, and underlying physiological effects might be different. Activation of myelinated nociceptors that generate first pain sensations appear to produce more inhibition than does direct stimulation of low-threshold myelinated afferents (Chung et al., 1984). Bypassing these receptors possibly avoids some of the antinociceptive early regulative mechanisms that could be activated by thermode or laser stimulation.

In conclusion, we were able to locate central representation of temporal summation in trigeminal pain processing to the pACC. This region may play an important role in the development of chronic pain disorders.

References

- Abler, B., Erk, S., Herwig, U., Walter, H., 2007. Anticipation of aversive stimuli activates extended amygdala in unipolar depression. *J. Psychiatr. Res.* 41, 511–522.
- Albe-Fessard, D., Berkley, K.J., Kruger, L., Ralston III, H.J., Willis Jr., W.D., 1985. Diencephalic mechanisms of pain sensation. *Brain Res.* 356, 217–296.

- Ayzenberg, I., Obermann, M., Nyhuis, P., Gastpar, M., Limmroth, V., Diener, H.C., Kaube, H., Katsarava, Z., 2006. Central sensitization of the trigeminal and somatic nociceptive systems in medication overuse headache mainly involves cerebral supraspinal structures. *Cephalalgia* 26, 1106–1114.
- Becerra, L.R., Breiter, H.C., Stojanovic, M., Fishman, S., Edwards, A., Comite, A.R., Gonzalez, R.G., Borsook, D., 1999. Human brain activation under controlled thermal stimulation and habituation to noxious heat: an fMRI study. *Magn. Reson. Med.* 41, 1044–1057.
- Borsook, D., Burstein, R., Becerra, L., 2004. Functional imaging of the human trigeminal system: opportunities for new insights into pain processing in health and disease. *J. Neurobiol.* 61, 107–125.
- Bouckoms, A.F., 1989. *Psychosurgery for Pain*. Churchill Livingstone, Edinburgh.
- Brett, M., Johnsrude, I.S., Owen, A.M., 2002. The problem of functional localization in the human brain. *Nat. Rev. Neurosci.* 3, 243–249.
- Buchel, C., Bornhoved, K., Quante, M., Glauche, V., Bromm, B., Weiller, C., 2002. Dissociable neural responses related to pain intensity, stimulus intensity, and stimulus awareness within the anterior cingulate cortex: a parametric single-trial laser functional magnetic resonance imaging study. *J. Neurosci.* 22, 970–976.
- Cardoner, N., Soriano-Mas, C., Pujol, J., Alonso, P., Harrison, B.J., Deus, J., Hernandez-Ribas, R., Menchon, J.M., Vallejo, J., 2007. Brain structural correlates of depressive comorbidity in obsessive-compulsive disorder. *NeuroImage* 38, 413–421.
- Casey, K.L., 2000. Concepts of pain mechanisms: the contribution of functional imaging of the human brain. *Prog. Brain Res.* 129, 277–287.
- Casey, K.L., Minoshima, S., Berger, K.L., Koeppe, R.A., Morrow, T.J., Frey, K.A., 1994. Positron emission tomographic analysis of cerebral structures activated specifically by repetitive noxious heat stimuli. *J. Neurophysiol.* 71, 802–807.
- Chung, J.M., Lee, K.H., Hori, Y., Endo, K., Willis, W.D., 1984. Factors influencing peripheral nerve stimulation produced inhibition of primate spinothalamic tract cells. *Pain* 19, 277–293.
- Coderre, T.J., Katz, J., Vaccarino, A.L., Melzack, R., 1993. Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. *Pain* 52, 259–285.
- Coghill, R.C., Sang, C.N., Maisog, J.M., Iadarola, M.J., 1999. Pain intensity processing within the human brain: a bilateral, distributed mechanism. *J. Neurophysiol.* 82, 1934–1943.
- Craig, A.D., Bushnell, M.C., Zhang, E.T., Blomqvist, A., 1994. A thalamic nucleus specific for pain and temperature sensation. *Nature* 372, 770–773.
