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Using fMRI to Quantify the Time Dependence of Remifentanil Analgesia in the Human Brain

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To understand and exploit centrally acting drugs requires reliable measures of their time course of action in the human brain. Functional magnetic resonance imaging (fMRI) is able to measure noninvasively, drug-induced changes in task-related brain activity. Here, we have characterized, in a specific region of the brain, the time of onset of action and the half-life of action of a clinically relevant dose of a potent opioid analgesic agent, remifentanil. These times were established from the temporal variation of the amplitude of the blood oxygen level-dependent response in the insular cortex contralateral to a painfully hot thermal stimulus, in volunteers receiving a remifentanil infusion. The insular cortex has repeatedly been reported as activated by noxious thermal stimulation. The times of onset and offset of drug action were each characterized by a half-life for changes in fMRI signal from within the insula. These characteristic times agreed with the observed drug-induced analgesia and previous pharmacokinetic–pharmacodynamic measurements for remifentanil. We have successfully measured, for the first time using fMRI, temporal pharmacological parameters for a CNS-active drug based on its effect on task-related activity in a specific brain region. Comparison of the time course of regional brain activity with pain perception could reveal those regions engaged in drug-induced analgesia.
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INTRODUCTION

Functional neuroimaging techniques such as functional magnetic resonance imaging (fMRI) have made it possible to study *in vivo* drug-induced changes in regional brain activity (Leslie and James, 2000; Stein, 2001; Tracey, 2001). In pharmacological fMRI studies to date, the temporal profile of the drug's effect has been assumed. The assumption has either been implicit in comparing fMRI-measured brain activity before and after administration (Breiter *et al*, 1997; Coull *et al*, 2001; Furey *et al*, 2000; Hariri *et al*, 2002; Honey *et al*, 1999; Kimberg *et al*, 2001; Kleinschmidt *et al*, 1999; Mattay *et al*, 2000; Thiel *et al*, 2001, 2002), or the assumption has been more explicit in incorporating pre-existing pharmacokinetic parameters into the analysis of fMRI data (Stein *et al*, 1998; Bloom *et al*, 1999; Wise *et al*, 2002). In either case, the aim has been to simply identify drug-induced changes in brain activity. fMRI must be developed beyond such studies if it is to be valuable for pharmacological assays. The development and assessment of novel centrally acting drugs requires a better understanding of their sites, mechanisms and time-courses of action. We suggest that fMRI has the potential to measure the *regional* time dependence of drug activity of certain compounds in the brain. This paper demonstrates that potential for the first time using painful thermal stimulation and remifentanil, a potent, short-acting, μ-selective opioid analgesic agent. The opportunity afforded by fMRI to compare the time-courses of drug effects in different brain regions with subtle behavioral changes, such as analgesia, could help to establish the networks critical for analgesia and hence the brain systems to be targeted for maximum therapeutic effect.

The rates of onset and offset of action of a drug are clinically important. They depend on the mechanism and site of action as well as the temporal variation of the drug concentration in the body, described by the drug's pharmacokinetics. The delay in onset of a centrally acting drug once in the blood can arise from the time taken for a significant concentration to be achieved at the notional effect site of the drug, for example brain. The time dependence of drug action may be measured from the

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effects of the drug on the body, known as the pharmacodynamics. In the case of remifentanil, pain threshold (Glass *et al*, 1993) has been used to characterize the time dependence of the analgesic effect. Additionally, less analgesia-relevant measures have been employed including spectral characteristics of the electroencephalogram (Egan *et al*, 1996; Hermann *et al*, 1999; Hoke *et al*, 1997; Minto *et al*, 1997a, b), which provides a measure of brain activity, but is not spatially localized, requires doses beyond the normal clinical range and more importantly is not directly associated with pain processing. PET can map the concentration or binding of pharmacological agents over time (Malizia *et al*, 1996; Pappata *et al*, 1996) or measure task or drug-induced changes in cerebral blood flow (Petrovic *et al*, 2002). However, PET is restricted as a tool for examining task-related brain activity by its relatively long measurement time and invasiveness. fMRI, however, noninvasively provides better spatial and temporal localization of brain activity during complex event-related tasks such as thermal pain (Peyron *et al*, 2000) that may be modulated by analgesic drugs including opioids (Tracey *et al*, 2000b; Tracey, 2001). We have previously identified from fMRI signal, remifentanil-induced regional modulation of pain-related brain activity (Wise *et al*, 2002) with the help of a model for drug effect site concentration based on EEG measurements (Minto *et al*, 1997a, b). The study presented here considerably extends this work by characterizing the time-course of drug effect within the brain using fMRI. We examine the temporal variation of thermal pain-related brain activity in the insular cortex during infusion of remifentanil. This is then used, for the first time, to calculate pharmacokinetic–pharmacodynamic parameters describing the time of onset and offset of drug action based on changes in pain-related brain function. Our fMRI measures are supported by their agreement with the time-course of reported analgesia during this study and previous crude EEG measurements (Minto *et al*, 1997a, b).

MATERIALS AND METHODS

The study was approved by the Oxfordshire Clinical Research Ethics Committee and volunteers gave informed written consent. Nine healthy male volunteers (mean age $\pm$ SD, 29 $\pm$ 9 years) were examined on three different occasions. The first examination was a medical screen to ensure that volunteers tolerated remifentanil, determined by an anesthetist monitoring blood pressure and oxygen saturation. The second and third examinations, randomized in order across subjects were (a) fMRI combined with painful stimulation and a remifentanil infusion to a maximum estimated plasma concentration of 1.0 ng/ml and (b) fMRI examination with painful stimulation and a saline infusion volumetrically equivalent to that of the remifentanil infusion.

