

## Acute opioid effects on human brain as revealed by functional magnetic resonance imaging

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**Functional magnetic resonance imaging has been widely used to study brain activation induced either by specific sensory stimulation or motor or cognitive task performance. We demonstrate that functional magnetic resonance imaging can provide information of brain regions involved in opioid-induced central nervous system effects. The reproducibility of the responses in the predefined regions of interest was confirmed by repeated boluses of ultra-short acting mu-opioid receptor agonist remifentanyl and saline. We report spatially and temporally detailed information after remifentanyl administration. Areas rich in mu-opioid receptors showed strong activations, whereas primary somatosensory cortex that has the lowest density of mu-opioid receptors showed negligible activation. The cingulate, orbitofrontal, posterior parietal and insular cortices, and amygdala showed activation, which was temporally closely related to most subjective sensations that were strongest at 80 to 90 s after drug administration. These areas belong to a circuitry that modulates the affective experience of sensory stimuli.**

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### Introduction

Exogenous opioids are used as analgesics. Their main adverse effects include sedation, respiratory depression, euphoria or dysphoria, nausea, and impaired cognitive function. The opioid system is also involved in the modulation of emotions, anxiety, reward, stress, learning, and memory acquisition. The physiological effects of opioids are mediated by activation of mu, delta, and kappa opioid receptors throughout the central and peripheral nervous systems. Post-mortem auto-radiographic and positron emission tomography (PET) studies in humans have shown that

the highest densities of mu-opioid receptors are found in the cingulate cortex, thalamus, periaqueductal gray, and caudate nucleus (Cross et al., 1987; Frost et al., 1985). The stimulation of opioid receptors further activates cholinergic, adrenergic, and dopaminergic neurotransmitter systems (Chiang and Zhuo, 1989; Hagelberg et al., 2004; Jackisch et al., 1986).

Modulation of neuronal activation by opioids has previously been studied with PET. The effects of a single intravenous fentanyl bolus (Firestone et al., 1996), fentanyl analgesia (Adler et al., 1997), remifentanyl infusions of different doses (Wagner et al., 2001), and remifentanyl analgesia (Petrovic et al., 2002) have caused significant changes in regional cerebral blood flow particularly in the anterior cingulate and orbitofrontal cortices. These areas have also been activated by pain, modulation of pain by hypnosis, and placebo analgesia (Baron et al., 1999; Iadarola et al., 1998; Petrovic et al., 2002; Rainville et al., 1997; Tölle et al., 1999).

Functional magnetic resonance imaging (fMRI) has been widely used to study brain activation induced either by specific sensory stimulation or motor or cognitive task performance. Recently, fMRI has also been used to analyze local changes in human brain activity resulting from drug administration. The term pharmacological MRI (phMRI) has been introduced (Honey and Bullmore, 2004). Most of the phMRI studies have combined the administration of a drug with cognitive task performance or sensory stimulation (Jokeit et al., 2001; Wise et al., 2004). The actual effects of a drug on brain activation in the absence of other modulating factors have been investigated only in a couple of phMRI studies. All such studies have been conducted in addicts using psychoactive drugs, e.g. cocaine (Breiter et al., 1997) and nicotine (Stein et al., 1998).

Pharmacokinetics present a particular challenge to fMRI studies of drug-induced changes in neuronal activation. Remifentanyl is a potent ultra-short acting mu-opioid receptor agonist (Rosow, 1999) that can be administered as repetitive intravenous boluses during one imaging period. In the present study, we used fMRI to assess

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the magnitude and distribution of brain activation caused by remifentanyl. The specific aim was to analyze the time course of the drug-induced blood oxygen level dependent (BOLD) signal intensity changes. The study was conducted in healthy, non-addict subjects in the absence of sensory stimuli such as pain or abstinence symptoms and craving of drugs that may activate the same brain areas as opioids. We anticipated that remifentanyl modulates the activation of brain areas that are known to have a high density of opioid receptors. We also expected to see some effects in brain areas that have been implicated to have a role in the processing of pain.

## Materials and methods

The study protocol was approved by the ethics committee of the Department of Surgery at the Helsinki University Central Hospital and by the Finnish National Agency for Medicines.

### Subjects

Eight healthy right handed subjects, four males and four females, aged 22–28 years (mean 24.9 years) participated in the behavioral pretest and the actual fMRI study. The behavioral pretest was performed to monitor individually the physiological and possible adverse effects of remifentanyl before the fMRI. The subjects had no pain and no history of drug or alcohol abuse, and they had no medication that could have affected the central nervous system and the test results. No analgesic medication was allowed on the day of the experiments, and the subjects fasted for at least 2 h before the behavioral pretest and the fMRI. Each subject gave a written consent for the participation in the study.

### Drug administration

An intravenous line was inserted into the antecubital vein of the left arm, and a slow infusion of Ringer's acetate was started. A remifentanyl (Ultiva®, GlaxoSmithKline) dose of 0.5 µg/kg was used both in the behavioral pretest and in the fMRI study. This dose was chosen on the basis of pilot experiments and a previous study that showed this dose to be analgesic (Petrovic et al., 2002). The drug was administered as a rapid intravenous bolus over 2 s and flushed with 10 ml of Ringer's acetate. Saline was used in the behavioral pretest and the fMRI study to control possible non-specific effects. For safety reasons, the anesthesiologist who was present during the behavioral pretest and the fMRI was not blinded to the agents injected.