- Craig, A.D., Reiman, E.M., Evans, A., Bushnell, M.C., 1996. Functional imaging of an illusion of pain. *Nature* 384, 258–260.
- Davis, K.D., Taylor, S.J., Crawley, A.P., Wood, M.L., Mikulis, D.J., 1997. Functional MRI of pain- and attention-related activations in the human cingulate cortex. *J. Neurophysiol.* 77, 3370–3380.
- Devinsky, O., Morrell, M.J., Vogt, B.A., 1995. Contributions of anterior cingulate cortex to behaviour. *Brain* 118 (Pt 1), 279–306.
- Dougherty, P.M., Palecek, J., Paleckova, V., Sorkin, L.S., Willis, W.D., 1992. The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli. *J. Neurosci.* 12, 3025–3041.
- Evans, A.C., Collins, D.L., Mills, S.P., Brown, E.D., Kelly, R.L., Peters, R.M., 1994. 3D statistical neuroanatomical models from 305 MRI volumes. *IEEE Nuclear Science Symposium and Medical Imaging Conference* pp. 1813–1817.
- Fairhurst, M., Wiech, K., Dunckley, P., Tracey, I., 2007. Anticipatory brainstem activity predicts neural processing of pain in humans. *Pain* 128, 101–110.
- Foltz, E.L., White, L.E., 1968. The role of rostral cingulotomy in pain relief. *Int. J. Neurol.* 6, 353–373.
- Friston, K.J., Ashburner, J., Poline, J.B., Frith, C.D., Heather, J.D., Frackowiak, R.S.J., 1995. Spatial registration and normalization of images. *Hum. Brain Mapp.* 2, 165–189.
- Giffin, N.J., Katsarava, Z., Pfundstein, A., Ellrich, J., Kaube, H., 2004. The effect of multiple stimuli on the modulation of the 'nociceptive' blink reflex. *Pain* 108, 124–128.
- Gitelman, D.R., Nobre, A.C., Parrish, T.B., LaBar, K.S., Kim, Y.H., Meyer, J.R., Mesulam, M., 1999. A large-scale distributed network for covert spatial attention: further anatomical delineation based on stringent behavioural and cognitive controls. *Brain* 122 (Pt 6), 1093–1106.
- Gracely, R.H., Lynch, S.A., Bennett, G.J., 1992. Painful neuropathy: altered central processing maintained dynamically by peripheral input. *Pain* 51, 175–194.
- Hutchinson, W.D., Davis, K.D., Lozano, A.M., Tasker, R.R., Dostrovsky, J.O., 1999. Pain-related neurons in the human cingulate cortex. *Nat. Neurosci.* 2, 403–405.
- Inghilleri, M., Berardelli, A., Cruccu, G., Priori, A., Manfredi, M., 1990. Motor potentials evoked by paired cortical stimuli. *Electroencephalogr. Clin. Neurophysiol.* 77, 382–389.
- Ji, R.R., Woolf, C.J., 2001. Neuronal plasticity and signal transduction in nociceptive neurons: implications for the initiation and maintenance of pathological pain. *Neurobiol. Dis.* 8, 1–10.
- Jones, A.K.P., Brown, W.D., Friston, K.J., Qi, L.Y., Frackowiak, R.S.J., 1991. Cortical and subcortical localization of response to pain in man using positron emission tomography. *Proc. R. Soc. Lond. B Sci.* 244, 39–44.
- Katsarava, Z., Lehnerdt, G., Duda, B., Ellrich, J., Diener, H.C., Kaube, H., 2002. Sensitization of trigeminal nociception specific for migraine but not pain of sinusitis. *Neurology* 59, 1450–1453.
- Katsarava, Z., Ayzenberg, I., Sack, F., Limmroth, V., Diener, H.C., Kaube, H., 2006. A novel method of eliciting pain-related potentials by transcutaneous electrical stimulation. *Headache* 46, 1511–1517.
