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Evidence for alterations in central noradrenergic signaling in irritable bowel syndrome*, ,**

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Abstract

Background/aims—Alterations in noradrenergic (NE) signaling have been implicated in the pathophysiology of irritable bowel syndrome (IBS), and adrenergic receptors are potential treatment targets.

Methods—To characterize central NE signaling in IBS, 11 patients and 11 healthy controls (HCs) were studied 3 times during an auditory oddball vigilance task after double-blind ingestion of the α2-adrenoreceptor (α2AR) antagonist yohimbine (YOH), the α2AR agonist clonidine (CLO), or placebo (PLA). Regional cerebral glucose metabolism was measured with [^{18}F] fluorodeoxyglucose (FDG) positron emission tomography (PET). Measures of anxiety, early-life trauma, plasma NE and blood pressure were acquired.

Results—Patients had higher plasma NE levels than HCs before and after ingestion of all drugs (all $p < 0.05$). YOH increased plasma NE and more anxiety in patients than in HCs. After YOH, NE levels directly correlated with drug-induced increases in anxiety in IBS patients ($r=0.61$), but not in HCs. IBS patients showed less YOH-mediated reduction of activity in a central arousal

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circuit, consistent with fewer functional presynaptic α2AR. In HCs, but not in patients, activation of amygdala and subgenual anterior cingulate cortex (sgACC) was inversely correlated with activation of anterior mid cingulate cortex (aMCC), and state anxiety covaried directly with activity in limbic and right frontotemporal cortices, but indirectly with activity in the left frontotemporal cortex. YOH-mediated reduction of activity in brainstem and amygdala inversely correlated with early life trauma.

Conclusions—IBS patients showed evidence for increased noradrenergic activity consistent with downregulation of presynaptic inhibitory α2ARs. Activity within central arousal circuits was biased toward greater excitability and reduced corticolimbic inhibition in IBS. Early life trauma may be one mediator of these abnormalities.

Keywords

Yohimbine; α2 Adrenergic receptors; Early-life trauma; Corticolimbic inhibition

Introduction

Irritable bowel syndrome (IBS) is a common functional gastrointestinal (GI) disorder characterized by recurrent abdominal pain associated with alterations in bowel habits (Longstreth et al., 2006). Symptom-related anxiety, and elevated trait anxiety are seen in the majority of affected patients (Tillisch et al., 2012), and the prevalence of disorders of mood and affect is more common both in patients, and non-healthcare seeking individuals meeting symptom criteria (Naliboff, 2009). A proposed neurobiological model of IBS shares considerable features with analogous models proposed for mood/affect disorders (Kennedy et al., 2012), and fibromyalgia (Verdu et al., 2008). A central feature of these models is upregulation of central arousal circuits involving noradrenergic (NE) and corticotropin releasing factor (CRF) signaling (Dickhaus et al., 2003; Hubbard et al., 2011). Analogous changes have been reported in animals with early life trauma (Al-Chaer and Weaver, 2009). In fact a history of aversive early life events, or a history of physical or sexual trauma, greatly enhances an individual's vulnerability for stress-related conditions such as IBS. Thus the likelihood of developing IBS in life is higher in patients with such aversive early life events as loss of the primary caregiver, divorce of their parents, or abuse (Al-Chaer and Weaver, 2009).

In preclinical models, alterations in NE pathways originating in the locus coeruleus complex (LCC) of the dorsal pons are implicated in IBS pathophysiology (Martinez and Taché, 2006), and recent studies have shown that antagonism of the CRF receptor type 1 (CRF-R1) is able to attenuate LCC responsiveness to a stressor in IBS (Hubbard et al., 2011). Enhanced perception of visceral signals may involve compromised ability to engage endogenous (descending) NE antinociceptive mechanisms (Chang et al., 2000b) and symptom-related anxiety and hypervigilance (Chang et al., 2000a; Coull et al., 2000; Naliboff et al., 1997), and may involve central (ascending) hyperarousal (Arnsten and Goldmanrakic, 1984; Valentino et al., 1999). However, few prior IBS studies directly assess central NE abnormalities in humans, let alone IBS patients.

NE released from ascending fibers interacts with 3 families of adrenergic receptors, the stimulatory α1 and β adrenoreceptors, and inhibitory α2 adrenoreceptor. NE has the highest affinity for α2 receptors, which have 3 subtypes. α_{2A} and α_{2C} receptors are predominantly presynaptic, although all 3 are found postsynaptically (Ramos and Arnsten, 2007). α_{2A} receptor agents have been used as pharmacological probes to presynaptically modulate central NE release. The α_{2A} receptor antagonist Idazoxan increased hippocampal NE release in stressed animals (Lim et al., 2010). Peripheral YOH stimulated NE and CRF releases, increased fear-potentiated startle (Bijlsma et al., 2010) and accentuated fear and anxiety (Meyerbroeker et al., 2012; Soeter and Kindt, 2011, 2012). Anxiety is the most common adverse effect of YOH.

Many IBS patients have subclinically elevated (Naliboff, 2009) and symptom-related anxiety (Labus et al., 2004), and there is considerable co-morbidity with anxiety disorders with evidence for increased central NE (Wong et al., 2000). In animal models, short-term chronic social stress downregulated α_{2A} adrenoreceptors (Flugge et al., 2003) in areas suggesting increased NE release. Dysregulations as a result of stressful levels such as early life trauma, increase the risk for the development of mood/affect disorders and persistent pain syndromes. In animals, it has been associated with downregulation of presynaptic α_2 adrenoreceptors, and increased sympathetic responsiveness (Ladd et al., 2000). Pharmacological evidence for downregulation of central α_{2A} adrenoreceptors in IBS has been demonstrated by desipramine challenge, which is thought to release growth hormone via *postsynaptic* hypothalamic α_2 receptors (Dinan et al., 1990). More recently, α_2 adrenoreceptor polymorphisms have been associated with IBS and somatization (Bremner et al., 1996a, 1996b). Preliminary clinical results support a possible therapeutic role for the α_2 adrenoreceptor agonist CLO(Camilleri et al.,2003).

We assessed whether IBS patients while performing a vigilance task show NE abnormalities related to early life trauma, increased anxiety, and hypervigilance toward potentially aversive stimuli by quantifying effects of adrenergic agents on peripheral NE, mood, and relative regional glucose metabolism, an index of local brain activity. In previous resting-state studies, neocortical metabolism decreased after YOH-induced NE increase, and increased after CLO-induced NE decrease (Bremner et al., 1997). By using YOH as a probe of central noradrenergic signaling, we specifically asked if IBS patients show: 1) evidence for a downregulation of presynaptic α_{2A} adrenoreceptors, and increased central NE release, as indexed by smaller reductions in brain metabolism, 2) altered connectivity within central arousal circuits, 3) correlation of these effects with early life trauma due to upregulation of arousal circuits involving NE and CRF common in early traumatic effects which in turn are prevalent among IBS, and 4) brain and behavioral responses to CLO in order to assess both agonists and antagonists in the same study.

