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Effects of psychological stress on the cerebral processing of visceral stimuli in healthy women

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Abstract *The aim of the study was to analyse effects of psychological stress on the neural processing of visceral stimuli in healthy women. The brain functional magnetic resonance imaging blood oxygen level-dependent response to non-painful and painful rectal distensions was recorded from 14 healthy women during acute psychological stress and a control condition. Acute stress was induced with a modified public speaking stress paradigm. State anxiety was assessed with the State-Trait-Anxiety Inventory; chronic stress was measured with the Perceived Stress Questionnaire. During non-painful distensions, activation was observed in the right posterior insular cortex (IC) and right S1. Painful stimuli revealed activation of the bilateral anterior IC, right S1, and right pregenual anterior cingulate cortex. Chronic stress score was correlated with activation of the bilateral amygdala, right posterior IC (post-IC), left periaqueductal grey (PAG), and right dorsal posterior cingulate gyrus (dPCC) during non-painful stimulation, and with activation of the right post-IC, right PAG, left thalamus (THA), and right dPCC during painful distensions. During acute stress, state anxiety was significantly higher and the acute stress – control contrast revealed activation of the right dPCC, left THA and right S1 during painful stimulation. This is the first study to demonstrate effects of acute stress on cerebral activation patterns during visceral pain in healthy women. Together with our finding that chronic stress was correlated wit the neural response to visceral stimuli, these results provide a framework for further studies addressing the role of chronic stress and emotional disturbances in the pathophysiology of visceral hyperalgesia.*

Keywords *anxiety, functional magnetic resonance imaging, stress, visceral pain.*

Psychological stress has been implicated in the pathophysiology of functional gastrointestinal disorders such as irritable bowel syndrome (IBS), but the mechanism(s) by which psychological stress causes and/or maintains chronic pain remain incompletely understood. The processing of sensory information, particularly of unpleasant or painful stimuli, has previously been found to have important cognitive, motivational, as well as emotional components, all of which are likely affected by psychological stress.[1] Imaging techniques have started to elucidate the neuro-anatomical connections and networks which mediate the influence of cognitions and emotions on the processing of sensory information.[2,3] The insular cortex (IC) and cingulate cortex appear to be of primary importance for the integration of sensory information with the emotional context.[4,5] Both areas are activated by emotional and motivational factors, for example during the recognition of negative emotions and in association with depression and sadness.[6,7] Via connections to the amygdala (AMY) and brain stem, the anterior cingulate cortex (ACC) and IC can elicit and coordinate affective and autonomic reactions.[8] This network may not only be responsible for the integration of sensory and emotional information and the coordination of affective reactions, but it may also play a role in the modulation of pain processing by

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psychological stress and stress-associated negative emotions. Support for this notion comes from previous functional magnetic resonance imaging (fMRI) studies conducted in the context of somatic pain indicating that the central processing of somatic pain stimuli is modulated by inter-individual differences in fear and anxiety.[9]

Little is known about the possible relevance of psychological stress and negative emotions in the context of the neural response to visceral pain. Support for effects of psychological stress on the neural response to visceral stimuli comes from evidence that IC and ACC activation in response to oesophageal distensions was greater when stimuli were presented in a negative emotional context.[5] Further, a recent fMRI study revealed that negative emotions of anxiety, anger and stress correlated negatively with anticipatory downregulation within the dorsal pons, AMY and ACC during anticipated visceral pain.[10] To address the hypothesis that psychological stress modulates the brain processing of visceral stimuli in healthy women, we chose two different experimental approaches. Firstly, we analysed the correlation of the brain response to non-painful and painful visceral stimuli and chronic stress, assessed with a validated questionnaire, in a control condition. Secondly, we assessed the brain response to non-painful and painful visceral stimuli in an acute stress compared to a control condition. Given the pivotal role of the IC and ACC in the processing of painful visceral stimuli, and the importance of the AMY for the response to aversive emotional stimuli such as fear and threat,[11] we hypothesized that both chronic and acute psychological stress would effect activation of these brain regions.

