

Supporting Online Material

Direct Evidence for Spinal Cord Involvement in Placebo Analgesia

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Subjects: 15 healthy male volunteers (mean age: 25 years; range: 21-30 years) participated in this study. All subjects had already participated in a previous placebo analgesia study (approximately 7 months earlier) that investigated cortical and subcortical responses during placebo analgesia (*S1*); subjects were only debriefed after participating in the current study. Data from two of the 15 subjects had to be discarded because of profound motion (exceeding 12mm, one subject) and failure of our automatic spatial normalization procedure (one subject). 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 magnetic resonance imaging (MRI) procedure, and the thermal stimulation. 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 upon debriefing directly after this study. In accordance with recently suggested ethical guidelines for deceptive research (*S2*), all subjects were given the possibility to withdraw their data upon being debriefed. However, none of the subjects decided to withdraw his data.

Study design: Subjects were told 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 anaesthetic") on spinal cord responses to painful stimulation. The design of this study followed a well-established placebo analgesia paradigm including both expectation and conditioning components (*S3-S10*; Fig. S1A).

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. To determine the temperatures that would elicit pain levels of 40, 60, and 80 on a visual analogue

scale (VAS; 0-100 with endpoints labelled “no pain” / “unbearable pain”) we applied several 20s long painful stimuli to their right forearm. As a starting point, we used the temperature that was employed in the previous study to elicit a pain level of 80 and then increased or decreased the temperature for the next stimulus in steps of 0.5 degrees until a pain level of 80 was reached; the other temperatures (pain level of 40 and 60) were adjusted using the obtained temperature difference to the previous study. The group averages of temperatures for pain levels of 40, 60, and 80 were (mean \pm standard error of the mean; in $^{\circ}\text{C}$): 46.3 ± 0.2 , 46.9 ± 0.2 , and 47.4 ± 0.2 . Subjects were then informed about the experimental procedures and five $4 \times 4 \text{cm}^2$ squares were drawn on their left radial volar forearm in order to stimulate dermatome C6. The two upper and lower squares were outlined in color and designated as the sites for later placebo cream / control cream stimulation. Thereby, red was chosen for the control cream and green for the placebo cream in order to enhance the association between skin patch and pain relief. The middle square was not used. We then treated subjects with two identical (pharmacologically inactive) creams, which were however presented as “lidocaine cream” and “control cream” and were kept in professionally labelled 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 minutes, because of “the time it takes for lidocaine to become fully effective”.

Afterwards, 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 6 trials each. Each trial consisted of anticipation, pain, pause, rating, and rest (Fig. S1B). At the start of the anticipation phase, a cross-hair changed from white to red, which signalled to the subjects that painful stimulation would soon follow. Subjects had to press a button with their middle finger as fast as possible when the cross-hair changed color. After a fixed delay (7.5s), a 20s painful thermal stimulus was administered ($\sim 1.5\text{s}$ ramp up, 17s plateau, $\sim 1.5\text{s}$ ramp down). A variable delay ($5 \pm 2\text{s}$) 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; $20 \pm 5\text{s}$) followed, during which a white

cross-hair 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°/s in the control condition to 0.7°/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)) and received a refreshment of the placebo and control cream.

After a waiting period of 10 minutes (to allow “lidocaine to become effective”), subjects were placed in the MR scanner again and 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, subjects again rated the analgesic efficacy of the placebo.

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 (*S11*, *S12*). Finally, which of the two placebo (or control) treated patches would be stimulated first in the manipulation session was also randomized. Similar to a previous study (*S9*), we used two patches for each condition to prevent stimulation of the same patch during manipulation and test.

Data acquisition: We used Presentation[®] software for stimulus presentation and recording of reaction times and pain ratings. Thermal stimulation was carried out using a TSA-II thermode (30x30mm² Peltier device).

MRI data were acquired on a 3 Tesla TRIO system. Subjects were positioned in a 12-channel head coil combined with a 4-channel neck coil (both receive-only) with the estimated target region of the spinal cord being centered in the neck coil and coinciding with the magnet’s isocenter. High-resolution (1x1x1mm³) T1-weighted anatomical images were acquired using a 3D-MPRAGE sequence (sagittal slice orientation, repetition time 2.3s, echo time 3.5ms,