Materials and methods

Participants

Eleven IBS patients meeting Rome II criteria (6 male; mean age: 40.5 sd 12.9; range 21–60) and age and sex matched HCs (mean age: 37.3 sd 10.6) recruited by local advertisements completed the study. Rome II diagnostic criteria for IBS in this study were based on the

definition of recurrent abdominal pain or discomfort for at least 3 days/month in the last 3 months and associated with two or more of the following: 1. improvement in defecation, 2. onset associated with a change in frequency of stool and 3. onset associated with a change in form (appearance) of stool. Structured clinical interviews were conducted and subjects were excluded if they had any major psychiatric disorders, including any anxiety and depression spectrum disorders within the past year, alcohol/substance abuse within two years, serious medical conditions, metal implants, or hearing loss. Although no drug screening was performed, subjects were excluded from the study if they were taking any medications except in the case of mild tricyclic antidepressants (TCAs) so long as there were no changes in the past 3 months. Informed consents were obtained from all subjects as approved by the Institutional Review Board at the Veterans Administration Greater Los Angeles Healthcare System and the University of California Los Angeles.

Questionnaires

During screening, patients completed the UCLA Bowel Symptom Questionnaire (BSQ) (Munakata et al., 1997), the Eysenck Personality Questionnaire-Revised (EPQR) (Eysenck and Eysenck, 1994), the Visceral Sensitivity Index (VSI) (Labus et al., 2007), the Hospital Anxiety and Depression Index (HAD) (Farhadi et al., 2001), and the Early Trauma Inventory Short Form (ETISF) (Bremner et al., 2007). The ETISF quantifies life traumas experienced before age 18 within four domains: general, physical, emotional and sexual. A summary score formed by adding the subscale scores measured overall childhood trauma. On scan days, subjects rated fatigue and anxiety levels using the Stress Symptom Rating scales (SSR) before and after drug administration (Naliboff et al., 1991).

Drugs

Yohimbine tablets at 40 mg were manufactured by the Clinical Research Pharmacy Coordinating Center, VA Cooperative Studies Program (Albuquerque, New Mexico). Placebo capsules were obtained through the professional Arts Pharmacy (Baltimore, MD). Clonidine tablets at 0.2 mg were on formulary at the VA Greater Los Angeles Healthcare System Pharmacy, which stored and dispensed all study drugs/placebo.

Study paradigm

The health assessment was followed by three drug sessions (median = 12±12 days apart) detailed in Supplementary Fig. S1. Groups were matched for the order of orally-administered identical (double-blind) tablets containing YOH, CLO and placebo (PLA). At each drug session, after fasting for 4 h (no alcohol for 24 h), subjects underwent a modified vigilance oddball task while blood pressure and EEG activity were recorded as previously described (Berman et al., 2002b). Preliminary EEG results have been reported (Berman et al., 2007). Thirty minutes after drug administration, participants received an intravenous injection containing 5 mCi of 2-[^{18}F]-fluoro-2-deoxy-D-glucose (FDG). Immediately thereafter, subjects began four 6-minute auditory oddball task blocks (Berman et al., 2002b). Subjects were first instructed in the task and completed a practice block. Each vigilance block required fixation on a small central cross on a computer monitor during a 5-minute period during which 128 tones (100 ms 90 dB SPL) varying randomly in pitch (1000 or 2000 Hz) and ear (right or left) were presented via air conduction earphones (Eartone type

3A). Intermixed with the tones, 16 single visual words were presented above the fixation cross for 200 ms. Half of the words were related to IBS symptoms. The others were matched for initial letter, length and frequency in print. Results from this part of the study were reported elsewhere (Vianna et al., 2009).

Inline Supplementary Fig. S1 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2012.08.028.

Positron emission tomography (PET)—Participants were then positioned in a GE Advance NXi PET camera and a 5-minute transmission scan was done as previously reported (Berman et al., 2002a). Emission data were acquired for 20 min and reconstructed using the OSEM method to generate an image of relative glucose metabolism during the auditory–visual vigilance task performance. A structural MR1 was acquired using a 1.5 Tesla Siemens Sonata.

Norepinephrine assays—NE assays were performed using a commercially-available Extraction and EL1SA kit (BA 10–0200, Rocky Mountain Diagnostics, Inc.; Colorado Springs, CO). The limit of detection for this assay was 44 pg/ml and intra- and inter-assay precisions were less than 16.1% and 15%, respectively.

Effects of each drug on NE, blood pressure and self-rated mood were quantified by within subject t-tests. Group differences were assessed with between-subject t-tests. All blood samples were lost for one control subject, and all values registered below the lowest level of detect-ability (about 50 ppm) for one patient, so these two individuals could not be included in the analyses of plasma NE. Levene's test for the homogeneity of variances was applied to examine variability in plasma levels of NE. Given violations in the assumption of equal variance, Welch's t test, an adaptation of the t-test when samples may have unequal variance, was applied to test for differences in mean plasma levels (Welch, 1947). Postdrug plasma NE levels were computed by averaging assays taken at 60 and 90 min after drug administration.

Data analysis

PET analysis—Each subject's PET image was co-registered to their anatomical MRI. Statistical Parametric Mapping software (SPM5: http://www.fil.ion.ucl.ac.uk/spm/software/spm5/) normalized all images into a standardized space (Montreal Neurological Institute), and applied the general linear model to identify regions where relative activity differed by group (IBS, Control), drug (YOH, CLO, PLA) and their interaction. Statistical parametric maps were thresholded at $p<0.005$ (uncorrected). To increase statistical power in areas of a priori interest, we used a region-of-interest (ROI) strategy focusing upon eight structures associated with pain or its modulation: anterior (aINS) and posterior insula (pINS), anterior cingulate gyrus (ACC) – divided into subgenual (sgACC), pregenual (pgACC), and mid cingulate (MCC) subdivisions, amygdala, dorsal brainstem, and ventrolateral prefrontal cortex (vIPFC). Preliminary evidence from other structures was corrected for the whole-brain volume. An alpha level of $p<0.05$ after volume correction was adopted, with $p<0.1$ findings noted as trends.