METHODS

Recruitment and screening

Healthy, right-handed women were recruited by public advertisement. The screening process included a personal interview, completion of questionnaires and standard physical examination with a manual rectal examination. Exclusion criteria included age <18 and >45 years, any clinical condition including gastrointestinal, neurological, psychological or psychiatric diagnoses and regular use of medications except contraceptive drugs or occasional use of pain killers or allergy medications. Subjects were screened for symptoms suggestive of IBS or other gastrointestinal conditions using a standardized questionnaire assessing frequency and severity of variety of gastrointestinal symptoms. All women were evaluated for possible internal anal tissue damage (e.g. painful haemorrhoids) which may interfere with balloon placement. To exclude clinically relevant levels of anxiety or depression, the German version [12] of the Hospital Anxiety and Depression Scale (HADS) was completed. Right-handedness was confirmed with a validated questionnaire.[13] A structural MRI scan was completed to exclude any brain tissue abnormality. Pregnancy was excluded using a urinary test. The study protocol was approved by the local Ethics Committee. All participants gave written informed consent prior to participation and were paid for their participation. Notably, the study public speaking stress protocol (see below) was not fully disclosed. Participants were only told they would be asked to complete a cognitive task during one set of distensions in the scanner. This was done to minimize anticipatory anxiety relating to the psychological stress paradigm.

Study design

Eligible subjects were scheduled for two study days which took place no more than 7 days apart. On the first study day, rectal sensory thresholds were determined using a pressure-controlled barostat device, and a structural MRI scan was completed. On the second study day, brain activation in response to non-painful and painful rectal distensions was measured with fMRI in a control condition and an acute stress condition, carried out in randomized order.

Psychological stress condition

To induce acute psychological stress in the scanning environment, we chose a modified public speaking stress model given our extensive experience with this paradigm and the fact that is demonstrably produces relatively pronounced emotional and neuroendocrine stress effects with comparatively little inter-individual variations.[14–16] Following a pilot study (data not shown), we adapted the stress paradigm for use in the scanner as follows: Following positioning on the fMRI platform and placement of the balloon, subjects were instructed to prepare a 5-min speech about a given topic which they were to present in front of a group of experts who would evaluate the speech for content and style. After a 5-min preparation time, five 'experts' in white laboratory coats entered the fMRI suite. The speech delivery phase followed, with termination after 1 min by a staged interruption: Another doctor rushed into the suite, addressing the main investigator claming a medial 'emergency' was on its way and the scanner would be needed within 10 min. After a staged short discussion, an 'agreement' was reached that the research subject's scan could still be completed first, but that the remaining parts of the speech would have to be delivered after the scan in an adjacent room. This was done to maintain elevated levels of stress and anxiety during scanning (rather than feeling of e.g. relief following disruption of the speech). Markedly elevated levels of state anxiety, measured prior to and after scanning using a validated questionnaire (see Methods section), document that this protocol was highly effective (Fig. 1).

Rectal balloon distensions

Rectal distensions were carried out with a pressure-controlled barostat system (modified ISOBAR 3 device; G & J Electronics, Ontario, Canada) as previously described.[16,17] Briefly, an infinitely compliant catheter-affixed polyethylene bag of cylindrical shape with a diameter of 10 cm and a maximal volume of 600 mL when fully inflated was attached to a rectal tube with an outer diameter of 5 mm. To carry out distensions, the balloon was inserted into the rectum after lubrication, with the distal bag margin 5 cm beyond the anal verge. Prior to the fMRI study, rectal perception

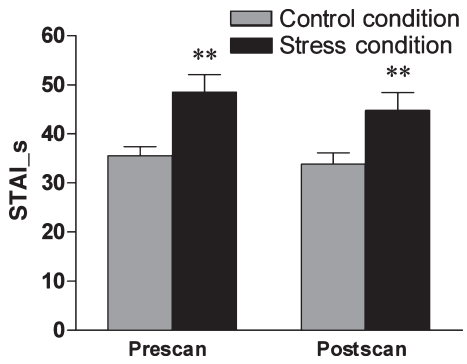

Figure 1 State anxiety during stress and in a control condition. State anxiety, assessed with the state version of the State-Trait-Anxiety Inventory (STAI-S), was measured prior to scanning (prescan) and after scanning (postscan) during acute stress and in a control condition. STAI-S scores were significantly higher, indicating greater state anxiety, in the acute stress condition (**$P < 0.001$, paired *t*-tests).

