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Imaging Human Cerebral Pain Modulation by Dose-dependent Opioid Analgesia

A Positron Emission Tomography Activation Study Using Remifentanil

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Background: **Previous imaging studies have demonstrated a number of cortical and subcortical brain structures to be activated during noxious stimulation and infusion of narcotic analgesics. This study used ^{15}O-water and positron emission tomography to investigate dose-dependent effects of the short-acting μ-selective opioid agonist remifentanil on regional cerebral blood flow during experimentally induced painful heat stimulation in healthy male volunteers.**

Methods: **Positron emission tomography measurements were performed with injection of 7 mCi ^{15}O-water during nonpainful heat and painful heat stimulation of the volar forearm. Three experimental conditions were used during both sensory stimuli: saline, 0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil, and 0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil. Cardiovascular and respiratory parameters were monitored noninvasively. Across the three conditions, dose-dependent effects of remifentanil on regional cerebral blood flow were analyzed on a pixel-wise basis using a statistical parametric mapping approach.**

Results: **During saline infusion, regional cerebral blood flow increased in response to noxious thermal stimulation in a number of brain regions as previously reported. There was a reduction in pain-related activations with increasing doses of remifentanil in the thalamus, insula, and anterior and posterior cingulate cortex. Increasing activation occurred in the cingulofrontal cortex (including the perigenual anterior cingulate cortex) and the periaqueductal gray.**

Conclusions: **Remifentanil induced regional cerebral blood flow increases in the cingulofrontal cortex and periaqueductal gray during pain stimulation, indicating that μ-opioidergic activation modulates activity in pain inhibitory circuitries. This provides direct evidence that opioidergic analgesia is mediated by activation of established descending antinociceptive pathways.**

This article is featured in "This Month in Anesthesiology." Please see this issue of ANESTHESIOLOGY, page 5A.

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PAIN perception can be modulated by both endogenous and exogenous mechanisms, comprising nonpharmacologic (*e.g.*, attention, stress, arousal, hypnosis, placebo) and pharmacologic factors.[1] Among the pharmacologic options, opioids and their receptors play a central role in every facet of modern pain treatment, anesthetic practice, and intensive care. Previous research focused on the specific antinociceptive action of single dosages of opioids (morphine, fentanyl, remifentanil) on neuronal activity and identified a number of sites of action within the brain. Morphine analgesia during cancer pain activated the prefrontal and temporal cortex, anterior cingulate cortex (ACC), striatum, and insula, whereas during experimental tonic heat pain and fentanyl administration, activations of the ACC and posterior cingulate cortex, motor cortex, thalamus, and temporal cortex have been reported.[2,3] Petrovic *et al.*[29] used an experimental pain model and positron emission tomography (PET) to study mechanisms of action of the short-acting μ-opioidergic agonist remifentanil. They found drug-induced activations of the rostral ACC, insula, orbitofrontal cortex, and brainstem areas. The latter overlapped with brain areas that have been implicated in pain modulation such as the periaqueductal gray (PAG). Interestingly, placebo analgesia acts similarly on the activity of these brain areas, presumably *via* endogenous opioid release.[4] Finally, functional magnetic resonance imaging and pharmacokinetic modeling data emphasized the role of the insular cortex and the PAG during opioid analgesia.[5,6]

Another approach used ligand PET with μ-specific (*e.g.*, carfentanil) as well as unspecific (*e.g.*, diprenorphine) opioid receptor ligands to identify brain regions with decreased exogenous opioid receptor binding after painful stimulation indicating an endogenous opioid receptor–mediated pain control system. These areas comprise the ACC, insular cortex, amygdalae, thalamus, and nucleus accumbens.[7–9] Hence, there is a growing body of literature about opioidergic mechanisms in pain processing and pain control; however, dose-dependent effects of synthetic opioids on experimental pain have not been investigated by means of neuroimaging techniques thus far. Because the most potent opioids used for clinical anesthesia, intensive care medicine, and pain therapy mediate analgesia by activation of the μ-opioid receptor, our investigation focused on the μ-selective

opioid agonist remifentanil. Its pharmacodynamic properties are comparable to other potent μ-opioid receptor agonists, while the pharmacokinetic profile provides fast and reproducible steady state concentrations.[10] We investigated *in vivo* the dose-dependent opioid induced alterations in cerebral activation in pain-coding and pain-inhibiting brain areas during μ-selective opioid analgesia using $H_2{}^{15}O$-PET.

Materials and Methods

Subjects

Seven right-handed male volunteers participated in this experiment. All subjects gave written informed consent acknowledging (1) that they would receive radioactive tracers, (2) that they would experience experimental pain stimuli and receive a potent analgesic in several dosages, (3) that all methods and procedures were clearly explained, and (4) that they were free to withdraw from the experiment at any time. Subjects were studied after all procedures were approved by the local institutional review board and the radiation protection authorities. The pain-free subjects ranged in age from 28 to 38 yr (mean ± SD, 32.7 ± 4.1 yr) and denied any previous or actual neurologic, psychological, and medical problems; history of any other severe disease (American Society of Anesthesiologists physical status I); or history of drug abuse.

