FMRI Measurement of CNS Responses to Naloxone Infusion and Subsequent Mild Noxious Thermal Stimuli in Healthy Volunteers

M. C. Borras,* L. Becerra,* A. Ploghaus, J. M. Gostic, A. DaSilva, R. G. Gonzalez, and D. Borsook

Center for Functional Pain Neuroimaging and Therapy Research, Athinoula A. Martinos Center for Biomedical Imaging, Departments of Radiology and Neurology, Massachusetts General Hospital, Harvard Medical School, Massachusetts 02129

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Borras, M. C., L. Becerra, A. Ploghaus, J. M. Gostic, A. DaSilva, R. G. Gonzalez, and D. Borsook. FMRI measurement of CNS responses to naloxone infusion and subsequent mild noxious thermal stimuli in healthy volunteers. J Neurophysiol 91: 2723–2733, 2004; 10.1152/jn.00249.2003. The aims of this study were to assess the effects of a μ-opioid antagonist, naloxone, on endogenous opioid systems and to evaluate the effect of naloxone on the CNS response to mild noxious heat. Doubled-blinded experiments were performed in a cross-over design in 10 healthy male volunteers. Functional magnetic resonance imaging (fMRI) data were collected before and during the infusion and also during thermal stimuli. Increased signal was observed in a number of cortical and subcortical brain regions for naloxone versus saline infusion. Cortical activation was induced in regions including cingulate, prefrontal cortex, and insula. Subcortical regions showing increased signal change included hippocampus and entorhinal cortex. A 46°C stimulus delivered to the back of the hand induced an overall increase in activation in a number of regions in the naloxone group that were not seen in the saline group (e.g., insula, orbitofrontal cortex, thalamus, and hippocampus). These results show that naloxone, even in the absence of psychophysical effects, produces activation in several brain regions that are known to have high levels of μ-opioid receptors and may be involved in endogenous analgesia. Our study is an example of how fMRI can measure subtle changes in brain activation induced by pharmacological agents without cognitive effects.

INTRODUCTION

Endogenous opioids are involved in a number of physiological processes in the peripheral and central nervous systems (PNS and CNS). Within the CNS, endogenous opioids have been reported to act during analgesia, feeding, neuroendocrine control (including stress) (Bodnar and Hadjimarkou 2003), and modulation of neural excitability. Opioid antagonists attenuate the behavioral effects of some classes of addictive drugs (e.g., amphetamine) (Schad et al. 2002) and suppress ethanol intake in rats (Shoemaker et al. 2002). Some of these behavioral effects may be the result of opioidergic effects on motivation and reward (Kornetsky 1995; Martin-Söelch et al. 2001; Ozaki et al. 2002). A number of studies have evaluated the effects of μ opioid agonists (fentanyl, morphine, and on CNS activation in humans using imaging) (Adler et al. 1997; Firestone et al. 1996; Petrovic et al. 2002; Wise et al. 2002). We have recently examined the effects of morphine on CNS activation in drug naïve subjects and found out that this opioid activates a number of regions, including putative reward regions such as the sublenticular extended amygdala (SLEA), ventral tegmentum/periaqueductal gray (VT/PAG), nucleus accumbens (NAC), and orbital gyrus (GOB) (Becerra et al. 2001a).

There are several opioid receptor subtypes. The μ opioid receptor is found throughout the CNS (Kieffer et al. 1995; Pasternak et al. 1995) and has been implicated in pain modulation (Fields 1994; Millan 2002), mood alteration (Emrich 1982, 1984; Markoff et al. 1982), stress (Borsook et al. 1994; Schlüger et al. 1998), functional illness (Davis et al. 1979), and opioid abuse potential (Roache 1991). Naloxone, predominantly a μ antagonist, produces few obvious behavioral effects in drug naïve subjects at doses of <0.25 mg/kg (Martin del Campo et al. 1994; Wolkowitz and Tinklenberg 1985). However, naloxone may have effects in conditions in which opioid receptors have been modulated. For example, naloxone induces rapid reversal of morphine effects, creating an opioid withdrawal state. Opioid withdrawal, often experienced by drug addicts, is an aversive condition that can include increased sensitivity to noxious stimuli (hyperalgesia) (Launlin et al. 1988; Li and Clark 2002). This effect of naloxone is dramatic; even after a single dose of morphine hyperalgesia can be elicited within 45 min of infusing naloxone (Heishman et al. 1989).

