

Activation of the Opioidergic Descending Pain Control System Underlies Placebo Analgesia

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SUMMARY

Placebo analgesia involves the endogenous opioid system, as administration of the opioid antagonist naloxone decreases placebo analgesia. To investigate the opioidergic mechanisms that underlie placebo analgesia, we combined naloxone administration with functional magnetic resonance imaging. Naloxone reduced both behavioral and neural placebo effects as well as placebo-induced responses in pain-modulatory cortical structures, such as the rostral anterior cingulate cortex (rACC). In a brainstem-specific analysis, we observed a similar naloxone modulation of placebo-induced responses in key structures of the descending pain control system, including the hypothalamus, the periaqueductal gray (PAG), and the rostral ventromedial medulla (RVM). Most importantly, naloxone abolished placebo-induced coupling between rACC and PAG, which predicted both neural and behavioral placebo effects as well as activation of the RVM. These findings show that opioidergic signaling in pain-modulating areas and the projections to downstream effectors of the descending pain control system are crucially important for placebo analgesia.

INTRODUCTION

Placebo effects are ubiquitous in modern medicine, not only as a control in randomized controlled trials but also as a subject of intense study in diverse clinical fields, such as Parkinson's disease, depression, immune function, and pain (Benedetti et al., 2005; Enck et al., 2008; Price et al., 2008). One of the best-studied placebo effects is placebo analgesia, where the administration of a pharmacologically inert substance has a pain-relieving effect, presumably due to the subject's expectation that a potent analgesic substance is being administered (Montgomery and Kirsch, 1997; Pascalis et al., 2002; Price et al., 1999; Vase et al., 2005).

Pharmacological challenge studies using the opioid antagonist naloxone (Amanzio and Benedetti, 1999; Grevert et al., 1983; Levine and Gordon, 1984; Levine et al., 1978) and positron emission tomography (PET) studies using μ -opioid selective tracers (Scott et al., 2008; Wager et al., 2007; Zubieta et al., 2005) have identified an important role of the endogenous opioid system in both clinical (Levine and Gordon, 1984; Levine et al., 1978) and experimental pain (Amanzio and Benedetti, 1999; Grevert et al., 1983; Scott et al., 2008; Wager et al., 2007; Zubieta et al., 2005). The tracer PET studies have demonstrated release of endogenous opioids under placebo analgesia in regions associated with pain modulation (Bingel and Tracey, 2008; Fields et al., 2006), including dorsolateral prefrontal cortex (DLPFC), rostral anterior cingulate cortex (rACC), and periaqueductal gray (PAG). Other (neurochemically nonspecific) imaging studies have shown enhanced responses in the same brain regions under placebo analgesia (Bingel et al., 2006; Kong et al., 2006; Petrovic et al., 2002; Wager et al., 2004). Importantly, several functional magnetic resonance imaging (fMRI) studies and one PET study have also demonstrated placebo-induced signal decreases in pain-sensitive brain regions, such as thalamus, insula, and dorsal anterior cingulate cortex (Bingel et al., 2006; Lieberman et al., 2004; Price et al., 2007; Wager et al., 2004), most likely related to the pain reduction experienced under placebo.

With regard to an underlying mechanism that can explain these observations, it has been hypothesized that placebo analgesia recruits the opioidergic descending pain control system (Basbaum and Fields, 1984; Fields et al., 2006; Millan, 2002), the activation of which leads to inhibition of nociceptive processing at the level of the spinal cord and thus to reduced neural responses in pain-responsive brain regions and a concurrently reduced pain experience. This pain control network presumably includes cortical structures, such as the DLPFC and rACC, but also includes the amygdala, hypothalamus, PAG, and rostral ventromedial medulla (RVM). The above-mentioned fMRI and tracer PET studies have provided evidence for DLPFC, rACC, and, in some cases, PAG involvement in placebo analgesia, and behavioral findings suggest a spinal component (Matre et al., 2006). However, conclusive evidence for the involvement of the lower opioid system (amygdala, hypothalamus, PAG, and RVM) and especially its functional relevance (i.e., relation

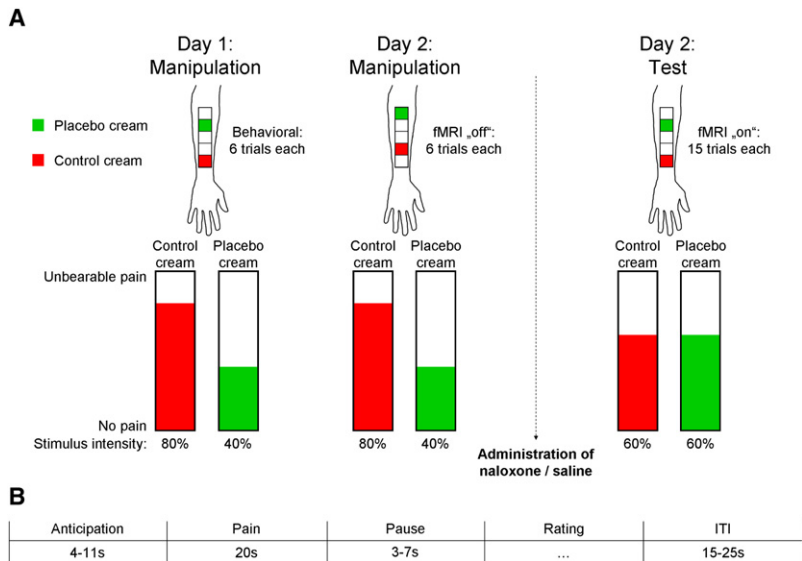


Figure 1. Experimental Paradigm

(A) The experiment took place on two consecutive days and consisted of three phases: manipulation day 1, manipulation day 2, and test day 2. Before each phase, subjects were treated with two identical creams on their left forearm. Subjects were told that one cream was a highly effective pain reliever, whereas the other served as sensory control. During the manipulation phases (which consisted of six trials under placebo cream and control cream, respectively), painful stimulation on the placebo-treated patch was surreptitiously lowered (from 80 [score on a visual analog scale (VAS)] under control to 40 under placebo) to convince the subjects that they had received a potent analgesic cream and to create expectations of future pain relief when treated with this cream. On day 2, the manipulation phase was carried out inside the (resting) MR scanner, to reactivate and strengthen the expectations of pain relief in this context. Before the test phase started, subjects either received an injection of saline or naloxone. fMRI data were collected during the test phase, which consisted of 15 trials under each condition. Importantly, the strength of painful stimulation was identical on both skin patches (60 on a VAS), in order to test for placebo analgesic effects.

(B) Each trial consisted of an anticipation phase (red crosshairs), the painful stimulation, a short pause, the pain rating, and an intertrial interval (ITI). During the ITI, subjects saw white crosshairs in the center of the screen, which changed color to red at the beginning of the anticipation phase, signaling to the subjects that the painful stimulation would soon follow. After the painful stimulation, subjects had to rate the pain intensity on a VAS.

to pain experience and neural responses in pain-sensitive areas) is scarce, also because the spatial resolution of previous studies was less sensitive for the analysis of these small structures.

