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Acute tryptophan depletion alters the effective connectivity of emotional arousal circuitry during visceral stimuli in healthy women

J S Labus¹, E A Mayer¹, J Jarcho¹, L A Kilpatrick¹, T O C Kilkens², E A T Evers³, W H Backes⁴, R-J M Brummer⁵, and M A van Nieuwenhoven⁵

¹Center for Neurobiology of Stress, Department of Medicine, Physiology and Psychiatry, David Geffen School of Medicine, UCLA, Los Angeles, California, USA ²Department of Psychiatry and Neuropsychology, University Hospital Maastricht, Maastricht, The Netherlands ³Department of Neuropsychology and Psychopharmacology, Maastricht University, Maastricht, The Netherlands ⁴Department of Radiology, University Hospital Maastricht, Maastricht, The Netherlands ⁵Department of Gastroenterology, University Hospital Örebro, Örebro, Sweden

Abstract

Objective—Alterations in serotonin signalling within the brain–gut axis have been implicated in the pathophysiology of irritable bowel syndrome (IBS) and is a treatment target. Acute tryptophan depletion (ATD) decreases brain serotonin (5-hydroxytryptamine; 5-HT) levels, and increases visceral perception and negative emotional bias in patients with IBS. The aim of the present study was to determine the effect of ATD on brain activity and connectivity during visceral stimuli in healthy women, and to compare the ATD-induced brain connectivity of an arousal circuit in female patients with IBS without ATD.

Methods—12 healthy females (19–25 years) were studied under placebo (PLA) conditions and ATD. Functional MRI measurements were performed during a rectal barostat protocol, consisting of random non-painful and maximal tolerable distensions. Partial least squares analyses and structural equation modelling were used to evaluate the effect of ATD on functional and effective brain connectivity during distension. Results in healthy controls under ATD were compared with the effective connectivity of brain responses to 45 mm Hg rectal distension in 14 female patients with constipation-predominant IBS (IBS-C) (24–50 years).

Results—In healthy controls, ATD resulted in increased response of an extensive brain network to balloon distension, including the amygdala and nodes of emotional arousal and homeostatic afferent networks. The effect was greater during high inflation, suggesting greater engagement of the central serotonin system with more aversive visceral stimuli. Effective connectivity analysis

Correspondence to Dr M A van Nieuwenhoven, Department of Medicine, Division of Gastroenterology, Örebro University Hospital, 701 85 Örebro, Sweden; m.vannieuwenhoven@versatel.nl.

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Ethics approval This study was conducted with the approval of the Ethics Committee of the University Hospital Maastricht.

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revealed a profound effect of ATD on coupling between emotional arousal network nodes, resulting in loss of negative feedback inhibition of the amygdala. A near-identical pattern was identified in the patients with IBS-C.

Conclusions—The findings are consistent with an ATD-induced disinhibition of and increased connectivity within an emotional arousal network during aversive stimulation. Together with the previous demonstration of ATD-induced visceral hyperalgesia in healthy controls, and the near-identical effective connectivity pattern observed in patients with IBS-C, these findings suggest that dysregulation of this brain network may play a role in central pain amplification and IBS pathophysiology.

INTRODUCTION

Preclinical and clinical evidence implicates the serotonin (5-hydroxytryptamine; 5-HT) signalling system as an important modulator of the brain–gut axis, and this signalling system may be altered in irritable bowel syndrome (IBS).¹ For example, drugs aimed at modulating the activity of 5-HT selectively (selective serotonin reuptake inhibitors, 5-HT₃ and 5-HT₄ receptor antagonists) or both 5-HT and norepinephrine (NE) systems (non-selective reuptake inhibitors and tricyclic antidepressants) have been used in the treatment of functional gastrointestinal (GI) disorders, as well as in other chronic pain conditions and psychiatric syndromes. For example, 5-HT₃ receptor (5-HT₃R) antagonists have been demonstrated to be one of the most effective treatments for patients with diarrhoea-predominant IBS (IBS-D) and have shown effectiveness in co-morbid chronic pain disorders.² Finally, the 5-HTTLPR polymorphism, and polymorphisms in 5-HT₃R subunit genes have been implicated as a vulnerability factor for this group of functional pain disorders.^{3–5}

Even though only 5% of the total 5-HT is located in the central nervous system (CNS; primarily the raphe nuclei and their widespread cortical and spinal projections), the raphe 5-HT system plays an important role in state-dependent regulation of emotional state, pain sensitivity and autonomic activity. In addition, there are close interactions of the central NE and 5-HT systems in modulating limbic brain and cortical brain regions.⁶ Low brain 5-HT levels have been implicated in various types of psychopathology, including depression and anxiety, and sex differences in brain 5-HT levels and 5-HT synthesis may explain the greater susceptibility of women to stress-related disorders.^{7,8} Tonic serotonergic input to the amygdala and locus coeruleus complex (LCC; the major noradrenergic nucleus in the brain) are thought to have predominantly inhibitory influences on regions of emotional arousal and stress circuits in the brain, and disturbances in these systems are central to major psychiatric disorders of mood and affect, and to chronic pain disorders.^{9,10}