and pain thresholds were determined using staircase distensions with random pressure increments of 2–10 mmHg. Each pressure was maintained for 30 s, then subjects were prompted with a light signal to rate the sensation by using a rating scale, i.e. one = no perception, two = doubtful perception, three = sure perception, four = distension, not too unpleasant, five = distension, unpleasant, six = distension, very unpleasant/painful). Subsequently, the barostat was deflated completely to a pressure of 0 mmHg. In-between distensions, pauses with complete balloon deflation lasting approximately 10 s were accomplished. For ethical reasons, the maximal distension pressure was set at 50 mmHg and inflation was discontinued whenever a subject rated the distension as very unpleasant/painful. The threshold for first perception was defined as the distending pressure when the subjects rating changed from 'doubtful perception' to 'sure perception'; the pain threshold was defined as the pressure at which subjects indicated 'very unpleasant/painful'.

As there are large inter-individual but relatively small intra-individual variations in rectal pain thresholds,[18,19] rectal distension pressures for the fMRI study were based on individual thresholds in accordance with the methodology applied in the majority of recent fMRI studies on visceral pain perception.[20] Hence, based on the thresholds for rectal perception and pain, two distension pressures were determined for each subject for application during the fMRI experiments, designed to create to two distinct perceptual intensities: (i) 'non-painful', i.e. distension is clearly perceived, but non-painful, corresponding to a rating of three (see above) and (ii) 'painful', i.e. distension is unpleasant, corresponding to a rating of five. For ethical reasons, the pressure corresponding to a rating of six was not utilized for repeated distensions in the scanner.

To accomplish rectal barostat distensions during scanning, participants were positioned in a supine position on the fMRI platform with a foam pad placed underneath the hip to prevent excessive bending of the tube connecting the balloon catheter. The barostat device was kept outside of the scanner suite and was connected to the bag by a 6 m polyethylene tube (5 mm outer diameter, 4 mm inner diameter). It has previously been demonstrated that the length of the tube connecting the barostat bag with the device affects the perception pressures (i.e. pressure are higher with the long tubing).[21] Therefore, all barostat distensions including those for the initial determination of perception and pain thresholds during the first study visit (see above) were carried out using the same connecting tube.

Questionnaires

Subjects completed the German version of the Perceived Stress Questionnaire (PSQ), which has previously been validated and applied in healthy adults and various clinical populations.[22,23] For the analyses herein, the total PSQ score was used as an indicator of overall perceived chronic stress.

The German state version[24] of the State-Trait-Anxiety Inventory (STAI-S) [25] was completed prior and postscanning to assess acute anxiety. In addition, 100 mm visual analogue scales (VAS) scales were completed after scanning to assess how painful subjects rated the distensions delivered during scanning, and how much urge to defecate they had experienced during distensions (zero = no pain/no urge to defecate, 100 = unbearable pain/unbearable urge to defecate).

fMRI: imaging and analyses

All MR images were acquired using a 1.5 T MR (Sonata; Siemens, Erlangen, Germany) with a standard head coil. A 3D FLASH sequence (relaxation time 10 ms, echo time 4.5 ms, flip angle 30°, field of view 240 mm, matrix 512, slice-thickness 1.5 mm) was acquired for individual coregistration of functional and structural images. The blood oxygen level-dependent contrast images were acquired using an echo-planar technique (TR 3100 ms, TE 50 ms, flip angle 90°, FOV 240 mm, and matrix 64) with 34 transversal slices angulated in direction of the corpus callosum with a thickness of 3 mm and a 0.3 mm slice gap. We implemented a block-design comprising phases of distension alternating with phases without distension as described below. Ten scans, each lasting 31 s, formed blocks of active and passive phases. Of note, in the active condition the first two scans were not regarded as active as the first 6 s were needed to inflate the balloon. This resulted in an active phase of 25 s. A total of 12 rectal distensions were applied, comprised of six non-painful distensions, followed by six painful distensions.

