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Corticotropin-releasing factor receptor 1 antagonist alters regional activation and effective connectivity in an emotional-arousal circuit during expectation of abdominal pain

Catherine S. Hubbard[1,2], **Jennifer S. Labus**[1,4], **Joshua Bueller**[1,2], **Jean Stains**[1,2], **Brandall Suyenobu**[1,2], **George E. Dukes**[6], **Dennis L. Kelleher**[6], **Kirsten Tillisch**[1,2], **Bruce D. Naliboff**[1,4,5], and **Emeran A. Mayer**[1,2,3,4]

[1]Center for the Neurobiology of Stress, UCLA, Los Angeles, CA

[2]Department of Medicine, UCLA, Los Angeles, CA

[3]Department of Physiology, UCLA, Los Angeles, CA

[4]Department of Psychiatry, UCLA, Los Angeles, CA

[5]VA Greater Los Angeles Healthcare Syst., Los Angeles, CA

[6]GlaxoSmithKline, Research Triangle Park, NC

Abstract

Alterations in corticotropin-releasing factor (CRF) signaling pathways have been implicated in irritable bowel syndrome (IBS) pathophysiology. We aimed to: 1) determine the effect of the selective CRF receptor 1 antagonist (CRF_1), GW876008, relative to placebo, on regional activation and effective connectivity of a stress-related emotional-arousal circuit during expectation of abdominal pain using functional magnetic resonance imaging (fMRI) in human subjects with a diagnosis of IBS and healthy controls (HCs), and 2) examine GW876008 effects on state-trait anxiety and hypothalamic-pituitary-adrenal (HPA) axis response. While there were no drug-related effects on peripheral HPA activity, significant central effects were observed in brain regions associated with the stress response. Effective connectivity analysis showed drug-induced normalizations between key regions of the emotional-arousal circuit in patients. During pain expectation, orally administered GW876008 relative to placebo produced significant blood oxygen level-dependent (BOLD) signal reductions in the amygdala, hippocampus, insula, anterior cingulate and orbitomedial prefrontal cortices across groups. Patients showed significantly greater BOLD responses in the left locus coeruleus and hypothalamus following placebo compared to HCs, and BOLD signal decreases in the left hypothalamus following drug. The inhibitory effects of GW876008 in the hypothalamus in patients were moderated by anxiety; patients having average and high levels of state anxiety showed drug-related BOLD decreases. GW876008 represents a novel tool for elucidating the neuronal mechanisms and circuitry underlying hyperactivation of CRF/CRF_1 signaling and its role in IBS pathophysiology. The unique state anxiety effects observed suggest a potential pathway for therapeutic benefit of CRF_1 receptor antagonism for patients with stress-sensitive disorders.

Corresponding Author: Emeran A. Mayer, M.D., Director, Center for Neurobiology of Stress, Division of Digestive Diseases, UCLA CHS 47-122, 10833 Le Conte Avenue, Los Angeles, CA 90095-7378, emayer@ucla.edu, Tel: (310) 206-0449, Fax: (310) 206-3343.

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Introduction

Corticotropin-releasing factor (CRF) is considered the principal regulator of the vertebrate stress response. In addition to its role in the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Vale et al., 1981), CRF targets extrahypothalamic sites to mediate behavioral, autonomic, and neurochemical responses to stress (Dunn and Berridge, 1990). Alterations of this complex system in humans have been linked to a variety of anxiety-related psychiatric disorders and stress-sensitive pain syndromes, including irritable bowel syndrome (IBS) (Arborelius et al., 1999; Fukudo, 2007).

IBS is a common gastrointestinal disorder, characterized by chronic abdominal pain, altered bowel habits, increased anxiety, and stress sensitivity of symptoms (Mayer, 2000; Longstreth et al., 2006). Although IBS pathophysiology remains incompletely understood, extensive preclinical and some clinical evidence suggests increased engagement of the CRF/CRF receptor 1 (CRF_1) signaling system (Martinez and Taché, 2006). In rodents, stress-induced release, or exogenously administered CRF increases anxiety-like behaviors, and stimulates colonic secretion, intestinal motility and visceral sensitivity (Taché et al., 2009). Deletion of the CRF_1 gene using transgenic models or intraventricular administered CRF_1 antagonists have anxiolytic affects and attenuate stress- and CRF-induced alterations in gastric and colonic motor function (Million et al., 2003; Trimble et al., 2007). Moreover, recent clinical investigations have shown that intravenously administered CRF increases gastrointestinal motility and visceral pain sensitivity in IBS patients compared to healthy controls (HCs), while administration of a non-selective CRF receptor antagonist ameliorated these responses (Lembo et al., 1996; Fukudo et al., 1998; Sagami et al., 2004). Taken together, these findings have spurred the development of novel and highly selective CRF_1 antagonists as candidate drugs for treatment of IBS (Zorrilla and Koob, 2010).