Acute tryptophan depletion (ATD) is a validated method to temporarily reduce 5-HT synthesis in the CNS (and presumably within the gut's enterochromaffin cells) by decreasing the availability of its precursor tryptophan through the administration of an amino acid mixture lacking tryptophan. Following ATD, a substantial reduction of brain 5-HT synthesis as well as decreased levels of tryptophan have been demonstrated in humans.^{8,11} ATD increased haemodynamic brain responses to emotional words, and attentional bias towards negative stimuli was strongly associated with resting state blood flow in the right amygdala.¹² ATD has been used to investigate the role of the central 5-HT system in healthy control subjects, in patients with affective disorders (depression and anxiety) and in their first-degree relatives.^{13–15}

The reported effects of acute lowering of brain 5-HT levels using ATD on the perception of rectal distension stimuli vary between studies, with some showing increased perception of a rectal stimulus and an increased memory bias, while others showed no effect.^{16,17} Acute increases of synaptic 5-HT levels by administration of the 5-HT uptake inhibitor citalopram

had no effect on the perception of rectal balloon distension in a mixed sample of patients with IBS-D, even though it positively affected emotional memory bias, while reducing perception of upper GI distension in healthy controls.^{18,19} Based on our earlier findings of enhanced visceral perception during ATD, we aimed to test the general hypotheses that (1) the intervention in healthy women results in an increased engagement of an emotional arousal circuit, including the amygdala, during experimental visceral stimulation and (2) that the induced changes are similar to those seen in female patients with IBS without any dietary interventions. To test this hypothesis, we applied functional and effective connectivity analysis to BOLD (blood oxygen level-dependent) responses acquired during low and high distension in healthy female subjects, during ATD and placebo (PLA), and compared the findings with those observed in female patients with constipation-predominant IBS (IBS-C).

METHODS

Subjects

Twelve right-handed healthy females (19–25 years) were included in the ATD protocol, and 14 additional female patients with IBS-C (24–50 year) were studied without ATD or placebo. To ensure hormonal stability and to avoid possible effects of the menstrual cycle, the subjects who did not use oral contraceptives were studied during the follicular phase of the menstrual cycle.²⁰ Female subjects were studied because (1) the prevalence of IBS is higher in females than in males²¹; (2) the rate of central 5-HT synthesis is lower in females than in males⁸; and (3) women are more prone to the effect of ATD.²²

Exclusion criteria in both groups comprised abdominal surgery (other than appendectomy and cholecystectomy), use of medications other than oral contraceptives within 14 days prior to testing, and lactose intolerance. In the healthy control group, a positive first-degree psychiatric family history, any history of psychiatric disease or use of psychoactive medication, or Hamilton Depression Scale (HADS) scores ≥ 8 were also exclusion criteria.^{23–25} The Medical Ethics Committee of the University Hospital Maastricht approved the study protocol and all subjects gave their informed written consent before the start of the study. Differences in HADS scores between the groups were determined using an unpaired t test.

Design

ATD study in healthy controls—The functional MRI (fMRI)-barostat protocol comprised the measurement of brain responses to rectal non-painful stimuli and maximal tolerable stimuli in a placebo-controlled double-blind randomised crossover design under a PLA condition and an ATD condition, respectively. The two test days were separated by at least 1 week. On the fMRI test days subjects ingested an amino acid mixture at T=0 h. At T=4 h the fMRI-barostat experiment was started.

Acute tryptophan depletion—The amino acid mixtures were prepared by the department of Pharmacy of the University Hospital, as previously described.²⁴ After an overnight fast, the subjects consumed the amino acid mixtures dissolved in 250 ml of water as quickly as possible, using a nose clamp to avoid the unpleasant smell of the mixtures. Water, decaffeinated tea and peppermint were available to neutralise the unpleasant taste. Tryptophan (TRP) and the ratio TRP/ Σ LNAA (sum of the large neutral amino acids tyrosine, leucine, phenylalanine, isoleucine and valine) were measured at T<0 and T=4h, using high-performance liquid chromatography (HPLC).²⁶

Determination of perception thresholds: barostat procedure—The determination of the non-painful distension pressures (low inflation (INF)) and maximal tolerable pressures (high INF) are described in the Supplementary data.

Rectal distension during fMRI scanning—High INF and low INF distension pressures were applied by a barostat, which was placed outside the MRI room, by means of long tubes (10 m), which were guided through the wave-guide of the MRI room wall. The rectal barostat stimulation/distension protocol consisted of six low INF distensions and six high INF distensions, respectively, in random order. Each distension lasted 30 s and was interleaved with a 30 s rest (0 mm Hg) interval. The distensions were presented in a block design. Epochs of eight scans during rectal distension were followed by eight scans without rectal distension (rest). This sequence was repeated up to the total of 202 scans.

Subjective ratings during fMRI scanning—No perceptual stimulus ratings were acquired during the MRI scanning protocol because we only applied two different rectal stimuli (high INF and low INF), which makes perceptual ratings unreliable. Moreover, it is not clear whether perceptual stimulus ratings obtained during MRI scanning are reliable, reproducible and comparable with a condition outside the MRI magnet.

fMRI data acquisition—See Supplementary data.

fMRI data processing and analyses—See Supplementary data.