For data analysis, SPM 05 software (Wellcome Department of Cognitive Neurology, London, UK) was used. Prior to statistical analysis, images were realigned using sinc interpolation and normalized to the standard stereotactic space corresponding to the template from the Montreal Neurological Institute (http://imaging.mrc-cbu.cam.ac.uk/imaging/MniTalairach). Bilinear interpolation was applied for normalization. The images were smoothed with an isotropic Gaussian kernel of 9 mm. A voxel-by-voxel comparison according to the general linear model was used to calculate differences of activation between active and resting condition. The model consisted of a box-car function convolved with the haemodynamic response function (hrf) and the corresponding temporal derivative. High-pass filtering with a cut-off frequency of 120 s and low-pass filtering with the hrf was applied. For group analysis, single subject contrast images were entered into a random effects model for group comparisons with the subjects being the random factor. Significant signal changes for each contrast were assessed by means of a *t*-statistics on a voxel-by-voxel basis.[26] The resulting set of voxel values for each contrast constituted a statistical parametric map of the *t*-statistic. In the group analyses using one-sample *t*-tests the threshold of the *t*-statistic was set to $P < 0.001$ uncorrected for multiple comparisons for the following predefined regions of interest. For rectal distension stimuli, the activation of the following brain regions has been reported in humans: ACC, IC, AMY, somatosensory and motor cortices, periaqueductal grey (PAG) and thalamus (THA).[1,4,27] ACC regions were determined based on Vogt.[28] Paired *t*-tests were performed between non-painful and pain stimuli. Additionally, the correlation of cortical

activation with the non-painful and pain conditions was performed; fMRI data were analysed with PSQ scores in a multiple regression.

Statistical analyses of questionnaire data

Comparisons of psychological data were accomplished using paired *t*-tests. Correlations were calculated by computing Pearson's *r*. The alpha level for significance was set at 0.05. All non-fMRI data are shown in the text as mean ± SEM.

RESULTS

Participants

Fourteen healthy, right-handed women (mean age: 32.78 ± 8.38 years) participated in this study. In no case was there any evidence of brain tissue abnormality on structural MRI. No subject had anxiety or depression scores, measured with the HADS, in either the subclinical (i.e. scores >8) or the clinical range (i.e. scores >11). Accordingly, mean HADS anxiety scores (4.57 ± 2.4) and depression scores (2.42 ± 2.59) were low. Mean total score on the PSQ was 22.12 ± 15.89 (maximum: 60, minimum: 1.66).

Rectal perception and pain thresholds; subjective assessment of distension stimuli

The basal median rectal perception threshold was 17.0 mmHg (mean: 17.8 mmHg; SEM: 1.0 mmHg; minimum: 10.0 mmHg; maximum: 23.0 mmHg) and the basal median rectal pain threshold was 34.0 mmHg (mean: 32.6 mmHg; SEM: 1.7 mmHg; minimum: 16.0 mmHg; maximum: 42.0 mmHg).

In the control condition, painful distensions were perceived as being significantly more painful (36.92 ± 3.12 mm) compared to perception stimuli (9.23 ± 11.04 mm) (paired *t*-test: $P < 0.01$). Similarly, painful distensions elicited significantly more urge to defecate (64.15 ± 28.98 mm) compared to perception stimuli (11.69 ± 12.57 mm) (paired *t*-test: $P < 0.001$).

Effects of acute psychological stress on state anxiety and subjective ratings

State anxiety was expectedly significantly higher in the acute stress condition as evidenced by higher scores both prior to and after scanning (both $P < 0.01$, Fig. 1). On the other hand, acute stress had no significant effect on the subjective assessment of distension stimuli with respect to the extent of pain and urge to defecate, assessed with VAS scales (data not shown).

Correlational findings (psychological measures)

Perceived Stress Questionnaire total score did not correlate significantly with either the rectal perceptual threshold ($r = -0.238$, $P = 0.41$) or with the rectal pain threshold ($r = 0.084$, $P = 0.78$). However, PSQ total score was significantly and highly correlated with trait anxiety ($r = 0.78$, $P < 0.01$), and with anticipatory anxiety, assessed with the STAI-S just prior to scanning (control condition: $r = 0.67$; stress condition: $r = 0.56$, both $P < 0.05$).