Experimental Setting

The volunteers had fasted for at least 6 h before the study. Electrocardiograms and arterial oxygen saturation were measured and continuously recorded (Capnomac Ultima; Datex, Helsinki, Finland). Noninvasive blood pressure measurements were performed at 5-min intervals (Dinamap 1846 SX; Criticon, Tampa, FL). End-tidal carbon dioxide concentrations were measured using a Capnomac Ultima monitor *via* a catheter placed at the nasopharyngeal border. Capillary carbon dioxide was measured immediately after every condition of drug administration by blood samples taken from a warm, nonheated fingertip.

During experimental pain stimulation, a total of three different drug infusion regimens were investigated in respect to regional cerebral blood flow (rCBF): saline ("control"), 0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil ("low-dose remifentanil"), and 0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil ("moderate-dose remifentanil"). According to its short half-life, remifentanil was delivered by an infusion pump (Combimat 2000; Döring, München, Germany) in a blinded, randomized order with a time interval of more than 30 min between the two remifentanil infusion rates.

To establish steady state plasma concentrations, remifentanil was administered *via* a separate intravenous line in a left antecubital vein to avoid bolus effects during ^{15}O-water injections. All PET scanning sessions were scheduled at similar times of the day in a quiet ambient environment. Subjects were instructed to remain in a supine position with their eyes closed, to concentrate on the pain stimuli, and not to move or say anything until termination of each PET scan.

After each stimulation and completion of each associated PET scan in the control condition and the two remifentanil dose conditions, subjects were asked to rate their individually experienced pain intensity on a visual analog scale (0–100; 0 = no pain, 100 = unbearable pain).

A semirandomized study protocol was used to overcome the problem of different dates of data acquisitions and possible residual remifentanil effects. Each of the seven subjects underwent two separate PET scan sessions, with at least 3 months between the scanning sessions. One group of subjects (n = 3) was first subjected to the "painful/nonpainful heat + control" condition (3 PET scans), whereas "painful/nonpainful heat + low-dose remifentanil" and "painful/nonpainful heat + moderate-dose remifentanil" (3 PET scans each) were performed on a second session. In the second group, subjects (n = 4) were first exposed to the painful/nonpainful heat + low-dose remifentanil and painful/nonpainful heat + moderate-dose remifentanil condition and at a second session to the painful/nonpainful heat + control condition (fig. 1). Therefore, each subject underwent a total of 18 PET scans, 9 during painful heat stimulation and 9 during nonpainful heat stimulation. With this protocol design, we were able to study the effects of remifentanil without the potential bias of interscan variability.

Pain Stimulation

A temperature-controlled contact thermode (surface area 1.6 × 3.6 cm; contact pressure 0.4 N/cm^2; PATHtester MPI 100; PHYWE, Göttingen, Germany) was used for the two stimulus conditions (nonpainful heat, painful heat) in the three drug conditions (control, low-dose remifentanil, moderate-dose remifentanil). The thermode was attached to the right volar forearm, and the position was changed in a clockwise direction after each scan to avoid habituation effects.

Determination of the thermal pain threshold was accomplished by an adjustment procedure, in which the subjects used a heating and a cooling button to adjust the temperature to what they perceived as just being barely painful starting from a baseline temperature of 37°C. Seven consecutive trials were performed, and the average temperature of the last six trials was considered as the pain threshold. This procedure for the detection of the individual pain threshold was performed twice (24 h and 1 h before the PET session), and the average value was used for the PET experiment.

Series of heat pulses were applied with a frequency of

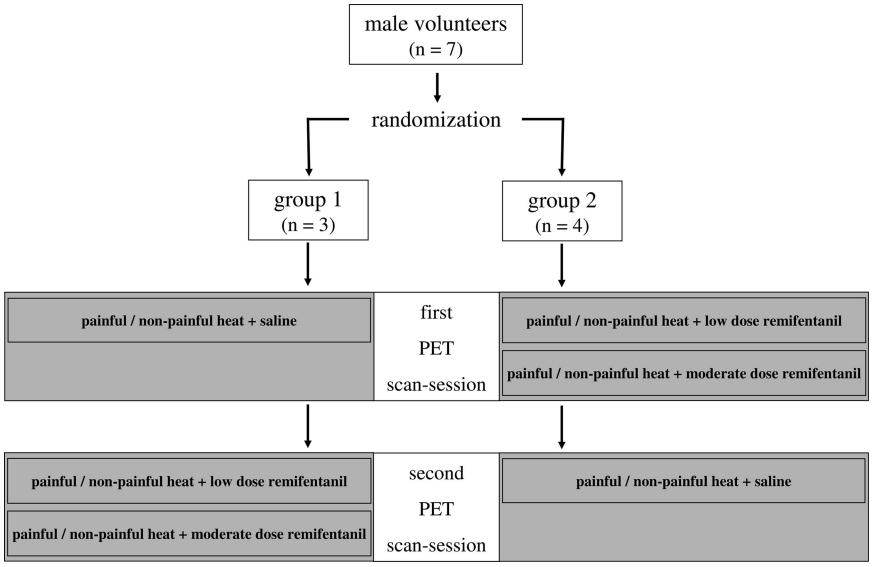

Fig. 1. Study protocol. PET = positron emission tomography.