Functional magnetic resonance imaging (fMRI) is ideally suited as a non-invasive tool for investigating the modulatory effects of CRF/CRF_1 signaling on stress-related emotional-arousal circuits in humans, most notable of which include the amygdala (AMYG), hippocampus (HPC), hypothalamus (HT), locus coeruleus complex (LCC), insular (INS), anterior cingulate (ACC) and orbitomedial prefrontal cortices (OFC) (Valentino et al., 1999; Pezawas et al., 2005; Stein et al., 2007; Labus et al., 2008). The well-established functional neuroanatomy of stress-related emotional-arousal circuits gleaned from neuroimaging studies, combined with the known distribution of CRF_1 and CRF-expressing neurons in rodent and non-human primate brains (Aguilera et al., 1987; Dunn and Berridge, 1990), allow for specific hypothesis-driven study designs to investigate the central effects of CRF_1 antagonism in IBS patients. Using a fMRI paradigm involving expectation of a painful electrical abdominal stimulus (Phelps et al., 2001; Naliboff et al., 2008; Kumari et al., 2009) to model abdominal pain-related anxiety in IBS patients, and acute oral doses of a selective CRF_1 antagonist, GW876008 (Di Fabio et al., 2008), this placebo (PLA) controlled study aimed to address the following questions: 1) Does GW876008 attenuate the reactivity and effective connectivity of nodes within an emotional-arousal circuit, and is this effect greater in IBS patients? 2) Is the drug effect on this circuit moderated by anxiety? 3) Does GW876008 attenuate behavioral and neuroendocrine measures of anxiety and HPA axis activity differentially in patients compared to HCs?

Materials and Methods

Subjects

An age-matched sample of 31 right-handed females recruited from the greater Los Angeles community, 14 of which were diagnosed with IBS (mean age = 35.50, ± 12.48 yrs) and 17 non-IBS HCs (mean age = 33.65, ± 15.87 yrs), participated in this study. The UCLA

Medical Institutional Review Board approved all procedures and each subject provided informed consent. Diagnosis of IBS was guided by history and clinical examination, using the Rome II criteria (Thompson et al., 2000), and assessed by a gastroenterologist or nurse practitioner trained in the diagnosis of functional bowel disease. All bowel habit subtypes (constipation, diarrhea, and alternating) were deemed eligible to participate in this study. Of the 14 IBS patients, 43% were diagnosed with constipation-predominant symptoms, 21% with diarrhea predominance, and the remaining 36% with alternating symptoms of constipation and/or diarrhea. Other eligibility criteria required that subjects tested negative for drugs of abuse in their urine, lacked any significant medical problems other than IBS, were free of past or present psychiatric illness as determined by the Mini International Neuropsychiatric Interview (Sheehan et al., 1998), and were not currently taking any medications with central nervous system effects. All subjects were tested in the follicular phase of their menstrual cycle defined as day 3-14 post-menses.