### Behavioral pretests

The behavioral pretests were performed in a post-anesthesia care unit. Heart rate, blood pressure, blood oxygen saturation (SpO<sub>2</sub>), and end-tidal carbon dioxide (ETCO<sub>2</sub>) were monitored using an AS3 Datex-Ohmeda monitor (Helsinki, Finland).

During the behavioral pretest, three remifentanyl and one saline bolus were administered intravenously in a single-blind and randomized fashion at 7-min intervals. At 1-min intervals, the subjects rated their subjective sensations and the adverse effects, and the readings of the SpO<sub>2</sub>, ETCO<sub>2</sub>, heart rate, and blood pressure were marked down. They were instructed to answer

questions about their sensations on a three-step scale (no effect–mild effect–strong effect, or in case of mood, dysphoria–no effect–euphoria). The subjects had been introduced to the questions before the experiment.

### MR imaging

The fMRI experiments were performed using a Siemens Vision (Erlangen, Germany) 1.5 T scanner and a standard head coil. The head was stabilized with a moldable vacuum cushion to minimize movement artifacts. During the scanning, the subjects were instructed to avoid movement and to maintain visual fixation of their eyes on the central fixation cross. At the beginning of each fMRI session, a structural image set was acquired using a T1-weighted three-dimensional MPRAGE sequence (180 sagittal slice planes with thickness of 1 mm, no gap, field of view 256 mm, 256 × 256 matrix, TR 9.7 ms, TE 4 ms, TI 20 ms, flip angle 10°). The fMRI measurements were performed using a gradient-echo echo-planar sequence (30 axial slice planes with a thickness of 4 mm each, no gap between slices, field of view 256 mm, 64 × 64 matrix, TE 60 ms, TR 3000 ms, flip angle 90°). The axial slices of the functional image set extended from the superior edge of the brain down to the basal parts of the cerebrum. The slices were parallel to a line from the base of the occipital lobe to the base of the frontal lobe in the midline sagittal localizer image. The total imaging time was about 40 min.

During the fMRI experiments, heart rate and SpO<sub>2</sub> were continuously monitored using a Datex-Ohmeda AS3 non-invasive patient monitoring system (Datex-Ohmeda, Finland) suitable for MR conditions. The readings were marked down at 1-min intervals. Three doses of remifentanyl 0.5 µg/kg and one saline control were administered. Half of the subjects received the saline control as the second and half as the third injection. The subjects were unaware of the order of any of the injections (remifentanyl or saline). Each dose was separated by 7 min as in the behavioral pretests. After the fMRI, the subjects filled in a questionnaire about their subjective sensations during the fMRI experiment. The subjects answered “yes” or “no” to questions regarding euphoria, dysphoria, difficulty in maintaining central eye fixation, visual and auditory hallucinations, dizziness, drowsiness, and muscle rigidity. Free space was available for describing other experienced sensations.

Physiological data collected both during the behavioral pretest (heart rate, blood oxygen saturation level, ETCO<sub>2</sub> level) and during the fMRI (heart rate, blood oxygen saturation level) were analyzed. The averaged data from the three 7-min periods in which remifentanyl was administered were compared with the 7-min saline period.

### Image analysis

The analysis was performed using tools from the FSL software package (<http://www.fmrib.ox.ac.uk/fsl/>). Motion correction was carried out using the MCFLIRT tool (Jenkinson et al., 2002). Mean-based intensity normalization of all volumes by the same factor and nonlinear high-pass temporal filtering (Gaussian-weighted local straight line fitting, with sigma = 315 s) was applied thereafter. Statistical analysis was carried out with IRVA (Inter-Repetition Variance Analysis) (Clare et al., 1999). IRVA was used as there was no prior information about the exact timing of the

CNS effects caused by a remifentanyl bolus of 0.5 µg/kg. Seven-minute periods (140 volumes) from the beginning of the three remifentanyl injections were analyzed with IRVA. The saline and remifentanyl responses were visually examined from the ROI averages. Group (simplified fixed effects) analysis was carried out using FEAT, the fMRI Expert Analysis Tool version 4.10. *Z* (Gaussianized *F*) statistic images were thresholded using the GRF-theory-based maximum height thresholding with a significance threshold of  $P = 0.01$  (Forman et al., 1995; Friston et al., 1995). Registration to the individual high resolution images and the standard image (ICBM152 T1-weighted template, International Consortium for Brain Mapping) (Collins et al., 1994) was carried out using the MCFLIRT tool (Jenkinson et al., 2002).

The anatomical locations of the cerebral voxel cluster local maxima in the averaged statistical group map were determined using the automated anatomical labeling of activations (Tzourio-Mazoyer et al., 2002). The anatomical locations of the local maxima in the cerebellar activation cluster in the averaged statistical group map were determined using the three-dimensional MRI atlas of the human cerebellum (Schmahmann et al., 2000). The coordinates of the local activation maxima were reported in the ICBM152 space.