Functional connectivity analysis—See Supplementary data.

Effective connectivity—Effective connectivity analysis examines neural interactions between regions simultaneously to explicitly quantify the effect brain structures exert on one another in a specific brain circuit or network and provides a means to test hypotheses regarding neural circuits/network functioning. This approach is hypotheses driven and restricts inferences to specific circuits or networks comprised of a limited number of brain regions.

Effective connectivity analysis of the ATD data was performed using path analysis within a structural equation modelling framework. Given expectations of engagement of a known emotional arousal network^{27–29} that includes the amygdala, rostral anterior cingulate cortex (rACC), infragenual ACC (iACC), orbital medial prefrontal cortex (omPFC) and dorsal pons/LCC (dpons/LCC),^{27–29} effective connectivity analysis was applied to quantify the neural activity in this specific emotional arousal network and to test for hypothesised differences induced by ATD (see figure 1). Literature searches from Pubmed (<http://www.ncbi.nlm.nih.gov/entrez>) and the Collations of Connectivity on the Macaque Brain (CoCoMac) database (<http://www.cocomac.org>) support the designated anatomical projections among the nodes of this network.³⁰ The activity in the brain regions selected a priori and comprising the emotional arousal circuit were represented by the voxel loading most reliably on the latent variable extracted from the spatiotemporal partial least squares (st-PLS). Given the time-dependent response of brain regions across the 26 s temporal window of inflation, we chose to quantify early response in this network averaging data from the first 8 s of the response (lag 0, lag 1) for entry into the analysis. Residual variances, representing external input into the system (eg, unspecified regions, psychological characteristics, hormonal milieu), were fixed at 35% of the observed regional variances within group and condition.³¹ ATD-induced differences in the effective connectivity of the network were tested using multigroup tests for invariance.³² Differences in each path in the model were calculated using one degree of freedom χ^2 statistics for group. Critical ratios for

path coefficients, representing the coupling between regions, were interpreted as significant at $p < 0.05$.

Study in IBS-C

Brain responses to 5, 25 and 45 mm Hg rectal distension were assessed by fMRI. Subjects completed four runs comprising five 45 mm Hg inflations, five 25 mm Hg inflations and five 5 mm Hg inflations presented in semi-random order. Each trial consisted of 18 s before balloon inflation, followed by 15 s of inflation at the designated pressure. The inflations were cued by a light 3–5 s before inflation. The tube length was ~10 m.

fMRI data acquisition—See Supplementary data.

Effective connectivity—As described above, path analysis was used to characterise the effective connectivity of the previously described emotional arousal network during the 45 mm Hg inflation in patients with IBS. Voxels or nodes of the network best representing the regions comprising the network (amygdala, rACC, iACC and omPFC) were identified from the results of both a task and seed PLS. See Supplementary data.

RESULTS

Subject characteristics

Twelve healthy female volunteers (mean age 22.7 ± 0.5 years) participated in the ATD study, and 14 female patients with IBS-C (mean age 36.1 ± 8.1 years) were also studied. In the ATD study, nine subjects were on oral contraceptives. In the IBS-C study, one subject was postmenopausal and five were on oral contraceptives. HADS anxiety (mean \pm SD) for the IBS subjects was: 7.4 ± 3.1 . HADS depression for the IBS subjects was: 3.4 ± 2.6 . The IBS group had three subjects with a score above 8 on HADS anxiety. HADS anxiety for the healthy subjects was: 2.3 ± 2.2 . HADS depression for the healthy subjects was: 0.3 ± 0.7 . The differences between the groups were significant for both anxiety and depression ($p < 0.01$).

ATD study

Rectal distension pressures—Individual distension pressures required to generate a subjective sensation of urge to defecate (low INF) or maximal tolerable pressure (high INF) are shown in figure 2. The mean values for low and high INF were 19.6 ± 6.6 and 52.1 ± 12.2 mm Hg, respectively.

Validation of ATD—No significant baseline differences between ATD and PLA were found for plasma tryptophan levels (34.4 ± 1.3 and 37.3 ± 1.8 $\mu\text{mol/l}$, respectively, $p = 0.21$) and the ratio TRP/ Σ LNAAs (0.15 ± 0.02 and 0.16 ± 0.05 , respectively, $p = 0.17$). The amino acid analysis showed that the depletion was successful. During ATD, plasma tryptophan levels (14.4 ± 1.1 $\mu\text{mol/l}$) and the TRP/ Σ LNAAs ratio (0.02 ± 0.01) decreased significantly (58% and 85%, $p < 0.001$ and $p < 0.001$, respectively), compared with baseline values. Values are given as the mean \pm SEM.

Effect of ATD on brain responses to rectal distension

Functional connectivity: St-PLS revealed a significant ($p < 0.01$, 73% variance) network (pattern) of brain regions, which showed an interaction between group (ATD, PLA) and condition (low, high INF) (figure 3). Although during both PLA and ATD there were differences in brain activity due to the level of balloon inflation, these distension-related differences were significantly enhanced during ATD.