Experimental design

This was a single center, randomized, double-blind, PLA-controlled, three-period crossover study of two single oral doses (20 mg or 200 mg) of the CRF_1 antagonist, GW876008, versus PLA. Study visits were conducted in the Center for Neurobiology of Stress Clinic and the Ahmanson-Lovelace Brain Mapping Center at UCLA. The study consisted of an initial screening visit (visit 1) and a familiarization visit (visit 2) wherein the subject was acclimated to the MRI environment (Fig. 1). During the familiarization visit, subjects with significant magnetic susceptibility-related artifacts were excluded. The familiarization visit was followed by three study treatment visits (visit 3, 4, and 5), each separated by approximately one month (Fig. 1). At each treatment visit, a subject was randomized to one of the three treatment groups 90 minutes prior to the start of the study test session and then given a single oral dose of GW876008 (20 mg or 200 mg) or PLA. Immediately prior to drug or PLA administration, all subjects completed a series of questionnaires, including The Hospital Anxiety and Depression (HAD) scale (Zigmond and Snaith, 1983), The Positive and Negative Affect Schedule (PANAS) subscales (Watson et al., 1988), and The State and Trait Anxiety Inventory (Spielberger, 1983). In addition, 90 min following drug or PLA treatment, subjects completed post-treatment measures of the PANAS and state anxiety subscales. Serial adrenocorticotropic hormone (ACTH) and cortisol blood samples were also collected prior to and following treatment at time points 0, 0.5, 1, 1.5, 2, and 4 hrs. Scanning commenced 120 min following administration of drug or PLA.

Drug, dosage and administration

GW876008 (GlaxoSmithKline) is a highly selective and potent antagonist for the G protein-coupled CRF_1 receptor subtype (Di Fabio et al., 2008). Based on phase II clinical trials in patients with IBS, a 20 mg and 200 mg dose of GW876008 was chosen in an attempt to provide a sufficient therapeutic range (Dukes et al., 2009; Thoua et al., 2009). PLA tablets were identical to the active GW876008 tablets in all respects with the exception of omission of the active ingredient. Subjects were assigned to study treatment in accordance with the randomization schedule provided by GlaxoSmithKline.

Pain threshold assessment procedure

Delivery of transcutaneous electrical stimulation to the abdomen was accomplished using a Digitimer constant current stimulator (Digitimer, Model DS7A; Hertfordshire, England) and two electrode stimulation pads placed 6 cm apart over subject's lower left abdomen in the region overlaying the sigmoid colon. Each stimulation to the abdomen consisted of a pulse train lasting 750 ms with a 2 ms pulse width and a frequency of 37 Hz. For each subject, a moderately intense but not intolerable pain threshold (in mA) was determined during study visit 2 (familiarization visit) and this level was then used on study treatment visits 3, 4, and 5

(Fig. 1). Threshold assessment utilized a method of limits procedure beginning with a current intensity of 1.0 mA which was increased in 0.5 mA steps until subject reported the stimulus was 'aversive but tolerable'. Following a brief rest period, each subject was given an additional stimulation at this threshold level and asked to rate the level of pain intensity and unpleasantness on separate validated 20-point verbal descriptor anchored visual analog scales, with higher scores reflecting greater degrees of intensity and unpleasantness, respectively (Gracely et al., 1978) (Table 1).

Expectation of abdominal pain paradigm

In order to model the characteristic hypervigilance and symptom-related fear often reported by IBS patients, we used a paradigm of expected pain to the left lower abdomen, a region many IBS patients refer their pain to, and which shows tenderness on physical exam. The threat of a pain experience in this body region would be expected to generate anticipatory anxiety and hypervigilance. Each subject was briefed on the experimental task immediately prior to the initiation of the experiment and then placed in the scanner bed in a supine position. Abdominal stimulation pads were attached and subject was fitted with a pair of goggles (Resonance Technology) that displayed the task stimuli using SuperLab Software (Cedrus; San Jose, CA). Prior to the start of the pain expectation protocol, each subject underwent an emotional reactivity task wherein fMRI blood oxygen level-dependent (BOLD) responses were acquired while a subject matched and labeled negatively-valenced emotions as well as identified the sex of human faces depicting angry or fearful expressions (data to be presented in a separate report). After completing the emotional reactivity task, a subject began the pain expectation paradigm following a 3 min rest period.