The BOLD signal time courses were investigated in several regions of interest (ROI). The ROIs included anatomical areas that have been shown to have high densities of opioid receptors (Cross et al., 1987; Frost et al., 1985; Pilapil et al., 1987) or that have been significantly activated in previous imaging studies (Firestone et al., 1996; Wagner et al., 2001) on opioids, pain perception, and attention (Baron et al., 1999; Iadarola et al., 1998; Petrovic et al., 2002; Rainville et al., 1997; Tölle et al., 1999). If the group data analyses indicated further areas of strong activation, these were included, too. The ROIs chosen were the rostral, mid-caudal, and caudal anterior cingulate cortex and the posterior cingulate cortex and the medial and the inferior frontal gyri. The insular cortex, cerebellum, and thalamus were chosen because these subcortical regions are also known to be activated during pain. The amygdala and hippocampus were included in the ROIs because previous fMRI and PET studies indicate that these limbic structures are involved in pain mediation (Becerra et al., 1999; Bingel et al., 2002; Derbyshire et al., 1997) and in the effects of psychoactive drugs (Breiter et al., 1997; Stein et al., 1998). The primary somatosensory cortex which has the lowest and the caudate nucleus which has the highest density of mu-opioid receptors in the human brain (Frost et al., 1985) were investigated as control areas. The transverse temporal gyrus and the precuneus showed strong activation in the group data analyses and were therefore included in the ROIs. Ellipsoid ROIs were drawn to cover the core area of the significant activation of the selected anatomical structures in the averaged statistical group map. Anatomically defined ROIs of the amygdala, the hippocampus, the caudate nucleus, and the thalamus were overlaid onto the individual maps. The anatomical ROIs were defined on the basis of individual structural images and a human brain atlas (Duvernoy, 1999). In the individual structural images, the omega-shaped region of the postcentral gyrus was identified in the left hemisphere, and an ellipsoid ROI was positioned on this area representing the primary somatosensory cortex.

The ROIs from the ICBM152 space and individual structural images were spatially transformed to the subject's EPI coordinate space (Jenkinson et al., 2002). The signal intensity time courses were extracted from these ROIs and averaged over the voxels in

the ROI. To obtain the average evoked response, the time courses were first averaged voxel-wise over the three repetitions of remifentanyl administration. The mean signal level of the five images preceding the bolus administration served as baseline. Thereafter, the evoked responses were normalized to percent changes relative to the baseline and averaged spatially over the voxels in the ROI. The individual evoked response time courses were averaged over the subjects (Fig. 2). Analyzers of the data were not blinded to the agent injected.

## Results

### Behavioral pretests

In two of the eight subjects, remifentanyl injections caused a short lasting and transient respiratory depression both in the behavioral pretest and in the fMRI. During fMRI, the blood oxygen saturation (SpO<sub>2</sub>) decreased to the lowest level of 88% in these subjects. Excessive head movement (over 2 mm) during the fMRI of these two subjects leads to their exclusion from the analysis.

In the remaining six subjects, changes in the arterial blood pressure and SpO<sub>2</sub> were negligible during the behavioral pretests. The mean systolic blood pressure decreased from a baseline level of 145 ± 12 mm Hg to 141 ± 17 mm Hg during the 7-min period after remifentanyl administration. The mean diastolic blood pressure decreased from 89 ± 10 mm Hg to 83 ± 8 mm Hg. The mean baseline SpO<sub>2</sub> was 98 ± 1% and reached its lowest level (97 ± 2%) at 4 min after remifentanyl administration. Before remifentanyl administration, the mean end-tidal carbon dioxide (ETCO<sub>2</sub>) was 5.2 ± 0.5% and reached its highest value 4 min after remifentanyl administration (5.3 ± 0.6%).

In the pretest, all six subjects reported various sensations after each remifentanyl bolus. These included difficulty to maintain central eye fixation ( $n = 6$ ), dizziness ( $n = 5$ ), drowsiness ( $n = 5$ ), muscle rigidity ( $n = 4$ ), euphoria ( $n = 3$ ), and shortness of breath ( $n = 2$ ). Some subjects also felt such sensations as itching, nausea, taste of plastic, and tinnitus. All subjective sensations peaked at 1 min after remifentanyl administration (Fig. 1). They were over within the 7-

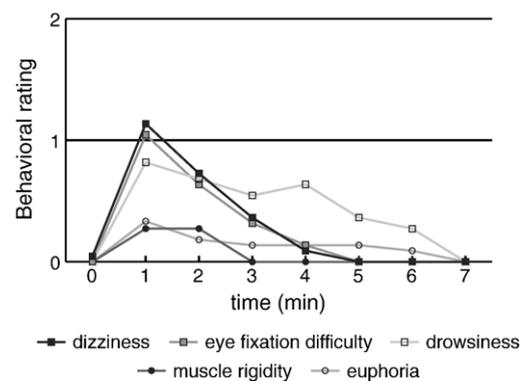


Fig. 1. Graph of the mean behavioral ratings of six subjects during the behavioral pretest. Dizziness, drowsiness, difficulties in maintaining central eye fixation, muscle rigidity, and euphoria were the major behavioral responses. The behavioral measures were obtained at 1-min intervals starting at the beginning of the injection (0 min). A verbal rating scale from 0 to 2 (0 = no effect, 1 = mild effect, 2 = strong effect) was used to measure the behavioral responses.

min period and before the administration of the next dose. Both the duration and intensity of the sensations varied among the subjects. None of the remifentanyl-associated symptoms mentioned above was reported after saline.