Because ATD resulted in strong effects in a large number of brain regions during INF, we summarise the main results below with a focus on the homeostatic afferent, emotional arousal, attentional and cognitive modulatory regions hypothesised to be key regions in response to interoceptive stimuli.³³ A detailed cluster report summarising the latent variable is presented in Table 1 in the Supplementary data.

In general, during high INF (compared with low INF), the ATD-sensitive network comprised statistically reliable activations within regions of the homeostatic afferent network (dorsal ACC subregions (rACC and mid-cingulate cortex (MCC)), insula subregions (anterior and posterior insula (a/p INS) and thalamus) and the emotional arousal network (pons/LCC), amygdala). Additional regions included the cerebellum and the substantia nigra/ventral tegmental area. Less activity in the ATD-sensitive network was observed during high INF as compared with low INF in prefrontal regions (dorsolateral PFC (BA 9), omPFC (BA 11)), and in regions of the default network (posterior cingulate cortex (PCC) and ACC) (Table 1, Supplement).

ATD affects brain responses preferentially during high INF: While most regions generally demonstrated sustained activity, higher activity during ATD as compared with PLA was observed transiently in the amygdala, a key brain region in the emotional arousal network, and the thalamus (which is part of the homeostatic afferent rather than the emotional arousal network). As this transient activation was only observed during the first 4 s (first scan) of the brain's response to balloon inflation, we examined the effective connectivity of the emotional arousal network only during the initial part of the response (first two scans, ~8 s). Activity for the regions comprising the emotional arousal network was extracted from the most reliable (ie, representative) voxels contributing to the latent variable extracted by the task st-PLS (table 1).

Effective connectivity: Effective connectivity analyses revealed that during high INF, subjects receiving ATD (compared with PLA) exhibited a stronger engagement of the emotional arousal circuitry (table 2, figure 4). For example, during PLA, a significant negative connectivity from the pons to the omPFC ($\beta=-2.44$), and from the omPFC to the amygdala ($\beta=-0.23$) was observed. The connectivity between these regions was altered during ATD—for example, the negative connectivity from the pons to the omPFC was greatly reduced ($\beta=-0.61$, $\Delta\chi^2=5.6$, $p<0.05$), and that from the omPFC to the amygdala became positive ($\beta=0.17$, $\Delta\chi^2=5.6$, $p<0.01$). In addition, during ATD, many more nodes of the emotional arousal circuitry were engaged, including: amygdala to the pons ($\beta=0.90$), rACC to the amygdala ($\beta=1.20$), and pons and iACC to the rACC ($\beta=0.43$). χ^2 Tests for differences between PLA and ATD during high INF indicated that connectivity was significantly altered in all but one connection (amygdala to iACC). Specifically, the emotional arousal circuitry was significantly and more positively engaged during ATD (iACC to rACC, $\Delta\chi^2=4.0$, $p<0.05$, rACC to amygdala, $\Delta\chi^2=6.3$, $p<0.05$, and amygdala to pons, $\Delta\chi^2=6.8$, $p<0.01$).

Distension study in patients with IBS—Representative voxels of the emotional arousal network identified in the PLS analyses are presented in table 3. Effective connectivity analysis of brain activity during 45 mm Hg distension in patients with IBS-C revealed a similar pattern of connectivity to healthy controls receiving ATD (table 4, figure 4). For example, nodes positively engaged during ATD were also positively engaged in IBS-C, including: omPFC to amygdala ($\beta=0.19$) and amygdala to iACC ($\beta=0.49$).

DISCUSSION

During controlled rectal balloon distension, acute lowering of central and peripheral 5-HT concentrations by ATD in healthy women without any personal or family history of psychiatric or GI symptoms resulted in an increased response of an extensive network of brain regions, all of which receive serotonergic projections from the dorsal and medial raphe nuclei.³⁴³⁵ Using an identical ATD protocol, we have previously shown that acute lowering of 5-HT can enhance visceral perception in healthy women, which may be in part influenced by an ATD-mediated increase in emotional response bias.¹⁶

In the current study, the observed ATD effect was greater during an inflation pressure close to the tolerance threshold (high INF), suggesting a greater engagement of the central 5-HT system with more aversive visceral stimuli. Effective connectivity analysis revealed a profound effect of ATD on the coupling between nodes of an emotional arousal network, consistent with a loss of feedback inhibition of the amygdala, and an ATD-related disinhibition of the entire network. Despite slightly different experimental conditions and analytical techniques, nearly identical changes in the same circuit were observed in female patients with IBS-C, without ATD. Together, these findings suggest that alterations in serotonergic modulation of an emotional arousal circuit may play a role in centrally mediated visceral hypersensitivity in IBS.

In the following, we will discuss these findings in the context of the ATD literature, and in regards to the concept of increased activity of emotional arousal circuits in the pathophysiology of IBS.