The general linear model was also applied to quantify how brain activity covaried with 1) plasma NE and anxiety, 2) activity within 4 mm radius spheres centered at 26, −6, −18 (amygdala) and − 4, 24, −10 (sgACC), and 3) childhood trauma score (for mean-corrected YOH-minus-PLA subtraction images). One IBS patient and five HCs were not included in the final analysis due to missing trauma scores.

Results

Patient characteristics

Subject characteristics are shown in Inline Supplementary Table S1 Patients rated their symptom severity as mild (n = 3), moderate (n = 2) or severe (n = 6), and had an average symptom duration of 14.9 years. The average 24 h severity rating was 10.8 (range 3–17) on a scale from 0 to 20. Patients had a higher symptom related anxiety (VS1) ($p<0.0001$), and neuroticism (trait anxiety) scores ($p =0.009$). State anxiety and depression scores (all subclinical) did not differ between groups. One-tailed t-tests for groups with unequal variance suggested greater childhood trauma in IBS patients on the total score ($p = 0.054$) and physical trauma subscale ($p =0.002$).

Inline Supplementary Table S1 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2012.08.028.

Autonomic, NE and behavioral assessments

Autonomic—Predrug systolic blood pressure did not differ between patients (118±15) and HCs (119±11). Systolic blood pressure 30 min after drug administration did not differ from predrug values. At 60 and 90 min, across all participants, blood pressure decreased after CLO, increased slightly after PLA, and increased markedly after YOH (Inline Supplementary Fig. S2A). There were no group differences in systolic blood pressure post-drug.

NE assays—Pre-drug NE levels were more variable in IBS, $F = 0.115$, $p = 0.002$, and mean plasma level was significantly higher by Welch's t test ($t(20) = 3.22$, $p =0.004$, 678±334 vs. 319 ±113). For all participants, post drug NE levels were higher after YOH (mean±SD = 907±1047), and lower after CLO (344±252), as compared to PLA (532±448; both $p<0.04$). Plasma NE levels, which were higher in patients than in HCs prior to drug administration, were also higher after the administration of all three study drugs (Inline Supplementary Fig. S2B). The increase in plasma NE following YOH ingestion was larger in patients than in HCs ($t = 1.83$, $p = 0.048$).

Anxiety and fatigue—Although patients reported more self-rated fatigue (4.62 ± 2.02 vs. 3.31 ±1.90) state anxiety (2.51 ±1.45 vs. 1.64±1.19) prior to drug ingestion, these differences did not attain significance (both $p>0.1$). Fatigue increased after the administration of all three drugs (all $p<0.01$), but more after CLO than PLA ($p = 0.02$). No group differences in fatigue were observed post-drug.

Across groups (n = 22), self-rated anxiety increased after administration of YOH ($p = 0.004$), but not after PLA or CLO (both $p>0.1$). YOH increased anxiety more in IBS patients

than in HCs ($t = 2.28$, $p = 0.042$). While anxiety prior to drug ingestion did not differ, anxiety post-YOH was higher in IBS patients than in HCs. ($t = 2.97$, $p = 0.011$). After ingestion of YOH, plasma NE level was directly correlated with drug-related anxiety increase in IBS patients ($r = 0.61$; $p<0.01$***), but not in HCs ($r=-0.53$; $p<0.1$*). Drug effects on self-rated anxiety and fatigue are shown in Inline Supplementary Fig. S2C

Inline Supplementary Fig. S2 can be found online at http://dx.doi.org/10.1016/j.neuroimage.2012.08.028.

Drug effects on brain activity

Across both groups, CLO was associated with more relative brain activity than YOH. Compared to PLA, CLO was also associated with more activity in most regions, and YOH with less activity in pgACC. However, YOH compared to PLA reduced brain activity in the ACC, amygdala, dorsal brainstem and pINS more in HCs than in patients. CLO increased activity in the sgACC more in patients than in HCs (Table 1 and Fig. 1).

Relationship between plasma NE and state anxiety with brain activity

NE, an inhibitory neurotransmitter, correlated negatively with activity in most of the brain. There were no group differences in the magnitude of this effect. In contrast, salient group differences in the relationship of anxiety with regional brain activity indicated that in HCs, but not in IBS patients, anxiety covaried positively with activity in limbic structures and the right frontotemporal cortex, and covaried negatively with activity in the left frontotemporal cortex (Table 2 and Fig. 2).

Functional connectivity analyses

Amygdala connectivity—Across all groups, there was support for the engagement of the emotional arousal circuit characterized by positive covariation of the amygdala with both dorsal brainstem, including the LCC, and sgACC. However, covariate by group interactions indicated connectivity differences between IBS patients and HCs in the brainstem, amygdala, sgACC and aMCC (Table 3).

sgACC connectivity—IBS patients had more positive covariation than HCs between the sgACC and the dorsal brainstem. HCs, but not IBS patients, were characterized by negative correlations between activity aMCC and activity in both the amygdala and sgACC (Fig. 3).

Correlation of brain response to YOH with early life trauma

We assessed the relationship between early life trauma and the ability of YOH to downregulate activity within an emotional arousal circuit (amygdala, sgACC, dorsal brainstem). Group differences in covariation could not be adequately assessed because only six HCs were administered the trauma questionnaire. More trauma correlated with a smaller YOH-mediated reduction of activity in the left dorsal brainstem (spatial-extent volume-corrected $p = 0.034$; Family-wise error corrected maximum effect voxel $p = 0.018$ @ $-6, -26, -24$, $t = 4.20$), with a similar trend in the left amygdala ($p = 0.088$; maximum effect voxel $p = 0.065$ @ $-30, -6, -24$, $t = 3.30$). These relationships were restricted to the

lateral but not medial amygdala and the pontine, but not midbrain portion of the dorsal brainstem (i.e. – LCC; see Fig. 4).

Discussion

In the current study, we show that pharmacological modulation of central NE circuits known to be involved in NE release, emotional arousal, attention and sympathetic nervous system activity differ between patients with IBS and HCs. These findings were not a consequence of comorbid anxiety disorders, since all patients were free of such diagnoses and rated state anxiety within the normal range.

Brain activity differences between groups were associated with differences in plasma NE and anxiety. In HCs, but not IBS, anxiety correlated positively with activity in limbic and right frontotemporal structures, but negatively with the left frontotemporal activity. Functional connectivity analysis of emotional arousal circuits revealed that only HCs showed expected negative correlations between ACC and limbic activity, consistent with compromised corticolimbic feedback inhibition in IBS (Mayer and Bushnell, 2009). These findings support previous reports of abnormalities in a central arousal circuit and its prefrontal modulation in IBS patients (Berman et al., 2008; Labus et al., 2008b).