Painful Thermal Stimulation

During each functional imaging session, 28 noxious thermal stimuli each of 3 s duration were applied to the dorsum of the left hand (Figure 1). A purpose built, MR compatible thermal resistor (1.5 $\times$ 2 cm) delivered the fast ramping (30–60°C in 0.8 s) painful thermal stimuli. This allowed a short stimulus to be delivered for event-related fMRI studies. Three seconds was an appropriate duration for a stimulus that was safe and immediately felt as hot without a significant initial component of warmth. The 3 s thermal stimulus has been shown in our previous studies of remifentanil (Tracey *et al*, 2000b) to give robust pain-related activity, which could be scored by volunteers for pain intensity. A brief stimulus is also advantageous to avoid significant change in remifentanil concentration during the stimulus itself. The mean interstimulus interval was 65.3 s. There was a pseudorandom sequence of interstimulus intervals approximately uniformly distributed in the range 61.5–70.5 s. This variation in interstimulus interval was adopted to reduce regularity of stimulus presentation and hence reduce the volunteers' ability to predict the arrival of the next stimulus. A relatively long interstimulus interval was employed to allow time for reporting of perceived pain intensity between stimuli and to reduce sensitization of the volunteer to the stimulus. Thermal stimuli delivered too rapidly in succession to a site on the skin may result in changes in perceived intensity over time of identical stimuli.

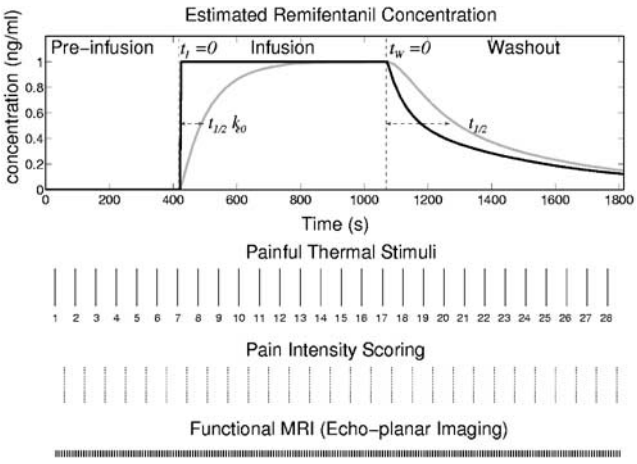

Figure 1 One imaging session lasting approximately 30 min. Intravenous infusion was performed with either remifentanil or saline. During saline infusions, remifentanil concentration remained zero. Painful thermal stimuli were of a constant temperature within one session. The heavy line in the plot indicates the estimated blood plasma remifentanil concentration. The half-tone line indicates the effect site concentration of remifentanil estimated from the pre-existing pharmacokinetic model based on EEG measurements (Minto *et al*, 1997a, b). The half-life of equilibration between the plasma compartment and the effect site ($t_{1/2}k_{e0}$) and the washout half-life of the effect site concentration ($t_{1/2}$) are shown schematically.

To improve the effective temporal sampling of the blood oxygen level-dependent (BOLD) hemodynamic response to the painful stimuli, consecutive noxious stimuli were offset in time by a whole number of interscan (TR) periods plus 1/2TR = 1.5 s. Before imaging while subjects lay in the MRI scanner, the thermal pain intensity threshold was determined for each subject. The stimulus temperature was adjusted iteratively to elicit a consistent rating of 3–4 indicating moderate–strong pain on a 5-point numerical rating scale (Jensen and Karoly, 2001). That temperature was used for each stimulus during the functional imaging session. During imaging, 21 s after the end of each thermal stimulus (Figure 1), the 5-point numerical pain intensity scale was shown to the subjects for 5 s. This prompted the

subjects to report the most recent stimulus for pain intensity by pressing one of five buttons, with the right hand. A pain scale of 5 points, eliciting a button press, was chosen because of the need to perform and record pain rating repeatedly and rapidly to provide minimal perturbation in the fMRI data associated with scoring.

fMRI

Imaging was performed at 3 T with an Oxford Magnet Technology, 1 m bore magnet. The magnet was driven by a Varian Unity Inova console. Whole-brain gradient-echo echo-planar imaging (EPI) was performed giving T2* weighting or BOLD contrast (image matrix 64×64; 4×4 mm pixels; echo time (TE) 30 ms). Each volume comprised 21 contiguous axial slices, 6 mm thick. Volumes were acquired continuously with a repetition time (TR) of 3 s. For each subject, a T1-weighted structural scan (64 contiguous 3 mm axial slices; in-plane field of view 256 mm, 1×1 mm pixels) was acquired. This was used for anatomical overlay of brain activation and to assist in placing individual subject's data into a common stereotactic space.

Drug Infusion

Remifentanil (at a solution concentration of 10 μg/ml) or saline was infused intravenously by a target controlled infusion (TCI) pump (Graseby 3500 TCI incorporating 'Diprifusor', SIMS Graseby Ltd, UK, supplied, preprogrammed with pharmacokinetic software, by Anaesthesia Technology Ltd, UK (Gray and Kenny, 1998)). The TCI pump controlled delivery of the remifentanil to achieve and maintain the desired remifentanil blood plasma concentration, based on the subject's sex, age, weight, and height incorporated into the existing published three-compartment pharmacokinetic model of remifentanil employed by the pump (Minto *et al*, 1997a, b). Subjects were blinded to the infusion of remifentanil or saline. Figure 1 illustrates the expected blood plasma concentration profile of remifentanil estimated from the existing pharmacokinetic model. This includes a sharp rise in plasma concentration at the start of infusion due to a loading dose administered by the TCI pump. The remifentanil plasma concentration profile is expected to differ from the remifentanil effect site concentration profile governing the fMRI response. The maximum plasma concentration of 1 ng/ml has previously been found to give a clear analgesic response to our painful thermal stimulus without excessive sedation or blood oxygen desaturation (Tracey *et al*, 2000b).