Testing the hypothesized mechanism of descending pain control during placebo analgesia requires an experimental approach that is sensitive to neural responses in pain-encoding and pain-modulating areas and to their dependence on endogenous opioid neurotransmission. Neither tracer PET nor fMRI alone can satisfy these two conditions, as tracer PET is not sensitive to increases or decreases in neural responses (or an equivalent thereof) and standard fMRI cannot make inferences about neurochemical events. We therefore employed pharmacological fMRI (Honey and Bullmore, 2004; Leslie and James, 2000) in two groups of subjects ($n = 48$): one receiving the opioid antagonist naloxone and one receiving saline. This allowed us to compare both behavioral and blood-oxygen-level-dependent (BOLD) responses under a “natural opioid state” and a “blocked opioid state.” To also allow investigation of BOLD responses in lower brainstem areas, we made use of higher spatial resolution in comparison to previous imaging studies on placebo-analgesia

and employed a brainstem-dedicated image preprocessing strategy. We used an established placebo analgesia paradigm that included an initial expectation manipulation phase and a later test phase (Price et al., 1999; Wager et al., 2004, 2007; Figure 1). In a double-blind procedure, naloxone was administered just before the test phase, in order to have the same treatment expectations in both groups.

RESULTS

Placebo Effects in Behavior and BOLD Responses

We first tested whether our experimental manipulation resulted in a behaviorally measurable placebo analgesic effect and whether this effect would be reduced in the naloxone group. Pain ratings showed a significant main effect of condition ($F_{(1,38)} = 30.76, p < 0.001$), as well as a significant group-by-condition interaction ($F_{(1,38)} = 5.53, p = 0.01$; Figure 2A). More specifically, while a placebo effect was evident across the whole sample (23% reduction in pain ratings under placebo compared to control), this effect was significantly stronger in the saline

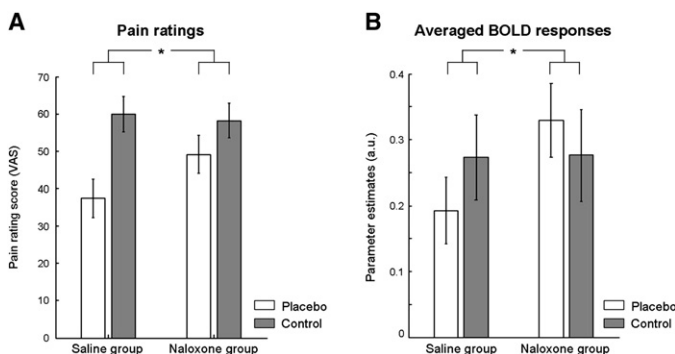


Figure 2. Behavioral and Neural Placebo Effects

(A) The pain ratings show that placebo effects are significantly weaker in the naloxone group. The influence of naloxone is specific for the placebo condition, as pain ratings under the control condition are almost identical across groups.

(B) Naloxone also blocks “neural placebo effects” (i.e., reduced BOLD responses under placebo compared to control), as seen in the parameter estimates averaged across all pain-responsive regions (see Table S1 for a list of included regions). *: significant group-by-condition interaction at $p \leq 0.05$. Error bars indicate SEM.

Table 1. BOLD Responses (Control > Placebo) in Pain-Responsive Regions

Region	x	y	z	t Value	p Value
Early Pain: Control > Placebo					
Saline Group					
Basal ganglia	18	8	8	3.47	p < 0.001
Insula	-34	0	-6	3.18	p < 0.001
Early Pain: Control > Placebo					
Saline Group > Naloxone Group					
Basal ganglia	12	14	14	3.41	p < 0.001
Basal ganglia	18	8	6	2.86	p < 0.003
Insula	28	24	-2	2.69	p < 0.004
Late Pain: Control > Placebo					
Saline Group					
dACC	0	28	22	3.43	p < 0.001*
Amygdala	28	-6	-14	3.33	p < 0.001
Insula	48	-4	16	2.96	p < 0.002
Basal ganglia	26	8	14	2.87	p < 0.003
Insula	-46	-18	0	2.83	p < 0.003
Basal ganglia	-28	10	-2	2.82	p < 0.003*
SI	22	-30	56	2.82	p < 0.003
Insula	-44	-2	4	2.80	p < 0.003
Insula	-36	2	-6	2.78	p < 0.003
Amygdala	-24	-2	-18	2.73	p < 0.004*
SI	-28	-28	62	2.71	p < 0.004
Pons	2	-32	-34	2.68	p < 0.004*
SI	-28	-28	50	2.65	p < 0.005
Late Pain: Control > Placebo					
Saline Group > Naloxone Group					
Thalamus	6	-16	6	3.81	p < 0.001
Insula	32	14	-6	3.45	p < 0.001
Amygdala	30	-6	-14	3.41	p < 0.001
Insula	46	18	12	3.25	p < 0.001
dACC	-4	26	20	3.14	p < 0.001+
Basal ganglia	8	14	-10	3.01	p < 0.002
dACC	4	24	22	2.99	p < 0.002
Thalamus	-12	-12	4	2.86	p < 0.003
SI	-28	-28	50	2.81	p < 0.003
dACC	8	34	18	2.79	p < 0.003
Basal ganglia	-10	10	-10	2.67	p < 0.005
SII	36	-18	26	2.64	p < 0.005
Thalamus	6	-6	2	2.64	p < 0.005+

Contrasts (bold typeface) are listed according to the appearance in the main text. Coordinates are denoted by x, y, z in mm (MNI-space), and strength of activation is expressed in t scores (df = 76). dACC, dorsal anterior cingulate cortex; SI, primary somatosensory cortex; SII, secondary somatosensory cortex. *p < 0.05 corrected; +p < 0.08 corrected for the dACC and p < 0.06 corrected for the thalamus.

group (36% reduction) than in the naloxone group (10% reduction). As can be seen in Figure 2A, the effect of naloxone was specific for the placebo condition, since the ratings for the

control condition did not differ between groups. Skin conductance responses (SCR) to the painful stimulation showed a significant group-by-condition interaction in the same direction as the pain ratings ($F_{(1,37)} = 2.91$, $p = 0.05$), again indicating that placebo analgesia was impaired by naloxone administration.

Next, we tested whether BOLD responses in pain-responsive brain regions would mirror the behavioral data by showing reduced activation under the placebo condition compared to the control condition and whether this difference would in turn be reduced by naloxone. Based on previous observations, which showed that placebo effects in BOLD responses are observed in a late phase of painful stimulation (Price et al., 2007; Wager et al., 2004), we expected this effect in the second half of the 20 s pain stimulus. To obtain a global estimate of naloxone influence on neural placebo effects, we identified pain-responsive brain regions across both groups by a conjunction analysis (Table S1) and averaged the parameter estimates across all identified regions for each subject and condition. This analysis resulted in a significant group-by-condition interaction during late pain ($F_{(1,38)} = 2.84$, $p = 0.05$; Figure 2B) but not during early pain ($F_{(1,38)} = 0.0003$, n.s.), indicating that naloxone significantly reduced neural placebo effects during late pain.