readout flip angle 9° , inversion time 1.1s, field-of-view $192 \times 240 \times 256 \text{mm}^3$). The field of view ranged from the top of the midbrain to the second thoracic vertebra. For this acquisition, both coils were used, whereas for the acquisition of functional images we only used the neck coil because no significant signal contributions of the target region could be expected from the head coil. Functional images were acquired using a gradient-echo echo-planar imaging (EPI) sequence (repetition time: 1.5s, echo time: 40ms, flip angle: 80° , field of view: $128 \times 128 \text{mm}^2$, matrix: 128×128 ; GRAPPA with a PAT-factor of 2 and 24 reference lines). The target volume covered an area from the beginning of the fifth cervical vertebra to the beginning of the first thoracic vertebra, approximately including the spinal segments C5-T1. We acquired 12 slices positioned perpendicular to the spinal cord, using a slice thickness of 5mm in order to achieve adequate signal-to-noise ratio despite our high in-plane resolution ($1 \times 1 \text{mm}^2$). To minimize sensitivity to flow effects, flow rephasing in slice direction and spatially-selective saturation pulse superior and inferior to the target volume were used and the images obtained with the individual coil channels were combined with a sum-of-squares algorithm. Furthermore, additional saturation pulses were applied posterior and anterior to the target region, i.e. in the phase-encoding direction, in order to minimize pulsatile blood flow artifacts. The adjustments prior to the functional acquisitions, e.g. the shim of the magnetic field, were performed for a manually defined volume of about $35 \times 30 \times 70 \text{mm}^3$ covering the target region in the spinal cord. The first ten volumes of each session were discarded in order to eliminate T1 saturation effects.

To allow for retrospective physiological noise correction (*S13*), which is critical in spinal fMRI (*S14*, *S15*), heart-rate and respiration-rate data were measured using the vendor-supplied pulse sensor and respiratory belt and were recorded on the scanner together with the trigger pulses preceding the acquisition of each volume to ensure timing accuracy (*S14*).

Data analysis - behavior: All behavioral data were analyzed in MATLAB[®] using a threshold of $p \leq 0.05$. To test for reduced pain ratings under placebo as compared to control, we used a paired t-test (one-tailed). To test for possible differences in reaction times under placebo as compared to control, we also used a paired t-test (two-tailed, as we had no a-priori hypothesis); in two subjects reaction times could not be recorded due to technical problems.

Data analysis – fMRI: fMRI data processing and statistical analyses were carried out using statistical parametric mapping (SPM5, Wellcome Trust Centre for Neuroimaging, London, UK). Motion correction was performed using a standard rigid body transformation as

employed in SPM (six degrees of freedom). To minimize the influence of neck and shoulder muscles on the motion correction procedure (*S16*), the cost function was weighted by an automatically created mask (using morphological procedures implemented in Matlab's Image Processing Toolbox) that only included the spinal cord and surrounding tissue. Within this mask, highly variant regions (i.e. CSF) were excluded, to base the registration procedure only on spinal cord movement.

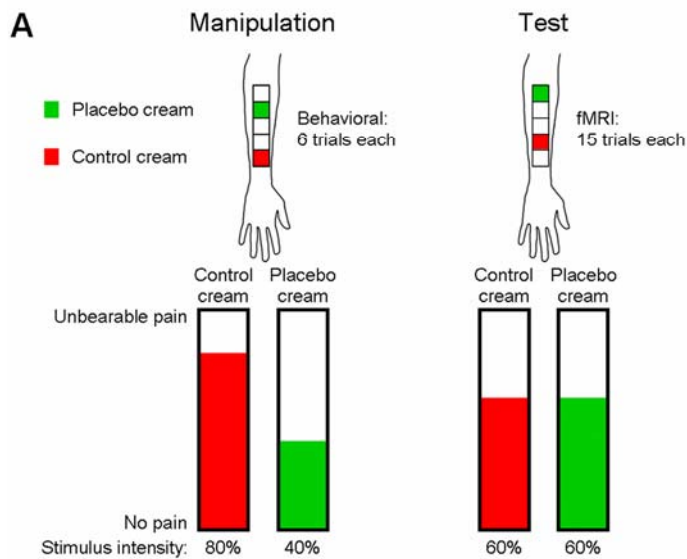
To bring all data sets into a common space, we also spatially normalized the data, which allowed us to perform group analyses. We first defined a template image by choosing the subject with the least anterior-posterior and left-right curvature of the spine (*S17*). We then used the standard SPM normalization algorithm (including both affine and non-linear terms) to normalize the T1-weighted images from all other subjects onto this subject's T1-weighted image. The obtained parameters were then applied to the functional images, which were resampled at a resolution of $1 \times 1 \times 1 \text{mm}^3$. The structural images were then averaged and the resulting mean image was used for localization of BOLD responses. Finally, the functional images were smoothed with a 2mm (FWHM) isotropic Gaussian kernel, which was used as a compromise to facilitate group analysis, but at the same time to retain the fine-scale spatial structure. Data were also subjected to temporal high-pass filtering (cutoff period: 128s).

