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Clinical

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Pain Catastrophizing is Associated with Dental Pain in a Stressful Context

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APPENDIX

APPENDIX METHODS

Electrical Stimulation

Electrical stimulation (square-wave, 10-msec duration, Grass Telefactor S88, W. Warwick, RI, USA) of the enamel surface of the right upper incisor was delivered *via* an electrode developed in-house. The cathode (silver, 2 x 2 mm) was embedded in a shell made of prosthetic material (Tempron, GC, Alsip, IL, USA) and light-cured resin (Filtek, 3M, St. Paul, MN, USA). The shell was fitted to the 2 upper incisors of each participant, ensuring a fixed electrode position on the tooth surface. The insulated wire leading to the cathode was made of copper. The anode was placed on the surface of the cheek (9 x 6 mm, model: 9013R0241, Medtronic Dantec, Copenhagen, Denmark). A drop of conductive gel was applied on the front enamel surface to be stimulated before the shell was attached. The discrete 11-point pain intensity scale with verbal anchors was used for psychophysical calibration, and stimulus evaluation during functional scanning ranged from 0 to 10 (0, no pain; 10, intolerable pain). The 2 stimulus levels to be applied during functional scans were then calibrated outside the scanner room for each participant by the method of ascending limits in 4 series (the first was discarded) and corresponded to mild-moderate pain (3 on scale; LI-level) and strong pain (6 on scale; HI-level). The stimulus levels were checked again inside the scanner. In case of substantial elevated pain ratings due to generalized anxiety, participants were allowed a resting period before stimulus levels were re-checked.

Imaging Data Acquisition and Processing

Data were acquired on a 3-Tesla imaging system (Bruker MedSpec S300, Kalsruhe, Germany) with a quadrature head coil. Patients' heads were placed in the scanner after being immobilized with a vacuum-bean pad. Functional data were acquired with a T2*-weighted gradient-echo EPI using blood-oxygenation-level-dependent contrast (TR/TE/θ = 2000 msec/50 msec/90°) with the parameters: matrix, 64 x 64 x 20; field of view (FOV), 230 x 230 mm^2 with 120-mm coverage in the slice direction (5-mm thickness plus 1-mm gap). For each session, 463 images and 455 images were acquired for the 2 stimulus sequences, respectively. The anatomic image was acquired with a T1-weighted, 3D gradient-echo pulse sequence (modified driven equilibrium Fourier transform: TR/TE/TI = 88.1 msec/4.12 msec/650 msec) with the following parameters: matrix, 256 x 256 x 192; FOV, 230 x 230 mm^2; slice thickness, 1.5 mm.

Functional imaging data were pre-processed and analyzed with statistical parametric mapping (SPM5 software from the Wellcome Department of Cognitive Neurology, London, UK). Scans were slice-time-corrected, re-aligned, and co-registered to the individual anatomic image before being normalized to standard space (Ashburner *et al.,* 1999). Scans were further re-sampled (2-mm^3 voxel), smoothed (8-mm), high-pass-filtered, and corrected for temporal serial correlations (Friston *et al*., 2000). For image statistics, onsets of cues, electrical stimuli, and scales were taken as individual events and modeled with a canonical hemodynamic response function with temporal derivatives. Head movement parameters as estimated from the re-alignment were included as regressors.

Behavioral Analysis

The rating of pain intensity and pain-related anxiety and the scores of psychological assessment all followed a Gaussian distribution (Kolmogorov-Smirnov test for normal distribution, $p > 0.1$), and, accordingly, parametric statistical analyses were performed. A significant increase in the anxiety rating in the unpredictable (UnP) conditions *vs*. predictable (P) conditions (one-tailed paired *t* test) suggested that unpredictability about intensity induced higher anxiety. A significant increase in the pain rating in the LI-UnP condition *vs*. LI-P condition (one-tailed paired *t* test) suggested that unpredictability about intensity induced stronger pain (*i.e., unpredictability effect*). In contrast, a significant increase in pain ratings in the HI-UnP condition *vs.* LI-UnP condition (one-tailed paired *t* test) would reveal increased pain modulated by the heightened nociceptive intensity of stimuli, with the level of anxiety matched (*i.e*., *intensity effect*).