The effects of naloxone are seen when endogenous opioid systems have been activated as well. Antagonism of endogenous μ opioids has been reported to modulate drug-induced CNS effects. For example, naloxone can attenuate the effect of cocaine (Bain and Kornetsky 1987) and precipitate morphine withdrawal (Lowe et al. 2002). Naloxone-induced antagonism of endogenous opioids also modulates the stress response (Drolet et al. 2001).

Naloxone effects on experimental pain have been widely reported. Some studies report that naloxone increased pain after noxious stimuli (Buchbaum et al. 1983), whereas others suggest that there is no alteration in pain threshold but an increase in pain-associated anxiety (Grevert and Goldstein 1977, 1978; Stacher et al. 1988). Naloxone administration also has effects on clinical pain: it both enhances baseline clinical pain and diminishes the analgesic effectiveness of a placebo (Grevert et al. 1983).

Although a few neuroimaging studies have reported activation in endogenous opioidergic systems in the brain after pain (Bencherif et al. 2002; Zubieta et al. 2001), no studies have examined the effects of the μ-antagonist, naloxone, on baseline CNS activity or on CNS activity in human subjects after...
painful stimuli. Our study addresses two fundamental questions. The first one is whether naloxone has any effect on normal tonic CNS activity as measured by fMRI in healthy volunteers with no history of addiction or opioid exposure. The second question addresses whether prior naloxone administration modulates the effect of mild noxious heat on the CNS.

In a previous study a using a similar noxious thermal paradigm in which heat was applied to the dorsum of the hand, more than one hemodynamic response could be temporally segregated into an early and late phase (Becerra et al. 2001). The two major responses displayed activation in primary sensory regions (classic pain circuitry) and regions involved in emotion (reward/aversion circuitry), respectively. Given that naloxone may affect both sensory and reward circuitry, we used this approach in this work.

METHODS

Ten healthy, right-handed, male volunteers (age = 31.5 ± 6.6 yr) with no known prior opioid exposure were recruited for this study. Informed consent was obtained from all subjects according to the protocol, which was approved by the Human Research Committee at the Massachusetts General Hospital. The study also complied with the guidelines of the Helsinki Accord and the IASP for experimental pain in humans.

Experimental paradigm

Subjects participated in two MRI scan sessions 1 wk apart. Naloxone or saline infusions were selected in a randomized, double-blinded, crossover design. Each session was subdivided into three functional acquisitions (Fig. 1A).

INFUSION SCAN. A 300-s baseline was acquired prior to the injection of a 10 ml solution of either saline or naloxone (4 mg) at 0.1 ml/s.

A Protocol

**Anatomical**

**Functional**

- Baseline
- Infusion
- Brush
- Heat
- Scan Time

**B Infusion Analysis**

- Infusion
- (3-block) Analysis
- Step function
- Scan Time

- Baseline
- 0
- 300
- 600
- 900
- 1200
- 21260

FIG. 1. A: experimental paradigm. Each functional magnetic resonance imaging (fMRI) session consisted of anatomical and functional scans. The functional scans had 3 parts: infusion, brush, and heat. During the infusion scan, which started by a 300-s baseline, either naloxone or saline solutions were injected during 100 s (●●●●●) after which the scanning session continued for 860 s (—). Brush and heat (46°C) scans consisted of 4 trials each. Each trial consisted of a 30-s stimulus (●●●●●) and a 25-s inter-stimulus. B: infusion analysis. The figure shows how the post infusion scan was analyzed in 3 blocks (see text).

The biological half-life of naloxone is ~1 h, and the onset of effects generally occurs within minutes of intravenous administration. The total scan time during this infusion experiment was 1,260 s.