In a second analysis, we tested which brain regions showed significant neural placebo effects (i.e., weaker BOLD responses in the placebo condition than in the control condition) in the saline group and in which regions naloxone reduced these effects. During early pain, such effects were only observed in the insula and the basal ganglia, whereas during late pain such effects were much more widespread (Table 1): during late pain, the saline group showed significant neural placebo effects in the dorsal anterior cingulate cortex (dACC), the amygdala, the insula, the basal ganglia, the somatosensory cortex, and the pons. A significant reduction of these effects by naloxone was observed in the thalamus, the insula, the amygdala, the dACC, the basal ganglia, and the somatosensory cortex.

Responses in the Descending Pain Control System

More importantly, we investigated whether regions implicated in descending pain control (DLPFC, rACC, amygdala, hypothalamus, PAG, and RVM) would show stronger responses under placebo as compared to control in the saline group and whether this difference would in turn be reduced by naloxone. The DLPFC showed significant activations under placebo compared to control (Table 2 and Figure 3a) as well as a modulation by naloxone. The rACC exhibited two types of responses: at a more ventral location in subgenual rACC, we observed an increase in activation under placebo as compared to control (Table 2 and Figure 3B), whereas in pregenual rACC, we observed a strong deactivation under placebo as compared to control (Table 2 and Figure 3C). Only the response in pregenual rACC was significantly affected by naloxone. While both types of rACC responses have been reported in previous fMRI studies on placebo analgesia (Bingel et al., 2006; Wager et al., 2004), specifically opioid-dependent deactivations in pregenual rACC have been linked to antinociceptive processes (Eippert et al., 2008). Note that the placebo-induced responses in DLPFC and rACC and their modulation by naloxone were only observed during early pain, not during late pain (Table 2).

Table 2. BOLD Responses (Placebo > Control) in Pain-Modulatory Cortical Regions

Region	x	y	z	t Value	p Value
Early Pain: Placebo > Control					
Saline Group					
DLPFC	22	12	38	3.78	p < 0.001*
Subgenual rACC	16	36	-12	3.70	p < 0.001*
Pregenual rACC	-12	38	0	3.44	p < 0.001*
DLPFC	34	12	48	3.03	p < 0.002
DLPFC	24	12	52	2.71	p < 0.004
Subgenual rACC	-16	34	-14	2.69	p < 0.004
Early Pain: Placebo > Control					
Saline Group > Naloxone Group					
DLPFC	22	16	38	3.50	p < 0.001 ⁺
Pregenual rACC	-12	38	0	3.26	p < 0.001 ⁺
DLPFC	-30	24	28	2.95	p < 0.002
Late Pain: Placebo > Control					
Saline Group					
—	—	—	—	—	—
Late Pain: Placebo > Control					
Saline Group > Naloxone Group					
—	—	—	—	—	—

Contrasts (bold typeface) are listed according to the appearance in the main text. Coordinates are denoted by x, y, z in mm (MNI-space), and strength of activation is expressed in t scores (df = 76). DLPFC, dorsolateral prefrontal cortex; rACC, rostral anterior cingulate cortex. *p < 0.05 corrected; ⁺p < 0.06 corrected for the DLPFC and p < 0.06 corrected for the pregenual rACC. Note that the pregenual rACC is listed here although it shows a strong deactivation under placebo.

To further investigate subcortical effects with higher sensitivity, we used an additional image preprocessing strategy optimized for deep-brain structures, including amygdala, hypothalamus, PAG, and RVM (see [Experimental Procedures](#) for details). In contrast to the responses in DLPFC and rACC, which were only present during the early pain phase in the saline group, we observed responses in the hypothalamus, PAG, and RVM that were similarly present during early and late pain ([Table S2](#)). Using a regressor that spanned the entire pain interval (i.e., 20 s length) resulted in significant activation in the hypothal-

amus (-3 -9 -7, $t_{(76)} = 3.34$, $p < 0.001$), the PAG (-3 -35 -17, $t_{(76)} = 2.97$, $p < 0.002$; -1 -37 -22, $t_{(76)} = 2.77$, $p < 0.004$), and the RVM (-5 -33 -39, $t_{(76)} = 2.99$, $p < 0.002$; corresponding to the reticular nuclei adjacent to the nucleus raphe magnus; [Figure 4](#)). Importantly, the responses in all three regions were significantly modulated by naloxone (hypothalamus: -3 -10 -7, $t_{(76)} = 2.97$, $p < 0.002$; PAG: 0 -38 -21, $t_{(76)} = 3.05$, $p < 0.002$; RVM: -6 -37 -40, $t_{(76)} = 3.58$, $p < 0.001$). Next, we tested whether the strength of activation of the descending pain control network was associated with a behavioral marker of placebo analgesia (i.e., pain ratings). In the saline group, we observed significant correlations between pain ratings and BOLD responses in the hypothalamus (-4 -9 -7, $t_{(36)} = 3.86$, $p < 0.001$; 1 -7 -11, $t_{(36)} = 3.52$, $p < 0.001$) and the PAG (-1 -33 -11, $t_{(36)} = 3.54$, $p < 0.001$), as well as in a region slightly more rostral to the previously observed RVM activation, possibly representing the nucleus raphe pontis (-2 -35 -29, $t_{(36)} = 2.68$, $p < 0.006$, trend-level). In all three regions, the correlation was significantly stronger in the saline group than in the naloxone group (hypothalamus: 1 -6 -11, $t_{(36)} = 3.88$, $p < 0.001$, -4 -10 -8, $t_{(36)} = 3.57$, $p < 0.001$; PAG: -1 -32 -11, $t_{(36)} = 3.14$, $p < 0.002$; nucleus raphe pontis: -1 -36 -32, $t_{(36)} = 3.07$, $p < 0.002$).

Connectivity between rACC and PAG

As previous studies have reported enhanced connectivity between rACC and PAG during placebo analgesia ([Bingel et al., 2006](#); [Petrovic et al., 2002](#); [Wager et al., 2007](#)), as well as during other forms of pain modulation ([Valet et al., 2004](#)), we investigated whether such connectivity is opioid dependent and can thus be blocked by naloxone. We observed that the intraindividual coupling between rACC and PAG showed a significant main effect of condition ($F_{(1,38)} = 5.57$, $p = 0.01$) and a significant group-by-condition interaction ($F_{(1,38)} = 7.73$, $p < 0.005$). More specifically, in the saline group, rACC-PAG coupling was enhanced under placebo as compared to control, whereas this difference was abolished in the naloxone group ([Figure 5A](#)).