Seven thermal stimuli preceded the infusion. Infusion began and continued while stimuli 8–17 were delivered during an estimated constant plasma concentration of remifentanil. Infusion was halted and remifentanil concentration decayed from the action of esterases in the blood plasma while stimuli 18–28 were delivered (Figure 1).

Image Analysis to Reveal Pain-Related Brain Activity

Representative group maps of pain-related activity were calculated. These were used to confirm the existence of an appropriate anatomical region, responsive to pain, for use in the further analysis of the time dependence of the drug effect.

Image analysis to reveal significant brain activity based on changes in BOLD signal was performed on each subject's data using FMRIB Expert Analysis Tool (FEAT, http://www.fmrib.ox.ac.uk/fsl) (Smith *et al*, 2001). The following processing was applied to each subject's time-series of fMRI volumes: motion correction using MCFLIRT (Bannister and Jenkinson, 2001; Jenkinson and Smith, 2001); spatial smoothing using a Gaussian kernel of full-width-half-maximum 5 mm; nonlinear highpass temporal filtering (Gaussian-weighted least-squares straight line fitting, with a high-pass filter cutoff of 70 s) and subtraction of the mean of each voxel time-course from that time-course. The fMRI signal was then linearly modelled on a voxel by voxel basis using FMRIB's Improved Linear Model (FILM) with local autocorrelation correction (Woolrich *et al*, 2001).

Each thermal stimulus was assumed to elicit a hemodynamic response described by the convolution of a square on-off function of 3 s duration with a generalized hemodynamic response function (gamma variate function). The significance of the model fit to each voxel time-series was calculated, yielding statistical parametric maps of pain-related activity for each subject and infusion. These were combined using FEAT in a second level random-effects group analysis following the coregistration of each scan to the standard of the MNI (Montreal Neurological Institute). This resulted in a *Z*-score map of statistically significant pain-related activity across all infusions (Figure 2). All infusions were included as this improved the power to detect pain-related activity. A cluster-based correction of the *Z*-statistic images was performed. The raw *Z*-statistic images from the group analysis were thresholded at *Z*-scores greater than 4.0. This conservative threshold was chosen to preserve anatomical clarity in the display of the brain activity maps (Figure 2). For each resulting cluster of spatially connected voxels surviving the Z threshold, a cluster probability threshold of $P = 0.01$ was applied to the computed significance of that cluster. The cluster-based significance thresholding at $P < 0.01$ provides a correction for the problem of multiple comparisons. The technique of cluster-based significance thresholding is described further

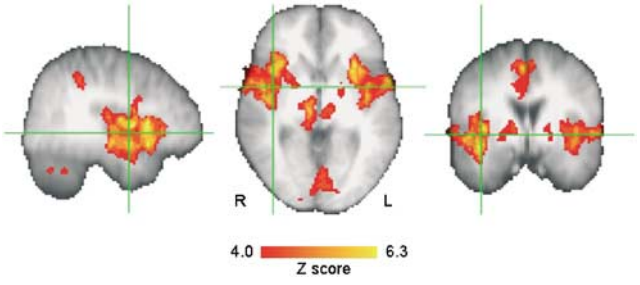

Figure 2 Representative group activity in response to painful thermal stimuli. Random effects group analysis from 18 datasets, group $Z > 4.0$ and cluster threshold $P < 0.01$. The background gray-scale image is the mean structural scan of all subjects after each scan was coregistered to the standard of the MNI. The cross-hairs indicate the right-sided insular cortex contralateral to the stimulus. This anatomical region was used in further analysis of the fMRI time-series to estimate the pharmacokinetic–pharmacodynamic parameters of remifentanil.

by Forman *et al* (1995), Friston *et al* (1994), and Worsley *et al* (1992).

A previous fMRI study of steady-state infusion of remifentanil has shown pain-related activity to be robust in the insular cortices and significantly modulated by the drug (Tracey *et al*, 2000b; Tracey, 2001). The right-sided insular cortex contralateral to the stimulus was therefore chosen for further time-series analysis, aimed not simply at detecting that modulation but aimed at describing its time-course. The boundaries of the insular cortex contralateral to the stimulus were manually defined from the MNI standard brain. Following coregistration of the functional scans to this standard, the BOLD signal time-course of the contralateral insula was extracted for each individual for further analysis.

Pharmacokinetic–Pharmacodynamic Parameters from fMRI

We have described in a previous study (Wise *et al*, 2002) analysis of data acquired in the experimental protocol given above confirming that the existing pharmacokinetic–pharmacodynamic model describing the magnitude of drug effect, previously established by EEG measurements, could be used to identify the regions whose pain-related activity was modulated by remifentanil. Importantly, the study presented here takes a different approach with the specific aim of characterizing the time-course of drug effect within the insular region in a novel manner using fMRI, without use of the pharmacokinetic–pharmacodynamic time constants derived from EEG measures of drug effect (Minto *et al*, 1997a, b). This study therefore develops and demonstrates the methods that reveal the potential of fMRI for determining pharmacokinetic–pharmacodynamic parameters based on function within a specific brain region.