0.6 Hz for the $H_2{}^{15}O$-PET activation studies. From the individual pain threshold (mean ± SD, 45.11° ± 0.73°C; range, 43.98°–46.2°C), the pulses changed between a maximum of 1°C above the pain threshold to a minimum of 0.3°C below the pain threshold for the painful heat stimulation (amplitude 1.3°C). For the nonpainful heat stimulation, the temperature undulated between a maximum of 1°C below and a minimum of 2.3°C below the individual pain threshold (amplitude 1.3°C). Each thermal stimulation was continued for 5 min; the PET scans were taken during the last 50 s of the painful or nonpainful heat stimulation. This kind of thermal stimulation was chosen to avoid skin damages.[11]

Imaging Data

Positron emission tomography was performed using a Siemens 951 R/31 PET scanner (CTI, Knoxville, TN) in three-dimensional mode with a total axial field of view of 10.5 cm and no interplane dead space. The patient's heads were positioned parallel to the canthomeatal line with the primary sensorimotor cortex covered within the field of view. Attenuation was corrected using a transmission scan (two-dimensional) with an external 68Ge/68Ga ring source before the tracer injection. For each PET scan, a semibolus of 7 mCi ^{15}O-water was administered intravenously *via* a second intravenous line in a left antecubital vein over 35 s using an infusion pump (SP22; Harvard Apparatus, South Natick, MA). The PET scan was initiated when the tracer bolus entered the brain, as indicated by an abrupt increase in the coincidence-counting rate of the tomograph. After correction for randoms, dead time, and scatter, images were three dimensionally reconstructed by filtered back-projection with a Hanning filter (cutoff frequency 0.4 cycles per projection element), resulting in 31 slices with a 128 × 128 pixel matrix (pixel size 2.0 mm) and interplane separation of 3.375 mm.

Statistical Analysis of PET Data

For observer-independent determination of changes in rCBF, images were preprocessed and statistically analyzed using the statistical parametric mapping approach (SPM99; Wellcome Department of Imaging Neuroscience, Institute of Neurology, University College London, London, United Kingdom). The emission scans were intraindividually realigned before transformation into a reference space according to the Montreal Neurological Institute template of SPM99 by normalization. This template has been determined from 305 magnetic resonance imaging scans of healthy subjects at the Montreal Neurological Institute (Montreal, Quebec, Canada).[12] As a final preprocessing step, the images were smoothed using an isotropic gaussian kernel (12-mm full-width at half-maximum).

Categorical comparisons were performed across conditions for each drug concentration between painful *versus* nonpainful heat conditions (painful heat > nonpainful heat for control, low-dose remifentanil, and moderate-dose remifentanil). All statistical parametric maps of the categorical comparisons were thresholded at $P < 0.05$, corrected for multiple comparisons with the false discovery rate approach.

Furthermore, analysis of antinociceptive effects of remifentanil (associated with increases in rCBF) on cerebral pain processing was conducted ($P < 0.001$, uncorrected). Thereby, an exclusive mask was applied to the subtraction analysis of rCBF increases due to remifentanil dose increase during painful heat [(0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and pain) minus (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and pain)]. As an exclusive mask, the remifentanil-induced activation during nonpainful heat [(0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and no pain) minus (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and no pain)] was used ($P < 0.01$ as masking threshold).

To identify pain processing regions that show decreas-

Table 1. Systemic Hemodynamic Parameters and Respiratory Values during Different Remifentanil Dosages

Parameter	Saline (Control)	0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$ Remifentanil (Low)	0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ Remifentanil (Moderate)
Systolic blood pressure, mmHg	143 ± 16.2	145 ± 22.2	144 ± 16.6
Diastolic blood pressure, mmHg	95 ± 15.6	71.0 ± 13.0	71.0 ± 13.4
MABP, mmHg	74 ± 14.0	92.0 ± 14.6	94 ± 14.3
HR, beats/min	63 ± 13.7	65 ± 9.0	68 ± 10.8
Oxygen saturation, %	99 ± 0.5	98 ± 1.3	97 ± 2.4
End-tidal carbon dioxide, mmHg	43 ± 2.0	43 ± 4.2	41 ± 4.8
Capillary carbon dioxide, mmHg	42 ± 2.1	41 ± 3.2	40 ± 3.0

Data are presented as mean ± SD. No significant differences were observed between conditions (paired *t* test, $P < 0.05$).
HR = heart rate; MABP = mean arterial blood pressure.

ing activation during remifentanil analgesia in a dose-dependent manner, negative covariation analysis (remifentanil dosage as covariate of interest) was performed ($P < 0.001$, uncorrected).