FMRI RESULTS

Brain regions activated during rectal distensions in the control condition

In response to non-painful distensions, we observed activation of the right posterior IC (post-IC) and right S1 (Table 1). Painful rectal distensions revealed activation of the bilateral anterior IC, right somatosensory cortex (S1) and right pregenual ACC (Table 1). Direct comparisons of brain activation during painful *vs* non-painful stimulation using paired *t*-tests with an uncorrected *P*-value of 0.001 revealed a superior activation in the post-IC (Talairach coordinates: 50, 10, 0; *t*-value = 5.65) in the pain condition. The vice versa contrast did not reveal any significant differences.

Correlation of brain activation to rectal distensions with chronic stress

To determine the relationship between chronic stress and the brain response to rectal stimuli, PSQ scores

Table 1 Peak Talairach coordinates of regions significantly activated during rectal distensions

	Talairach coordinates					
	OST				Cluster level	
Regions of interest	H	*x*	*y*	*z*	*t*-value	K_E
Non-painful						
Posterior insula	R	46	−1	15	5.49	12
S1	R*	40	−46	50	3.75	16
Painful						
Anterior insula	R	38	12	3	5.59	108
Anterior insula	L*	−40	2	5	4.43	24
S1	R	51	−42	56	7.53	208
pACC	R*	4	38	17	4.73	46

All coordinates were converted from MNI to Talairach space.
pACC, pregenual anterior cingulate cortex; OST, one sample *t*-test; MNI, Montreal Neurological Institute (one sample *t*-test; $P < 0.001$, uncorrected; *$P < 0.005$, uncorrected).

Table 2 Peak Talairach coordinates of regions significantly activated performing a multiple regression with chronic stress score (PSQ)

	Talairach coordinates											
	Non-painful				Cluster level		Painful				Cluster level	
Regions of interest	H	*x*	*y*	*z*	*t*-value	K_E	H	*x*	*y*	*z*	*t*-value	K_E
Amygdala	R	32	−7	−15	4.87	10	–	–	–	–	–	–
Amygdala	L*	−32	1	−13	7.46	20	–	–	–	–	–	–
Posterior insula	R*	48	−9	12	3.46	5	R	46	−21	5	5.11	21
PAG	L*	0	−28	−25	4.43	17	R*	2	−26	−22	4.55	12
Thalamus	–	–	–	–	–	–	L*	−6	−11	6	3.80	11
dPCC	R*	12	−37	35	4.26	28	R*	2	−21	49	4.34	55

All coordinates were converted from MNI to Talairach space ($P < 0.001$, uncorrected; *$P < 0.005$, uncorrected). PSQ, Perceived Stress Questionnaire; PAG, periaqueductal grey; dPCC, dorsal posterior cingulate gyrus.

were included in a multiple regression. For non-painful stimuli, PSQ score was significantly associated with activation of the bilateral AMY, right post-IC, left PAG and right dorsal posterior cingulate gyrus (dPCC) (Table 2, Fig. 2A). For painful rectal distensions, significant correlations were observed for activation of the right post-IC, right PAG, left THA and right dPCC (Table 2, Fig. 2B).

Effects of acute psychological stress

To determine the effect of acute psychological stress on the brain response to rectal stimuli, direct comparisons of brain activation during acute stress *vs* control were accomplished with paired *t*-tests. Whereas no differences between conditions were observed for non-painful stimuli, during painful distensions activation of the right dPCC, left THA and right S1 were significant in the stress condition compared to control (Table 3, Fig. 3).

DISCUSSION

In summary, our findings in healthy women showed a correlation of chronic stress and rectal distension-induced brain activation in multiple brain regions. Specifically, during non-painful visceral stimulation, activation of the post-IC, AMY, PAG and dPCC were associated, and during painful stimulation, the post-IC, dPCC, PAG and THA correlated with chronic stress score. Furthermore, experimental stress expectedly led to increased state anxiety, but also to greater activation of the dPCC, THA and S1 in response to painful visceral stimuli.