Pharmacological modulation of the adrenergic system

We used the α_{2A} receptor antagonist YOH and the α_{2A} receptor agonist CLO to manipulate brain circuits involved in emotional arousal and associated autonomic and behavioral responses. Pharmacological manipulation of central α_{2A} receptors with YOH and CLO have been used to study the NE modulation of brain activity in both humans and animals (Bremner et al., 1996a, 1996b). Although their effects are not specific for presynaptic α_{2A} receptors, both drugs have been used to modulate presynaptic inhibitory α_{2A} autoreceptors on LCC or projection neurons to stimulate (YOH) or inhibit (CLO) NE release, thereby regulating cortical/subcortical levels of NE. Since no peripheral visceral stimulus (e.g. bowel distension) was employed, it is unlikely that peripheral drug effects (Camilleri et al., 2003) can explain the behavioral and brain group differences we observed.

Peripheral and behavioral measures

There is evidence of increased sympathetic and sympathoadrenal activity in IBS patients from abnormal cardio autonomic responses (Adeyemi et al., 1999; Aggarwal et al., 1994), urine catecholamine levels (Heitkemper et al., 1996) and plasma NE levels (Wong et al., 2006). Consistent with these reports, IBS plasma NE levels were higher than HCs before pharmacological modulation, and after all drugs. IBS patients had greater YOH-induced increase in NE, consistent with greater reactivity of central sympathetic regulation. In contrast to plasma NE levels, no significant group differences were observed in blood pressure, despite the combined sample showing expected blood pressure increases after YOH, and decreases after CLO.

When viewed together, these findings support the concept of increased sympathetic tone and reactivity in IBS patients, as indexed by basal and YOH-induced plasma NE levels, without a corresponding abnormality in noradrenergic vascular regulation of systolic blood pressure

(regulated by α1 and βARs). They also demonstrate that the oral doses used were sufficient to produce significant pharmacological effects without side effects. There were insignificant differences in self-rated anxiety and fatigue at baseline, but the greater YOH-induced increase in anxiety in patients and its correlation with the greater increase in plasma NE levels is also consistent with greater reactivity in IBS.

Drug effects on brain circuits involved in emotional arousal

Consistent with previous reports (Bremner et al., 1997) and with the presumed mechanism of NE on target neurons, YOH administration, as compared to PLA, reduced brain activity in MCC, while CLO increased activity in most ROIs. NE from ascending projection neurons acts on postsynaptic α2, αl and β receptors, with α2 receptors showing the highest affinity. Increasing extracellular NE inhibits spontaneous activity, presumably via α2 receptors, while sparing evoked responses to a variety of sensory stimuli, increasing the signal-to-noise ratio for specific stimuli (Ramos and Arnsten, 2007). This may be responsible for NE increase releasing CRF from limbic areas with noradrenergic input, including LCC and bed nucleus striae terminalis, thereby increasing anxiety and autonomic responses. By increasing the responsiveness of cortical attentional regions to visceral stimuli, it may also contribute to hypervigilance.

Compared to HCs, IBS patients had *reduced* YOH-mediated inhibition of brain activity in the ACC, amygdala, dorsal brainstem and pINS. The study was specifically designed not to record brain activity during a resting state, but during attentional vigilance to a validated oddball task. Although other interpretations are possible, these findings are consistent with reduced stimulatory effect of YOH on central NE release, possibly through reduced availability of presynaptic α_{2A} receptors. Reduction of α_{2A}Rs has been reported in chronically stressed tree shrews, and this was associated with increased central NE (Fluegge et al., 2003). However, the findings may also reflect decreased postsynaptic inhibitory α_{2A}Rs, as previously suggested (Dinan et al., 1990).

Correlation of brain activity with subjective anxiety

The majority of ascending NE pathways originate from the pontine LCC, which in turn is a major recipient of afferents from limbic forebrain, including amygdala and PFC. Feed forward interactions between NE neurons in LCC and CRF – containing neurons in the hypothalamus and amygdala are implicated in central stress circuitry (Koob, 1999). Ascending NE pathways alter the responsiveness of emotional arousal and attentional mechanisms. Animal data suggest that ascending NE pathways modulate nociceptive response in subregions of the ACC (Vogt et al., 2009). Enhanced NE release from ascending projection neurons in vPFC and aMCC could therefore alter the ability of frontal regions to inhibit emotional arousal. Together, NE-related dysregulation of attentional and emotional circuits could play a role in the characteristic IBS features of hypervigilance and anxiety, by modulating the emotional arousal circuits (Mayer and Bushnell, 2009).

Our results suggest that the relationship of anxiety to the laterality of frontotemporal cortical activity is abnormal in IBS patients (Fig. 2). HCs displayed a strong *positive* covariation of self-rated anxiety with activity in the limbic system and *right* frontotemporal cortex, and an

equally strong *negative* covariation with *left* frontotemporal cortex activity. These findings are consistent with prominent models of emotional processing postulating that positive emotions are associated with left anterior cortical activity and negative emotions with right anterior cortical and limbic activity (Coan and Allen, 2004; Davidson, 2002). There was no relationship between anxiety and corticolimbic activity or laterality in IBS patients. Fig. 2 suggests that cortical inhibition of the limbic stress circuit may be deficient in IBS, similar to a failure of depressed individuals to demonstrate left> right frontal cortex activation during cognitive downregulation of negative emotions (Johnstone et al., 2007). More generally, the absence of normal anterior cortical asymmetry of emotional regulation in both IBS and depression may be related to the asymmetric frontotemporal dendritic remodeling and suppression of cytogenesis that have been induced by chronic stress in animal models (Cullen et al., 2006; Czeh et al., 2008).

Evidence for altered connectivity in a central arousal circuit

Neuroanatomical and functional imaging evidences support the existence of an emotional arousal circuit involving the amygdala, sgACC and pgACC, in which projections from the pgACC to the amygdala provide negative feedback inhibition (Pezawas et al., 2005). The circuit receives multiple ascending noradrenergic inputs from LCC (Vogt et al., 2009), and inhibitory input from dorsal and vlPFC (McNaughton and Corr, 2004). Evidence for alterations in the connectivity of this arousal circuit in HCs based on the 5-HTTLPR polymorphism of the SERT (Kilpatrick et al., 2011), and in IBS patients (Labus et al., 2008a, 2011; Truong et al., 2008) has been reported. Herein, we confirmed our earlier findings that compared to HCs, IBS patients showed a lack of negative correlations between activity both in the amygdala and correlated sgACC with the aMCC/pgACC, consistent with a compromised negative feedback inhibition (Fig. 3). Supporting an extensive preclinical literature on the effects of aversive early life events on the expression of receptors and responsiveness of CRF/NE brain circuits (Meaney, 2001) (Caldji et al., 2000), we found that early life trauma was associated with smaller YOH-mediated reduction of brain activity in key components of the emotional arousal circuit, the LCC and amygdala (Fig. 4).