*fMRI study*

The three remifentanyl boluses caused negligible changes in SpO<sub>2</sub> during the fMRI experiments in the six subjects. Before remifentanyl administration, the mean SpO<sub>2</sub> was 99 ± 1%, and it was at its lowest level 3 min after remifentanyl administration (97 ± 2%).

After the imaging session, the volunteers evaluated their subjective sensations using a questionnaire. During the image

acquisition, the subjects experienced drowsiness (*n* = 6), dizziness (*n* = 4), euphoria (*n* = 4), difficulty to maintain central eye fixation (*n* = 3), muscle rigidity (*n* = 3), and dysphoria (*n* = 1). Five subjects reported that the noise of the scanner became temporarily muffled and less disturbing during scanning.

*Areas of activation*

At the group level, remifentanyl induced multiple cortical and subcortical BOLD signal intensity changes (Fig. 2, Table 1). These BOLD signal changes were mostly bilateral and were mainly located on the medial walls of the hemispheres. The BOLD signal increased bilaterally in the rostral, mid-caudal, and caudal parts of

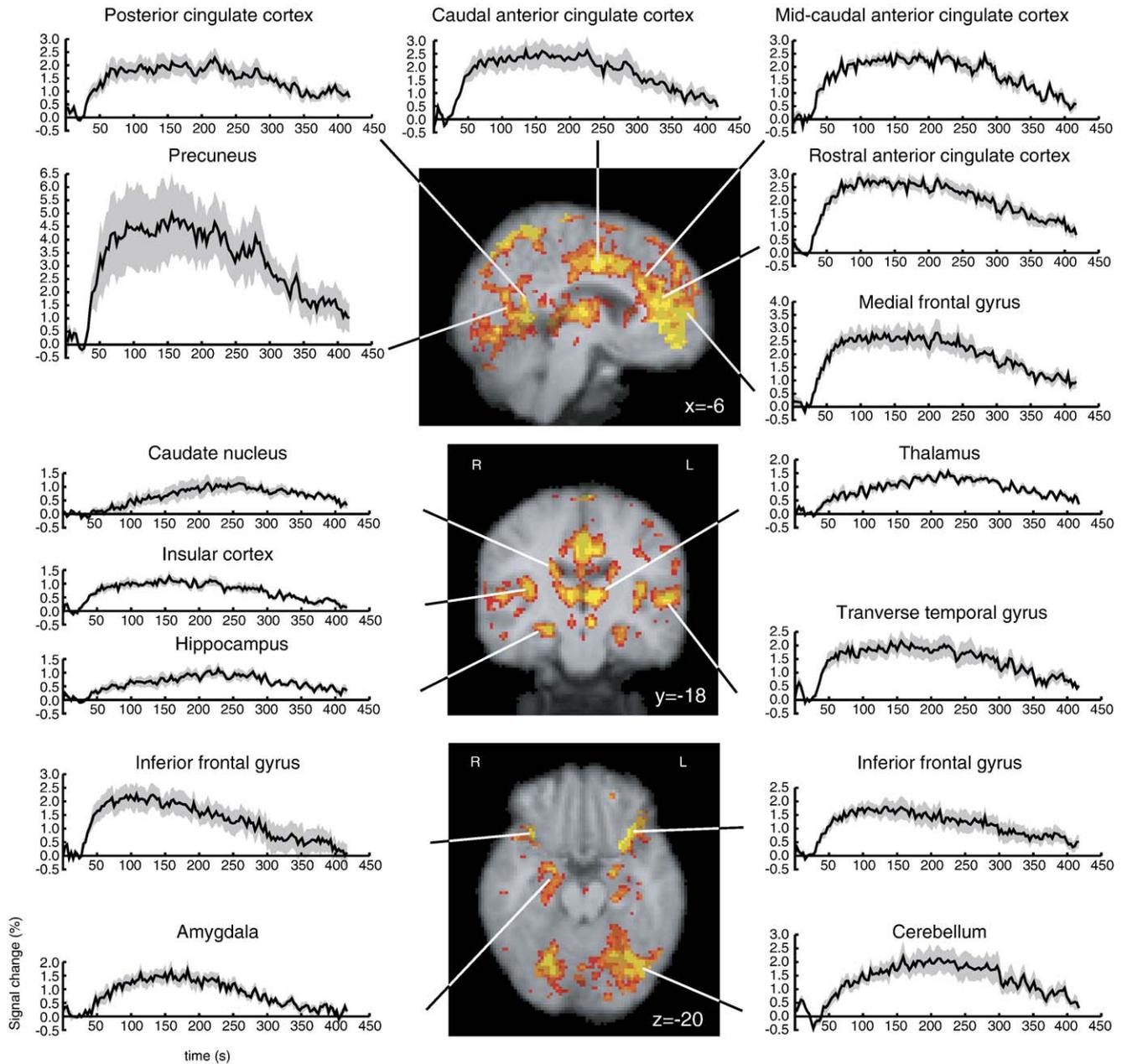


Fig. 2. Group average (fixed effect) activation maps and time courses of signals (±1 SEM, gray area) in response to intravenous remifentanyl boluses. The time courses show the average signal changes over the three repetitions of remifentanyl administration and voxels within the ROI in six subjects. The intravenous injection of the drug occurred at 0 s.