Image Data Analysis

The acquired imaging data were pre-processed with SPM8 (http://www.fil.ion.ucl.ac.uk/spm/software/spm8/) to create the following images:

(iii) three *baseline* images, which, respectively, showed BOLD activation during painful electrical stimulation (*i.e*., during the *pain phase* of a trial, see Fig. 1B in the main paper) in the 3 experimental conditions (LI-P, LI-UnP, and HI-UnP); and

(iv) a *contrast* image that compared the baseline image LI-UnP with the baseline image LI-P. This contrast image would show the brain regions with increased activation in response to increased unpredictability (*i.e*., showing the brain regions modulated by the unpredictability effect). To investigate the brain regions that reflected the individual variations in PCS scores, we performed a voxel-by-voxel regression analysis by calculating the correlation between PCS scores and the degree of activation, across all participants.

Two analyses were performed for testing our imaging hypothesis regarding the role of the hippocampus, as follows.

Region-of-Interest (ROI) Analysis

We hypothesized that, in a stressful context, a higher degree of catastrophizing is associated with increased hippocampal activation. The hippocampus consists of functionally heterogeneous subregions: the anterior hippocampus, related to emotional processing, such as fear and anxiety; and the posterior hippocampus, related to memory and learning of aversive stimuli (Goosens, 2011). We thus selected the anterior and posterior hippocampus as the ROIs and confined the regression analysis to only the voxels within the ROI (Poldrack, 2007). The results were corrected by the small-volume correction approach (SVC, controlled for family-wise error, $P_{FWE} = 0.05$). Voxels exceeding the criteria were considered significant (see below for the definition of the ROI). The ROI analysis was performed for the contrast image.

Whole-brain Exploratory Analysis

For exploratory purposes, we searched the whole brain for regions in which activation positively correlated with the PCS score. The whole-brain analysis was performed separately for the 3 baseline images and the contrast image. The resulting voxels with $p < 0.005$ (uncorrected for multiple comparison) and a cluster extent > 20 voxels were considered significant.

ROI Definition

The hippocampal ROI was defined based on the Harvard-Oxford cortical structural atlas (http://www.fmrib.ox.ac.uk/fsl/fslview/index.html), a probability map consisting of the anatomic brain images of 37 healthy individuals, based on the following 3 steps:

We extracted the area labeled 'Hippocampus, Cornu Amonis' (*i.e*., the hippocampus proper) with a stringent threshold of 75% to form the basic hippocampus ROI, separately for both hemispheres.

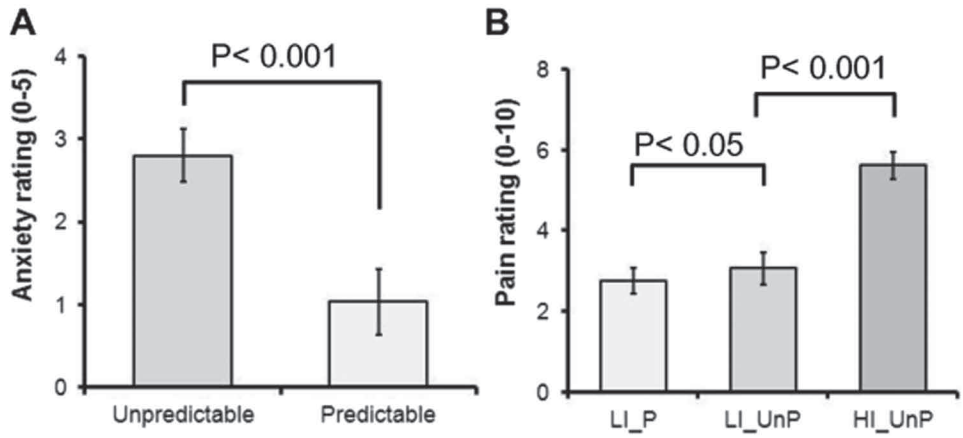
Because there have been no universal criteria regarding the definition of the anterior and posterior subregions of the hippocampus, we followed the approach recently reported by Satpute *et al.* (2012). We selected the most anterior and the most posterior voxels located in the basic hippocampus ROI, until each of them occupied 1/3 of the basic ROI. The middle 1/3 was not included in either the anterior or the posterior subregion because of lack of a clear boundary in this transitional zone.