The minimal cluster extension (number of activated voxels) was set at 15 or more contiguous voxels passing the significance threshold for all analyses. Small volume correction was applied on the PAG according to the hypothesis of opioidergic pain modulation by PAG activation and its relatively small spatial extension.

Results

Cardiorespiratory Parameters

Cardiorespiratory parameters showed no significant differences during all conditions and are presented in table 1. Especially the end-tidal and capillary carbon dioxide values did not show a statistically detectable difference, nor did an oxygen desaturation occur.

Pain Rating

All volunteers rated the nonpainful heat stimulation during the control condition on the 0–100 visual analog scale as 0, whereas the painful heat stimulus was rated as 68 ± SEM 5.

Remifentanil significantly reduced the subjective perception of pain: Volunteers rated the painful heat stimulus on the visual analog scale during the low-dose remifentanil condition as 42 ± SEM 7 and during the moderate-dose remifentanil condition as 29 ± SEM 6 (fig. 2). All visual analog scale rating changes were statistically significant (paired *t* test) across the three conditions (control *vs.* low-dose remifentanil, $P < 0.0003$; control *vs.* moderate-dose remifentanil, $P < 0.00002$; and low- *vs.* moderate-dose remifentanil, $P < 0.007$).

$H_2{}^{15}O$-PET

Painful heat stimulation during saline infusion induced brain activation in areas that have been previously described to be activated during experimental painful heat (thalamus, insula, ACC, S2, frontal cortex; fig. 3A and table 2), whereas remifentanil administration at both dosages suppressed all detectable activations at the chosen threshold ($P < 0.05$, false discovery rate corrected; fig. 3B).

With increasing remifentanil dosage [(0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and pain) minus (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and pain)], increases in rCBF during painful heat stimulation were detected in the PAG (with small volume correction) and cingulofrontal cortex. Detailed results of this voxel-wise statistical analysis are depicted in figure 4 and table 3.

The thalamus, prefrontal cortex, S2 cortex, insula, temporal cortex, basal ganglia, and parahippocampal and occipital cortex showed decreases in rCBF during painful heat stimulation while remifentanil dosage was increased (covariation analysis, fig. 5 and table 4).

Discussion

Remifentanil had subjective analgesic effects and changed the pain-related rCBF pattern in human volunteers. In fact, we observed that brain regions were decreasingly activated by pain stimulation during remifentanil analgesia in a dose-dependent manner. Furthermore, the ACC and PAG seemed to be increasingly activated by remifentanil analgesia during a painful *versus* a nonpainful stimulus.

Neuronal activity is reflected by rCBF changes and can be investigated *in vivo* in the awake human brain by PET. Experimental noxious stimuli alter rCBF in a number of cortical and subcortical regions.[13–17] Thereby the

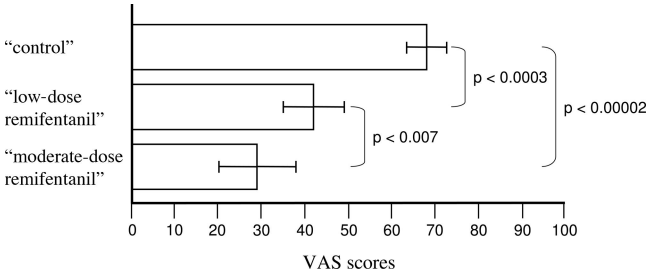

Fig. 2. Visual analog scale (VAS) ratings during the control (saline), low-dose remifentanil (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$), and moderate-dose remifentanil (0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$) conditions. The differences between each of the experimental conditions were statistically significant (paired *t* test).