BRUSH SCAN. Four brush stimuli were applied to the dorsum of the hand within a premarked area that corresponded to the heat probe using the brush side of a 1-cm-wide Velcro at a frequency of 1 Hz over a 25-s interval, each preceded by an inter-stimulus interval of 30 s. This functional scan was used as a control.

HEAT SCAN. Heat stimuli were delivered to the subjects using the FDA approved controlled thermal sensory analyzer (TSA; MEDOC, Haifa, Israel). The probe (3 × 3 cm) was strapped to the dorsum of the left hand with an elastic holder. The stimulus had a ramp rate of 4°C/s and produced a trapezoid-wave stimulus (Becerra et al. 1999). Four moderately painful stimuli (46°C, 25 s) were applied. Each thermal stimuli was preceded by a 30-s interstimulus interval at baseline temperature (35°C).

Physiological monitoring

Heart rate, respiratory rate, O2 saturation, and end tidal CO2 were monitored during the infusion scan and each subsequent scan for safety purposes. A laser Doppler system (Moore Instruments, Wilmington, DE) recorded cutaneous blood flow (BF) during all experiments to detect changes in autonomic function during drug infusion and noxious heat. The probe, attached to a fiberoptic cable was fed from outside the magnet and attached using double-sided tape to the fingertip.

Psychophysical data

Psychophysical data for hedonics and pain ratings were measured during either the infusion (hedonics) or sensory (heat, brush) stimuli. Subjects rated their sensations using a dial connected to a visual analog scale (VAS) on a projected screen throughout the experiment. Stimuli presentation and data collection were both controlled by a Macintosh LAB-VIEW system.

HEDONICS. Participants were informed of the identity of each infusion and told that they might experience a euphoric “high” (scale = 0 to +5) or dysphoric “low” (scale = 0 to –5).

HEAT AND BRUSH. During heat and brush stimuli, subjects rated pain unpleasantness and pain intensity, alternately, every 5 s. During the painful stimuli, the left end of the scale was labeled “no pain” and the right side of the scale as “maximum pain.” For unpleasantness, the left end was labeled “not unpleasant” and the right end as “maximum unpleasantness.” The difference between pain unpleasantness and pain intensity was explained to subjects prior to experiments using the approach reported previously (Price et al. 1980).

Imaging

A 3 T Siemens scanner with a quadrature head-coil was used to obtain brain images during anatomical and functional scans. The following parameters were used for the anatomical scans: TE = 6.6 ms, TR = 3.0 s, in plain resolution = 1 mm, slice thickness = 1 mm, and flip angle = 10°. Functional scans were acquired continuously in coronal orientation, perpendicular to the AC-PC line and centered around the AC point, with in plain resolution = 3.125 mm, slice thickness = 4 mm, and flip angle = 90°. For infusion scans, TE = 30 ms and TR = 4.3 s were used. Heat scans were acquired with TE = 30 ms and TR = 2.5 s. The field of view covered during functional scans included the entire brain except for the occipital lobe and the posterior cerebellum.
Image processing and statistical analysis

Statistical analysis was carried out using FSL 3.4 software (Center for Functional Magnetic Resonance Imaging of the Brain, University of Oxford, UK; www.fmrib.ox.ac.uk/fsl). Within FSL, BET (Brain Extraction Tool) was applied to all data files with a Gaussian spatial smoothing of 6 mm and high-pass temporal filtering. Each individual functional scan was registered to its high-resolution anatomical scan and Talairach-transformed using FLIRT (fMRI linear Image Registration Tool). Statistical maps were generated using FEAT within FSL; FEAT uses a generalized linear model approach.

INFUSION SCAN. We utilized a three-block design (see Fig. 1B). Each block was 1/3 of the post infusion time, and each block was compared with baseline. The rationale for this approach was to capture changes over time (i.e., initial changes induced by drug vs. ongoing changes). Each block was convolved with a gamma function (peak time = 3 s, mean lag = 6 s).

BRUSH SCAN. The model for this scan was a convolution of a square wave representing the four stimuli with the same gamma function used for infusion.