ATD effect on functional connectivity of brain regions during visceral stimulation

Using multivariate analysis techniques, we identified an extensive network of brain regions which differentiated low and high INF conditions, with greater engagement of this network during ATD, compared with PLA. The ATD-sensitive network included (but was not limited to) brain regions involved in the processing of interoceptive information, including INS, dACC and thalamus, as well as key regions of an emotional arousal circuit, including the amygdala and ACC subregions. These findings are consistent with a recent report by Roiser *et al* showing differentially affected BOLD responses in healthy controls in an 'extended visceromotor network' which participates in the processing of emotional information.¹²³⁶ The fact that the ATD effect was more pronounced during the high INF condition suggests that in the healthy brain, ascending 5-HT pathways arising from pontine serotonergic nuclei show greater engagement with more painful signals from the viscera.

The approach of acutely reducing the activity of the brain 5-HT signalling system by diet-induced tryptophan depletion is a well-established technique to study the role of 5-HT signalling in the brain. It has been used previously in healthy controls, in patients with major depression and in patients with IBS during different types of experimental stimuli.⁹¹⁶¹⁷³⁷³⁸ ATD has been validated by demonstrating that the technique in healthy controls was associated with a marked reduction in 5-HT synthesis and a reduction of 5-HT metabolites in the cerebrospinal fluid (CSF) consistent with lowering of brain 5-HT concentrations.⁸¹¹ Similarly, as already demonstrated previously by others, we showed in the current study that the dietary manipulation resulted in a significant lowering of plasma tryptophan levels, and the TRP/ Σ LNAA ratio, the best peripheral index of tryptophan availability to the brain for 5-HT synthesis.¹²¹⁶³⁹

In general, biological effects of ATD, including those on brain activity and on neurotransmitters in the CSF, have been more consistent than effects on subjective measures, such as mood, perceptual or pain ratings. For example, a recent fMRI study in

healthy women showed that ATD greatly increased brain haemodynamic responses to emotional words even though no effect on subjective mood ratings was observed.¹² One of the most consistent findings in healthy controls, and in patients with depression and those with IBS has been the increase in negative emotional bias.^{12,16,40} Even though perceptual responses were not measured in the current study, greater ATD-induced responses within interoceptive brain regions, in particular the aINS, during an aversive rectal distension would be expected to be associated with increased perceptual ratings of such a stimulus. In support of this expectation, we have previously shown that ATD in healthy controls resulted in increased pain perception at higher distension pressures, making them more similar to ratings by patients with IBS.¹⁶

ATD affects effective connectivity within an emotional arousal network

Consistent with the close interaction of the 5-HT system with central stress circuits, ATD resulted in higher corticotrophin-releasing factor (CRF) levels compared with a control condition, without an associated change in subjective mood.⁹ Positive feedback regulation between the pontine noradrenergic LCC and the amygdala plays an important role in central CRF release, and both LCC and amygdala activity are modulated by 5-HT pathways.^{64,42} For example, 5-HT₃Rs, the target of a novel, effective IBS drug, are found presynaptically and postsynaptically, and activation can facilitate the release of a variety of excitatory and inhibitory neurotransmitters.⁴³ We have previously demonstrated reductions in amygdala and brainstem activity during 5-HT₃R antagonism in patients with IBS during expectation of an aversive rectal stimulus, consistent with a role for these receptors in mediating the release of an excitatory neurotransmitter in these brain regions.⁴⁴ It has been suggested that the connectivity within an emotional arousal network including the amygdala, and infragenua and supragenua subregions of the ACC may be in part determined by 5-HT mechanisms, since healthy controls who are s carriers of the 5-HTTLPR gene polymorphism showed evidence for structural changes within these regions, and alterations in functional connectivity between two nodes of this circuit.²⁷⁵ In the current study, ATD resulted in a profound change in nearly all connections between the nodes of this arousal circuit during the initial stages of the rectal distension, in particular in the pathways from omPFC and rACC which under normal conditions provide inhibitory input to the amygdala. Inhibitory input from these regions was either lost or weakened (became more positive) by ATD in healthy controls. These changes would be expected to result in greater amygdala activation, as observed during the high INF condition. The pattern of connectivity observed in healthy controls after ATD were nearly identical to the pattern of connectivity observed in a group of women with IBS-C, who were studied without ATD or PLA. These findings are consistent with the hypothesis that the increased engagement of this circuit in patients with IBS is in part related to reduced serotonergic modulation.

Limitations of the study

No simultaneous recording of perceptual ratings during MRI scanning were made because we only used two different distension pressures (high INF and low INF) which makes determination of differences in perceptual ratings unreliable. Moreover, little is known about the reproducibility of perceptual ratings during MRI scanning. However, we have previously demonstrated the effect of ATD on perceptual ratings of the same rectal stimulus in healthy women. We showed mild but significant effects of ATD in the highest distension pressures (painful distensions).¹⁶ Individual distension pressures to elicit mild urge and tolerance, respectively, were determined in an interval between 2 and 14 days prior to the fMRI procedure. It is conceivable that perceptual sensitivity may have changed during the time of the threshold determination and the fMRI study. However, perception thresholds in healthy controls are fairly stable between two distension sessions and, given the lack of overlap of distension pressures for the low and high INF condition, it is highly unlikely that individuals