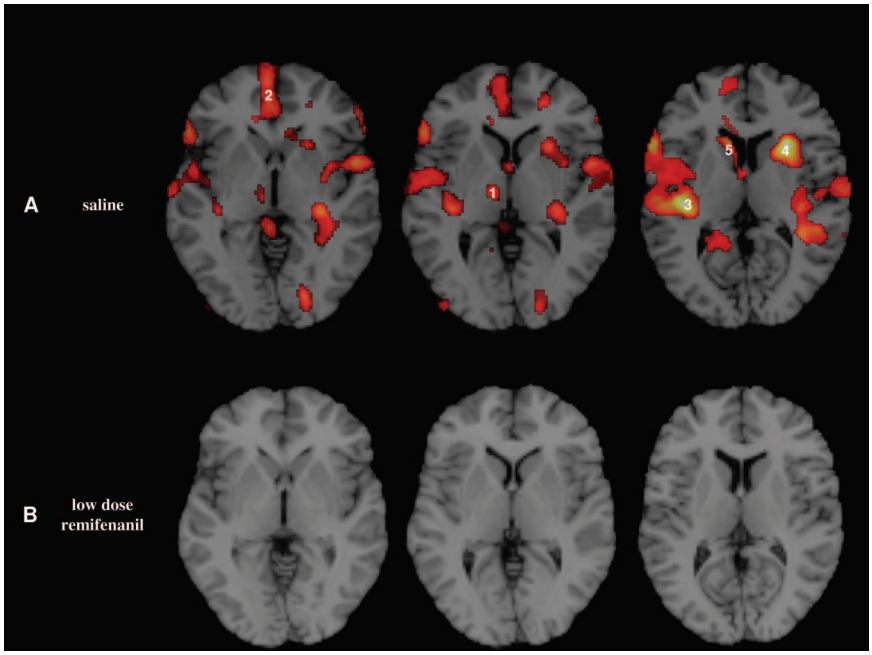

Fig. 3. Voxel-wise statistical analysis of $H_2{}^{15}O$-PET data during administration of saline (*A*) and low-dose remifentanil ($0.05\ \mu g \cdot kg^{-1} \cdot min^{-1}$; *B*). Pain induced increases in brain activation are shown (categorical comparison: painful heat > nonpainful heat). The statistical parametric maps (thresholded at $P < 0.05$, false discovery rate corrected) are overlaid on skull-stripped normalized structural magnetic resonance images in sagittal planes (average of 27 T1-weighted scans of the same individual from the Montreal Neurological Institute) as provided by SPM99. The results of the moderate-dose remifentanil condition ($0.15\ \mu g \cdot kg^{-1} \cdot min^{-1}$) are not shown because no significant activation clusters could be identified. 1 = Thalamus; 2 = cingulofrontal cortex; 3 = secondary somatosensory cortex; 4 = insula; 5 = caudate nucleus.

ACC; the prefrontal, insular, inferior parietal, and somatosensory cortices; and the thalamus were most consistently activated in previous studies.[18,19]

Analgesia is a dose-dependent phenomenon, but the neuronal correlate of this clinical observation has not been investigated so far. Therefore, we used experimental painful heat and PET to analyze the multifocal activity of supraspinal pain processing brain regions including the descending inhibitory system in response to increasing remifentanil analgesia.

Regarding data interpretation, it is appreciable that some brain regions of the complex pain network might contribute to the individual pain perception, whereas others are rather involved in pain modulation. It might thereby be expected that remifentanil suppresses activity in brain areas promoting the different aspects con-

Table 2. Brain Activations Induced by Painful Heat Stimulation

Region	MNI Coordinates			Z Score of Peak Activation
	x	y	z	
Contralateral thalamus	−10	−12	4	3.66
Ipsilateral insula	30	18	10	Infinite
	40	4	−8	4.66
Ipsilateral secondary somatosensory cortex	40	−10	14	4.14
Contralateral secondary somatosensory cortex	−40	−20	10	Infinite
Cingulofrontal cortex (midline)	0	42	−2	4.43
	−2	64	−4	4.98
Contralateral posterior cingulate cortex	−12	−48	10	3.62
Anterior cingulate cortex	−8	18	42	4.98
Ipsilateral superior frontal gyrus	6	14	66	5.68
Ipsilateral temporal cortex	36	−40	−6	5.45
Ipsilateral parietal lobe	16	−58	68	4.52
Ipsilateral occipital cortex	22	−82	0	4.48
Contralateral caudate nucleus	−8	20	10	5.74
Contralateral basal ganglia (striatum)	−18	16	−10	4.30
Ipsilateral basal ganglia (striatum)	14	22	−6	4.18
Ipsilateral brainstem	8	−24	−14	5.93
Ipsilateral cerebellum	16	−70	−14	5.56
	18	−38	−22	4.16
Contralateral cerebellum	−2	−36	−2	4.94

The x-axis runs medial–lateral relative to the midline (positive = right); the y-axis is anterior–posterior relative to the anterior commissure (positive = anterior); the z-axis is superior–inferior relative to commissural line (positive = superior). False discovery rate corrected at $P < 0.05$.

MNI = Montreal Neurological Institute.