Some limitations of the study and considerations for future work included the following. We had a small sample size in the current study, and although an in depth structured clinical interview was conducted to exclude subjects with drug abuse and use histories, a formal drug screen test was not conducted. Additionally, an in depth clinical interview was conducted to rule out major psychiatric disorders, but due to the high prevalence of mood disorders such as anxiety and depression among IBS patients, it is possible that potential co-morbidities exist especially with anxiety spectrum disorders in this sample before the study began. Therefore, the results need to be interpreted accordingly. Another aspect to consider in future work would be to quantify plasma levels for the agonist clonidine and the antagonist yohimbine during the PET scan in order to better determine the effects of both in altering noradrenergic signaling in IBS.

Summary and conclusions

Congruent with earlier studies of PTSD (Bremner et al., 1996b), a syndrome frequently comorbid with IBS, our findings are consistent with alterations in the noradrenergic modulation of central arousal circuits in IBS patients. These alternations may be related to increased tonic firing of the LCC (Vogt et al., 2009), to a downregulation of pre and possibly postsynaptic α_{2A} receptors (Fluegge et al., 2003) and/or a genetically determined alteration in the responsiveness of these receptors (Park and Camilleri, 2005). Persisting changes in the noradrenergic system due to adverse early life events, and/or chronic disease-related stress may be involved in these alterations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

NE	noradrenergic
HCs	healthy controls
α2AR	α2-adrenoreceptor
YOH	yohimbine
CLO	clonidine
PLA	placebo
FDG	[18F]-fluoro-2-deoxy-D-glucose
PET	positron emission tomography
MCC	mid cingulate cortex
sgACC	subgenual cingulate cortex
pgACC	pregenual cingulate cortex
INS	insula
IBS	irritable bowel syndrome
GI	gastrointestinal
CRF	corticotropin releasing factor
LCC	locus coeruleus complex
BSQ	UCLA Bowel Symptom Questionnaire
EPQR	Eysenck Personality Questionnaire-Revised

VSI	Visceral Sensitivity Index
HAD	Hospital Anxiety and Depression Index
ETISF	Early Trauma Inventory Short Form
SSR	Stress Symptom Rating scales
SPM	Statistical Parametric Mapping software
MNI	Montreal Neurological Institute
ROI	region-of-interest

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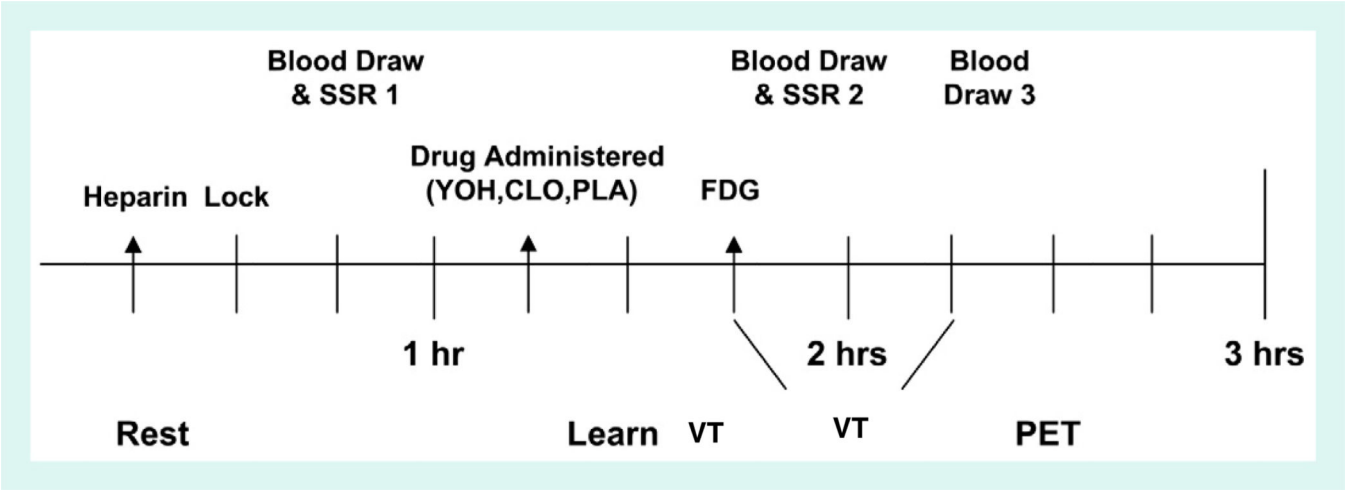

Fig. 1.

Drug and group effects on relative glucose metabolism. Upper panel depicts $p<0.005$ statistical interactions in the regions of interest (ROIs – outlined in green) between group and drug effects (compared to placebo), superimposed on a structural MRI representing atlas space. Lower panel depicts individual group effects responsible for the interaction above. Other possible interactions and contributing group effects were insignificant. Yohimbine effects are depicted on slices 6 mm to the left, 2 mm anterior, and 14 mm inferior to the anterior commissure (i.e., MNI coordinates −6, 2, −14). Clonidine effects are depicted at MNI −4, 24, −8. All figures depict neurological orientation (left=left). sgACC = subgenual cingulate, pgACC = pregenual cingulate, MCC = mid cingulate cortex, aINS = anterior insula, pINS = posterior insula, amyg = amygdala, DBS = dorsal brainstem, and vlPFC = ventrolateral prefrontal cortex.