With regard to the functional relevance of this finding, we observed that the strength of coupling under placebo showed a significantly more positive correlation with the behavioral placebo effect in the saline group than in the naloxone group ($r_{\text{saline}} = 0.28$, $r_{\text{naloxone}} = -0.33$; $Z = 1.83$; $p = 0.03$), i.e., the stronger the placebo-dependent coupling between rACC and

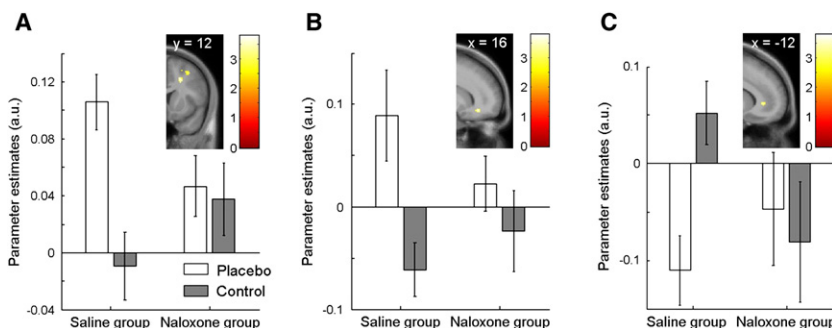


Figure 3. BOLD Responses in Cortical Pain Modulatory Regions

Activation maps (contrast “early pain: placebo > control [saline group]”) and peak voxel parameter estimates show that BOLD responses in (A) the DLPFC and (B) the subgenual rACC are significantly stronger under placebo compared to control in the saline group. This difference is strongly reduced in the naloxone group. (C) Conversely, the pregenual rACC shows a strong deactivation under placebo as compared to control in the saline group, and this response pattern is significantly different in the naloxone group. The visualization threshold for all images is set to $p < 0.005$ uncorrected and activation maps are displayed on the average structural image over all subjects. Error bars indicate SEM.

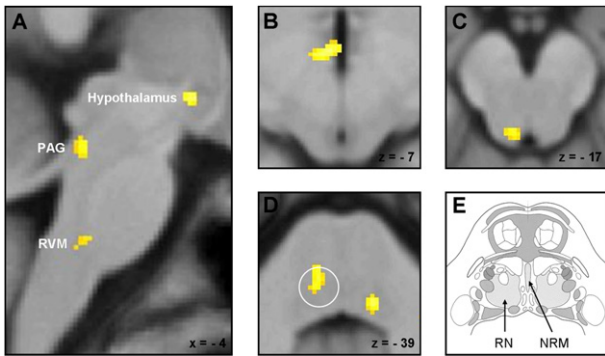


Figure 4. Midbrain and Brainstem BOLD Responses in Areas of the Descending Pain Control System

(A) The sagittal slice (contrast “pain: placebo > control [saline group]”) shows placebo-enhanced responses in the hypothalamus, the PAG, and the RVM, all of which are significantly reduced by naloxone. The three transverse slices show the location of these responses in more detail: (B) hypothalamus, (C) PAG, (D) RVM. The response in the RVM corresponds to the reticular nuclei (RN; approximately indicated by the white circle) adjacent to the nucleus raphe magnus (NRM), which consist of the nucleus reticularis gigantocellularis (more medial) and the nucleus reticularis parvocellularis (more lateral). (E) The anatomical drawing indicates the location of these structures (modified from Naidich et al. [2009]; with permission). The visualization threshold is set to $p < 0.01$ uncorrected, and the activation maps are displayed on the average structural image over all subjects.

PAG, the stronger the reported pain reduction in the saline group. Complementary to that, the strength of rACC-PAG coupling under placebo predicted placebo-dependent reductions in contralateral secondary somatosensory cortex BOLD responses in the saline group ($44 -18 -30$, $t_{(36)} = 3.40$, $p < 0.001$; $38 -16 -26$, $t_{(36)} = 3.13$, $p < 0.002$; Figure 5B), and this relationship was significantly stronger than in the naloxone group ($44 -18 28$, $t_{(36)} = 4.80$, $p < 0.001$ [$p < 0.01$ corrected]; $46 -14 26$, $t_{(36)} = 3.96$, $p < 0.001$ [$p < 0.03$ corrected]).

Finally, if rACC-PAG coupling is involved in descending pain control, the strength of this coupling should also influence the activity of the RVM, which directly controls nociceptive processing in the dorsal horn. In line with this assumption, the strength of rACC-PAG coupling under placebo predicted stronger RVM BOLD responses under placebo compared to control in the saline group ($-5 -40 -40$, $t_{(36)} = 3.16$, $p < 0.002$), and this relationship was significantly stronger than in the naloxone group ($-5 -39 -39$, $t_{(36)} = 3.74$, $p < 0.001$).

DISCUSSION

In summary, we observed that naloxone impaired placebo analgesia on both the behavioral and neural level, blocked placebo-induced responses in DLPFC, rACC, hypothalamus, PAG, and RVM, and abolished placebo-enhanced coupling between rACC and PAG, which predicted both behavioral and neural placebo effects in the saline group as well as opioid-dependent activation of the RVM. Our results thus delineate specific opioidergic mechanisms that configure placebo analgesia and that are disrupted by naloxone.

Previous behavioral studies have shown naloxone administration to impair placebo-dependent pain reduction (Amanzio and Benedetti, 1999; Grevert et al., 1983; Levine and Gordon, 1984; Levine et al., 1978). We observed a similar behavioral effect of naloxone, which we found to be manifested neurally by a blockade of placebo-induced decreases in BOLD responses in pain-sensitive brain regions. It is interesting to note that naloxone did not block subjective placebo effects (i.e., decrease in pain ratings) completely. This might either imply an additional nonopioidergic component (Amanzio and Benedetti, 1999; Gracely et al., 1983; Grevert et al., 1983; Vase et al., 2005), in line with recent imaging and behavioral data implicating dopamine in experimental placebo analgesia (Schweinhart et al., 2009; Scott et al., 2007, 2008), or it could be related to self-consistency bias and related cognitive processes (Kong

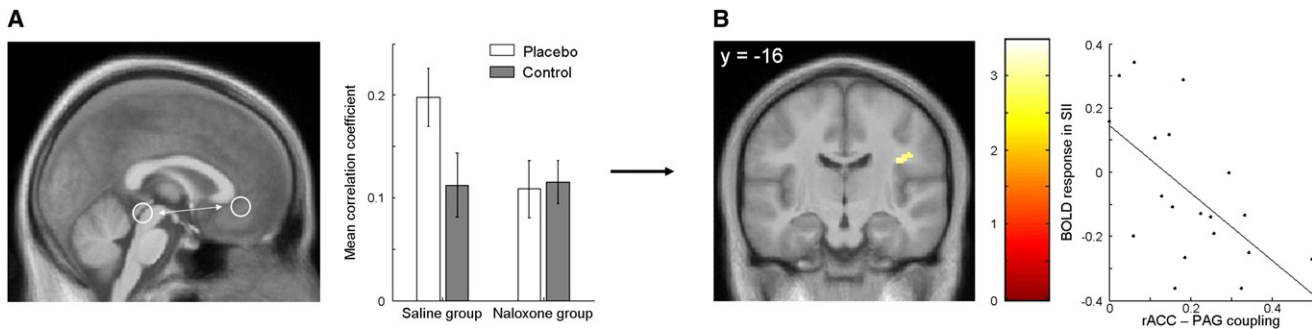


Figure 5. Connectivity of rACC and PAG

(A) The midline sagittal slice (left) depicts the approximate location of rACC and PAG. The intraindividual coupling between rACC and PAG (as represented by the correlation between the respective BOLD time courses) is significantly stronger under placebo than under control in the saline group, whereas this difference is abolished in the naloxone group. Error bars indicate SEM.