HEAT SCAN. In a previous study (Becerra et al. 2001), we described the hemodynamic response to painful stimuli as composed of two sequential phases (early and late) of ~12-s duration each. The same approach was used here. Briefly, each thermal stimulus was divided into two components (early and late phases) of equal duration (12 s) and convoluted with a gamma function similar to the one used for the infusion analysis. Statistical maps were generated for each phase.

Group analysis

A multi-subject statistical analysis was used to obtain activation maps for each experimental stimulus. The naloxone group was compared with the saline group using random effect analysis. Uncorrected thresholding, P < 0.05 (Phelps et al. 2001), was used to render the infusion activation statistical maps reported below. Both positive and negative activation maps were obtained and displayed using MEDx (Sensor Systems).

Global BOLD signal comparison between naloxone and saline

Raw signals taken over the whole brain were compared in the naloxone and saline infusion data to determine whether global perfusion changes were potentially influencing the focal fMRI activations. Each whole brain signal was demeaned using its corresponding pre-

![Figure 2](image_url)

**FIG. 2.** Skin blood flow, in arbitrary units, for infusion (A) and heat stimuli (B) after infusion. Both plots show (black solid) naloxone and (gray dotted) saline. In each plot, the times during which infusion or stimuli were applied are delimited by gray rectangles. No significant differences (P > 0.05) were found between naloxone and saline sessions in neither infusion nor heat measurements. Data in A have been smoothed for display.
infusion baseline value. A Student t-test was used to determine whether naloxone and saline infusion scans showed significant differences in global BOLD signal.

RESULTS

Skin blood flow
INFUSION. Infusion of saline or naloxone had little or no effect on skin blood flow (Fig. 2A) as measured by the laser Doppler (paired, 2-tailed, t-test: P > 0.05).

HEAT STIMULI. During heat administration, there is a decrease in skin blood flow in most cases for both saline- and naloxone-treated subjects (Fig. 2B; heat 1–heat 4). No significant differences were obtained between the two infusions (paired, 2-tailed, t-test: P > 0.05).

Psychophysical ratings
HEDONICS. No significant differences in the on-line hedonic scale were observed between saline and naloxone during the course of the infusion.

PAIN INTENSITY AND PAIN UNPLEASANTNESS RATINGS. During the heat stimuli, subjects rated pain intensity and unpleasantness on a VAS scale (see METHODS). The average VAS ratings during these stimuli are shown in Fig. 3A, where pain and unpleasantness values are represented alternately every 5–7 s (Fig. 3, B and C). Note that VAS levels increase to between 3/10 and 5/10 four separate times, corresponding with the application of the four noxious thermal stimuli. Pain and unpleasantness ratings were analyzed separately, and comparisons of naloxone with saline were performed using paired, Student’s t-test, for both pain intensity and unpleasantness (Tables 1 and 2). Note from the data presented in the tables, that there is a consistency between pain unpleasantness and pain intensity. In Table 1, there is no significant difference between early and late phases for intensity versus unpleasantness for either saline or naloxone. However, as shown in Table 2, there is a significant difference in the late phase after heat for both pain intensity and pain unpleasantness for naloxone versus saline.

<table>
<thead>
<tr>
<th>TABLE 1. Intensity vs. unpleasantness</th>
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<tr>
<td></td>
</tr>
<tr>
<td>---------------------------------------</td>
</tr>
<tr>
<td>Early</td>
</tr>
<tr>
<td>Late</td>
</tr>
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</table>

FIG. 3. Visual analog scale (VAS) rating for heat after infusion. A: averaged VAS among 8 subjects, where pain and unpleasantness are rated every 5 s for naloxone (black solid) and saline (dotted gray). The times during which heat stimuli were applied are delimited by dotted (early phase) and gray (late phase) rectangles. B: pain intensity ratings for saline (circles) and naloxone (triangles) taken every 5 s. Significant differences (P < 0.05) were found between naloxone and saline sessions only for the late phase (see Tables 1 and 2). C: pain unpleasantness ratings for saline (circles) and naloxone (triangles) taken every 5–7 s. Significant differences (P < 0.05) were found between naloxone and saline sessions only for the late phase (see Tables 1 and 2).
fMRI results

GLOBAL BOLD SIGNAL COMPARISON BETWEEN NALOXONE AND SALINE. The raw fMRI signal from naloxone and saline was not statistically different across matched time points (Student’s t-test; \( P > 0.05 \)). See Fig. 4.