had changed their subjective ratings sufficiently to be given an inappropriate stimulus intensity during the two conditions.⁴⁵ Our subjects were not characterised in terms of trait anxiety, and no genetic information on the serotonin transporter gene (SERT) polymorphism in participants was obtained. Given the limited sample size, it is conceivable that over-representation of one of these subgroups may have influenced our results. A possible effect of ATD on mechanoelastic properties of the rectum cannot be ruled out, which in turn might indirectly affect brain responses to rectal distension. An increase in 5-hydroxyindoleacetic acid metabolites with the ATD procedure has previously been reported, consistent with a lowering of peripheral 5-HT concentrations. Such peripheral effects would be expected to affect the gut-based 5-HT signalling system, which plays an important role in mediating and modulating intestinal reflexes and motility, in addition to providing a major input to subsets of vagal afferents to the brain.¹ However, we have previously demonstrated that pressure–volume relationships before and after ATD were not different in healthy subjects.¹⁶ These findings are consistent with the known greater effect of ATD on the availability of tryptophan in the brain, in particular the cortex, compared with peripheral tissues.⁴⁶

The two groups (healthy controls and patients with IBS) were studied under slightly different experimental conditions and analysed by slightly different approaches. However, despite these differences, findings in the two groups are remarkably similar, and consistent with earlier results using the connectivity model.²⁸ Finally, functional connectivity can be modulated by experimental conditions and it is unknown how the observed differences in connectivity during rectal distension relate to potential differences in resting state connectivity.

Summary and implications for IBS pathophysiology

Acute lowering of central (and peripheral) 5-HT availability in healthy women, which has previously been shown to result in enhanced visceral perception, is associated with changes in a large number of brain regions, including an emotional arousal circuit during rectal distension. Nearly identical changes were seen in a group of female patients with IBS-C. Female patients with IBS have previously been shown to have greater amygdala activation and greater engagement of the emotional arousal circuit during the expectation of an aversive rectal stimulus when compared with male patients,^{28,47} and the BOLD responses to expectation of such a stimulus in emotional arousal regions were correlated with altered brain responses to the actual distension.⁴⁸ Furthermore, differences in rectal distension-induced brain responses between female patients with IBS and healthy controls were highly correlated with anxiety ratings.⁴⁹ Pharmacological reduction in the activity of nodes of the emotional arousal circuit with a 5-HT₃R antagonist, which has anxiolytic properties, has previously been shown to be associated with IBS symptom reduction.⁴⁴ Hence, altered processing and perception of visceral afferent signals in patients with IBS appears to be closely correlated with central mechanisms of anxiety. When viewed together, our findings suggest that acute lowering of central 5-HT concentrations in healthy women has an IBS-like effect both on brain responsiveness in emotional arousal regions and on the perception of visceral stimuli.

One may speculate that acute or chronic variations in central 5-HT concentrations may play a role in IBS symptom generation/exacerbation, in particular in female patients. Given the lower brain 5-HT synthesis that has been described in women, and the lower serotonin transporter expression that has been postulated in carriers of the s-allele of the 5-HTTLPR polymorphism, female s carriers may be particularly susceptible to developing IBS symptoms under conditions of low 5-HT availability.⁸

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Significance of this study

What is already known about this subject?

- ▶ The serotonin (5-hydroxytryptamine; 5-HT) signalling system plays an important role in modulating brain–gut interactions.
- ▶ Pharmacological manipulation of the 5-HT signalling system has a beneficial effect on irritable bowel syndrome (IBS) symptoms.
- ▶ Acute tryptophan depletion (ATD) is a validated technique to acutely lower central and peripheral 5-HT concentrations.
- ▶ ATD in healthy women is associated with an increased visceral sensitivity and negative emotional bias.

What are the new findings?

- ▶ ATD in healthy women results in an increased response of an extensive network of brain regions to an aversive visceral stimulus, including increased responses of the amygdala.
- ▶ ATD alters the coupling within an emotional arousal circuit, resulting in reduced feedback inhibition of the amygdala during an aversive visceral stimulus. An identical pattern of effective connectivity within the emotional arousal network was observed in female patients with IBS-C.

How might it impact on clinical practice in the foreseeable future?

- ▶ Reduced central 5-HT levels as seen in women, s carriers of the 5-HTTLPR gene polymorphism, sleep deprivation, depression and chronic stress may be responsible for the increased IBS symptom prevalence in these populations and conditions.
- ▶ Pharmacological interventions in populations and conditions with reduced central 5-HT levels aimed at normalising 5-HT signalling may be of therapeutic value.