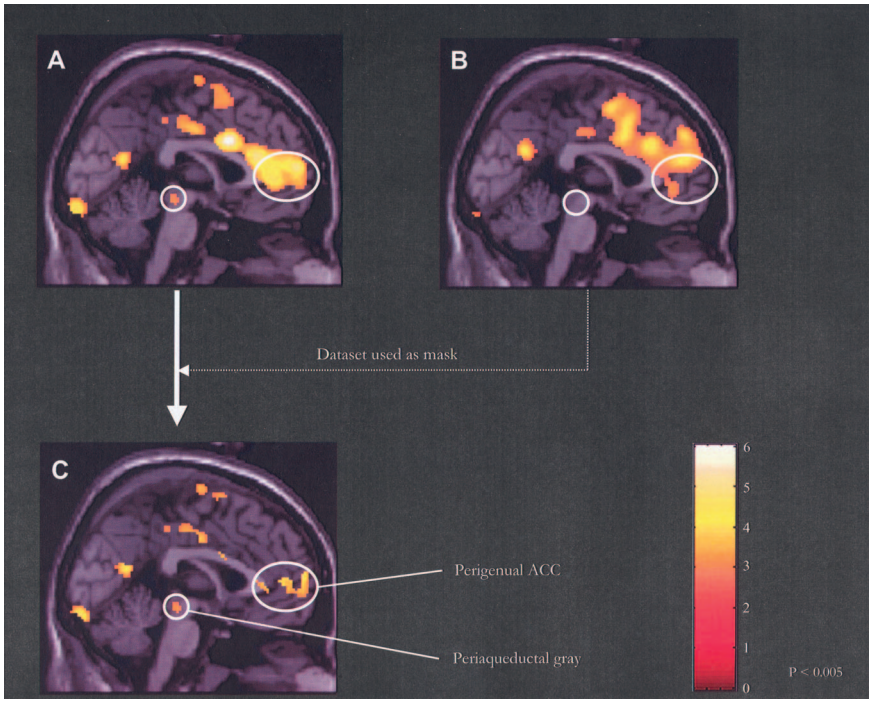

Fig. 4. Voxel-wise SPM99 analysis of $H_2{}^{15}O$–positron emission tomography data. The statistical parametric maps (threshold at $P < 0.005$ for descriptive purposes) are overlaid on normalized structural magnetic resonance images in sagittal planes (average of 27 T1-weighted scans of the same individual from the Montreal Neurological Institute) as provided by SPM99. (*A*) Remifentanil-induced regional cerebral blood flow increases during painful heat stimulation are shown [(0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and pain) minus (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and pain)]. (*B*) Remifentanil-induced regional cerebral blood flow increases during nonpainful heat stimulation are shown [(0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and no pain) minus (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$ remifentanil and no pain)]. (*C*) This modified analysis of the data already shown in *A* was intended to separate pain-specific from -nonspecific (nonpainful heat-related) effects of remifentanil. Thereby, the data from *B* were used as an exclusive mask of the analysis shown in *A*, *i.e.*, voxels that were significant in *B*, were excluded from this analysis. ACC = anterior cingulate cortex.

tributing to the complex sensation of pain. Indeed, we found decreases in activation in a number of brain areas such as the thalamus and somatosensory cortex (S2), which have been previously reported to process the pain experience.[20]

Contrarily, brain areas that would rather contribute to pain modulation would not necessarily be expected to decrease their activity, but to differentially change their activity to jointly suppress pain-related activity in the various regions. In fact, such an activity pattern was observed in the PAG and cingulofrontal cortex.

The distinct role of the most important regions evidencing altered activity in the context of various degrees of remifentanil analgesia will be discussed in greater detail in the following sections.

Periaqueductal Gray

Our finding of an activation of the PAG by remifentanil demonstrates the importance of descending inhibition of nociceptive transmission as part of the "brain defense system."[21,22] Its potential to significantly act in an antinociceptive way has been demonstrated in experimental as well as clinical settings.[23] Implantation of electrodes and electrical stimulation of the PAG induces inhibition of nociceptive dorsal horn neurons and profound analgesia in humans and animals.[24–26] This analgesic effect is thought to derive from a release of endogenous opioids, because the effects are reversible by the administration of the opioid antagonist naloxone.[24,27]

The PAG controls nociceptive transmission indirectly by means of connections through neurons in the rostral ventromedial medulla and the dorsolateral pontine tegmentum. These two regions project to the spinal cord dorsolateral funiculus and control pain by selectively influencing primary afferent nociceptor terminals and somata of dorsal horn neurons responding to noxious stimulation. Supraspinal input to the PAG originates from the hypothalamus and from the limbic forebrain (ACC), including several regions

Table 3. Regional Activations due to Increased Remifentanil during Painful Heat

Region	MNI Coordinates x	y	z	Z Score of Peak Activation
Periaqueductal gray matter	0	−26	−10	2.81*
Cingulofrontal cortex	6	44	8	4.33
Occipital cortex	−4	−96	−16	5.00

Effects (activations) of remifentanil dose increase during nonpainful heat were used as exclusive mask (false discovery rate correction [$P < 0.05$], exclusive masking threshold $P < 0.01$). The x-axis runs medial–lateral relative to midline (positive = right); the y-axis is anterior–posterior relative to the anterior commissure (positive = anterior); the z-axis is superior–inferior relative to commissural line (positive = superior).

* Small volume corrected using a 10 mm diameter sphere.

MNI = Montreal Neurological Institute.

