**Supplemental Material**
Manuscript number: PAINREPORTS-D-17-0007
Title: The impact of pain-related fear on neural pathways of pain modulation in chronic low back pain

*Subject Recruitment and characteristics*

Subjects were recruited via in local chiropractic and physiotherapy centers as well as via online advertisements. Inclusion criteria for chronic LBP patients were low back pain of at least 6 months duration and age ≥18 years. Exclusion criteria were a history of psychiatric or neurological disorders and specific causes for the pain that were ruled out by the chiropractor and/or manual therapist. For the healthy controls exclusion criteria were past chronic pain episodes, acute and/or recurrent back pain within the last 6 months, a history of psychiatric or neurological disorders and age ≤18 years. The groups were gender- and age-matched (Pearson Chi-Square test for gender: x2 = 2.506, p > .05, two-sample t-test for age: *t*(38) = 1.874, p > .05).

*Questionnaires*

Pain-related fear in chronic LBP patients was assessed by using two questionnaires focusing on potentially different conceptual definitions of pain-related fear, either on fear of movement/(re)injury/kinesiophobia (Tampa Scale of Kinesiophobia questionnaire, TSK[4,11]) or fear avoidance beliefs (Fear Avoidance Beliefs Questionnaire, FABQ [12]). The 17-item German version of the TSK has a satisfactory internal consistency (Cronbach’s α = 0.76–0.84) and contains statements focusing on fear of physical activity which were rated by the subjects on a 4-point Likert scale from 1 = “strongly disagree” to 4 = “strongly agree” [1,9]. Due to further abbreviated and established versions of original 17-item TSK questionnaire, we also calculated the questionnaire scores of the 13- and 11-item TSK versions. Both versions were previously validated by using confirmatory factor analysis and demonstrate acceptable levels of internal consistency [3,10]. In addition, the chronic LBP patients completed the German version of the FABQ (FABQ-D [8]) which includes two subscales, namely beliefs about work and beliefs about physical activity with internal consistencies of α = 0.88 and α = 0.77, respectively [12]. The FABQ-D consists of 16 items with which subjects rate their agreement on a 7-point rating scale (0 = “completely disagree” to 6 = “completely agree”). Furthermore, the chronic LBP patients were asked to fill out the painDETECT (PD-Q) questionnaire [2] that includes three 11-point numeric rating scales (NRS), with 0 being “no pain” and 10 being the “worst imaginable pain”. Mean scores were *M* = 3.77 (SD = 3.77) for current pain, *M* = 6.15 (SD = 2.16), for strongest and *M* = 3.75 (SD = 1.88) for average pain intensity in the previous 4 weeks.

*MR scanning parameters*

The MR measurements were conducted using a 3-T whole-body MRI system (Philips Achieva, Best, Netherlands), equipped with a 32-element receiving head coil and MultiTransmit parallel RF transmission. Each imaging session consisted of a survey scan, a B1 calibration scan (for MultiTransmit), a SENSE reference scan, a high resolution T1-weighted anatomical scan and an echo planar imaging (EPI) sequence for blood oxygen level-dependent (BOLD) functional MRI (fMRI) data acquisition. The whole-brain EPI sequence including 365 volumes with 37 slices in the axial orientation using the following parameters: field of view (FOV) = 240 × 240 mm2; acquisition matrix = 96 × 96; slice thickness = 2.8 mm without slice gap; repetition time (TR) = 2100 ms; echo time (TE) = 30 ms; SENSE factor = 2.5; flip angle 80°. Anatomical data were obtained with a 3D T1-weighted turbo field echo scan consisting of 145 slices in sagittal orientation with the following parameters: FOV = 230 × 226 mm2; slice thickness = 1.2 mm; acquisition matrix = 208 × 203; TR = 6.8 ms; TE = 3.1 ms; flip angle = 9°; number of signal averages = 1.

*Experimental protocol and stimulus ratings*

The stimuli consisted of video clips of 4 s duration showing daily activities selected from the short electronic version of the Photograph Series of Daily Activities (PHODA) that has established a fear hierarchy of daily activities based on ratings of perceived harmfulness in chronic LBP patients [5]. The videos were recorded from a 3rd person perspective and included scenes showing potentially harmful activities for the back such as shoveling soil with a bent back, lifting a flowerpot with slightly bent back and vacuum cleaning under a coffee table with a bent back. Neutral activities involved walking up and down the stairs and walking on even ground (please see Figure 1 in [7]).

In the MRI scanner, subjects wore MR-compatible goggles (Resonance Technology, Northridge, CA, USA) connected to a computer running Presentation® software (Neurobehavioral Systems, Davis, CA, USA) that displayed the video clips in a pseudo-randomized order (no more than two identical consecutive trials). Subjects were asked to carefully observe the video clips during fMRI data acquisition. The experiment comprised 30 trials in total (the three harmful and three neutral activities were each presented five times and pooled for each condition in the subsequent psychophysiological interaction (PPI) analysis). After each video clip, subjects were asked to rate the perceived harmfulness of the activity on a visual analog scale (VAS) by means of a MR compatible track ball (Current Designs, Philadelphia, PA, USA) that moved an indicator on the VAS. The VAS was anchored with the endpoints “not harmful at all” (0) and “extremely harmful” (10) and was shown for 4 s. The duration of the inter-stimulus interval (ISI, after the VAS rating, black screen with a green fixation cross) was jittered between 6 and 8 s. The VAS ratings were analyzed using a repeated-measures ANOVA with within-subject factor “video type” and between-subject factor “group”. The repeated measures ANOVA revealed a significant main effect for “video type” (*F*(1,38) = 137.83, p = .001 / Overall mean ratings: harmful activities *M* = 5.28, SD = 2.28, neutral activities *M* = 1.25, SD = 1.33) but no effect of “group” (*F*(1,38) = 0.127 p = .72) nor an interaction effect “group × video type” (*F*(1,38) = 0.14, p = .71).

*fMRI analysis*

FMRI analysis was performed using SPM12, v6470 (http://www.fil.ion.ucl.ac.uk/spm/software/spm12/). Image pre-processing included head motion correction, spatial normalization to the Montreal Neurological Institute (MNI) space and spatial smoothing with a 8-mm Gaussian kernel [6]. A high-pass filter with a cut-off of 128 s was applied on the pre-processed data to remove low frequency noise. Trials were modeled as boxcar regressors and convolved with the standard canonical hemodynamic response function (HRF) without model derivatives as implemented in SPM12. A general linear model (GLM) was fitted for each subject by a design matrix composed of the onsets and duration (4 s) of the harmful and neutral video clips. The serial autocorrelation of the BOLD time series was modeled with a first-order autoregressive model (AR1). A PPI analysis was used to assess the temporal covariance between the amygdala and the PAG as described in the main body of the manuscript.

References

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