Data analysis was performed using a general linear model approach. The first-level design matrix of each subject included the following regressors: anticipation placebo, pain placebo, rating placebo, anticipation control, pain control, rating control, estimated movement parameters (3 translations and 3 rotations) for both sessions and two session constants. Anticipation was modelled by convolving a delta function (at anticipation onset) with the canonical hemodynamic response function (HRF), pain was modelled by convolving a 20s boxcar function (starting at pain onset) with the canonical HRF, and rating was modelled by convolving delta functions (representing each button press) with the canonical HRF. We then defined contrasts to test for a) reaction time button presses (anticipation placebo + anticipation control), b) the main effect of pain (pain placebo + pain control), and c) the effect of placebo on pain (pain control – pain placebo). After model estimation, the ensuing first-level contrast images from each subject were used for second-level analysis using one-sample t-tests.

As the influence of physiological noise, which mainly arises from cardiac and respiratory sources, is much stronger in the spinal cord than in the brain (*S14, S15, S18, S19*), we also corrected for this potential confound. We used the selective averaging method described by Deckers et al. (*S20*) to generate regressors representing cardiac and respiratory effects (using

10 bins for cardiac and respiratory effects, respectively). This method is similar to the often employed retrospective image correction (RETROICOR) approach (S13), but is model-free (i.e. does not assume a specific periodic function). Following the procedures outlined in Brooks et al. (S14), we also aimed to correct for low-frequency noise by using the average CSF signal as a regressor; our procedure only differed from that of Brooks et al. (S14) in that we included voxels whose variance lay in the top 25th percentile. Regressors representing cardiac, respiratory, and low frequency effects (21 for each session) were thus also included in the first-level design matrix of each subject.

In analogy to previous fMRI studies on placebo analgesia (S9), data are reported at an uncorrected threshold of $p < 0.005$. Note that our analysis for placebo effects on BOLD responses does not suffer from problems of circularity, as the contrast weight vector defined for identifying the main effect of pain is orthogonal to the contrast weight vector testing for a differential effect at this voxel (which is unproblematic at the second level (S21)).



B

Anticipation	Pain	Pause	Rating	ITI
7.5s	20s	3-7s	...	15-25s

Fig. S1. Experimental paradigm. **A)** The experiment consisted of two phases: manipulation and test. Before each phase, subjects were treated with two identical (pharmacologically inactive) 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 phase (which consisted of 6 trials under placebo cream and control cream, respectively), painful stimulation on the placebo-treated patch was surreptitiously lowered (from 80 (score on a visual analogue 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. 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 cross-hair), the painful stimulation, a short pause, the pain rating and an inter-trial interval (ITI). During the ITI, subjects saw a white cross-hair in the center of the screen, which changed color to red at the beginning of the anticipation phase, signalling to the subjects that the painful stimulation would soon follow. Subjects also had to press a button as fast as possible when they detected the color change (reaction time task, used to probe motor-related spinal cord BOLD responses). After the painful stimulation, subjects had to rate the pain intensity on a VAS.

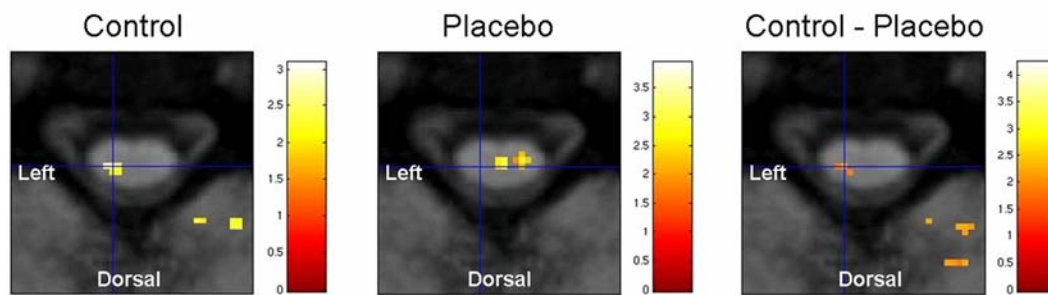


Fig. S2. Pain-related BOLD responses under control and placebo. Transverse slices descriptively ($p < 0.05$ uncorrected) show the BOLD responses to painful stimulation at the same segmental level as in the main text (C6, approximately at the junction with C5). Under control (left panel), we observed a distinct cluster in the ipsilateral dorsal horn, which could not be detected under placebo (middle panel; blue cross-hair). As expected when contrasting control with placebo (right panel), we observed a distinct cluster in the ipsilateral dorsal horn. We also observed contralateral responses under placebo, but unfortunately the design of our current study (unilateral stimulation only) does not allow to clarify the functional relevance of this observation, e.g. with respect to somatotopy (S23). Signal changes outside the spinal canal are most likely due to residual physiological noise. All activation maps are displayed on the average structural image and color bars denote t-values.