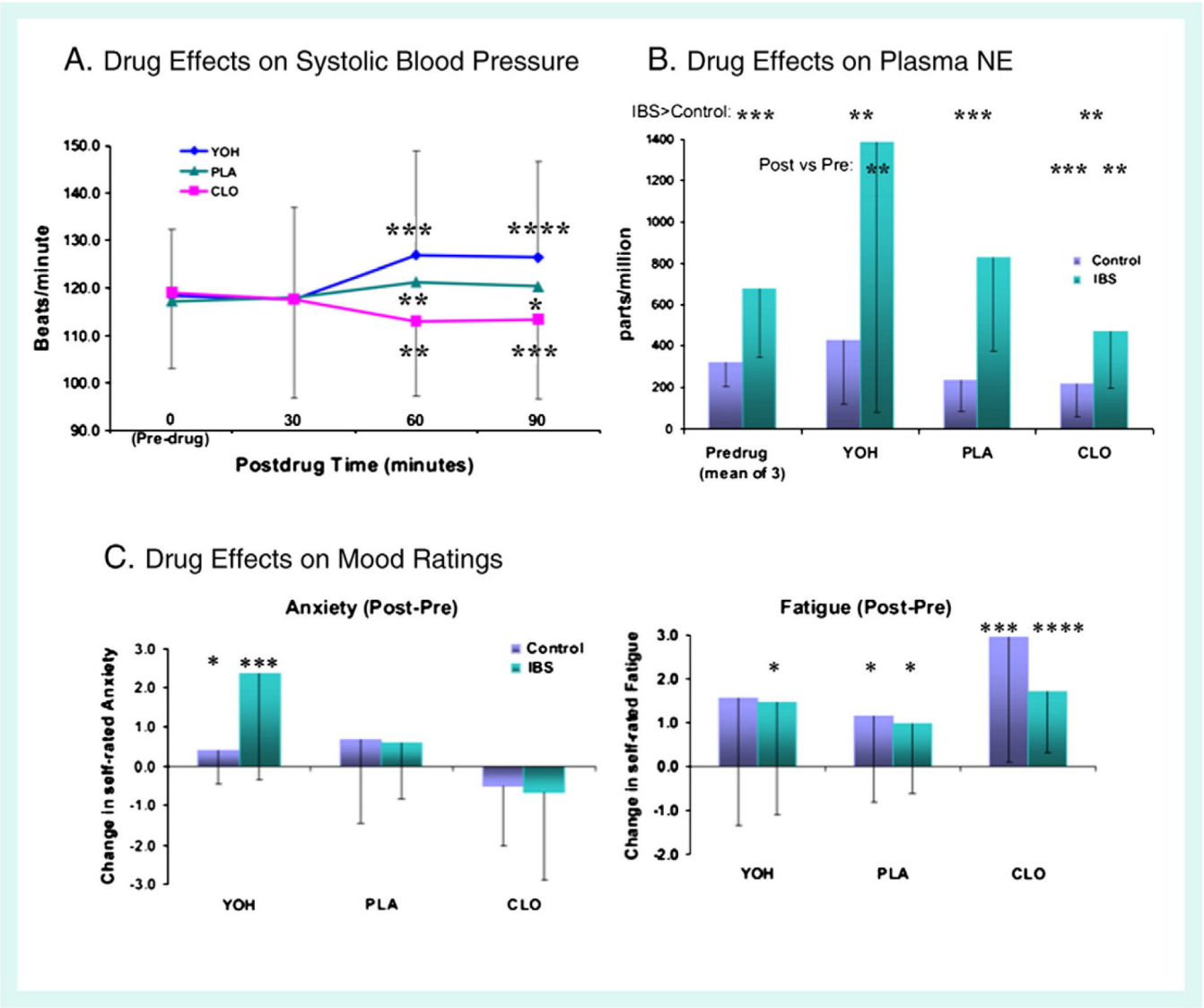

Fig. 2.

Covariance of relative glucose metabolism with self-rated anxiety by group. Upper panel depicts areas of $p<0.005$ statistical interaction in the ROIs between the slopes of covariation between metabolism and anxiety in the two groups. Lower panel depicts positive and negative covariations with anxiety in the control group, the major contributor to these interactions (see Table 2 for effects, Fig. 1 for format and abbreviations).

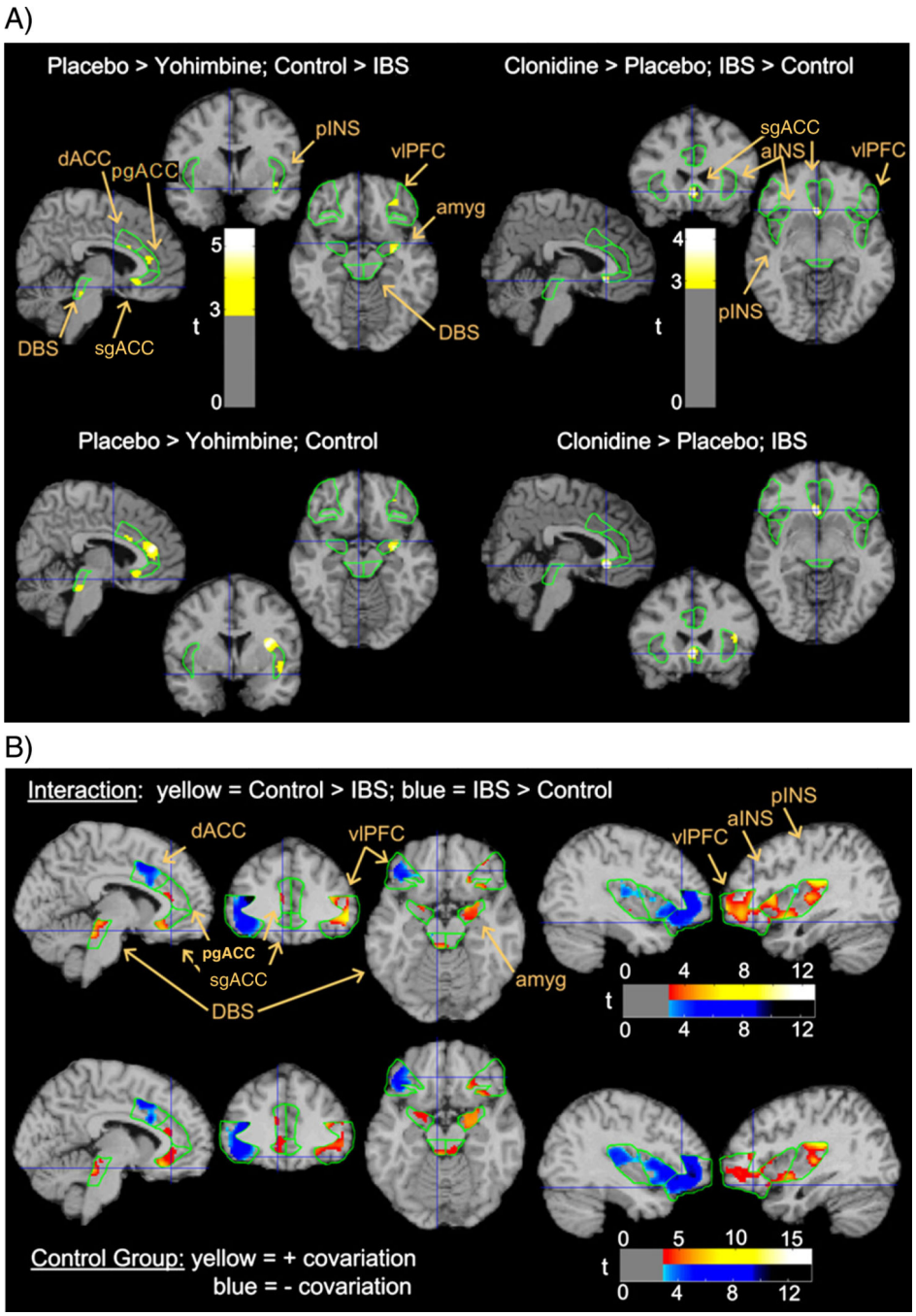

Fig. 3.