By examination of the fMRI time-series in the contralateral insula, parameters describing the time-of-action of remifentanil were estimated. A region of interest analysis was deemed necessary to achieve an adequate signal-to-noise ratio in the time-series data.

The characteristics of the hemodynamic response on and off remifentanil, during the different epochs of the experiment, were examined to identify a suitable measure of drug effect (pharmacodynamics) in the fMRI signal. The spatial average of each subject's fMRI time-series was calculated over the region of interest for each infusion. For remifentanil and separately saline infusions, this time-series was then averaged across stimuli and finally across subjects for epochs of stimulation preinfusion (stimuli 1–7), during infusion when plasma concentration was expected to be constant (stimuli 11–17) and during the washout period (18–28) (Figure 3). The primary drug-dependent parameter of the BOLD hemodynamic response to the stimuli appeared to be the response amplitude. A model was developed to describe this response amplitude for each stimulus from the fMRI signal.

The hemodynamic response to each stimulus was described by a separate regressor formed by convolving the input stimulus function with a generalized gamma variate hemodynamic response function of full-width at half-maximum 6 s (FMRIB Expert Analysis Tool, http://www.fmrib.ox.ac.uk/fsl). The mean lag of the hemodynamic

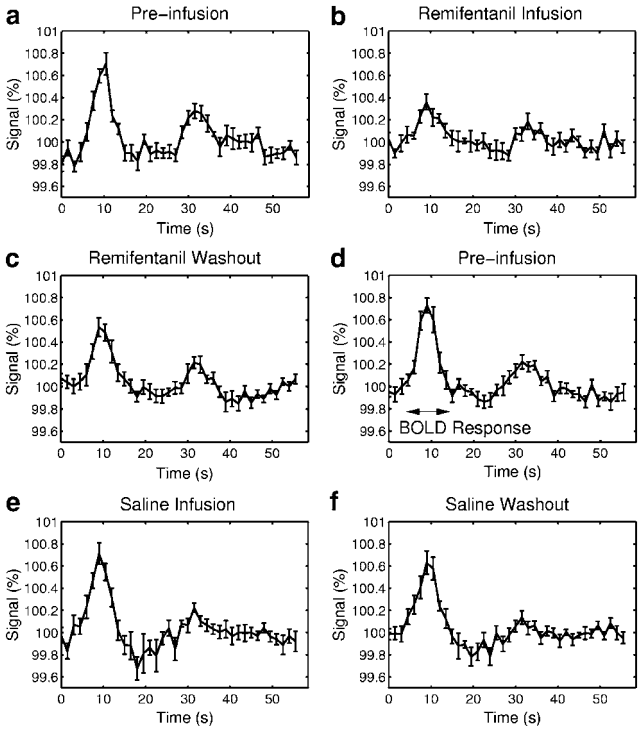

Figure 3 Mean fMRI signal over stimulus cycles and subjects in the insular region of interest following painful stimulation. The peak centered at a delay of approximately 9 s, represents the BOLD response to the brief noxious stimulation. Time = 0 s, indicates the start of the 3 s painful stimulus. Error bars indicate the SE over subjects. (a–c) are derived from the remifentanil infusions, while (d–f) are derived from the saline infusions. (a, d) are the mean fMRI signal over the first stimulus cycles 1–7 during the preinfusion period. (b, e) are the mean fMRI signal over stimulus cycles 11–17 during the steady infusion period (1 ng/ml estimated plasma concentration). (c, f) are the mean fMRI signal over stimulus cycles 18–28 during the remifentanil (or saline) washout period.

response function was chosen as 7.5 s after comparison with the group mean insular BOLD response to the painful stimulation, from the saline infusions only. This lag ensured a modeled response that peaked at the same time as the BOLD response to pain and provided a visually reasonable representation of it. Only the data from the saline infusions, and not the remifentanil infusions, was used to verify the suitability of the hemodynamic response function in order to reduce the possibility of overfitting the remifentanil data used for pharmacokinetic modeling. Least-squares linear regression between the insular time-series and the model was performed. Each regression coefficient therefore represented the amplitude of a BOLD response to pain.

To characterize the pharmacodynamic effects of remifentanil, BOLD response amplitude and perceived pain intensity were tested for a significant decrease between saline and remifentanil infusion sessions and between the two portions of the experiment (Figure 4): preinfusion (stimuli 1–7) and the period of steady infusion (stimuli 11–17). Significance was tested with a paired one-tailed Student's t-test at $P < 0.05$.

BOLD response amplitudes to each stimulus were pooled (averaged) across subjects to achieve adequate signal-to-noise for the parameter estimation (Figure 5). A further model was constructed to describe the temporal variation of