NALOXONE VERSUS SALINE INFUSION. Statistical maps for each block were generated. Significant changes were only observed in the first block. This block correlates with the onset of the drug effect on the brain (the intravenous injection was administered over 6 min). When compared with the saline infusion, the naloxone infusion produced a number of significant activations in cortical and subcortical regions. The statistical maps are shown in Fig. 5, A and B, and Tables 3 and 4 for positive and negative activations, respectively.

Cortical activations. Naloxone produced positive significant activations in a number of cortical regions. These included: perigenual cingulate (GC), dorsolateral prefrontal cortex (GFi), claustrum (Cl), insula (INS), entorhinal cortex (ENT), and parahippocampal cortex (GH; Table 3). Negative activation (decreased signal change) in cortical regions was obtained just at the perigenual cingulate (GC) and orbitofrontal gyrus (GOb; Table 4; Fig. 5, A and B).

Subcortical activations. The same group analysis of naloxone versus saline infusions revealed positive activation after naloxone in the following subcortical regions: head of caudate (NC), nucleus accumbens (NAC), nucleus subthalamicus/substantia nigra (Ns/SN), hippocampus (Hi), lingual gyrus (GL), and cerebellar vermis. Significant decrease signal change in subcortical regions was only observed in the hypothalamus. Most of these regions show small changes in signal (see Tables 3 and 4), but GC, GFi, NC, Hi, GH, GL, and Hy were significantly activated with a \( Z \) value >2.3 (\( P < 0.01 \); Fig. 5, A and B).

NALOXONE EFFECTS ON NOXIOUS HEAT. The statistical maps of the analysis are shown in Fig. 6, A and B, and summarized in Tables 5 and 6. The levels of activation during heat stimuli were all beyond the threshold of \( Z = 2.3 \) (i.e., greater than the scores obtained during just infusion). Areas activated included both cortical and subcortical regions.

Cortical regions. When activation after heat was compared in naloxone-infused subjects versus saline-infused subjects, the following regions showed a significantly larger increase in activation with naloxone: GFi, lateral prefrontal cortex (GFm), globus pallidus (GP), gyrus fusiformis (GF), GH, GL, GOb, GC, and INS. No negative activations were observed with these thresholds.

Subcortical regions. Thalamus, NC, claustrum, and hippocampus were activated at the subcortical level. This last region, Hi, experienced the strongest of all activations induced by heat after infusion. No significant subcortical negative activations were observed.

NALOXONE EFFECTS ON BRUSH. Brush stimuli were used as control when comparing naloxone with saline infusions because the drug has no known effects on nonnoxious mechanical stimuli. Following our subtraction analysis, no significant activation was observed in the cortical regions corresponding to the SI area. Furthermore, no significant activation was found for the brush stimulus in other primary somatosensory pathways (i.e., lateral thalamus) supporting the result for the lack of cortical activation. However, activation was observed in the parahippocampus and entorhinal cortex with a \( Z \) value >2.3 (\( P < 0.01 \)).

DISCUSSION

In a cohort of 10 subjects, we observed that naloxone produces changes in BOLD activity in CNS pathways and significantly modulates the CNS response to a mild noxious heat stimulus. No significant changes in hedonics recorded during infusion were observed, demonstrating, as in prior studies, that naloxone, at low doses, has no effect on mood (Zacny et al. 1994). In response to a mild noxious heat stimulus, significant differences between pain intensity and pain unpleasantness were observed (see following text).

FIG. 4. BOLD for whole brain after infusion of (black solid) naloxone and (gray dotted) saline. The mean blood flow of preinfusion baseline (0–300 s) has been subtracted from each infusion. No significant difference was found between naloxone and saline fMRI signals (\( t \)-test, \( P > 0.05 \)). Data have been smoothed for display.