(B) The intraindividual rACC-PAG coupling strength under placebo was used to predict decreases in BOLD responses in pain-sensitive regions under placebo compared to control. The activation map on the left shows that contralateral SII BOLD responses are negatively influenced by rACC-PAG coupling. The plot on the right illustrates that the stronger the coupling between rACC and PAG, the stronger the reduction in BOLD responses in SII under placebo compared to control in the saline group (this relationship is significantly different from the naloxone group); the y axis represents difference scores (placebo-control). The visualization threshold is set to $p < 0.005$ uncorrected, and the activation map is displayed on the average structural image over all subjects.

et al., 2007; Wager et al., 2006). Our data point toward the latter interpretation because physiological placebo effects (SCR and BOLD) were not evident in the naloxone group. We observed that neural placebo effects (i.e., placebo-dependent reduction of pain-related BOLD responses) and their reversal by naloxone were most evident in the late pain phase. This suggests that early heat-pain responses are rather opioid insensitive, an idea supported by both behavioral data (Borrás et al., 2004) and the rather slow dynamics of opioidergic neurotransmission (Padlunaya et al., 2006).

The placebo-dependent reduction of BOLD responses and its reversal by naloxone was most evident in dorsal anterior cingulate cortex. A modulation of this region has been shown not only in studies on placebo analgesia (Lieberman et al., 2004; Price et al., 2007; Wager et al., 2004) but also in various other conditions, such as attentional manipulations (Bantick et al., 2002), expectation manipulations (Keltner et al., 2006), and hypnosis (Rainville et al., 1997). In line with a recent study on conditioned hypoalgesia (Eippert et al., 2008), our results suggest that a downregulation of dorsal anterior cingulate cortex depends on opioidergic neurotransmission. The widespread reductions of BOLD responses in pain-sensitive regions (both subcortical and cortical) under placebo and their reversal by naloxone go along with effects observed under exogenous opiate administration (Casey et al., 2000; Wagner et al., 2007), suggesting that afferent inhibition at the level of the spinal cord via descending pain control might be one mechanism underlying placebo analgesia. This is in line with recent behavioral studies showing that expectations regarding pain can alter spinal nociceptive processing (Goffaux et al., 2007; Matre et al., 2006).

With regard to areas involved in pain modulation and descending pain control (Basbaum and Fields, 1984; Bingel and Tracey, 2008; Fields et al., 2006; Millan, 2002), we observed placebo-enhanced DLPFC as well as rACC responses during painful stimulation that were reduced by naloxone. As placebo analgesia is most likely the result of several different neurobiological mechanisms (Kong et al., 2006, 2007), it is entirely possible that cortico-cortical interactions (i.e., direct influences from DLPFC or rACC on pain-sensitive areas; see also Craggs et al. [2007]) underlie the observed neural and behavioral placebo effects. However, in addition to opioid-dependent DLPFC and rACC activation, we also observed placebo-induced BOLD responses in the hypothalamus, the PAG, and the RVM, which taken together constitute a phylogenetically conserved system of descending pain control acting at the level of the spinal cord (Basbaum and Fields, 1984; Fields et al., 2006; Millan, 2002). The opioid-dependent responses in these areas do not merely represent an epiphenomenon, but are of functional relevance, as responses in these areas were related to behavioral placebo effects.

As we mapped the whole hierarchy of descending pain control structures (from DLPFC to RVM), we also attempted to link cortical and brainstem responses. Since there is direct anterior cingulate input to the PAG (An et al., 1998; Floyd et al., 2000) and as previous studies observed heightened rACC-PAG functional connectivity under placebo analgesia (Bingel et al., 2006; Petrovic et al., 2002; Wager et al., 2007), we wanted to test whether these connections are opioid dependent. We observed

placebo-enhanced coupling between the rACC and the PAG, which was abolished by naloxone. In agreement with the finding that electrical PAG stimulation leads to profound opioid-dependent analgesia in humans (Hosobuchi et al., 1977), we could show that placebo-enhanced rACC-PAG connectivity is of functional relevance, as it predicted both behavioral (as measured by pain ratings) and neural placebo effects (as measured by BOLD responses in secondary somatosensory cortex). The main route of descending control from the PAG is via the RVM to the dorsal horn of the spinal cord (e.g., via direct PAG connections to spinally projecting RVM neurons [Morgan et al., 2008]). We therefore tested whether rACC-PAG coupling would drive RVM BOLD responses and observed that the strength of rACC-PAG coupling indeed predicted opioid-dependent RVM activation.

With regard to the temporal occurrence of responses in regions of the descending pain control system, it is interesting to note that cortical responses in DLPFC and rACC were present during early pain but not during late pain, and thus preceded placebo-induced reductions of BOLD responses in the pain matrix. Subcortical responses in the hypothalamus, PAG, and RVM were however present during both early and late pain. In combination with the observed effects on rACC-PAG coupling, we tentatively suggest the following mechanism. Upon painful stimulation, treatment expectations regarding the analgesic efficacy of the placebo cream need to be “kept in mind” and pain needs to be “kept out of mind” (Bunge et al., 2001; Lorenz et al., 2003). Such maintenance and selection processes have extensively been associated with the DLPFC (Miller, 2000; Miller and Cohen, 2001). Via opioid-dependent signaling, the DLPFC will recruit areas such as rACC that can engage the lower parts of the descending pain control system through their projections. The lower part of the descending pain control system (hypothalamus, PAG, and RVM) will then exert an opioid-dependent inhibitory influence on spinal nociceptive processing, leading to reduced nociceptive input to thalamic and cortical areas and thus to a reduced pain experience. This inhibitory brainstem control, once initiated via cortical input, will persist until the nociceptive input is terminated, possibly supported by feedback signals relayed via ascending spinobulbar or spinohypothalamic projections.

It will be interesting to see whether opioid-dependent activation of the descending pain control system is a common feature of different forms of pain modulation, such as hypnosis, attentional distraction, reappraisal, and placebo analgesia, which share some common neuroanatomical features (Benedetti et al., 2005; Ochsner and Gross, 2005; Petrovic and Ingvar, 2002; Wiech et al., 2008) and act inhibitory (Kiernan et al., 1995; Matre et al., 2006; Willer et al., 1979) on nociceptive processing in the spinal cord.