- Kaube, H., Katsarava, Z., Käufer, T., Diener, H.C., Ellrich, J., 1999. A new method to increase nociception specificity of the human blink reflex. *Clin. Neurophysiol.* 111, 413–416.
- Kaube, H., Katsarava, Z., Käufer, T., Diener, H., Ellrich, J., 2000. A new method to increase nociception specificity of the human blink reflex. *Clin. Neurophysiol.* 111, 413–416.
- Kaube, H., Katsarava, Z., Przywara, S., Drepper, J., Ellrich, J., Diener, H.C., 2002. Acute migraine headache: possible sensitization of neurons in the spinal trigeminal nucleus? *Neurology* 58, 1234–1238.
- Keltner, J.R., Furst, A., Fan, C., 2006. Isolating the modulatory effect of expectation on pain transmission: a functional magnetic resonance imaging study. *J. Neurosci.* 26, 4437–4443.
- Kong, J., White, N.S., Kwong, K.K., Vangel, M.G., Rosman, I.S., Gracely, R.H., Gollub, R.L., 2006. Using fMRI to dissociate sensory encoding from cognitive evaluation of heat pain intensity. *Hum. Brain Mapp.* 27, 715–721.
- Kwan, C.L., Crawley, A.P., Mikulis, D.J., Davis, K.D., 2000. An fMRI study of the anterior cingulate cortex and surrounding medial wall activations evoked by noxious cutaneous heat and cold stimuli. *Pain* 85, 359–374.
- Li, J., Simone, D.A., Larson, A.A., 1999. Windup leads to characteristics of central sensitization. *Pain* 79, 75–82.
- Marini, G., Pianca, L., Tredici, G., 1996. Thalamic projection from the parafascicular nucleus to layer V pyramidal cells in frontal and cingulate areas of the rat. *Neurosci. Lett.* 203, 81–84.
- Musil, S.Y., Olson, C.R., 1988. Organization of cortical and subcortical projections to anterior cingulate cortex in the cat. *J. Comp. Neurol.* 272, 203–218.
- Obermann, M., Yoon, M.S., Ese, D., Maschke, M., Kaube, H., Diener, H.C., Katsarava, Z., 2007. Impaired trigeminal nociceptive processing in patients with trigeminal neuralgia. *Neurology* 69, 835–841.
- Paus, T., Koski, L., Caramanos, Z., Westbury, C., 1998. Regional differences in the effects of task difficulty and motor output on blood flow response in the human anterior cingulate cortex: a review of 107 PET activation studies. *NeuroReport* 9, R37–47.
- Petit, L., Courtney, S.M., Ungerleider, L.G., Haxby, J.V., 1998. Sustained activity in the medial wall during working memory delays. *J. Neurosci.* 18, 9429–9437.
- Peyron, R., Garcia-Larrea, L., Gregoire, M.C., Costes, N., Convers, P., Lavenne, F., Mauguere, F., Michel, D., Laurent, B., 1999. Haemodynamic brain responses to acute pain in humans: sensory and attentional networks. *Brain* 122 (Pt 9), 1765–1780.
- Picard, N., Strick, P.L., 1996. Motor areas of the medial wall: a review of their location and functional activation. *Cereb. Cortex* 6, 342–353.
- Ploghaus, A., Tracey, I., Gati, J.S., Clare, S., Menon, R.S., Matthews, P.M., Rawlins, J.N., 1999. Dissociating pain from its anticipation in the human brain. *Science* 284, 1979–1981.
- Porro, C.A., Cettolo, V., Francescato, M.P., Baraldi, P., 1998. Temporal and intensity coding of pain in human cortex. *J. Neurophysiol.* 80, 3312–3320.
- Price, D.D., Hull, C.D., Buchwald, N.A., 1971. Intracellular responses of dorsal horn cells to cutaneous and sural nerve A and C fiber stimuli. *Exp. Neurol.* 33, 291–309.
- Price, D.D., Mao, J., Mayer, D.J., 1994. Central neural mechanisms of normal and abnormal pain states. In: Fields, H.L., Liebeskind, J.C. (Eds.), *Progress in Pain Research and Management*. IASP Press, Washington, pp. 61–84.