<table>
<thead>
<tr>
<th>Intensity</th>
<th>Unpleasantness</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early</td>
<td>0.6596</td>
</tr>
<tr>
<td>Late</td>
<td>&lt;0.0001 (( t = 6.3 ))</td>
</tr>
</tbody>
</table>

TABLE 2. Naloxone vs. saline
Naloxone induces activation in the CNS

Naloxone-specific activation changes were found in a number of cortical regions including the frontal cortex (positive activations in GFi and GFm; negative activation in GOb), the anterior insula, and the entorhinal and parahippocampal cortices (positive activations). Significant subcortical activations were observed in the substantia nigra/subthalamic nucleus, the head of the caudate, and the nucleus accumbens. Some additional activation was observed in the cerebellum.

The middle orbitofrontal cortex and the lateral prefrontal cortex are involved in processing aversive and rewarding stimuli, respectively (O’Doherty et al. 2001). Most studies reporting activation in these regions evaluate responses to stimuli that are clearly rewarding or aversive. Here we observe activation in these areas following a stimulus with no effects discernable to the subject. Such activation may represent a subtle resetting of the threshold for activation of aversive/reward circuitry.

Naloxone has been reported to enhance blood flow within the hippocampus/entorhinal cortex via afferent projections from the septal region (Nishimura et al. 1992). The entorhinal cortex primes responses that are adaptive to an aversive input, such as the motor response necessary for escape from a threatening environment. Enhanced activation in this region after naloxone infusion indicates a shifted baseline, potentially lowering the threshold for activation of adaptive responses.
TABLE 3. Group analysis of positive activation for infusion (naloxone vs. saline)

<table>
<thead>
<tr>
<th>Region</th>
<th>Talaraich Coordinates</th>
<th>Z Score</th>
<th>P Value (×10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical areas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC (32), perigenu cingulate</td>
<td>-13 41 2</td>
<td>2.66</td>
<td>3.9</td>
</tr>
<tr>
<td>GFi (46), dorsolat. prefront. CX</td>
<td>-34 37 10</td>
<td>2.55</td>
<td>5.4</td>
</tr>
<tr>
<td>CI/INS, claustrum/ant. insula</td>
<td>-32 4 -12</td>
<td>2.28</td>
<td>11</td>
</tr>
<tr>
<td>GH (35), parahippocampal CX</td>
<td>-13 -44 -1</td>
<td>3.03</td>
<td>1.2</td>
</tr>
<tr>
<td>Subcortical areas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NS/SN, subthal. nucl./substan. nigra</td>
<td>6 -15 -12</td>
<td>2.15</td>
<td>16</td>
</tr>
<tr>
<td>NC, head of caudate</td>
<td>-25 -15 -8</td>
<td>2.53</td>
<td>5.7</td>
</tr>
<tr>
<td>NAccs, nucleus accumbens</td>
<td>8 8 -5</td>
<td>1.66</td>
<td>48</td>
</tr>
<tr>
<td>Hi, hippocampus</td>
<td>-22 -25 -14</td>
<td>2.6</td>
<td>4.7</td>
</tr>
<tr>
<td>ENT (28), entorhinal CX</td>
<td>19 -25 -16</td>
<td>2.02</td>
<td>22</td>
</tr>
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<td>GL (19), lingual gyrus</td>
<td>-20 -52 -8</td>
<td>3.59</td>
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</tr>
<tr>
<td>Ver, cerebellar vermis</td>
<td>-2 -52 -22</td>
<td>2.28</td>
<td>11</td>
</tr>
</tbody>
</table>

Brodman Areas are in parentheses.