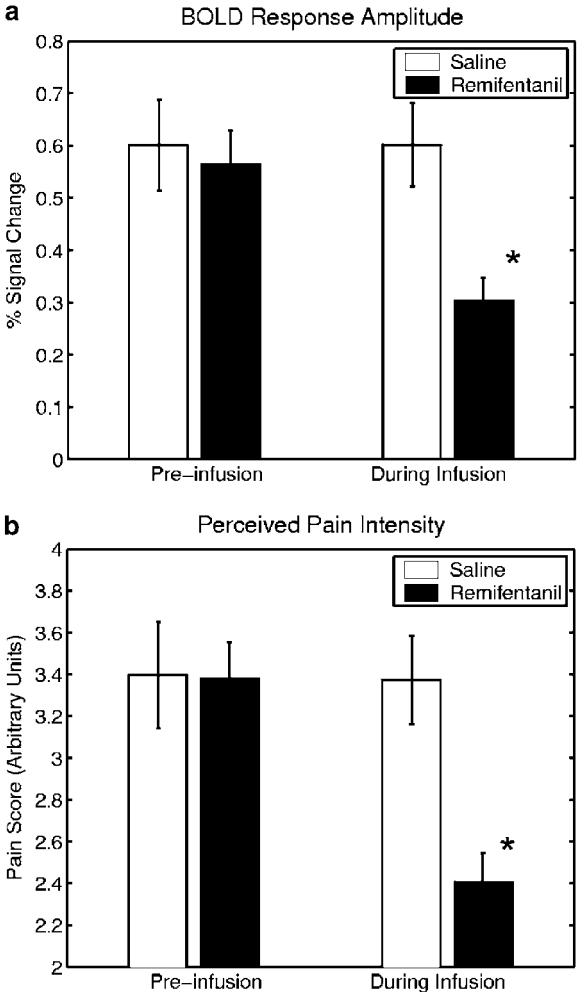

Figure 4 fMRI measured brain activity and perceived pain intensity. Mean amplitudes of the BOLD response to pain (a) from the insular region of interest and mean perceived pain intensity scores (b) from blocks of painful stimulation preceding infusion (stimuli 1–7) and during steady infusion (stimuli 11–17). Error bars indicate the SE of the mean across subjects. Filled bars indicate remifentanil infusion. Unfilled bars indicate saline infusion. *Indicates a significant reduction of BOLD amplitude and perceived pain intensity on infusion of remifentanil compared to preinfusion and also a significant reduction in these quantities during remifentanil infusion compared to saline infusion. Significance was tested with a paired one-tailed Student's *t*-test at $P<0.05$.

the BOLD response amplitudes for the remifentanil infusions only. Modeling of the time dependence of the BOLD amplitude and perceived pain intensity scores was not performed for the saline infusion because no significant change in amplitude of BOLD response or of pain scores was detected. For the purposes of estimating the time of action of remifentanil, the variation of BOLD response amplitude with remifentanil effect site concentration was assumed to be linear over the small range of concentrations applied.

Considering first the period of infusion of remifentanil and assuming a step change in remifentanil plasma concentration at $t_I = 0$ (Figure 1), the amplitude of the BOLD response to stimulation was described by

$$S(t_I) = S_0 - (S_0 - S_I)[1 - \exp(-k_I t_I)] \quad (1)$$

where k_I is the plasma-BOLD effect equilibration rate constant and the associated half life of equilibration is given by $t_{1/2I} = \ln 2/k_I$.S_0, the BOLD response amplitude at zero remifentanil concentration, was taken as the mean BOLD response amplitude for the stimuli 1–7 preceding remifentanil infusion. $t_{1/2I}$ was substituted into Eq. (1) and was estimated along with S_I (BOLD response amplitude at 1 ng/ml effect site concentration) by fitting as described below. S_0 is shown in Figure 4, whereas S_I was estimated by fitting to be 0.27% signal change with 95% confidence intervals of 0.08 and 0.46%.

Considering now the period, beginning at $t_W = 0$ (Figure 1), when remifentanil infusion was halted, the pain-related BOLD responses were modeled by the following decay function:

$$S(t_W) = S_0 - (S_0 - S_W)\exp(-k_W t_W) \quad (2)$$

where S_W was the BOLD response amplitude when infusion was halted, estimated from Eq. (1). k_W is the 'washout' rate constant and the associated washout half-life of remifentanil is given by $t_{1/2W} = \ln 2/k_W$. This forms our estimate of the time taken for the effect of the remifentanil on the fMRI BOLD signal in the insular cortex to reduce by 50%. $t_{1/2W}$ was substituted into Eq. (2) and estimated by fitting.

The pharmacokinetic parameters were estimated by nonlinear least-squares fitting of the fMRI determined BOLD response amplitudes to Eqs. (1) and (2). Fitting was performed with a Gauss–Newton algorithm with Levenberg–Marquardt modifications for global convergence implemented using MATLAB software (The Mathworks, Inc., MA). The 95% confidence intervals were estimated on the parameters $t_{1/2I}$ and $t_{1/2W}$ (Table 1).

To allow comparison of fMRI with an alternative pharmocodynamic effect of remifentanil, the same pharmacokinetic analysis was applied to group mean perceived pain intensity scores. Pain scores instead of BOLD response amplitudes were applied in Eqs. (1) and (2) (Figure 5) to estimate $t_{1/2I}$ and $t_{1/2W}$ (Table 1).

RESULTS

Pain-Related Brain Activity

Throughout any one imaging session, painful stimuli of 3 s duration were presented at a constant maximum temperature. The mean $\pm$ SD was $56.4 \pm 1.4^{\circ}$C, and stimulus temperature was applied across nine subjects and two sessions. A network of brain regions was observed to activate in response to the brief painful thermal stimuli (Figure 2). The group average map from a random effects analysis incorporates scans taken during remifentanil and saline infusions, from all subjects ($n = 9$). Regions including the bilateral insular cortices, the anterior cingulate cortex, the cerebellum, the bilateral secondary sensory region (SII) and the bilateral thalamus were active at this statistical level ($Z > 4.0$ and activated cluster significance $P < 0.01$). This pattern of activity is consistent with previous reports of thermal pain-related brain activity (Becerra *et al*, 1999; Jones *et al*, 1991a; Peyron *et al*, 2000; Talbot *et al*, 1991; Tracey *et al*, 2000a). The right-sided insular cortex (contralateral to the stimulus), defined from the MNI standard brain, was chosen for further time-series analysis.