Finally, we examined the sizes of the newly created anterior and posterior subregion ROIs. The ROIs showed slight differences in their size (the right hemisphere, anterior/posterior/basic hippocampus = 87/88/262 voxels; the left hemisphere, anterior/posterior/basic hippocampus = 71/72/216 voxels).

APPENDIX RESULTS

Anxiety rating was significantly higher in the unpredictable condition compared with the predictable condition (two-tailed paired *t* test, UnP > P, $t = 7.57$, $p < 0.001$) (Appendix Fig.). Pain rating was significantly higher in the LI-UnP condition compared with the LI-P condition (one-tailed paired *t* test, LI-UnP > LI-P, $t = 2.32$, $p = 0.018$), and in the HI-UnP condition compared with the LI-UnP condition (one-tailed paired *t* test, HI-UnP > LI-UnP, $t = 9.13$, $p < 0.001$) (Appendix Fig.). The findings indicate that increased unpredictability induced anxiety and exacerbated pain, in line with previous findings (Ploghaus *et al*., 2001; Brown and Jones, 2008).

The participants rated increased pain and pain-related anxiety in the unpredictable condition, compared with the predictable condition. Multiple-regression analysis revealed the PCS score as the only variable significantly correlated with $\Delta P_{unpredictability}$ ($t = 3.75$, $p = 0.002$, zero-order $r = 0.72$) (Fig. 2A, main paper) (see Appendix Table for results of psychological assessment). In contrast, none of the variables significantly predicted $\Delta P_{intensity}$. The PCS score was not correlated with $\Delta P_{intensity}$ (Fig. 2A, main paper). The finding confirmed our behavioral hypothesis that pain catastrophizing predicted the increased pain modulated by unpredictability; in contrast, it did not predict the increased pain modulated by increased nociceptive intensity. Notably, individual variations in increased anxiety (*i.e.,* anxiety rating in the unpredictable condition *vs*. the predictable condition) did not correlate with the increased pain ($p = 0.37$), and the PCS score did not significantly correlate with increased anxiety ($p = 0.98$). The findings suggest that the changing anxiety *per se* did not account for the changing pain experience. The participants felt stronger pain and anxiety in the stressful (unpredictable) context, but the degree of heightened pain was predicted by pain catastrophizing, rather than pain-related anxiety. We performed the multiple-regression analysis described in the 'Statistical Analysis', with each of the PCS subscale scores (rumination, magnification, and helplessness) as the predictor. We found that all the PCS subscale scores significantly correlated with $\Delta P_{unpredictability}$ ($t = 4.11$, $p = 0.001$, zero-order $r = 0.75$ for rumination; $t = 2.88$, $p = 0.013$, zero-order $r = 0.62$ for magnification; $t = 2.49$, $p = 0.027$, zero-order $r = 0.57$ for helplessness) but not with $\Delta P_{intensity}$. The finding suggested that all 3 psychological dimensions of the PCS contribute to increased



Appendix Figure. Anxiety and pain mediated by unpredictability about pain. **(A)** The participants reported a higher anxiety rating when the stimuli were delivered in the unpredictable condition compared with the predictable condition (two-tailed paired *t* test, $p < 0.001$). **(B)** The participants reported a higher pain rating when the low-intensity stimuli were delivered in the unpredictable condition (LI-UnP) compared with the predictable condition (LI-P) (two-tailed paired *t* test, $p < 0.05$), and when the high-intensity stimuli were delivered in the unpredictable condition (HI-UnP) compared with the LI-UnP condition (two-tailed paired *t* test, $p < 0.001$).

Appendix Table. Psychological assessment. (A) The mean, standard deviation (SD), minimum (Min), and maximum (Max) of the scores of each general and dental-specific trait assessment. The DBS and the PCS scores were calculated as the total scores from all the respective subscales. Note the distribution of PCS scores from the current participants is consistent with the results previously reported (mean ± SD = 17.3 ± 7.9, Sullivan et al., 1995). (B) Correlation analyses (Pearson's correlation) revealed no significant correlation between each of pairs of the scores.