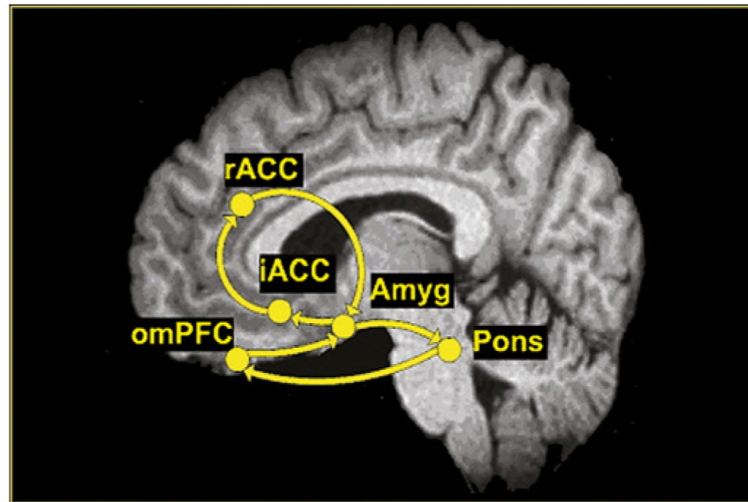


Figure 1. Hypothesised emotional arousal network. Anatomical projections among the nodes of the emotional arousal network are represented by unidirectional arrows. The model is based on Pezawas *et al.*,²⁷ Labus *et al.*²⁸ and Stein *et al.*²⁹ Amyg, amygdala; iACC, infragenual cingulate cortex; omPFC, orbital medial prefrontal cortex; sACC, supragenual anterior cingulate cortex.

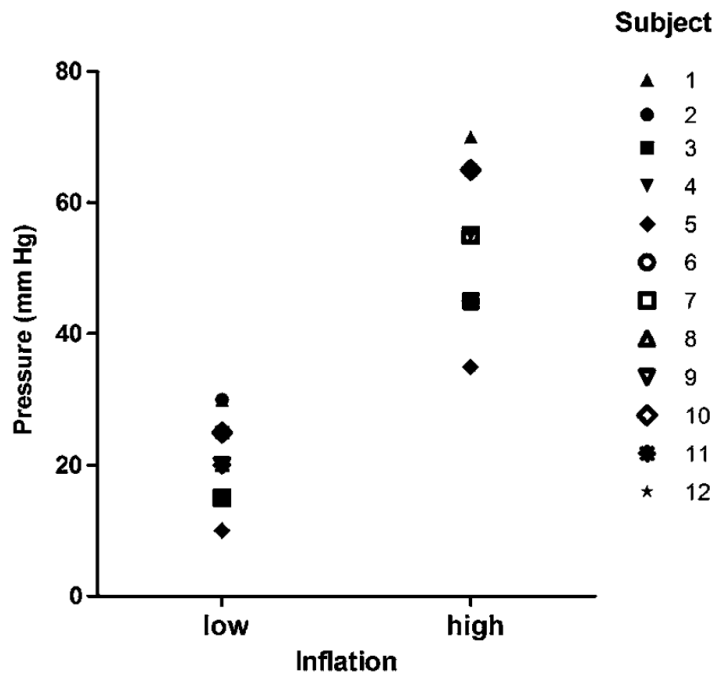


Figure 2. Individual rectal pressure values required to produce a sensation of mild urge (low) and maximal tolerable (high) during controlled rectal distension. Despite interindividual variation of individual thresholds, there was no overlap between low and high ratings.

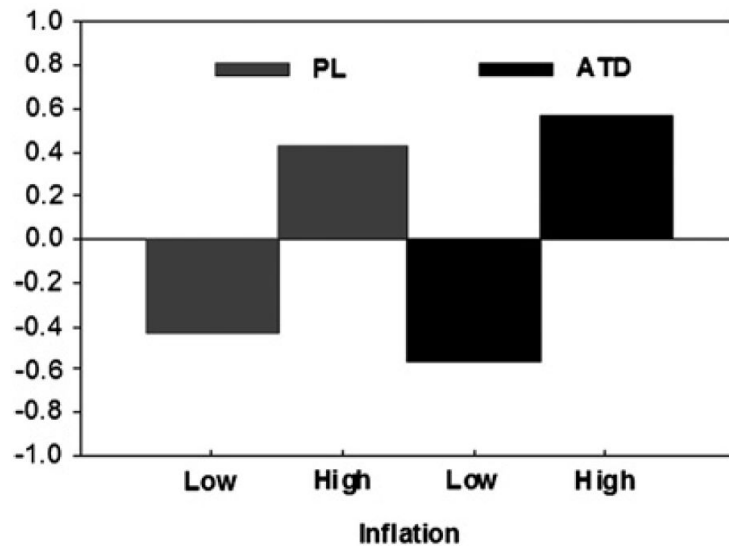


Figure 3.

Plot of the linear contrast weights (or design saliences) for the significant latent variable of the brain network. The y-axis represents the numerical weights for the contrast. Inflation conditions for placebo (PL) and during acute tryptophan depletion (ATD) are shown on the x-axis. The design saliences indicate that the network is characterised by an interaction between group (ATD, PL) and condition (low, high inflation). Although differences in brain activity due to the level of balloon inflation were observed in both groups, these differences were greater during ATD compared with PL. For details of the analysis, see the Methods section.