EXPERIMENTAL PROCEDURES

Subjects

Forty-eight German male volunteers (mean age: 26.13 years; range: 20–40 years) were assigned to two groups on a randomized double-blind basis. The experimental group received the opioid antagonist naloxone (“naloxone group”), whereas the control group received saline (“saline group”). Groups did not differ with respect to age, weight, personality scores, and basic pain sensitivity (see Supplemental Experimental Procedures and Results). Data

from eight of the 48 subjects had to be excluded (see [Supplemental Experimental Procedures and Results](#)). The Ethics Committee of the Medical Board in Hamburg, Germany, approved the study and all subjects gave written informed consent. The consent form included information about the experimental procedures, the MR procedure, the thermal stimulation, and the possible adverse effects of naloxone. The consent form did not include statements that subjects would be deceived and that the purpose of the study was to investigate placebo analgesia. Subjects were informed about these important aspects only during debriefing.

Drug Administration

At ~15 min before the start of the test phase, we administered a bolus dose of 0.15 mg/kg naloxone (Naloxon-ratiopharm, Ratiopharm, Ulm, Germany) or saline via an intravenous line inserted into the antecubital vein of the left arm. Because naloxone has a relatively short half-life (~70 min in blood plasma; Summary of Product Characteristics, Ratiopharm) and its clinically effective duration of action can be even shorter ([Gutstein and Akil, 2006](#)), we also administered an intravenous infusion dose of 0.2 mg/kg/hr naloxone or saline (diluted in 250 ml of saline), starting shortly after bolus administration. This dosing regime leads to a stable concentration of naloxone in blood plasma over the length of the experiment (E.D.S. et al., unpublished data) and is sufficient to block central opioid receptors completely ([Mayberg and Frost, 1990](#)).

Subjects were informed about naloxone, including its pharmacological properties, its general clinical use, and its possible side effects. Subjects were also informed that they would most likely not notice that they had received naloxone, as (in the dose employed here) it generally does not have effects on mood and experimental phasic pain stimulation ([Grevert and Goldstein, 1978](#); [Petrovic et al., 2008](#)). Naturally, we could not inform subjects about the true purpose of naloxone administration in this study (this was done during debriefing). We therefore told them that they would receive naloxone because its pharmacological properties allowed us to visualize where in the brain pain-specific responses would occur.

The experimenters who interacted with the subjects (U.B. and J.Y. administered the drug [i.e., either naloxone or saline]; F.E. carried out the experiments) were blinded as to which drug was given. E.D.S. and two research assistants, who did not interact with the subjects, carried out the assignment of subjects to the two groups and the naloxone handling. Unblinding occurred only after the experiment.

Study Design

We used a between-subjects design, because placebo effects are also shaped by prior experience ([Colloca and Benedetti, 2006](#)), which could strongly confound results obtained in a within-subjects design. Subjects were recruited with the understanding that this study investigated the influence of an analgesic cream (“lidocaine [2%], an extremely effective pain killer, which at higher doses even acts as a local anesthetic”) on brain responses to painful stimulation. The design of this study followed a well-established placebo analgesia paradigm including both expectation and conditioning components ([Figure 1A](#)) ([Colloca and Benedetti, 2006](#); [Colloca et al., 2008](#); [Klinger et al., 2007](#); [Montgomery and Kirsch, 1997](#); [Price et al., 1999](#); [Wager et al., 2004](#)). On day 1, subjects came into the building housing the MR scanner (situated on the campus of the university hospital) and were greeted by the experimenter wearing a white lab coat. They were then led into a medical examination room and were familiarized with the thermal stimulation by presenting them with several stimuli of varying duration and temperature on their right forearm. Subsequently, they were informed about the experimental procedures, and five 4 × 4 cm squares were drawn on their left volar forearm. The two upper/lower squares were outlined in green/red color and designated as the site for later placebo cream/control cream stimulation; the coloring was chosen to enhance the association between skin patch and pain relief. The middle square was used for calibrating the thermal stimulation, i.e., to find temperatures corresponding to 40, 60, and 80 on a visual analog scale (VAS; 100 parts; endpoints labeled with “no pain” and “unbearable pain”); subjects were stimulated with a pseudorandom sequence of 20 s thermal stimuli with different intensities and were asked to rate the intensity of each stimulus. We then used regression analysis to estimate the temperatures corresponding to intensity levels of 40, 60, and 80 on the VAS. This ensured that each subject would

be stimulated with individually tailored stimulus intensities that are however comparable across subjects on the VAS.

Upon termination of the calibration procedure, we treated subjects with two identical creams, which were however presented as “lidocaine cream” and “control cream” and were kept in professionally labeled tubes. We told subjects that they would receive lidocaine cream on the skin areas outlined in green and that they would receive a completely inactive sensory control cream on the skin areas outlined in red. They were furthermore told that the experiment would only start after 10 min, because of “the time it takes for lidocaine to become fully effective.” During these 10 min, subject filled out three questionnaires (Beck Depression Inventory, State-Trait Anxiety Inventory, and Crowne-Marlowe Social Desirability Scale).

Afterward, the manipulation phase started. Subjects were told that they would be stimulated on both skin patches (placebo cream, control cream) with 80% of their pain tolerance (i.e., right-hand end of VAS), but unbeknownst to them the temperature was lowered to 40% of their pain tolerance during the placebo condition. This served to convince subjects that the placebo cream is an effective analgesic substance against heat pain stimulation and to enhance their expectations regarding future treatment with the placebo cream. The manipulation phase consisted of two sessions (stimulation of skin patch treated with control cream and stimulation of skin patch treated with placebo cream) with six trials each. Each trial consisted of anticipation, pain, pause, rating, and rest ([Figure 1B](#)). At the start of the anticipation phase, a white crosshair changed color to red, which signaled to the subjects that painful stimulation would follow soon. Subjects had to press a button as fast as possible when the crosshair changed color. After a variable delay (7.5 ± 3.5 s), a 20 s painful thermal stimulus was administered (~1.5 s ramp up, 17 s plateau, ~1.5 s ramp down). A variable delay (5 ± 2 s) followed the thermal stimulation, before subjects had to rate the level of pain present on that trial using a VAS. A variable intertrial interval (ITI; 20 ± 5 s) followed, during which a white crosshair was displayed. Before each session, pain thresholds were assessed using the method of limits. We slowed down the rise time of the thermode on the placebo-treated skin patch (from $1.2^\circ/\text{s}$ in the control condition to $0.7^\circ/\text{s}$ in the placebo condition), in order to give the subjects a first hint of the efficacy of the placebo cream. After the manipulation phase, subjects had to indicate how effective they perceived the “lidocaine cream” to be for pain reduction (on a scale from 0 [no pain reduction] to 5 [extremely strong pain reduction]).