The pain expectation protocol consisted of two conditions; a SAFE condition and a THREAT condition (Fig. 1). In the SAFE condition, subjects saw a blue circle indicating they would not receive stimulation to their abdomen. In the THREAT condition, subjects viewed a red circle indicating they may receive a painful, but tolerable, stimulation to their abdomen at any time. For each trial, subjects also viewed a moving bar, incrementally filled with a gradient of color, indicating how much time was left in the current trial. For THREAT trials, the color started as yellow and went to red as the trial proceeded in time, whereas for the SAFE trials, the color started as purple and went to blue. Each subject received a total of seven THREAT trials and six SAFE trials per run and each run was repeated twice (Run 1, Run 2). Each trial lasted 30 s with 15 s rest periods between trials. At the start and end of each run, subjects viewed a crosshair in the center of the screen for a 30 s period. Although subjects were instructed they could receive abdominal stimulation at any time during the THREAT condition, in actuality abdominal stimulation was only delivered once per run; in the later half of Run 1 and in the earlier half of Run 2. This experimental design was chosen to elicit the maximal arousal response based on previous research and extensive piloting (Naliboff et al., 2008).

fMRI acquisition and image processing

All brain imaging was conducted with a Siemens 3 Tesla Trio MRI scanner. For each subject, a high-resolution structural T2-weighted echo-planar imaging volume (spin-echo; repetition time = 5000 ms; echo time = 33 ms; matrix size 128 × 128; 36 axial slices; field of view = 20-cm; 3-mm thick, skip 1-mm) was obtained coplanar with functional scans. Two functional BOLD runs were acquired (echo planar T2-weighted gradient-echo, repetition time = 3000 ms, echo time = 28 ms, flip angle = 90°, matrix size 64 × 64, 36 axial slices, field of view = 20-cm; 3-mm thick, skip 1-mm), each lasting approximately 10 min. A total of 432 BOLD volumes were collected during each functional run and the first two images of each run were discarded to account for instability of signal in these early scans. In addition, threat trials that contained abdominal stimulation were also excluded for analysis purposes

due to movement based artifacts. A high-resolution T1-weighted magnetization prepared rapid acquisition gradient echo MRI was acquired to aid in the registration of functional images and locate gross anatomical abnormalities.

All imaging analyses and summaries were generated using Statistical Parametric Mapping 5 (SPM5; Wellcome Trust Centre for the Study of Cognitive Neurology, London, UK) and Statistical Package for the Social Sciences (v 17) software. Images were converted from DICOM into NIFTI format, adjusted for slice timing, and realigned to control for superfluous motion. An average of the first 10 realigned fMRI images for each subject was co-registered with the subject's high-resolution echo-planar image, and then transformed into standard Montreal Neurological Institute (MNI) stereotactic coordinates (resolution = 2 mm isotropic) and smoothed with an 8 mm isotropic Gaussian kernel.

Statistical analyses

A random-effects general linear model was employed for statistical analyses of imaging data in SPM5. The primary analysis comprised of linear contrasts between the CRF_1 antagonist, GW876008, at 20 mg and 200 mg doses, versus PLA, and subsequent alterations in BOLD signal for *a priori* defined regions of interest (ROIs) as measured by fMRI during the pain expectation protocol in patients and HCs. Stimulus timings were convolved with the canonical hemodynamic response function provided in SPM5. Treatment effects of GW876008 at low (20 mg) and high (200 mg) doses compared to PLA were examined within anatomically defined ROIs (left: L and right: R) for corticolimbic-pontine structures comprising an emotional-arousal circuit which included the AMYG, HPC, HT, INS, LCC, anterior cingulate cortical subregions [anterior midcingulate (aMCC) and subgenual (sgACC) anterior cingulate cortices], and OFC cortex (Valentino et al., 1999; Pezawas et al., 2005; Stein et al., 2007; Labus et al., 2008). Due to the small spatial extent and diffuse nature of brainstem nuclei that comprise the LCC, as well as the inherent limitations to spatial resolution of fMRI, we used binary template maps (±1 standard deviation) previously validated *in vivo* (Keren et al., 2009) in standard neuroimaging space (MNI) to anatomically identify ROIs for the left and right LCC (http://www.eckertlab.org/LC). Given the limiting spatial resolving power, the term, LCC, refers to the LCC region, and not to any specific nucleus. Brain activity indexing expectation of pain for each ROI was defined by contrast beta images representing signal changes between experimental conditions (THREAT - SAFE). Due to the rapid activation of subcortical and brainstem regions (e.g., AMYG, LCC) during anticipatory pain, only the first 10 s of each trial was included in the analysis. Response to expectation of pain was then analyzed in a second level, 2 (Group: IBS, HCs) × 3 (Treatment: PLA, 20 mg and 200 mg dose of GW876008) general linear model, specifying subject as a random effect and controlling for order. For ROI analysis, activated and deactivated voxels were identified using an α level < 0.05, corrected for multiple comparisons with false-discovery rate (FDR). Peak activity in representative voxels was extracted for secondary analyses of behavioral and neuroendocrine interactions and for effective connectivity modeling.