Table 1  
Local maxima of the statistical parameter map of activation in response to the administration of intravenous remifentanyl (0.5 µg/kg)

Region	Z	Left x, y, z	Z	Right x, y, z
<i>Frontal lobe</i>				
Inferior frontal gyrus opercular section	4.98	-52, 12, 20	7.14	62, 14, 14
Inferior frontal gyrus orbital section	5.55	-46, 44, -16	7.2	52, 40, -14
Inferior frontal gyrus triangular section	6.61	-52, 30, 22	6.35	58, 36, 4
Middle frontal gyrus	7.38	-32, 8, 62	6.37	44, 22, 46
Middle frontal gyrus orbital section	5.81	-38, 44, -10	5.81	44, 48, -14
Superior frontal gyrus	6.93	-24, -4, 68	6.3	16, -12, 74
Medial superior frontal gyrus	5.1	-14, 64, 10		
Superior frontal gyrus orbital section	4.93	-16, 56, -14	4.84	18, 54, -10
Supplementary motor area	7.55	-12, -10, 74		
Precentral gyrus	8.56	-56, 4, 38	6.2	22, -26, 74
<i>Parietal lobe</i>				
Inferior parietal gyrus	7.57	-42, -56, 54	5.98	40, -38, 50
Paracentral lobule			5.42	2, -34, 72
Precuneus	16.8	0, -68, 54	16.8	0, -68, 54
Postcentral gyrus	7.41	-50, -30, 52	6.67	60, -8, 34
Rolandic operculum			5.03	62, 8, 14
Superior parietal gyrus	6.09	-22, -62, 54	4.97	32, -60, 56
Supramarginal gyrus	5.86	-66, -24, 24	4.7	68, -20, 26
<i>Temporal lobe</i>				
Angular gyrus	6.58	-56, -56, 24	6.46	44, -66, 30
Fusiform gyrus	5.29	-32, -4, -30	5.35	24, -64, -8
Inferior temporal gyrus			6.21	48, -66, -6
Insular cortex	4.7	-26, 26, 8		
Middle temporal gyrus	6.12	-56, -28, -8	7.51	50, -64, 2
Superior temporal gyrus	5.01	-42, -12, -10	6.28	66, -36, 16
Temporal pole			5.49	52, 16, -22
<i>Medial paralimbic cortices</i>				
Anterior cingulate cortex	15	0, 22, 32	15	0, 12, 36
<i>Occipital lobe</i>				
Calcarine gyrus	5.42	-14, -78, 4	6.45	14, -98, -2
Cuneus	5.93	-4, -94, 14	5.54	6, -88, 28
Lingual gyrus	4.81	-14, -74-12	4.92	20, -92, -12
Inferior occipital gyrus	4.96	-26, -88, -8	5.84	40, -84, -12
Middle occipital gyrus	8.08	-26, -80, 26	5.61	30, -72, 30
Superior occipital gyrus	6.61	-18, -90, 22	6.77	28, -78, 36
<i>Subcortical gray structures</i>				
Caudate nucleus			6.72	4, 10, -6
Pallidum	5.24	-22, -2, -2		
Putamen	5.79	-26, -6, 0		
<i>Cerebellum</i>				
Cerebellum lobule VI	15.4	-40, -50, -26		
Cerebellum lobules IV and V			5.82	26, -42, -26
Crus II cerebellum			7.3	4, -80, -30
Vermis	4.96	-2, -46, -24		

Coordinates are in the ICBM152 space. In addition to the anatomical areas corresponding to the local maxima, activation extended also to areas not reported in Table 1 as can be seen in Fig. 2.

the anterior cingulate cortex, medial and inferior frontal gyri, and in the anterior part of the cerebellum. A large activation was found in the precuneus with a 4.5% BOLD signal change in the averaged group data. The transverse temporal gyrus and posterior cingulate cortex showed a 1.5 to 1.9% change in the BOLD signal. The BOLD signal change in the thalamus, caudate nucleus, insular cortex, hippocampus, and amygdala ranged from 0.8 to 1.4%. The strongest activation of the thalamus was located in the dorsomedial nucleus.

#### Time courses of the signal changes

The signal started to rise after a delay of 20 to 30 s from the remifentanyl bolus. The signal increased, rapidly reaching a maximum between 80 and 90 s after remifentanyl administration in the anterior and posterior cingulate cortex, precuneus, insular cortex, amygdala, transverse temporal gyrus, and inferior and medial frontal gyri (Fig. 2). The signal was then sustained at the maximum plateau level for 2 to 4 min. However, in the cerebellum, hippocampus, thalamus, and the caudate nucleus, the BOLD signal intensity increased slowly and reached a peak at about 3 to 4 min after remifentanyl injection (Fig. 2). Following the plateau period, the BOLD signal slowly declined and returned close to the baseline by the end of the 7-min time period. The BOLD signal variations among the six subjects were most prominent in the precuneus. Interindividual differences and possible differences in the responses between consecutive remifentanyl injections will be reported elsewhere. Fig. 3 illustrates the different patterns of activation and activation in the primary somatosensory cortex where only a minute signal intensity increase was detected after remifentanyl injection. After saline administration, there was only a negligible signal intensity increase (Fig. 3). Fig. 4 shows the effects of three doses of remifentanyl and one saline injection on the signal intensity in a