Functional connectivity of relative glucose metabolism in other regions of interest with activity in sgACC, and amygdala. Depicts areas of covariation across all scans between metabolism in other ROIs and seed regions within (A) sgACC depicted on sagittal slices 4 mm to the right of the anterior commissure, and (B) amygdala depicted on sagittal slices 6 mm to the right of the anterior commissure (see Fig. 1 for format and abbreviations).

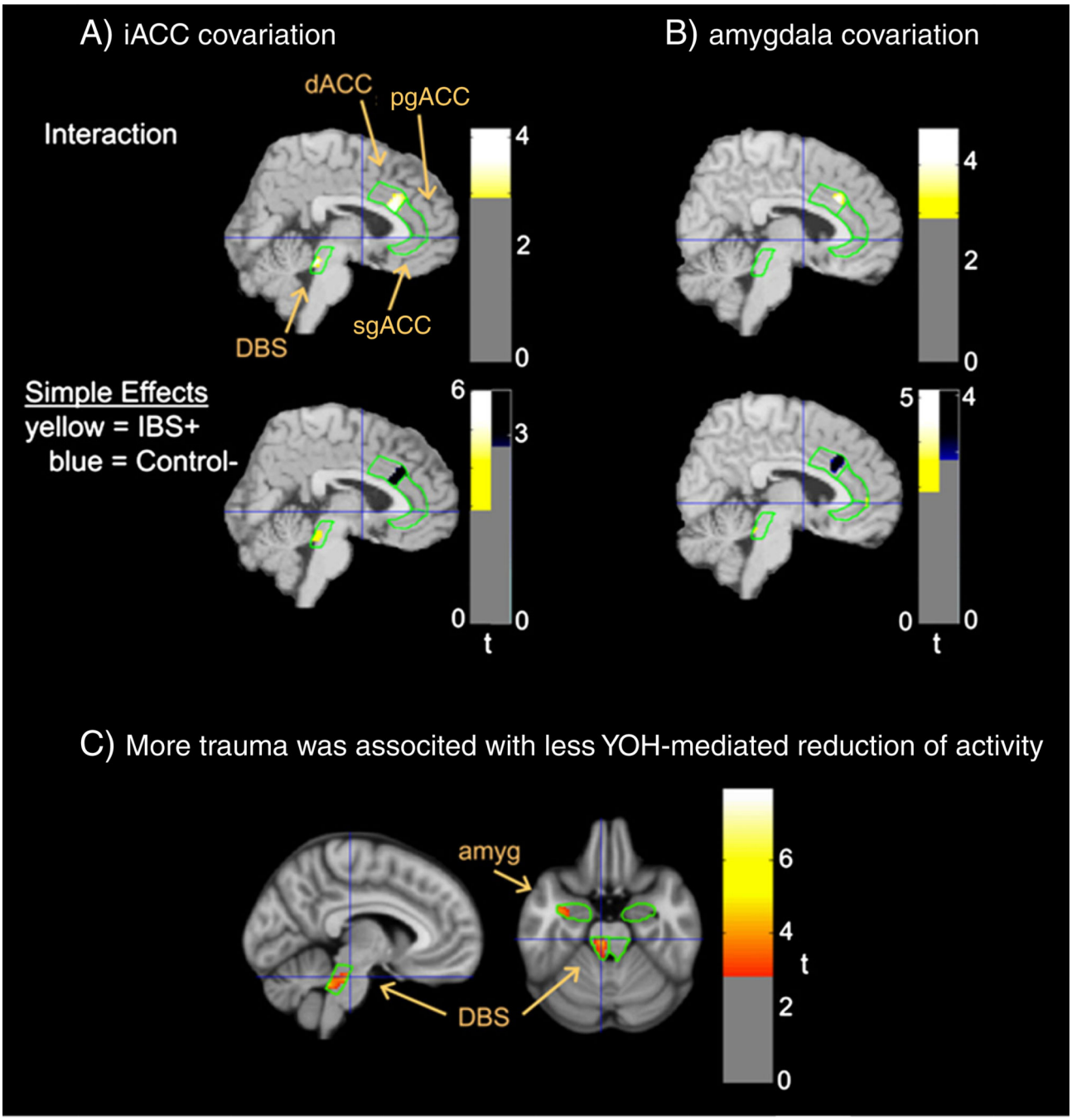

Fig. 4.

Covariation ($p<0.005$) of early life trauma with Yohimbine effects on relative glucose metabolism. Covariance between early life trauma and the ability of YOH, as compared to placebo, to downregulate relative glucose metabolism is depicted at coordinates −6, − 26, −24 (see Fig. 1 for format and abbreviations).

Table 1

Effects of drugs and IBS on brain activity. Group×drug interactions (individual group effects needed for interpretation).

		Cluster p	k	Voxel p (FWE)	T	X	y	z
PLA>YOH; HC>IBS								
Dorsal brainstem	L	0.066	13	0.060	3.05	−8	−30	−22
	R	NS		NS				
Amygdala	L	NS		NS				
	R	0.038	32	0.001 *	4.94	28	−6	−16
Subgenual ACC	L	0.028	60	0.005	4.22	−2	24	−10
	R	0.031	50	0.010	3.88	8	42	−10
Pregenual ACC	L	0.058	48	0.046	3.50	−6	36	14
	R	NS		NS				
Posterior insula	L	NS		NS				
	R	0.087	38	0.069	3.41	38	0	−10
HC:PIA>YOH								
Dorsal brainstem	L	0.035	42	0.037	3.29	−8	−30	−24
	R	NS		NS				
Amygdala	L	NS		NS				
	R	0.026	51	<0.0005 *	5.07	28	−6	−16
Subgenual ACC	L	0.027	62	0.005	4.21	−2	24	−10
	R	0.019	79	0.002 *	4.44	10	42	−10
Pregenual ACC	L	0.001*	376	0.001 *	5.13	−4	38	14
	R	0.053	51	0.055	3.40	16	44	14
Posterior insula	L	0.081	53	0.055	3.62	−38	−16	−6
	R	0.050	70	0.050	3.56	38	4	16
CLO>PIA, IBS>HC								
Subgenual ACC	L	0.045	34	0.008	4.01	−4	24	−8
	R	NS		NS				
IBS: CLO>PLA								
Subgenual ACC	L	0.023	73	0.001 *	4.74	−4	24	−10
	R	0.028	55	0.007	4.01	10	40	−12

* significant after Bonferroni correction, 0.05/16 = 0.0031, k = # of voxels. Con = control, PLA = placebo, YOH = yohimbine, FWE = family wise error corrected, ACC = anterior cingulate cortex, PFC = prefrontal cortex, NS=p>0.1.