(A)

	Mean	SD	Min	Max
BDI	6.3	3.0	2	13
DBS	5.7	2.8	2	11
MDAS	9.5	2.6	5	14
MPQ	9.6	4.0	4	16
PCS	19.2	8.1	9	38

(B)

	DBS	MDAS	MPQ	PCS
BDI	0.12	0.26	–0.39	0.38
DBS		0.30	–0.40	–0.48
MDAS			–0.28	0.16
MPQ				0.36

pain, while rumination, the tendency to ruminate pain-related experience, may play a critical role in shaping pain in a stressful dental setting.

APPENDIX DISCUSSION

We have noticed a different activation pattern in the LI-P condition, which may be related to processing of the salience network (Seminowicz and Davis, 2006; Taylor *et al*., 2009; Wiech *et al.,* 2010). We found that, in the LI-P conditions, there were more regions showing significant correlation with PCS scores, including the anterior insula and the anterior cingulate cortex, both critical regions of the salience network (Taylor *et al*., 2009; Wiech *et al*., 2010). The finding was consistent with those from a previous report in which mild pain was delivered to healthy participants (Seminowicz and Davis, 2006). The changing activation pattern suggested that (i) in a context-dependent modulation of catastrophizing in a stressful context, as mentioned above, the hippocampal activation is associated with the acquisition of threat information; while in contrast, (ii) in a less-threatening or less stressful context (*i.e*., when the stimuli intensity is low and predictable), activation of the salience network is associated with the detection of a threat, with high-catastrophizers being more prone to perceive that 'pain is there'.

In the current study, we did not investigate the effect of a predictable high-intensity (HI-P) pain for experimental reasons. It is noteworthy that an interactional effect between predictability and stimulus intensity may exist. As demonstrated by Brown and Jones (2008), when the stimulus intensity was low, an unpredictable stimulus would evoke stronger pain than a predictable stimulus, as seen in our study. In contrast, when the stimulus intensity was high, a predictable stimulus would evoke stronger pain than an unpredictable stimulus (Brown and Jones, 2008). This finding suggests that anxiety (feeling about uncertainty) and fear (anticipation of predictable threat), both commonly seen in a dental setting, may be related to different pain-modulatory mechanisms. We did not include the high-intensity predictable condition in our paradigm for 2 reasons. First, we thought that a predictable high-intensity stimulus may lead to too much fear for the participants and result in a higher withdrawal rate and failure of image acquisition. When strong pain (as a potential threat) is predictable, the participants may involuntarily change the position of the body or the head in preparation for receiving the stimulus. This significant movement may lower the quality of the acquired image. Second, including an additional condition (increasing from 3 to 4 conditions)

would have meant a prolonged duration of the fMRI scan, which could lead to further general distress for the participants.

APPENDIX REFERENCES

Ashburner J, Andersson JL, Friston KJ (1999). High-dimensional image registration using symmetric priors. *Neuroimage* 9(6 Pt 1):619-628.

Brown CA, Jones AK (2008). A role for midcingulate cortex in the interruptive effects of pain anticipation on attention. *Clin Neurophysiol* 119:2370-2379.

Friston KJ, Josephs O, Zarahn E, Holmes AP, Rouquette S, Poline J (2000). To smooth or not to smooth? Bias and efficiency in fMRI time-series analysis. *Neuroimage* 12:196-208.

Goosens KA (2011). Hippocampal regulation of aversive memories. *Curr Opin Neurobiol* 21:460-466.

Ploghaus A, Narain C, Beckmann CF, Clare S, Bantick S, Wise R, *et al.* (2001). Exacerbation of pain by anxiety is associated with activity in a hippocampal network. *J Neurosci* 21:9896-9903.

Poldrack RA (2007). Region of interest analysis for fMRI. *Soc Cogn Affect Neurosci* 2:67-70.

Satpute AB, Mumford JA, Naliboff BD, Poldrack RA (2012). Human anterior and posterior hippocampus respond distinctly to state and trait anxiety. *Emotion* 12:58-68.

Seminowicz DA, Davis KD (2006). Cortical responses to pain in healthy individuals depend on pain catastrophizing. *Pain* 120:297-306.

Taylor KS, Seminowicz DA, Davis KD (2009). Two systems of resting state connectivity between the insula and cingulate cortex. *Hum Brain Mapp* 30:2731-2745.

Wiech K, Lin CS, Brodersen KH, Bingel U, Ploner M, Tracey I (2010). Anterior insula integrates information about salience into perceptual decisions about pain. *J Neurosci* 30:16324-16331.