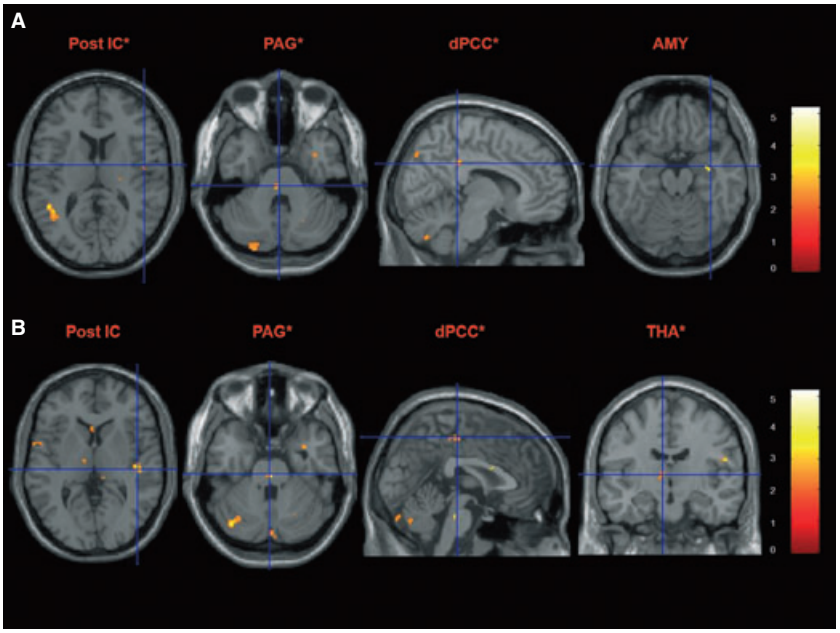

Figure 2 Correlation of chronic stress with rectal distension-induced brain activation. (A) During non-painful rectal stimulation, chronic stress correlated with activation of the bilateral amygdala (AMY), right posterior IC (post-IC), left periaqueductal grey (PAG) and right dorsal posterior cingulate cortex (dPCC). (B) During painful rectal stimulation, chronic stress correlated with activation of the right post-IC, right PAG, left thalamus (THA) and right dPCC. Task-related increase in MR signal are superimposed on sections of standard 3D T1-weighted anatomical brain images. The statistically uncorrected threshold was $P < 0.001$ and *$P < 0.005$ respectively.

Table 3 Peak Talairach coordinates of activation during acute stress compared to control condition

	Talairach coordinates					
	Acute stress – control contrast				Cluster level	
Regions of interest	H	*x*	*y*	*z*	*t*-value	K_E
Painful						
dPCC	R*	10	−39	44	5.35	177
Thalamus	L*	16	−23	12	4.51	29
S1	R	8	−53	63	7.39	103

All coordinates were converted from MNI to Talairach space (paired *t*-test; $P < 0.001$, uncorrected; *$P < 0.005$, uncorrected). dPCC, dorsal posterior cingulate gyrus.

Given the role of the ACC in the affective dimension of pain, and previous evidence that the ACC encodes both emotional and cognitive demands,[29,30] we hypothesized that psychological stress would affect brain activation in this anterior region of the cingulate cortex. However, we observed effects of both chronic and acute stress on activation of a posterior cingulate region, i.e. the dPCC, during visceral stimulation. The specific functions of the dPCC remain incompletely understood. Together with the posterior midcingulate cortex, the dPCC appears to be involved in orienting the body in response to sensory stimuli, including but not limited to nociceptive stimuli.[28] Interestingly, both regions dominate activity in areas of the cingulate cortex. We indeed observed that IC activation was associated with chronic stress, consistent with our hypothesis, and the notion that the IC is highly relevant for the processing of visceral stimulation as part of the network responsible for the integration of sensory and emotional information and the coordination of affective reactions.[8] However, we did not detect any effect of acute stress on IC activation, suggesting that its activation may be modulated by long-term rather than acute emotional states.

Our results extend previous findings, including evidence that non-painful oesophageal distensions induced greater activation in ACC and IC when stimuli were presented in a negative emotional context.[31] Furthermore, during the anticipation of visceral pain, negative emotions of anxiety, anger and stress correlated negatively with anticipatory downregulation within the dorsal pons, AMY and ACC.[10] In addition, PAG activity correlated with anxiety during visceral but not somatic stimulation.[32] Finally, during the experience of noxious thermal stimulation, pain-related anticipatory anxiety and fear of pain were associated with activation of the cingulate cortex as well as with medial prefrontal and ventral lateral frontal activation.[9] Together with our results, these findings clearly support the notion that both chronic and acute psychological stress affects the central processing of aversive sensory information, including visceral stimuli, in healthy subjects.