- Rainville, P., Duncan, G.H., Price, D.D., Carrier, B., Bushnell, M.C., 1997. Pain affect encoded in human anterior cingulate but not somatosensory cortex. *Science* 277, 968–971.
- Reitter, B.F., Johannsen, S., 1982. Neuromuscular reaction to paired stimuli. *Muscle Nerve* 5, 593–603.
- Robertson, R.T., Kaiz, S.S., 1981. Thalamic connections with limbic cortex. I. Thalamic projections. *J. Comp. Neurol.* 195, 501–525.
- Sawamoto, N., Honda, M., Okada, T., Hanakawa, T., Kanda, M., Fukuyama, H., Konishi, J., Shibasaki, H., 2000. Expectation of pain enhances responses to nonpainful somatosensory stimulation in the anterior cingulate cortex and parietal operculum/posterior insula: an event-related functional magnetic resonance imaging study. *J. Neurosci.* 20, 7438–7445.
- Sikes, R.W., Vogt, B.A., 1992. Nociceptive neurons in area 24 of rabbit cingulate cortex. *J. Neurophysiol.* 68, 1720–1732.
- Sikes, R.W., Vogt, L.J., Vogt, B.A., 2008. Distribution and properties of visceral nociceptive neurons in rabbit cingulate cortex. *Pain* 135 (1–2), 160–174 (2007 Nov 19 Electronic publication ahead of print).
- Staud, R., Vierck, C.J., Cannon, R.L., Mauderli, A.P., Price, D.D., 2001. Abnormal sensitization and temporal summation of second pain (wind-up) in patients with fibromyalgia syndrome. *Pain* 91, 165–175.
- Staud, R., Price, D.D., Robinson, M.E., Mauderli, A.P., Vierck, C.J., 2004. Maintenance of windup of second pain requires less frequent stimulation in fibromyalgia patients compared to normal controls. *Pain* 110, 689–696.
- Staud, R., Craggs, J.G., Robinson, M.E., Perlstein, W.M., Price, D.D., 2007. Brain activity related to temporal summation of C-fiber evoked pain. *Pain* 129, 130–142.
- Talairach, J., Tournoux, P., 1988. *Co-planar Stereotaxic Atlas of the Human Brain*. Thieme, Stuttgart.
- Talbot, J.D., Marret, S., Evans, A.C., Meyer, E., Bushnell, M.C., Duncan, G.H., 1991. Multiple representations of pain in human cerebral cortex. *Science* 251, 1355–1358.
- Talbot, J.D., Villemure, J.G., Bushnell, M.C., Duncan, G.H., 1995. Evaluation of pain perception after anterior capsulotomy: a case report. *Somatosens. Motor Res.* 12, 115–126.
- Taylor, B.A., Fennelly, M.E., Taylor, A., Farrell, J., 1993. Temporal summation—the key to motor evoked potential spinal cord monitoring in humans. *J. Neurol. Neurosurg. Psychiatry* 56, 104–106.
- Tracey, I., Iannetti, G.D., 2006. Brainstem functional imaging in humans. *Suppl. Clin. Neurophysiol.* 58, 52–67.
- Vierck Jr., C.J., Cannon, R.L., Fry, G., Maixner, W., Whitsel, B.L., 1997. Characteristics of temporal summation of second pain sensations elicited by brief contact of glabrous skin by a preheated thermode. *J. Neurophysiol.* 78, 992–1002.
- Vogt, B.A., Pandya, D.N., Rosene, D.L., 1987. Cingulate cortex of the rhesus monkey: I. Cytoarchitecture and thalamic afferents. *J. Comp. Neurol.* 262, 256–270.
- Yasui, Y., Itoh, K., Kamiya, H., Ino, T., Mizuno, N., 1988. Cingulate gyrus of the cat receives projection fibers from the thalamic region ventral to the ventral border of the ventrobasal complex. *J. Comp. Neurol.* 274, 91–100.