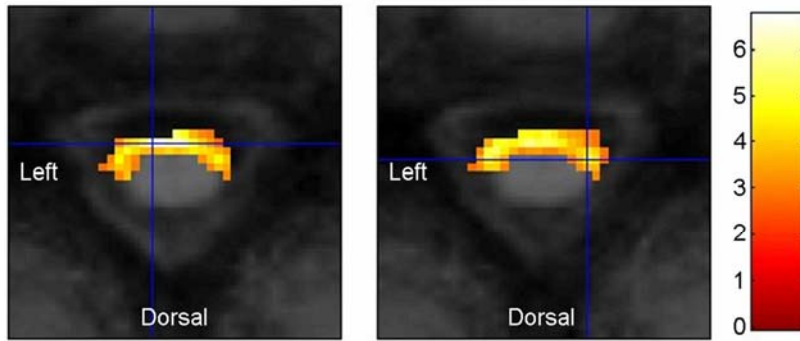


Fig. S3. Motor-related BOLD responses during the reaction time task. Data from both conditions (placebo and control) were pooled, because we observed no significant differences in reaction times between the two conditions ($t_{(10)} = 1.05$, $p = 0.31$). The location of these responses (segments C7/C8) is in line with the functional neuroanatomy of the motor system, as a middle finger (right hand) button press will mainly involve the flexor digitorum muscles (superficial and profundus), which are innervated by the median nerve as well as the lumbrical muscles of the hand innervated by the ulnar nerve. We did not observe a clear-cut laterality of motor-related BOLD responses, as both a midline / contralateral peak ($t_{(12)} = 6.77$, $p < 0.001$; left panel) and an ipsilateral peak ($t_{(12)} = 4.58$, $p < 0.001$; right panel) were present. Bilateral spinal cord BOLD responses during voluntary movements have previously been observed and hypothesized to relate to interneuronal activity (*S23*, *S24*). The visualization threshold is $p < 0.01$ uncorrected and all activation maps are displayed on the average structural image; the color bar denotes t-values.

- S1. F. Eippert *et al.*, *Neuron* **63**, 533 (Aug, 2009).
- S2. F. G. Miller, T. J. Kaptchuk, *J Neurosci* **28**, 4841 (May, 2008).
- S3. L. Colloca, F. Benedetti, *Pain* **124**, 126 (Sep, 2006).
- S4. L. Colloca *et al.*, *Pain* **139**, 306 (Oct, 2008).
- S5. R. Klingner, S. Soost, H. Flor, M. Worm, *Pain* **128**, 31 (Mar, 2007).
- S6. G. H. Montgomery, I. Kirsch, *Pain* **72**, 107 (Aug, 1997).
- S7. D. D. Price *et al.*, *Pain* **83**, 147 (Nov, 1999).
- S8. N. J. Voudouris, C. L. Peck, G. Coleman, *Pain* **38**, 109 (Jul, 1989).
- S9. T. D. Wager *et al.*, *Science* **303**, 1162 (Feb, 2004).
- S10. T. D. Wager, D. J. Scott, J.-K. Zubieta, *Proc Natl Acad Sci U S A* **104**, 11056 (Jun, 2007).
- S11. T. D. Wager, D. Matre, K. L. Casey, *Brain Behav Immun* **20**, 219 (May, 2006).
- S12. D. D. Price, J. Craggs, G. N. Verne, W. M. Perlstein, M. E. Robinson, *Pain* **127**, 63 (Jan, 2007).
- S13. G. H. Glover, T. Q. Li, D. Ress, *Magn Reson Med* **44**, 162 (Jul, 2000).
- S14. J. C. W. Brooks *et al.*, *Neuroimage* **39**, 680 (Jan, 2008).
- S15. M. Piche *et al.*, *Magn Reson Imaging* (Sep, 2008).
- S16. P. W. Stroman, P. W. Nance, L. N. Ryner, *Magn Reson Med* **42**, 571 (Sep, 1999).
- S17. P. W. Stroman, C. R. Figley, C. M. Cahill, *Magn Reson Imaging* **26**, 809 (Jul, 2008).
- S18. P. W. Stroman, *Clin Med Res* **3**, 146 (Aug, 2005).
- S19. F. Giove *et al.*, *Magn Reson Imaging* **22**, 1505 (Dec, 2004).
- S20. R. H. R. Deckers *et al.*, *Neuroimage* **33**, 1072 (Dec, 2006).
- S21. N. Kriegeskorte, W. K. Simmons, P. S. F. Bellgowan, C. I. Baker, *Nat Neurosci* **12**, 535 (May, 2009).
- S22. F. Benedetti, C. Arduino, M. Amanzio, *J Neurosci* **19**, 3639 (May, 1999).
- S23. M. Maieron *et al.*, *J Neurosci* **27**, 4182 (Apr, 2007).
- S24. M.-C. Ng *et al.*, *Neuroimage* **39**, 825 (Jan, 2008).