The assignment of placebo cream or control cream to the upper or lower patches was randomized across subjects, such that half of the subjects received the placebo cream on the upper patches and the other half received placebo cream on the lower patches. Similarly, which patch (placebo cream or control cream) was stimulated first was also randomized, to prevent a confounding effect of order ([Wager et al., 2006](#); [Price et al., 2007](#)). Finally, which of the two placebo (or control) treated patches would be stimulated in the first manipulation session was also randomized. Similar to a previous study ([Wager et al., 2004](#)), we used two patches for each condition to prevent stimulation of the same patch during manipulation and test on day 2.

On day 2, subjects were first tested for current alcohol and drug use (including THC and opiates) using commercially available saliva and urine tests (Diagnostik-Nord, Schwerin, Germany). Subjects then received the two creams on the respective skin areas, which had again been outlined by green and red color. After a waiting period of 10 min (to allow “lidocaine to become effective”), they were placed inside the MR scanner, where a further manipulation phase with six trials for each condition took place without scanning. Subjects were told that this phase served to make them comfortable with being in the MR scanner, whereas in reality it mainly served to reactivate and strengthen expectations of pain relief due to the placebo cream (i.e., to maximize the positive experience of placebo prior to test [[Colloca and Benedetti, 2006](#); [Colloca et al., 2008](#)]). After this manipulation phase, subjects rated the analgesic efficacy of the placebo cream, received a refreshment of the placebo and control cream, and then received a bolus injection of either naloxone or saline. Subjects were placed in the MR scanner again, the infusion of naloxone or saline was started, and ~15 min after the bolus administration, the test phase began. Similar to the manipulation sessions, each test session was preceded by pain threshold estimation. The test phase consisted of two sessions (15 trials each) during which fMRI data were recorded: in one session the skin part treated with placebo cream was stimulated, whereas in the other

session the skin part treated with control cream was stimulated. Importantly, in both sessions subjects were stimulated with the same temperature (equivalent to 60 on the VAS). This physically identical stimulation allowed for the assessment of placebo effects (i.e., reduced pain ratings under placebo cream compared to control cream).

After the experiment, we removed the i.v. line and subjects again rated the analgesic efficacy of the placebo cream and answered two questionnaires regarding (1) experienced adverse-effects of naloxone and (2) current mood (see Supplemental Experimental Procedures and Results).

Data Acquisition

We used Presentation software (Neurobehavioral Systems, Albany, CA, USA) for stimulus presentation and recording of reaction times and pain ratings. Thermal stimulation was carried out using a thermode (a 30 × 30 mm Peltier device; TSAII, Medoc, Tel Aviv, Israel).

Skin conductance responses (SCR) were acquired using MRI-safe electrodes (2700 CLEARTRACE², CONMED, Utica, NY, USA) attached to the hypothenar of the subject's left hand. The same dermatome (C8) was chosen for both electrodes to control for possible recording differences between dermatomes. We used a CED 2502 to amplify the skin conductance signal, a CED micro1401 mkII to digitize the signal at 1000 Hz, and Spike2 software to record the data (all equipment by Cambridge Electronic Design, Cambridge, UK). Functional magnetic resonance imaging (fMRI) data were acquired on a 3 Tesla system (Siemens Magnetom Trio, Siemens, Erlangen, Germany) equipped with a 12 channel head coil. Forty-four transversal slices (slice thickness, 2 mm; gap, 1 mm) were acquired in each volume (repetition time: 2.62 s, echo time: 25 ms, flip angle: 90°, field of view: 208 × 208 mm, matrix: 104 × 104; GRAPPA with PAT-factor 2 and 48 reference lines) using T2*-weighted echo-planar imaging (EPI). Slice orientation was tilted by -39°, which allowed coverage of areas as ventral as the medulla. The first five volumes of each session were discarded to allow for T1 saturation. We also acquired high-resolution (1 × 1 × 1 mm voxel size) T1-weighted images for each subject using a MP-RAGE sequence (in two subjects, high-resolution images could not be acquired due to time constraints).

Data Analysis: Behavior

All behavioral data were analyzed in MATLAB (The MathWorks, Natick, MA, USA) or STATISTICA (StatSoft, Tulsa, OK, USA), using a threshold of $p \leq 0.05$ (one-tailed in cases of a priori hypotheses). Pain ratings were analyzed using a two-way analysis of variance (ANOVA) with between-subject factor group (saline and naloxone) and within-subject factor condition (control and placebo). We used separate ANOVAs for pain ratings in the first manipulation session, the second manipulation session, and the test session. Postexperimental ratings regarding the analgesic efficacy of the placebo cream were analyzed using a two-sample t test.

Skin conductance responses (SCR) could not be acquired from one subject due to technical problems. Data from the remaining subjects were resampled to 10 Hz and smoothed with a 1 s (full width at half maximum, FWHM) Gaussian kernel. For SCR analysis during pain, we used a time interval of 25 s, starting at pain onset. Amplitudes were determined as the maximum in the analysis interval in relation to a preceding minimum in the analysis interval. Before statistical analysis, amplitudes were z transformed. SCR amplitudes were analyzed using a two-way ANOVA with between-subject factor group (saline and naloxone) and within-subject factor condition (control and placebo).

Data Analysis: fMRI

fMRI data processing and statistical analyses were carried out using statistical parametric mapping (SPM5, Wellcome Trust Centre for Neuroimaging, London, UK). Data processing consisted of slice timing (correction for differences in slice acquisition time), realignment (motion correction), spatial normalization to a standard EPI template, and smoothing with an 8 mm (FWHM) isotropic Gaussian kernel. Data were also subjected to high-pass filtering (cutoff period: 128 s) and correction for temporal autocorrelations (based on a first-order autoregressive model).

Data analysis was performed using a general linear model approach. The first-level design matrix of each subject included ten regressors (five in each session): anticipation placebo, anticipation control, pain-early placebo, pain-

early control, pain-late placebo, pain-late control, rating placebo, rating control, and two session constants. Anticipation was modeled by convolving a delta function (at anticipation onset) with the canonical hemodynamic response function (HRF), pain-early was modeled by convolving a 10 s boxcar function (starting at pain onset) with the canonical HRF, pain-late was modeled by convolving a 10 s boxcar function (starting 11 s after pain onset) with the canonical HRF, and rating was modeled by convolving delta functions (representing each button press during rating) with the canonical HRF. The painful stimulation was divided into early and late periods based on previous results regarding neural placebo effects (Price et al., 2007; Wager et al., 2004) and naloxone function (Borras et al., 2004). After model estimation, the ensuing first-level contrast images from each subject were used for second-level analysis. Second-level design matrices were configured using SPMs "full factorial" model and included four regressors (e.g., saline group: pain-early placebo, saline group: pain-early control, naloxone group: pain-early placebo, naloxone group: pain-early control). We corrected for possible nonsphericity of the error term (dependence of conditions and possible unequal variance between groups). In the following, we list analyses as they appear in the main text. When speaking of neural placebo effects, we refer to reduced BOLD responses under the placebo condition as compared to the control condition. Note that the main interest of this study concerned responses during the pain period and data from the anticipation phase are therefore presented in the Supplemental Experimental Procedures and Results.