Several mechanisms may underlie these effects, which are likely mediated by multiple brain regions. Psychological stress may affect pain processing by reducing the ability to cope with pain, which may be reflected by impaired pain inhibition involving the PAG and its connections.[10] Indeed, in our study PAG activation during visceral stimulation correlated with chronic stress score. High levels of stress may also result in more aversive emotional responses, i.e. greater anxiety during the anticipation and/or the actual experience of aversive bodily sensations, involving the AMY and its connections.[33] In our study, chronic stress score was not only correlated with anticipatory anxiety but also with bilateral AMY activation during visceral stimulation. Furthermore, increased chronic stress may represent a relatively stable personality characteristic, closely related to trait

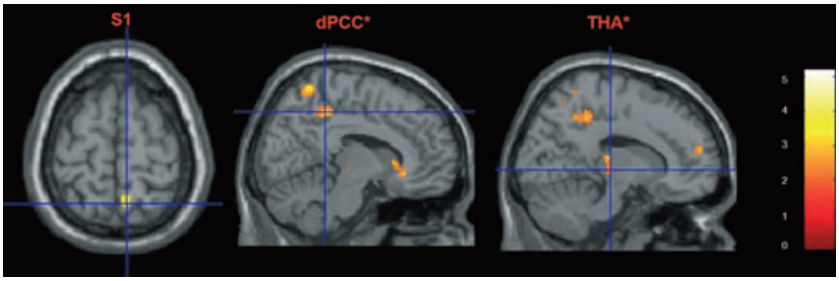

Figure 3 Comparison of brain activation during painful rectal distension in the acute stress *vs* control condition. Brain activation during painful rectal distensions in the acute stress condition compared to the control condition. The acute stress – control contrast revealed activation in the right somatosensory area (S1), right dorsal posterior cingulate cortex (dPCC) and left thalamus (THA). Task-related increase in MR signal are superimposed on sections of standard 3D T1-weighted anatomical brain images. The statistically uncorrected threshold was $P < 0.001$ and $^*P < 0.005$ respectively.

anxiety and/or neuroticism. This is supported by our observation that chronic stress score was significantly correlated with trait anxiety; even though overall levels of anxiety were low in this sample of healthy subjects. A possible role of personality traits is supported by evidence that the response to emotional stimuli is determined by personality factors including trait anxiety and neuroticism.[33–36] Even in healthy subjects without psychiatric conditions, trait anxiety and other personality characteristics suggestive of high anxiety proneness (e.g. neuroticism, anxiety sensitivity) were reportedly associated with AMY and IC to emotional stimuli not involving a pain component, e.g. looking at emotional faces.[31,33–36]

Our findings have implications for conditions involving chronic visceral pain, such as IBS, as well as other 'somatization syndromes' characterized by psychological disturbances, including high levels of chronic stress and greater anxiety. Several previous fMRI studies have documented alterations in the brain response to visceral stimulation in IBS patients, including differences in ACC and IC activation.[1,37] Based on our findings in healthy women, one could speculate that disturbed neural processing of visceral stimuli in these patients may at least in part be related to higher levels of chronic stress and greater anxiety responses to the experimental situation.

In conclusion, the present results suggest an influence of chronic and acute stress on cerebral activation patterns during painful and non-painful visceral stimuli, involving the PCC, IC, AMY and PAG. Our results lend support to the notion that long-term stress may affect currently experienced negative emotions and/or increase vigilance for aversive stimuli that carry the characteristic of a threat, such as visceral pain stimuli. Furthermore, inter-individual differences in the anxiety response to the experimental situation, which is demonstrably higher in patients with IBS[15] may increase the variance in visceral pain studies, and contribute to group differences between patients and controls. Together, these results provide a framework for designing future studies addressing the neural processes underlying the role of stress and anxiety in functional gastrointestinal disorders.

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