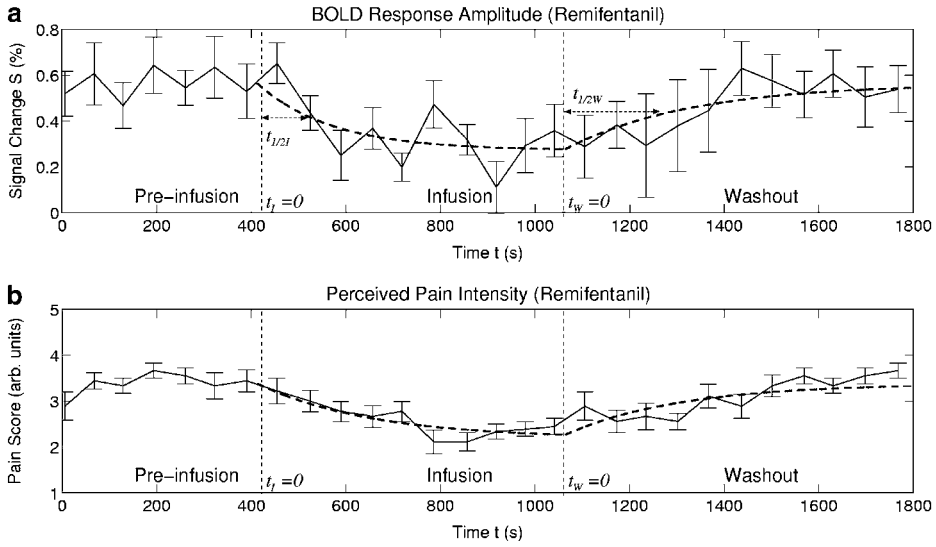

Figure 5 Time-course of fMRI and perceived pain intensity. Mean amplitude of the BOLD response to pain (solid line, a) and mean perceived pain intensity scores (solid line, b) during the course of the remifentanil sessions. Each point represents the response to one stimulus. Error bars indicate the SE of the mean across subjects. $t_I = 0$ and $t_W = 0$ indicate the onset and cessation of remifentanil infusion, respectively. The heavy broken lines indicate the fitted exponential models from which the onset equilibration half-life ($t_{1/2I}$) and the washout half-life of drug activity ($t_{1/2W}$) were estimated.

Table 1 Pharmacokinetic–Pharmacodynamic Parameters of Remifentanil Derived from fMRI BOLD Response Amplitude and Reported Pain Intensity

	fMRI BOLD response	Perceived pain intensity
Equilibration half-life ($t_{1/2I}$) (min)	1.83 (−2.17, 5.83)	2.80 (−0.12, 5.73)
'Washout' half-life ($t_{1/2W}$) (min)	3.07 (1.09, 5.05)	2.80 (1.12, 4.48)

Figures in parentheses indicate 95% confidence intervals for the estimated parameters.

Of the regions listed, this region shows significant modulation of pain-related activity resulting from remifentanil infusion (Tracey, 2001; Wise *et al*, 2002), and is observed to be one of the most robustly pain-active regions (Peyron *et al*, 2000). These factors would suggest that, given the limited signal-to-noise ratio of drug-induced changes in the fMRI signal, the likelihood of extracting meaningful pharmacokinetic data on drug effect was highest for this chosen region of interest.

Hemodynamic Response

It was necessary to establish an appropriate pharmacodynamic measure (a measure of drug effect) from within the fMRI signal. Figure 3 illustrates the average form of the hemodynamic BOLD response in the right insular region of interest, for stimuli within the three epochs of the experiment: preinfusion, steady infusion and washout. FMRI signal samples are spaced apart by 1/2TR = 1.5 s, made possible by jittering the stimulus onset with respect to slice acquisition timing. The time scale (abscissa) covers approximately one interstimulus period.

The consistent signal peak at approximately 9 s after the onset of the painful stimulation represents the principal hemodynamic response to the thermal stimulus. The second smaller peak at about 32 s after the start of the painful stimulus may arise from the pain intensity rating and subsequent button-press. This is consistent with the insula's established role in somatosensory processing (Burton *et al*, 1993), involvement in non-nociceptive touch (Johansen-Berg *et al*, 2000) and also previously reported activity during button pressing (Johansen-Berg and Matthews, 2002).

The form of the hemodynamic response during saline infusions (Figure 3d–f) remains similar throughout the course of the imaging session. The amplitude of response is consistent (approximately 0.6%) and the width (full-width at half-maximum) appears consistent across epochs. However, the amplitude of the response appears reduced during remifentanil infusion (Figure 3b) compared to the preinfusion period (Figure 3a). The mean response during the washout period (Figure 3c) shows a recovery of amplitude. Figure 3 demonstrates the suitability of the BOLD response amplitude as a marker of the effect of remifentanil on pain-related brain activity. The amplitude was established by comparing the fMRI signal after each stimulus to a hemodynamic response function.

Time Dependence of Pharmacological Effects

To identify a drug effect, BOLD response amplitude and perceived pain intensity were compared during two portions of the experiment (Figure 4): preinfusion (stimuli 1–7) and during the period of steady infusion (stimuli 11–17) having allowed several minutes for a period of blood–brain equilibration (Figure 1). The mean BOLD response amplitude and the reported pain intensity arising from stimulation were not significantly altered by saline infusion. However, remifentanil infusion significantly decreased BOLD response amplitude and perceived pain intensity within session and also in comparison to saline infusion

($P<0.05$, Figure 4). Remifentanil at an estimated 1 ng/ml plasma concentration reduced BOLD response amplitude by approximately half on average in the insula.