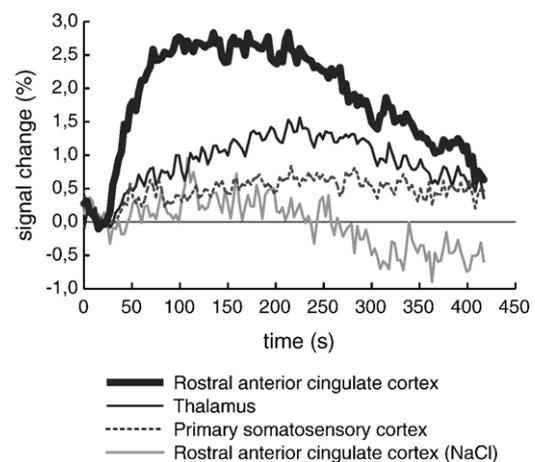


Fig. 3. Group fMRI time courses of BOLD signal changes in response to intravenous remifentanyl boluses in the rostral anterior cingulate cortex, thalamus, and primary somatosensory cortex. A rapid and intense maximum effect that occurred approximately 90 s after remifentanyl administration is seen in the rostral anterior cingulate cortex. In the thalamus, the BOLD signal intensity increased, slowly reaching its peak after about 220 s from the remifentanyl injection. The primary somatosensory cortex shows only a minor change in the BOLD signal with no clear peak. The rostral anterior cingulate cortex showed no change in the BOLD signal after a saline injection.

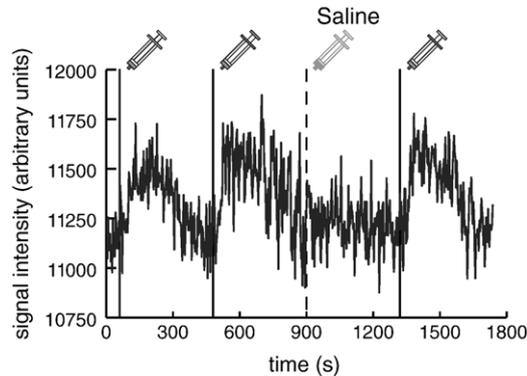


Fig. 4. The fMRI signal intensity time course of a single voxel in the rostral anterior cingulate cortex of a single subject. Markedly increased signal intensity is seen after the three intravenous remifentanyl ( $0.5 \mu\text{g}/\text{kg}$ ) boluses (solid line) but not after the saline injection (dashed line).

single voxel in the rostral anterior cingulate cortex of one subject.

## Discussion

Pharmacological fMRI is becoming an exciting method to study the effects of psychoactive drugs in different patient groups. The opioid system is particularly interesting as it modulates several physiologic functions including mood, learning, reward, and analgesia. It has been suggested to play a role in several disorders such as depression, anxiety, chronic pain, and addictive behavior. The function of the opioid system is affected by gender and several genetic polymorphisms, the polymorphism of the COMT enzyme being one example (Zubieta et al., 2003). Various diseases such as neuropathic pain and long-term treatment with opioids or their abuse may also modify the function of the opioid system.

To our knowledge, this is the first study in which the fMRI was used to investigate the effects of opioids in healthy volunteers in the absence of behavioral tasks or experimentally induced sensory or motor stimulation. Time-dependent changes in the BOLD signal induced by several opioid injections and a saline control have not been studied before either. The unusually short washout time of remifentanyl enabled the assessment of repeated responses to the drug.

Compared with saline, intravenous remifentanyl resulted in significant local increases in the BOLD signal. The areas rich in opioid receptors showed strong signal intensity increases, whereas the somatosensory cortical area primary somatosensory cortex that has a very low level of opioid receptors (Frost et al., 1985) was negligibly activated following the remifentanyl injections (Fig. 3).

### *Spatial patterns of the opioid-induced activation*

The distribution of activation in the brain following a remifentanyl injection can be explained to result from the physiologic and affective effects of this psychoactive drug or by the distribution of the opioid receptors in the brain or by both.

The regions that were most strongly activated by remifentanyl included the rostral, mid-caudal, and caudal parts of the anterior cingulate cortex, medial and inferior frontal gyri, and precuneus. These findings are in agreement with previous PET studies of opioid effects on brain activation (Adler et al., 1997; Firestone et

al., 1996; Petrovic et al., 2002; Wagner et al., 2001). The anterior cingulate cortex, prefrontal cortex, insula, and amygdala are parts of the opioid circuitry that is involved in the affective experience of pain and other sensations. A recent fMRI study (Rolls et al., 2003) suggests that the rostral anterior cingulate cortex is activated by pleasant touch, whereas the caudal anterior cingulate cortex is activated preferentially by painful touch. The activation of the rostral anterior cingulate cortex in the present study may be related to the pleasant effects of opioids. Bilateral activation of the posterior cingulate cortex has also been related to the processing of emotional stimuli, both pleasant and unpleasant (Maddock et al., 2003). The orbitofrontal cortex has been shown to be activated by pleasant rather than by neutral touch (Rolls et al., 2003).