In subcortical regions, there are three areas of interest with significant naloxone activation. These are the ventral tegmen-
tum/substantia nigra, the head of the caudate, and the nucleus accumbens. Activations in these regions may reflect alterations in dopaminergic input from the ventral striatum. The nucleus accumbens has μ receptors (Zubieta et al. 2001), thus changes in activation may be due to direct effects of μ receptor block-
ade in this region; however, indirect inputs may also be in-
volved. Stimulation of μ-receptors in the ventral tegmental area, the site of origin of A10 dopaminergic neurons projecting to the accumbens, increases dopamine release in the accumbens. Blockade of these receptors produces the opposite effect (Benjamin et al. 1993; Taber et al. 1998). Tonic activation of μ and kappa receptors is required for the maintenance of basal dopamine release in the nucleus accumbens (Spanagel et al. 1992), thus naloxone likely alters dopamine levels in the NAc.

The caudate nucleus has high densities of opioid receptors and opioid peptides (Delfs et al. 1994; Mansour et al. 1994). Specific connections are found between the entorhinal cortex and the caudate nucleus (Totterdell and Meredith 1997). Thus the naloxone activation seen in the ENT may also contribute to activation in the caudate. The caudate is involved in information-processing tasks, including the representation of goal-directed behaviors (Hollerman et al. 2000). The entorhinal cortex may prime activation in the caudate as part of a program to plan activity to avoid an aversive event.

Naloxone effects on CNS activation by thermal pain

In most pain studies, very little difference between pain intensity and pain unpleasantness have been shown for noxious heat (Price et al. 1980). If these two categories can be separated in nonpathological states (i.e., by pharmacological) manipulation, insights into CNS circuits that define these two psycho-
logical dimensions may be dissected. In this experiment, we measured these two psychological variables on-line. For the mild thermal stimulus, we found that naloxone produced a significant difference in the late phase for both pain intensity and pain unpleasantness (Table 2). Given that during this phase, predominantly sensory discriminative activation patterns are observed (Becerra et al. 2001) we interpret this as an effect of predominantly μ opioid blockade producing increased sensitivity within these circuits to a painful stimulus. The reason that these do not become uncoupled and that pain unpleasantness is not increased during the early phase is un-
clear because this measure may be expected to be correlated with emotional circuitry.

Heat-induced BOLD signal activation in a number of re-
gions was specifically altered by the presence of naloxone. In the current work, when the CNS response to noxious heat is divided into two temporally and functionally discrete phases (i.e., early and late), we observe that naloxone produces activation preferentially in pathways involved in emotional/motiva-
tional evaluation of sensory information.

Early phase activations were observed in frontal regions including the GFi and Gfm, the globus pallidus (GB), and the parahippocampal cortex (GH). Subcortical activations were observed in the caudate nucleus (NC) and thalamus (Th). Thalamic activation was in the mediodorsal and dorsolateral regions of the structure but not in the primary sensory pathways. Although activation differences were observed, there

TABLE 4. Group analysis of negative activation for infusion (naloxone vs. saline)

<table>
<thead>
<tr>
<th>Region</th>
<th>Talaraich Coordinates</th>
<th>Z Score</th>
<th>P Value (×10^-3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical areas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GC (24), perigenu cingulate</td>
<td>6 30 10</td>
<td>2.01</td>
<td>22</td>
</tr>
<tr>
<td>GOb (11), orbitofrontal gyrus</td>
<td>-18 30 -9</td>
<td>2.55</td>
<td>5.4</td>
</tr>
<tr>
<td>Subcortical areas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hy, hypothalamus</td>
<td>4 -1 -10</td>
<td>2.6</td>
<td>4.7</td>
</tr>
</tbody>
</table>

Brodman Areas are in parentheses.
was no difference in VAS scores for pain intensity during the early phase of the noxious stimulus. The latter suggests that naloxone effects may be primarily on nonsensory emotional pathways.

In the late phase, fewer regions showed significant naloxone-specific modulation of noxious heat-induced activations. The regions included the orbitofrontal cortex (GOb), the perigenual cingulate (GC), the insula (INS), and the hippocampus. The hippocampus and orbitofrontal cortex showed the most significant changes.