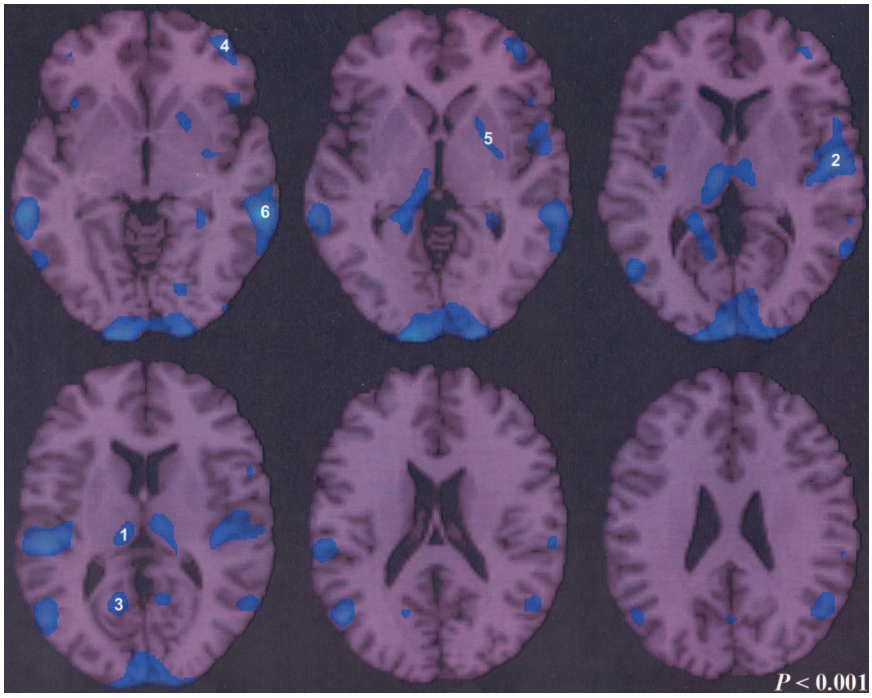

Fig. 5. Voxel-wise negative covariation analysis (remifentanil dosage as covariate of interest) of $H_2{}^{15}O$–positron emission tomography data during administration of saline, low-dose remifentanil (0.05 $\mu g \cdot kg^{-1} \cdot min^{-1}$), and moderate-dose remifentanil (0.15 $\mu g \cdot kg^{-1} \cdot min^{-1}$). Decreases of regional cerebral blood flow with increasing remifentanil dosage are shown during painful stimulation. The statistical parametric maps (thresholded at $P < 0.001$) are overlaid on normalized structural magnetic resonance images in axial planes (average of 27 T1-weighted scans of the same individual from the Montreal Neurological Institute) as provided by SPM99. 1 = Thalamus; 2 = insula/secondary somatosensory cortex; 3 = parahippocampal cortex; 4 = prefrontal cortex; 5 = basal ganglia; 6 = temporal cortex.

of the frontal neocortex and the central nucleus of the amygdalae, while projecting to spinothalamic pathways. Together with the perigenual ACC and the orbitofrontal cortex, the PAG seems to play a key role in pain modulation during distraction as an experimental pain stimulus and a simultaneous distraction task reduces pain while an increase in activation in the PAG was observed.[1,28] The functional interaction between cingulofrontal areas and the midbrain/PAG and posterior thalamus has been underlined using functional magnetic resonance imaging and functional connectivity analysis.[28] Moreover, Petrovic *et al.*[29] detected functional interactions on covariation analysis between the rostral ACC and the brainstem/PAG during both opioid and placebo analgesia.

The endogenous opioid that mediates these antinociceptive effects has not been identified. Results of experiments using microinjections of μ-opioid receptor agonists whose analgesic effects were reversed by μ-opioid receptor antagonists implicate that the μ-opioid receptor and enkephalins, as endogenous agonists, play a fundamental role in this native endogenous pain control system.[21] Furthermore, the importance of the μ-opioid receptor is underlined by its presence in the nuclei of pain-modulating circuits.

Table 4. Decreases of Pain-related Activity by Remifentanil

Region	MNI Coordinates			Z Score of Peak Activation
	x	y	z	
Contralateral thalamus	−6	−18	4	4.75
Ipsilateral thalamus	12	−16	10	4.25
Contralateral transition zone/parahippocampal	−10	−58	10	4.01
Ipsilateral transition zone/parahippocampal	14	−56	12	3.93
Contralateral S2	−50	26	14	5.88
Ipsilateral S2	56	−8	6	4.91
Ipsilateral basal ganglia	22	12	−4	3.90
Contralateral temporal cortex	−62	−40	−4	5.23
Ipsilateral temporal cortex	66	−38	−10	5.80
Ipsilateral prefrontal cortex	42	52	−8	5.25
Contralateral anterior insula	−34	22	−6	3.47
Ipsilateral anterior insula	48	21	−6	3.35
Contralateral occipital cortex	−14	106	0	5.49
Ipsilateral occipital cortex	24	104	4	5.12

Statistically significant decreases of pain-related brain activity by remifentanil as measured by $H_2{}^{15}O$–positron emission tomography (negative covariation analysis, extent threshold: 15 voxels; $P < 0.001$ uncorrected). The x-axis runs medial–lateral relative to midline (positive = right); the y-axis is anterior–posterior relative to the anterior commissure (positive = anterior); the z-axis is superior–inferior relative to commissural line (positive = superior).

MNI = Montreal Neurological Institute; S2 = secondary somatosensory cortex.

Henderson *et al.*[30] have revealed the existence of strong ventrolateral PAG projections to cardiovascular depressor regions within the caudal medulla which likely contribute to ventrolateral PAG-mediated hypotension and bradycardia. Hypotension and bradycardia are well-known phenomena accompanying remifentanil administration. Especially after rapidly changing infusion rates and bolus injections of remifentanil, these cardiovascular effects are frequent. Because we omitted these factors in conjunction with only moderate infusion rates to counteract respiratory depression, we did not see these cardiovascular changes in our experiment, and PAG activity can therefore be explained by its antinociceptive action.