In the present study, extensive activation of the insular cortex was observed. The activation was centered in the postero-superior part. The insula has a role in the identification of the emotional significance of environmental stimuli, production of affective states, and regulation of autonomic responses to emotive stimuli (Phillips et al., 2003). It has been suggested that the insula participates in the evaluation of distress (Reiman et al., 1997) and that it is involved in the evaluative, experiential, or expressive aspects of self-induced or internally generated emotions (Phan et al., 2002). This may suggest that as well as being involved in the affective experience of sensory stimuli, the activation of the insular cortex in our study may also reflect the role of the insula in the evaluation of remifentanyl-induced affective sensations.

The posterior parietal cortex is involved in spatial perception, association of sensory signals, and direction of attention. This region, which includes the precuneus, has also been reported to be activated by painful stimulation (Peyron et al., 1999). The posterior parietal cortex may help to localize the actual source of the pain in the body. In the present study, remifentanyl produced a strong activation in the precuneus, although no somatosensory stimuli were introduced. Peyron et al. (1999) have suggested that posterior parietal cortex activation might be related to stimulus evaluation. The subjects in our study were aware that they would be asked about the feelings and symptoms the opioid administration would cause during the scanning period. The large activation of the precuneus might thus be related to the evaluation of the effects of remifentanyl. Another reason for the large activation of the precuneus could be related to eye movements. All six subjects reported having difficulties in central eye fixation. Nystagmus and saccadic eye movements are a typical effect after a rapid intravenous administration of opioids (Rottach et al., 2002). The precuneus has been shown to be activated during saccades (Berman et al., 1999).

A significant BOLD signal increase was found in the transverse temporal gyrus. This activation may be related to the subjects' report that the noise of the MRI scanner became temporarily muffled and less disturbing after the remifentanyl injection. Previously, Crabb et al. (1996) have reported that remifentanyl reduces auditory evoked potentials during anesthesia. These results indicate that remifentanyl modulates the function of the auditory cortex. The finding that remifentanyl rendered the noise subjectively less unpleasant or disturbing may suggest that the opioid system of the temporal gyrus participates in the affective experience of auditory stimuli.

In the present study, remifentanyl injections induced bilateral activations in the amygdala. Activation of the amygdala may be partially related to the affective evaluative circuitry described above. The amygdala is activated not only by aversive but also by

other salient arousing stimuli (Breiter et al., 1997; Stein et al., 1998). It has also been associated with fear (Morris et al., 1998). Cocaine infusion caused either a BOLD signal increase or decrease in the amygdala of cocaine addicts (Breiter et al., 1997), whereas intravenous nicotine increased the BOLD signal in the amygdala of cigarette smokers (Stein et al., 1998). In a recent fMRI study on laser-evoked pain, activation was seen bilaterally in the amygdala (Bingel et al., 2002). The amygdala, thalamus, and basal ganglia including the caudate nucleus are part of the opioid reward circuitry. Activation of the basal ganglia has been reported in a variety of fMRI studies of emotion, pain, and addiction (Breiter et al., 1997; Phan et al., 2002; Rolls et al., 2003).

The medial thalamus and the caudate nucleus have the highest densities of mu-opioid receptors in the human brain (Frost et al., 1985). In the present study, the activation in the thalamus was centered in the dorsomedial nucleus, a structure that has input from the amygdala, and the inferotemporal cortex and has extensive connections with the frontal cortex and the anterior cingulate cortex. The activation of the thalamus may thus modulate the function of the limbic circuitry and have a role in the rewarding effects of remifentanyl.

Four of the six subjects reported muscle rigidity which is a well-known adverse effect following rapid intravenous injection of opioids. The activation of the basal ganglia could therefore also be related to muscle rigidity. Havemann et al. (1980) have demonstrated that the caudate nucleus is one of the regions involved in mu-opioid receptor agonist induced muscle rigidity.

Although no cognitive or memory tasks were performed during the fMRI experiment, hippocampal activation was noticed after remifentanyl. It is possible that the hippocampal activation in our study was due to the recognition of potential emotionally salient and/or novel feelings evoked by the remifentanyl injection. The hippocampus is also part of the reward circuitry.

The human cerebellum has mu-opioid receptors in the cortex, vermis, and dentate nuclei (Schadrack et al., 1999). Previous PET studies have shown either decreases (Firestone et al., 1996; Wagner et al., 2001) or increases (Petrovic et al., 2002) in the regional cerebral blood flow in the cerebellum after opioid administration. The cerebellum is also activated after painful stimuli (Peyron et al., 2000). In our study, remifentanyl caused BOLD signal increases bilaterally in the anterior parts of the cerebellum. Dizziness and difficulties in maintaining eye fixation on a stationary target are the typical acute effects of opioids. Rottach et al. (2002) showed that 0.1 mg of intravenous fentanyl caused disturbed eye fixation with downbeat nystagmus and a range of saccadic intrusions and oscillations. The subjects also developed mild symptoms of cerebellar dysfunction with intention tremor, ataxia, and dysarthria within 1 min from the injection of the drug. The difficulty to maintain central eye fixation after remifentanyl in the present study could be related to cerebellar dysfunction. The opioidergic systems in the cerebellum may have wider implications as the cerebellum is involved not only in motor control but also in cognitive and emotional processes (Schmahmann and Sherman, 1998).