To explore the moderating effects of state and trait anxiety (pre-treatment) on BOLD signal reductions by drug during expectation of abdominal pain in IBS and HCs, covariate analyses with a 2 (Group: IBS, HCs) × 3 (Treatment: PLA, 20 mg and 200 mg GW876008) repeated measures general linear mixed-effects model were performed for LCC and HT activity. Moderator effects were examined graphically by displaying parameter estimates and 95% normal confidence intervals for High (+1 SD above the Mean), Average (Mean) and Low (-1 SD below the Mean) values for anxiety (Holroyd et al., 2009).

In addition, we examined the effects of a 20 mg and 200 mg dose of GW876008 versus PLA on pre- and post-treatment measures of the PANAS and state anxiety subscales, as well as

plasma cortisol and ACTH levels via repeated measures general linear mixed-effects model. In each instance, specifying a heterogeneous autoregressive error-covariance matrix structure yielded the best fit among the commonly used covariance structures as indicated by Akaike's Information Criteria.

Of the 31 subjects in our sample, three individuals were excluded from the analysis due to BOLD signal loss in ROIs across all three fMRI study treatment visits. Three additional subjects were removed from cortical and subcortical ROI analysis due to signal dropout during one of the three study treatment visits. However, these same subjects were included in the ROI analysis for the LCC since this region remained intact and unaffected by signal drop-out. Presumably, signal dropout was caused by movement related artifacts and/or air pockets trapped in the sinuses resulting in significant signal distortions (Buxton, 2002).

Effective connectivity analysis was applied to test the hypotheses that GW876008 would differentially alter the strength of connectivity within a stress-related emotional-arousal circuit in IBS and HCs during expectation of abdominal pain. The network of interest encompassed unilateral brain regions localized to the left hemisphere (Fig. 2), including the AMYG, HPC, HT, LCC, aMCC, sgACC, OFC and ventral subregions of the anterior insula (aINS) (Pezawas et al., 2005; Stein et al., 2007; Labus et al., 2008). The spatial location of the voxels used to represent the regions or nodes of the circuit were selected from the primary SPM analyses. After specifying the structural model, path analysis using a structural equation modeling framework was performed with Amos 18.0 conducting full information likelihood estimation. Standard errors for parameter estimates were obtained via 200 bootstrapped samples and used to calculate 95% confidence intervals for parameter estimates based on the normal distribution.

Residual variances, representing external input into the system (e.g., unspecified regions, psychological characteristics, hormonal milieu), were fixed at 35% (McIntosh and Gonzalez-Lima, 1994) of the observed regional variances within group and treatment conditions. Drug treatment effects on the effective connectivity of the emotional-arousal network in IBS and HCs were tested using multi-group tests for invariance (Joreskog, 1971). Differences in the circuitry of the network were localized with pair-wise comparisons between an unconstrained and partially constrained model using chi-square differences with 1 degree of freedom where a chi-square difference value of 3.84 represented a $p < 0.05$. The 200 mg dose of GW876008 was chosen for the effective connectivity analysis based on results demonstrating no significant treatment differences for ROI activation following administration of GW876008 at low (20 mg) versus high (200 mg) doses.

Results

Clinical sample characteristics

Table 1 provides the descriptive and inferential statistics for clinical characteristics of the two groups, assessed prior to randomization. Significant group differences for the dependent variables were only observed for the trait anxiety measure [$F(1,25) = 6.43$, $p = 0.018$]. Prior to drug or PLA treatment, IBS patients had significantly higher levels of trait anxiety (mean ± SD: IBS, 35.3 ± 7.95; HC, 27.3 ± 8.28), but not state anxiety (mean ± SD: IBS, 31.8 ± 9.17; HC, 27.1 ± 6.90) compared to HCs (Table 1).