Table 2

Group differences in covariation of regional activity with anxiety.

		Cluster p	k	Voxel p (FWE)	T	X	Y	z
HC>IBS								
Dorsal brainstem	L	0.007	142	<0.0005 *	6.33	−2	−36	−18
	R	0.036	41	0.001 *	4.75	4	−34	−20
Amygdala	L	0.070	10	0.006	4.09	−18	0	−18
	R	0.003 *	193	<0.0005 *	5.68	28	4	−22
Subgenual ACC	L	0.016	92	<0.0005 *	5.34	−4	28	−12
	R	NS		NS				
Pregenual ACC	L	0.046	60	0.031	3.76	−10	36	16
	R	NS	16	0.005	4.54	14	32	22
Anterior insula	L	NS		NS				
	R	<0.0005 *	547	<0.0005 *	6.83	36	20	8
Posterior insula	L	NS	11	0.081	3.50	−40	−22	−4
	R	0.000 *	554	<0.0005 *	7.51	36	−22	20
Ventrolateral PFC	L	NS		NS				
	R	<0.0005 *	991	<0.0005 *	7.86	42	28	10
Positive covariation in the HC group								
Dorsal brainstem	L	0.010	102	<0.0005 *	7.73	−2	−36	−18
	R	0.043	31	<0.0005 *	7.23	16	−22	−6
Amygdala	L	0.021	58	0.002 *	5.58	−16	−2	−20
	R	0.002 *	218	<0.0005 *	7.85	26	2	−24
Subgenual ACC	L	0.005	146	<0.0005 *	7.24	−4	28	−12
	R	NS		NS				
Pregenual ACC	L	0.037	66	0.023	4.60	−12	36	18
	R	NS		NS				
Anterior insula	L	NS		NS				
	R	0.004	208	<0.0005 *	8.32	36	20	12

		Cluster p	k	Voxel p (FWE)	T	X	Y	z
Posterior insula	L	NS		NS				
	R	0.003 *	236	<0.0005 *	12.02	32	−18	8
Ventrolateral PFC	L	NS		NS				
	R	<0.0005 *	974	<0.0005 *	9.18	40	26	10
Negative covariation in the IBS group								
Posterior insula	L	NS		NS				
	R	NS	12	0.037	4.47	48	−8	0
IBS>HC								
MCC	L	0.009	200	<0.0005 *	5.79	−6	16	42
	R	NS	23	0.020	4.14	12	26	38
Anterior insula	L	<0.0005 *	489	<0.0005 *	8.25	−36	30	−8
	R	NS		NS				
Posterior insula	L	0.007	229	0.001 *	5.58	−44	−20	12
	R	NS		NS				
Ventrolateral PFC	L	<0.0005 *	1204	<0.0005 *	11.03	−36	36	12
	R	NS		NS				
Negative covariation in the HC group								
MCC	L	0.008	179	0.005	6.26	−10	18	42
	R	0.057	61	0.021	6.10	12	20	40
Anterior insula	L	<0.0005 *	678	<0.0005 *	9.05	−36	30	−8
	R	NS		NS				
Posterior insula	L	<0.0005 *	553	<0.0005 *	7.82	−42	−22	20
	R	NS		NS				
Ventrolateral PFC	L	<0.0005 *	1299	<0.0005 *	9.97	−38	46	−4
	R	NS		NS				

* significant after Bonferroni correction, 0.05/16 = 0.0031, k = # of voxels.

FWE = family wise error corrected, ACC = anterior cingulate cortex, PFC = prefrontal cortex, and MCC = mid cingulate cortex.

Table 3

Covariation within a limbic stress circuit (amygdala, dorsal brainstem, 3 ACC regions).

		Cluster p	k	Voxel p (FWE)	T	X	y	z
Covariation with subgemal ACC positive covariation								
Amygdala	L	0.041	30	0.024	3.49	−18	−6	−16
	R	0.020	66	0.031	3.37	26	−8	−18
Pregenual ACC	L	NS		NS				
	R	0.026	106	0.023	3.98	8	46	−2
IBS>HC								
Dorsal brainstem	L	NS		NS				
	R	0.056	21	0.012	3.83	4	−34	−18
MCC	L	NS		NS				
	R	0.023	128	0.030	3.94	4	22	22
Positive covariation in the IBS group								
Dorsal brainstem	L	NS		NS				
	R	0.046	30	0.008	4.02	4	−34	−18
Amygdala	L	0.037	35	0.019	3.60	−18	−6	−14
	R	0.037	33	0.019	3.60	22	0	−24
Pregenual ACC	L	NS		NS				
	R	0.041	77	0.009	4.40	12	56	−2
Negative covariation in the HC group								
MCC	L	NS		NS				
	R	0.030	110	0.050	3.70	2	34	32
Covariation with amygdala positive covariation (main effect)								
Dorsal brainstem	L	0.054	21	0.036	3.34	−6	−34	−14
	R	NS		NS				
Subgenual ACC	L	0.016	92	0.008	4.09	−4	26	−12
	R	0.005 *	173	0.002 *	4.72	10	42	−8
Pregenual ACC	L	NS		NS				
	R	0.014	153	0.007	4.49	6	46	0
IBS>HC group								

		Cluster p	k	Voxel p (FWE)	T	X	y	z
MCC	L	NS		NS				
	R	0.026	120	0.011	4.40	6	26	30
Positive covariation in the IBS group								
Subgenual ACC	L	0.019	83	0.005 *	4.32	−4	26	−10
	R	0.027	57	0.013	3.81	10	42	−10
Negative covariation in the HC group								
MCC	L	NS		NS				
	R	0.034	100	0.023	4.07	8	26	32

* significant after Bonferroni correction, 0.05/8 ROIs = 0.0063, k = # of voxels. Pla = placebo, FWE = family wise error corrected, ACC = anterior cingulate cortex, and MCC = mid cingulate cortex.