The pharmacokinetic model of remifentanil predicted a step change in blood plasma concentration of the drug at the start of infusion ($t_I = 0$, Figure 1), followed by a period of steady plasma concentration. We would expect a lag between the increase in plasma concentration and the effect of the drug, governed by its concentration at the effect site, assumed to be in the brain. This lag is conventionally described by a blood plasma–effect site equilibration rate constant (k_{e0}) and associated half-life of equilibration ($t_{1/2}k_{e0} = \ln 2/k_{e0}$) (Figure 1). This time constant represents the time of onset of action of the drug. We suggest that the effect of the equilibration time is evident in the fMRI signal. An fMRI-based group-mean equilibration time that we will refer to as $t_{1/2I}$, was estimated (Table 1) from the temporal profile of decrease in the BOLD response amplitudes, characterized by a single exponential function (Figure 5), following the commencement of infusion.

Following the end of infusion at 1064 s into the imaging session ($t_W = 0$, Figure 1), the effect of remifentanil was expected to decay with what is often termed the context-sensitive half-life (Hughes *et al*, 1992). A half-life of drug activity may be defined based on the fMRI signal and in this paper is labeled by $t_{1/2W}$ (a pharmacodynamic context-sensitive half-life). During the 'washout' period, we assumed a simple exponential recovery of the BOLD response amplitude towards the amplitude estimated during the preinfusion period (Figure 5). In this way, $t_{1/2W}$ was estimated (Table 1) as a group-mean pharmacokinetic–pharmacodynamic parameter based on changes in brain function within a specific region of brain (the insula).

Since there was no significant modulation of the BOLD response amplitude or perceived pain intensity by saline infusion, it was not possible to estimate the blood–effect site equilibration half-life and washout half-life for saline.

Group-mean perceived pain intensities followed a similar time-course to the changes in pain-related BOLD response amplitude in the contralateral insular region of interest (Figure 5) (correlation coefficient $r = 0.65$, $P<0.01$). Figure 5 shows that these time-courses are qualitatively similar to the profile of the estimated effect site concentration of remifentanil predicted from the exisiting pharmacokinetic–pharmacodynamic model (Minto *et al*, 1997a, b) (Figure 1). The parameters $t_{1/2I}$ and $t_{1/2W}$ were estimated (Figure 5, Table 1) from the temporal variation in pain intensity scores, used as a marker of drug effect, by the same approach as adopted for the analysis of BOLD response amplitude.

DISCUSSION

We have determined using fMRI the times of onset and offset of remifentanil's action. To our knowledge this is the first use of fMRI to establish pharmacokinetic–pharmacodynamic parameters based on drug-induced changes in task-related brain function. We have previously demonstrated using fMRI the dose dependence and specificity of remifentanil's effect in reducing pain-related activity (Tracey *et al*, 2000b; Tracey, 2001). We have further investigated regional modulation of pain-related brain activity by employing the pre-existing pharmacokinetic–pharmacodynamic model of the remifentanil effect site (brain) concentration in the analysis of fMRI data (Wise *et al*, 2002). The present study extends this work considerably by estimating the time-course of the pharmacodynamic effect in the brain from the fMRI data under the assumption of known pharmacokinetics of blood plasma concentration at a spatial resolution that is meaningful in terms of targeted drug discovery developments.

One brain region, activated by the noxious stimulus, was selected for analysis of time-varying drug activity: the contralateral (right sided) insula. In addition to showing opioid-induced modulation of fMRI-measured activity, the insular is consistently one of the most significantly pain-active regions in this experiment and in previous studies (Peyron *et al*, 2000). Detection of drug modulation of pain activity is most likely where the stimulus-related activity is initially most significant. Furthermore, the posterior insula has recently been implicated as a thermosensory cortex (Craig *et al*, 2000). Peyron *et al* (1999) suggested the role of insula in the sensory discriminative dimension of pain intensity encoding. PET ligand binding studies have shown the insula to posses a high concentration of μ-opioid receptors (Jones *et al*, 1991b, c), suggesting the insula as a potential site of analgesic action for remifentanil, a μ-opioid agonist. It was therefore an ideal anatomical region to select for our pharmacokinetic–pharmacodynamic modeling. The change in amplitude of the stimulus-induced BOLD response proved to be a sensitive pharmacodynamic marker with a halving of amplitude by the infusion of 1 ng/ml plasma concentration of remifentanil. The sensitivity of fMRI to a given dose of remifentanil is greater than for EEG spectral edge measurements. A concentration of 11.2 ng/ml, which is larger than typically used clinically, was required to provide a 50% decrease of the spectral edge frequency (Minto *et al*, 1997a). The concentration adopted in this study of 1 ng/ml is at the low end of the clinical dose range. fMRI may therefore be more appropriate tool for examining the cerebral effects of clinically relevant doses.

The BOLD response amplitude followed a similar temporal profile to the behavioral measures of perceived pain intensity. The simultaneous variation of remifentanil concentration, perceived pain intensity and BOLD response amplitude, and the differences in these quantities between remifentanil and saline infusions, suggest that changes in the BOLD signal reflect the analgesic effect of remifentanil on pain-related brain activity. The experiments do not prove that remifentanil is acting selectively and only at the neuronal level within the insula. Vascular effects of opioids may occur although a global hemodynamic confound is unlikely as our previous study revealed that brain activity in the occipital cortex arising from a visual stimulus did not exhibit a remifentanil dose-dependent effect (Tracey *et al*, 2000b; Tracey, 2001). Neuronal opioid effects may also occur at a lower level in the brainstem, spinal cord and periphery (Lorenz *et al*, 2000; Wagner *et al*, 2001). However, we believe based upon our previous work that the major effect we are measuring is likely to result from the direct action of remifentanil at the insular site.