#### *Temporal patterns of BOLD signal changes*

A rapid peak BOLD response within 80 to 90 s after intravenous remifentanyl administration was noticed (Figs. 3 and 4). The areas showing this pattern included the rostral, mid-caudal, and caudal anterior cingulate cortex, posterior cingulate cortex, precuneus, medial and inferior frontal gyri, insular cortex,

amygdala, and transverse temporal gyrus. In regions including the thalamus, cerebellum, caudate nucleus, and hippocampus, the peak developed slowly between 3 and 4 min after remifentanyl administration.

The temporal pattern of remifentanyl-induced activation in the regions with rapid BOLD peak responses was consistent with the temporal pattern of the subjective sensations observed in the pretests. It could be speculated that the rapid peak BOLD responses correlate with the activation of the opioid receptors and the related neural circuitry, resulting in the affective experience evoked by the drug.

All temporal patterns had a slow decrease in the activity resembling the time course of the subjective feeling of drowsiness. It has been suggested that bilateral thalamus activation is due to non-specific subcortical arousal (Peyron et al., 1999) or attention (Portas et al., 1998). The latter study indicated that activation in the thalamus was increased more by attention during a low arousal state (sleep deprivation) than in a high arousal state (use of caffeine). This implies a compensatory mechanism and may be related to a subjective experience of greater mental effort. In the present study, the effect of intravenous remifentanyl injection may be comparable to a low arousal state accompanied by a greater mental effort to stay alert and to maintain central eye fixation. The slow BOLD responses might reflect the neuronal activity related to the cognitive effort needed to adjust to the effects of remifentanyl, e.g. when the subject is required to sustain central eye fixation.

#### *Methodological aspects*

A rapid intravenous injection of remifentanyl may cause respiratory depression resulting in hypoxia and CO<sub>2</sub> retention (Babenco et al., 2000). Hypercapnia is known to have effects on the BOLD signal (Posse et al., 2001). In an earlier study, the highest BOLD signal increases induced by hypercapnia during alternating periods of breath holding and self-paced normal breathing were found in the cerebellum and the visual cortex (Kastrup et al., 1999). In our study, two subjects showed short lasting and transient respiratory depression as the oxygen saturation decreased both during the behavioral pretest and during the fMRI. They also had head movements, which lead to the exclusion of these two subjects. Only negligible respiratory or hemodynamic changes occurred in the remaining six subjects during either the behavioral pretests or fMRI. No global BOLD signal changes were observed. Therefore, it is unlikely that the observed changes in the BOLD signal were the result of cardiorespiratory factors. One limitation of the study was that blood pressure and ET<sub>CO</sub><sub>2</sub> were not measured during fMRI. These were not monitored because the somatosensory stimulation resulting from measuring ET<sub>CO</sub><sub>2</sub> (a nasal suction system) and blood pressure (pressure of the manometer) during fMRI was expected to introduce confounding effects.

All subjects participated in a behavioral pretest before the fMRI. This was in order to find out whether the subjects would experience any major adverse effects after remifentanyl administration. No major adverse effects were observed after remifentanyl administration. However, the subjects were exposed to the effects of remifentanyl before the fMRI. Therefore, it is possible that they were not totally blinded to the saline control and the impact of expectance of opioid effects cannot be ruled out.

Two subjects had to be excluded because of head movement. Furthermore, previous fMRI studies on psychoactive drugs have

demonstrated that the drug infusion can cause significant head motion that may result in artifacts (Breiter et al., 1997). All other subjects exhibited only minor head movement (<2 mm) during the fMRI experiment. In our study, the subjects were opioid naive prior to the experiments. A limited verbal rating scale from 0 to 2 (0 = no effect, 1 = mild effect, 2 = strong effect) was used to describe the behavioral responses because a more extensive verbal rating scale was expected to cause difficulties in quantifying reliably differences among various subjective sensations.

The activation magnitudes following intravenous remifentanyl injections were comparable to those reported after cognitive stimuli in fMRI studies. The activation in the precuneus was strikingly strong, 4.5%. The cortical activations were relatively stronger than the activations in subcortical areas that, however, have the highest mu-opioid receptor densities (medial thalamus, caudate nucleus). The volumes of the subcortical structures are relatively small in relation to the imaging resolution used. The partial volume effect, i.e. the inclusion of neighboring anatomical structures in the voxels within the ROI, may also partly explain the relatively low activation in the subcortical structures of smaller volume. The subcortical ROIs were drawn manually to minimize this effect.

Several other areas like the periaqueductal gray and many brain stem nuclei are rich in opioid receptors and have an important role in many opioid effects, such as pain and respiration. These areas, however, were not imaged in the present study.

## Conclusion

For the first time, we have used fMRI to report spatially and temporally detailed information after opioid administration in the absence of behavioral tasks or experimentally induced sensory or motor stimulation. This study sets basis for further research exploring functional changes in the opioid system resulting from genetic differences and/or disease or treatment.

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