The observed naloxone activations may represent CNS responses due to fear, stress, or any other emotional reaction to the applied aversive thermal stimulus. Naloxone has been shown to increase aversive responses in rat models (Eichenberger et al. 2002; Shippenberg and Bals-Kubick 1995) and to inhibit descending control-diffuse inhibitory effects in humans.

**TABLE 5.** Group analysis of positive activation for early phase of heat after infusion (naloxone vs. saline)

<table>
<thead>
<tr>
<th>Region</th>
<th>Talaraich Coordinates</th>
<th>Z Score</th>
<th>P Value ($\times 10^{-3}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cortical areas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GFi (47), orbitofrontal CX</td>
<td>31 37 -9</td>
<td>3.05</td>
<td>1.14</td>
</tr>
<tr>
<td>GFm (46), lateral prefrontal CX</td>
<td>38 30 24</td>
<td>3.29</td>
<td>0.50</td>
</tr>
<tr>
<td>GP, globus pallidus</td>
<td>-15 1 -1</td>
<td>2.90</td>
<td>1.87</td>
</tr>
<tr>
<td>GF, gyrus fusiformis</td>
<td>-36 -13 -31</td>
<td>2.84</td>
<td>2.26</td>
</tr>
<tr>
<td>GH (36), post. parahippocampal CX</td>
<td>24 -23 -19</td>
<td>2.60</td>
<td>4.70</td>
</tr>
<tr>
<td>GL (37), lingual gyrus</td>
<td>22 -50 -12</td>
<td>3.38</td>
<td>0.36</td>
</tr>
<tr>
<td>Subcortical areas</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NC, nucleus caudatus</td>
<td>-11 18 -1</td>
<td>3.22</td>
<td>0.64</td>
</tr>
<tr>
<td>Th, thalamus</td>
<td>-10 -13 10</td>
<td>2.40</td>
<td>8.20</td>
</tr>
</tbody>
</table>

Brodman Areas in parentheses.
effect on blockade of endogenous opioids, including interactions between opioids and GABA (Haefely 1983) and between opioids and tachykinin receptors (Ribeiro et al. 1998). Areas of activation by naloxone observed in this study, such as the hippocampal gyrus, have been implicated in stress responses, including unconditional fear (Kjelstrup et al. 2002) and anxiety (Ploghaus et al. 2001). Furthermore, there is evidence of an interaction between endogenous opioid peptides and the dopaminergic mesocorticolimbic system in behavioral responses to stress. Frontal brain regions are also implicated in this interaction.

In an attempt to document autonomic changes that occur during noxious stimulation, we measured ipsilateral blood flow during the heat stimulus as we have done previously (Papanicolas et al. 1999). Skin blood flow displayed a consistent decrease throughout the stimulus and returned to “baseline” levels after the stimulus was terminated. The finding that changes in blood flow occur during noxious heat even at a site distant from the stimulus is consistent with a number of studies using laser Doppler to correlate changes in skin blood flow with painful stimuli (Danilov et al. 1994; Kurvers et al. 1997; Ochoa et al. 1993). Such changes reflect systemic autonomic function. We did observe changes in the hypothalamus during each heat stimulus with both naloxone and saline; however, no differences in these regions were observed between the two infusion types.

Caveats

Naloxone is devoid of agonist properties; however, it is most likely a direct consequence of the drug. Further studies are required to evaluate the effects of higher doses of naloxone.

Conclusion

The outcome of our study can be summarized in four points: neural activation produced by a drug without “cognitive” effects (when given at low doses) in the normal brain can be measured using fMRI, and fMRI analysis may be useful in studying the effects of drugs that are given chronically and require a significant lead time (i.e., weeks) to become effective; naloxone affects circuits where μ-opioid neurons, or their projections, are present; regions commonly activated by rewarding stimuli (including opioids), such as the prefrontal cortex and Nac, are activated by naloxone; and the effect of naloxone on perceived pain intensity and BOLD signals in response to noxious thermal stimuli supports the contention that endogenous opioids regulate CNS processing of pain input. It is likely that the CNS regions where activation by noxious heat is modulated by naloxone are the sites of action of endogenous opioid pathways involved in regulating CNS response to aversive stimuli.

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