In a first analysis testing for "global placebo effects," we investigated the overall influence of naloxone on neural placebo effects. Therefore, we identified all regions that showed significant BOLD responses to painful stimulation during the control condition in both groups via a conjunction analysis (Nichols et al., 2005). We then extracted parameter estimates from each region (6 mm sphere around peak voxel) for both conditions (control and placebo) from each subject, averaged the parameter estimates across all regions on a subject-by-subject basis and finally calculated a two-way ANOVA with between-subject factor group (saline and naloxone) and within-subject factor condition (control and placebo) over the averaged parameter estimates. This analysis was carried out separately for early-pain (first 10 s of 20 s pain period) and late-pain (last 10 s of 20 s pain period).

In a second analysis, we tested for regionally specific neural placebo effects and their modulation by naloxone. We thus used the contrast "saline group: control > placebo" to test for effects in the saline group and the interaction contrast "(saline group: control > placebo) > (naloxone group: control > placebo)" to test for a group-by-condition interaction. These analyses were also carried out separately for early-pain and late-pain.

We also investigated placebo-enhanced BOLD responses in pain modulatory structures and their modulation by naloxone. To this end, we used the contrast "saline group: placebo > control" to test for effects in the saline group and the contrast "(saline group: placebo > control) > (naloxone group: placebo > control)" to test for a group-by-condition interaction. Again, these analyses were carried out separately for early pain and late pain.

To further investigate such effects at the subcortical level with higher sensitivity, we used an additional image preprocessing strategy optimized for deep-brain structures, including amygdala, hypothalamus, PAG, and RVM. We first created a mask (box) of the following dimensions (-30:30, -60:0, -72:0, x y z in mm). Voxels in the mask were set to one, and voxels outside the mask were set to zero. To optimize the normalization procedure for the lower structures (especially the brainstem), we weighted the normalization cost function with this mask; only affine transformations were used. A similar procedure (Napadow et al., 2006) has been shown to significantly improve brainstem coregistration accuracy. Normalized images were resliced at a resolution of 1 × 1 × 1 mm and were smoothed with a 4 mm FWHM isotropic Gaussian kernel to preserve the fine-scale structure. Note that weighting the motion-correction cost-function with the same box (also combined with increasing the spatial sampling rate and masking out highly variant regions, such as blood-vessels) did not lead to a consistent increase in sensitivity and was therefore omitted. Instead we used standard motion-correction as done in the original analysis. Statistical analyses were carried out as described above, but also using a regressor that spanned the entire pain interval (20 s). We also investigated whether behavioral placebo effects (i.e., difference in pain ratings) would predict placebo-induced increases in BOLD responses in the

amygdala, hypothalamus, PAG, and RVM. Therefore, we used the difference in ratings (control-placebo) as a covariate in a two-sample t test on the second level, testing whether a greater rating difference would lead to stronger BOLD responses (placebo compared to control) in the saline group, but not in the naloxone group.

To test for rACC-PAG connectivity, we first anatomically defined a seed voxel in the center of the PAG, as identified on the average high-resolution image of our subjects. The PAG was used for seed voxel identification because in contrast to rACC, the PAG borders can readily be identified on a structural scan, which allows easy identification of the geometric center of this structure. The following coordinates were found to be the geometric center of the PAG: $x = 0$, $y = -32$, $z = -10$. We then set a sphere of 6 mm radius around this coordinate and extracted the mean time series for each subject under each condition. These time series were then used as sole predictors in subject-specific design matrices and contrasts were computed that tested for placebo > control. Contrast images were subsequently raised to the second level, where we observed a significant effect in the saline group (16 44 0, $t_{(76)} = 4.04$, $p < 0.001$ [$p = 0.01$ corrected]) as well as a significant group-by-condition interaction in rACC (16 44 0, $t_{(76)} = 3.47$, $p < 0.001$ [$p = 0.05$ corrected]). However, as such an analysis is not necessarily symmetric (Friston et al., 1997) we also extracted the mean time series of a 6 mm sphere around the observed rACC coordinate and then correlated the two time series. The resulting correlation coefficients for each condition in each subject were Fisher-z transformed and subjected to a two-way ANOVA with between-subject factor group (saline and naloxone) and within-subject factor condition (control and placebo). To test for the functional relevance of this coupling, we correlated the coupling strength under placebo (Fisher-z transformed correlation coefficients) with the behavioral placebo effect (difference between control and placebo for each subject) and tested whether the saline group would show a significantly more positive correlation than the naloxone group. A similar analysis was carried out with the fMRI data, where we used the coupling strength under placebo as a covariate in a two-sample t test on the second level, investigating whether greater coupling would lead to stronger reductions in BOLD responses (placebo compared to control) in the saline group, but not in the naloxone group (only tested for late pain). Finally, we used the coupling strength under placebo as a covariate in a two-sample t test on the second level, investigating whether greater coupling would lead to stronger RVM BOLD responses (placebo compared to control) in the saline group, but not in the naloxone group.

We used an initial height threshold of $p < 0.005$ uncorrected (similar to previous studies on placebo analgesia [Wager et al., 2004]). We also report corrected p values as obtained from small volume correction in a priori regions of interest at a level of $p \leq 0.05$. Correction was based on peak coordinates (ignoring laterality) obtained from previous studies on pain processing and pain modulation. The DLPFC (Zubieta et al., 2005) was corrected using a sphere of 15 mm radius. The primary somatosensory cortex (Bingel et al., 2007), the secondary somatosensory cortex (Bingel et al., 2007), the insula (Bingel et al., 2007), the dACC (Büchel et al., 2002), the rACC (pregenual part [Eippert et al., 2008]; subgenual part [Bingel et al., 2007]) were corrected using spheres of 12 mm radius. The basal ganglia (Bingel et al., 2002), the amygdala (Zubieta et al., 2005), the thalamus (Zubieta et al., 2001), and the pons (Petrovic et al., 2004) were corrected using spheres of 6 mm radius. Corrected p values are not reported for the results of the brainstem-specific analysis (hypothalamus, PAG, and RVM [which is defined as the nucleus raphe magnus and adjacent reticular nuclei (Millan, 2002)]), since the descending pain control system has not been investigated at this level with sufficient spatial resolution, and coordinates for small-volume correction are thus lacking.

SUPPLEMENTAL DATA

Supplemental Data include Supplemental Experimental Procedures, Supplemental Results, four tables, and one figure and can be found with this article online at [http://www.cell.com/neuron/supplemental/S0896-6273\(09\)00543-1](http://www.cell.com/neuron/supplemental/S0896-6273(09)00543-1).

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