The pain-related BOLD response amplitude depends on the concentration of remifentanil at the site of drug action,

often termed the effect site concentration. Existing pharmacokinetic–pharmacodynamic relationships for drugs such as remifentanil (Egan, 1995; Patel and Spencer, 1996) often express the characteristic times of onset or offset of drug action in terms of the temporal variation in the notional effect site concentration. That variation is studied via a pharmacodynamic effect, for example EEG spectral changes, that is related to the effect site concentration by a model (Shargel and Yu, 1999). However, in this study, we have directly examined the temporal variation of the pharmacodynamic effects on the BOLD signal and the perceived pain intensity. We have adopted the same easily implemented nonlinear model for the time dependence of the perceived pain intensity and the BOLD response, described by a single amplitude parameter for each stimulus. While a nonlinear model could be used to describe the complete fMRI signal, this would be more complex to implement and our consistent and simple approach to the fMRI and behavioral data allows us to easily compare the time dependence of pain intensity and an fMRI index of brain activity.

We have characterized the drug effects by a half-life of onset ($t_{1/2I}$) and offset ($t_{1/2W}$) (Table 1) of changes in the BOLD signal and reported pain intensity. Under the assumption, supported by the present study, of a linear relationship between the change in BOLD response amplitude or reported pain intensity and the remifentanil effect site concentration, these functionally defined half-lives are equivalent to the equilibration half-life ($t_{1/2}k_{e0}$) and context sensitive half-life ($t_{1/2}$) for effect site concentration established in previous studies of remifentanil.

The plasma–effect site (brain) equilibration half-life $t_{1/2}k_{e0}$ (min) (Figure 1), describing the onset delay, has been estimated from EEG measurements as 1.03 min (Minto *et al*, 1997a), 1.41 ± 1.48 min (Egan *et al*, 1994), 1.23 ± 0.96 min (Hermann *et al*, 1999) and 1.6 ± 0.9 min (Egan *et al*, 1996). The ventilatory response to carbon dioxide has estimated the equilibration half-life to be 2.04 min (−0.24, 4.32 min: 95% confidence intervals) (Babenco *et al*, 2000). Analgesic measurements have estimated 1.31 ± 1.5 min (Glass *et al*, 1993) These previous estimates by alternative techniques are in agreement with our estimates of $t_{1/2I} = 1.83$ min (−2.17, 5.83 min: 95% confidence intervals) from the insular cortical BOLD response and $t_{1/2I} = 2.80$ min (−0.12, 5.73 min: 95% confidence intervals) for reported pain intensity.

The characteristic time of offset of action or context-sensitive half-life ($t_{1/2}$) (Hughes *et al*, 1992) is the time taken for the effect site concentration to fall by 50% following a variable length infusion. Owing to the rapid breakdown of remifentanil in the blood and tissues, this half-life does not vary with the duration of infusion (Minto *et al*, 1997a, b). EEG measurements have yielded a context-sensitive half-life of 3–5 min (estimated at 3.7 min from the population model, for volunteers in this study) (Minto *et al*, 1997b), in agreement with our fMRI estimate from the insular cortex $t_{1/2W} = 3.07$ min (1.09, 5.05 min: 95% confidence intervals), and from reported pain intensity $t_{1/2W} = 2.80$ min (1.12, 4.48 min: 95% confidence intervals). Half-life measurements based on concentration data give 3.2 ± 0.9 min (Kapila *et al*, 1995) and based on a pharmacodynamic measure of ventilation give 5.4 ± 1.8 min (Kapila *et al*, 1995).

In our analysis to yield characteristic times of action for remifentanil, we have assumed plasma remifentanil concentrations predicted from previously published pharmacokinetic data (Minto *et al*, 1997a), rather than measuring remifentanil concentration directly. However, given linear superposition of doses under first-order pharmacokinetics, estimates of half-lives would be little affected by a scaling of concentration between volunteers. Furthermore, the unwanted influence of interindividual variability in actual plasma levels was likely reduced by the use of pooled data from the sample population to perform modeling. The agreement, within estimated confidence intervals, of our pharmacokinetic parameters with previous measurements from other techniques, supports our simple fMRI-pharmacokinetic modeling procedure. However, the confidence and precision of our pharmacokinetic estimates might be improved by using a noxious stimulus for which a shorter interstimulus interval is possible, hence improving the time resolution.

Ventilatory changes and analgesia that have been used to characterize remifentanil in past studies are clinically relevant pharmacodynamic effects although they give no information about where the neural correlates of analgesia may occur. The clinical relevance of the change in spectral characteristics of the EEG arising from cortical neurons, on infusion of remifentanil, however is less clear. Unlike EEG spectral measurements, the fMRI data presented in this study relates specifically to the processing of pain signals that can be correlated with reported pain perception. Furthermore, the spatial localization and depth of penetration of EEG is poor compared to fMRI. Unlike EEG, fMRI has the potential to differentiate and visualize the influence of pharmacological agents on small brain structures, deep brain structures and hidden cortical areas such as the insular cortex. While PET is capable of revealing drug receptor occupancy, fMRI is better suited for studying complex paradigms such as those involving pain that may be modulated by drugs. The potential of fMRI for identifying the temporal dynamics of drug activity through clinically relevant changes in regional brain function has been demonstrated. Further experimentation to compare the temporal behavior of pain-related brain activity across many regions with subtle changes in pain perception may reveal those regions most critical for drug-induced analgesia.

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