Cingulofrontal Cortex

Positron emission tomography studies using the opioidergic ligands diprenorphine and carfentanil were able to demonstrate a high opiate receptor density in the cingulofrontal region.[8,31] With the anatomical linkage of the ACC to the PAG, the former is anatomically closely connected to the opioid-mediated pain-modulatory circuit.[32]

Although opioid analgesia specifically attenuates cerebral responses to painful stimulation, the cingulofrontal cortex was increasingly activated in our study during painful stimulation and increased remifentanil administration. As noted above, participation of this area in pain modulation was recently supposed due to the results of functional magnetic resonance imaging studies investigating effects of distraction and placebo on pain perception and processing.[28,33] Taken together with the activation patterns of other neuroimaging studies and opioidergic ligand PET results, the antinociceptive effect of exogenous opioids as well as placebo analgesia is likely to be mediated *via* opioidergic neurotransmission in the ACC.[2,9,29,34,35]

The properties of the cingulofrontal region are however not limited to pain processing, but an involvement in the processing and modulation of emotional contents, such as fear and anxiety, is well recognized.[36] In view of these complex functions, attentional and also emotional control of pain processing is suggested as a major role of the cingulofrontal cortex.[37,38]

Methodologic Considerations

The interpretation of our results bases on general assumptions that underlie changes in rCBF in a $H_2{}^{15}O$-PET activation study. These underlying mechanisms are complex and related to factors acting in parallel as well as in series.[39] Among these factors, the following three possibilities seem to be fundamental for changes in rCBF: (1) rCBF might be related to increases in lactate concentration (released by astrocytes), (2) it might be triggered by products of neuronal spiking, and (3) the blood vessels themselves might be involved in the rCBF changes.[40,41] These considerations emphasize the difficulties in interpreting changes in PET signal although considerable advances have been made during recent years. Finally, a spatial mismatch between the actual μ-opioid receptor effect and the source of the PET signal cannot be excluded.[2]

Starting PET scanning for each condition after an interval of greater than 30 min of continuous infusion of remifentanil guaranteed steady state blood concentration of remifentanil.[42,43] This zero-order infusion and the avoidance of an additional bolus prolonged the experimental duration but provided a safe setting and omitted possible adverse and confounding reactions of the volunteers, such as nausea and vomiting. Furthermore, no cardiovascular side effects or respiratory depression were noticed. This may be caused by the relatively small increments of remifentanil over a relatively long period, thus allowing physical adaptation. Because residual nonanalgesic but psychomimetic effects persist as long as 60 min after remifentanil infusion, we used a semirandomized study protocol with a step-up infusion rate to overcome the problem of extended study time.[44]

The pharmacodynamic effects of the μ-opioidergic drug within the chosen concentrations cannot be blinded in practice. Therefore, the identification of the different experimental conditions was relatively simple for the volunteers. Because we believe that the condition with infusion of saline was for this reason not a true placebo condition, we chose to term the saline condition as "control" instead of "placebo." However, we acknowledge that a partial placebo effect might have contributed to the results of the control condition.

The highest dose of remifentanil used in our study was $0.15\ \mu g \cdot kg^{-1} \cdot min^{-1}$, providing adequate analgesia in our as well as other experimental and clinical pain conditions.[45–47] Higher remifentanil infusion rates would be of further theoretical interest. This is inherent with the need of additional scans; either reduction of tracer activity or a reduction of scans per condition due to radiation protection would result in less robust statistics and would therefore not be beneficial. Furthermore, in a study paradigm with spontaneous breathing volunteers, the maximal dose of remifentanil is limited because the occurrence of unacceptable side effects (*e.g.*, respiratory depression, nausea and vomiting) would interfere with the interpretation of our findings.[45,48]

We clearly showed that remifentanil induced dose-dependent decreases in multiple supraspinal brain areas of the pain neuromatrix (fig. 5). However, one could wonder whether these dose-dependent effects might be in contradiction to the categorical analyses, where both remifentanil dosages abolished all detectable pain-induced brain activations. In our view, this discrepancy is a consequence of the statistical thresholding. We chose thresholding with correction for multiple comparisons (false discovery rate) for the categorical comparisons to reduce the occurrence of false-positive results. If we

would have chosen a less strict threshold resulting in limited reliability (*e.g.*, 0.05 uncorrected for multiple comparisons), the categorical comparisons would evidence the dose-dependent nature of the remifentanil-induced decrease in brain activation, which was also clearly reflected by the clinical pain ratings (fig. 2).

In conclusion, our data reveal the neuroanatomical targets of the μ-opioidergic receptor agonist remifentanil during an experimental pain condition by PET. The specific modulation of pain processing structures by increasing dosages of remifentanil provides further insight into the cerebral mechanisms of exogenous opioid analgesia. On the basis of the presented data, especially the role of the brainstem and the cingulofrontal cortex in remifentanil analgesia is underlined.

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