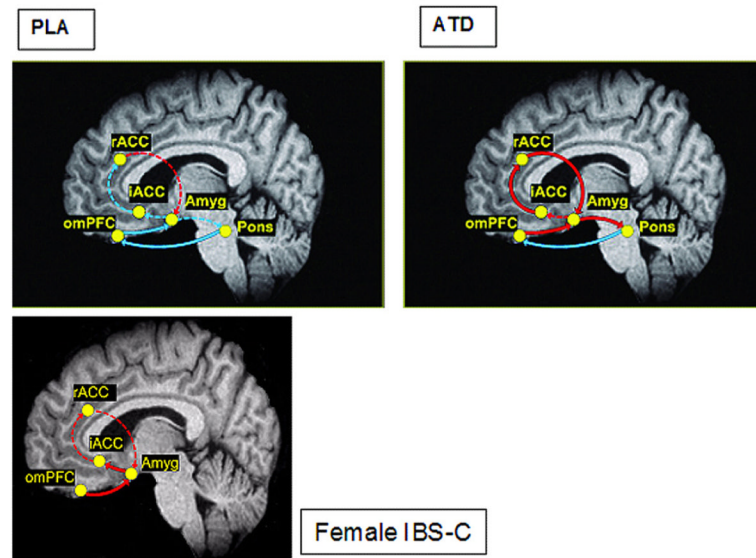


Figure 4. Estimated effective connectivity of the initial response in the hypothesised emotional arousal circuit during high inflation pressure with placebo (PLA) and acute tryptophan depletion (ATD) and in patients with constipation-predominant irritable bowel syndrome (IBS-C). The β coefficients (calculated in effective connectivity analysis) are depicted by the style and colour of the arrows. Solid arrows represent a parameter estimate that was considered statistically significantly different from zero, whereas dashed lines represent non-significant coefficients. Red arrows represent positive coupling whereas blue arrows represent negative coupling. The magnitude of the coefficients associated with each thickness is depicted. Amyg, amygdala; iACC, infragenual cingulate cortex; omPFC, orbital medial prefrontal cortex; sACC, supragenual anterior cingulate cortex. For details of the analysis, see the Methods section. For actual values of β coefficients, see tables 2 and 4.

Table 1

Spatial location of voxels in MNI space of the voxel identified from the the spatiotemporal partial least squares and considered as representative nodes for the emotional arousal network regions

Network node	X	Y	Z
Amyg	-16	2	-20
Pons	-6	-32	-18
rACC	-4	20	30
iACC	-2	20	-6
omPFC	-16	54	-16

Amyg, amygdala; iACC, infragenual anterior cingulate cortex; omPFC, orbital medial prefrontal cortex; rACC, rostral anterior cingulate cortex.

Table 2

Results of the effective connectivity analysis for the ATD study

Emotional arousal circuits			PLA		ATD		$\Delta \chi^2$
			β	SE	β	SE	
omPFC	→	Amyg	-0.23	0.11	0.17	0.08	5.6
Amyg	→	iACC	-0.04	0.17	0.07	0.11	0.2
iACC	→	rACC	-0.15	0.25	0.43	0.15	4.0
rACC	→	Amyg	0.27	0.31	1.20	0.21	6.0
Amyg	→	Pons	-0.75	0.44	0.90	0.10	6.8
Pons	→	omPFC	-2.44	0.66	-0.61	0.19	6.3

β Values (path coefficients) represent coupling between network regions and demonstrate differences in the effective connectivity between PLA and ATD. The χ^2 difference statistic quantifies the differences in connectivity between PLA and ATD. χ^2 difference values in bold are significant at $p < 0.05$. Path coefficients were interpreted as significant at $p < 0.05$ and are in bold in the table.

Amyg, amygdala; ATD, acute dietary tryptophan depletion; iACC, infragenua anterior cingulate cortex; omPFC, orbital medial prefrontal cortex; PLA, placebo; rACC, rostral anterior cingulate cortex; $\Delta \chi^2$, χ^2 difference statistic.

Table 3

Spatial location of voxels in MNI space identified from the partial least squares analysis of patients with IBS-C and considered as representative nodes for the emotional arousal network regions

Networknode	X	Y	Z
Amyg	24	-8	-20
rACC	10	34	32
iACC	4	30	-6
omPFC	4	32	-22

Amyg, amygdala; iACC, infragenual anterior cingulate cortex; IBS-C, constipation-predominant irritable bowel syndrome; omPFC, orbital medial prefrontal cortex; rACC, rostral anterior cingulate cortex.

Table 4

Results of the effective connectivity analysis for patients with IBS-C

		IBS-C	
Emotional arousal circuits		β	SE
omPFC	→ Amyg	0.19	0.08
Amyg	→ iACC	0.49	0.21
iACC	→ rACC	0.17	0.11
rACC	→ Amyg	0.174	0.28

β values (path coefficients) representing the coupling between regions were considered significant at $p < 0.05$ and are in bold in the table.

Amyg, amygdala; ATD, acute dietary tryptophan depletion; iACC, infragenual anterior cingulate cortex; IBS-C, constipation-predominant irritable bowel syndrome; omPFC, orbital medial prefrontal cortex; PLA, placebo; rACC, rostral anterior cingulate cortex; $\Delta\chi^2$, χ^2 difference statistic.