Effect of GW876008 on behavioral and neuroendocrine measures

Analysis of the PANAS subscales (negative and positive affect, fear, hostility and serenity) demonstrated no significant differences in mood due to drug treatment for either group. No significant drug effects were seen for pre- versus post-treatment state anxiety scores within or between groups. For ACTH pre-treatment levels, there was a significant main effect for

Group [F(1, 100) = 4.48, $p = 0.037$], with patients (mean ± SD: 19.88 PG/mL ± 29.43) showing overall lower ACTH levels across baseline compared to HCs (mean ± SD: 102.31 PG/mL ± 25.48). In contrast, no significant Group effect was found for plasma cortisol levels, nor did we find any significant Treatment effects or Group × Treatment interactions for plasma cortisol or ACTH.

Effects of GW876008 versus PLA on BOLD signal responses during expectation of abdominal pain

Main effects of treatment (PLA vs. drug)—Significant main effects for Treatment (PLA, 20 mg and 200 mg GW876008) were observed lateralized to the L sgACC [F = 14.24, $p = 0.02$], L OFC [F = 6.56, $p = 0.028$], and L posterior INS [F = 22.97, $p = 0.038$], as well as bilaterally for the HPC [L: F = 16.89, $p = 0.036$; R: F = 20.13, $p = 0.006$]. Trends toward significant effects for Treatment were also seen for the AMYG [L: F = 8.50, $p = 0.094$; R: F = 10.65, $p = 0.084$], and R sgACC [F = 13.32, $p = 0.067$]. Planned contrasts revealed significant drug-induced reductions (20 mg and 200 mg doses of GW876008 compared to PLA) in fMRI BOLD signal during pain expectation (Threat – Safe) for the bilateral sgACC, as well as unilaterally, for the AMYG, HPC, OFC and posterior INS (all in L hemisphere; Table 2). Other ROIs, including the R AMYG and R HPC showed significant attenuation in BOLD signal responses during pain expectation following administration of high (200 mg), but not low (20 mg) doses of GW876008 compared to PLA (Table 2).

Group × treatment interactions—ROI analysis revealed significant Group × Treatment interactions for the L HT [F = 15.82, $p = 0.004$] and L LCC [F = 6.88, $p = 0.043$] during pain expectation. Significant group differences were found in response to administration of a 20 mg [IBS(20 mg - PLA) – HC(20 mg - PLA)] or 200 mg [IBS(200 mg - PLA) – HC(200 mg - PLA)] dose of GW876008 relative to PLA for both the L HT and L LCC (Table 3). Following PLA administration, patients showed significantly greater BOLD signal activity in the L HT [t = 6.06, $p < 0.001$] and L LCC [t = 3.37, $p = 0.002$] during expectation of pain (Threat – Safe) compared to HCs, while this difference was not observed for drug treatment conditions (Figs. 3 and 4). Patients showed significant BOLD signal reductions in the L HT following administration of the 20 mg [t = 4.02, $p = 0.003$] and the 200 mg dose [t = 4.09, $p = 0.002$] of GW876008 compared to PLA, whereas HCs showed no significant treatment effects (Fig. 3). Conversely, in the L LCC, HCs but not IBS patients showed significant increases in BOLD signal responses following treatment with either the 20 mg [t = 2.41, $p = 0.035$] or the 200 mg [t = 2.86, $p = 0.018$] dose of drug relative to PLA (Fig. 4).

Given the pre-treatment group differences in trait anxiety (Table 1), we re-examined these differences using between-group contrasts for the L HT and L LCC while controlling for this variable using SPM5 t-tests specifying trait anxiety as a covariate of no interest at the second level. Following inclusion of trait anxiety as a covariate into the model, significant group effects for the L LCC remained for both the 20 mg (t = 2.65, $p = 0.049$) and the 200 mg (t = 2.44, $p = 0.027$) doses of GW876008 compared to PLA. For the L HT, between group contrasts remained significant at the low (20 mg; t = 3.08, $p = 0.045$) drug dose and approached significance at the high (200 mg; t = 2.93, $p = 0.07$) drug dose relative to PLA treatment.

Effects of GW876008 versus PLA on network connectivity of an emotional-arousal circuit in IBS and HCs

As can be seen in Table 4, in comparison to PLA, administration of a CRF_1 antagonist led to significant alterations in effective connectivity in the emotional-arousal circuit in IBS patients and HCs. Both patients and HCs showed drug-induced increases in positive effective connectivity for paths from ventral aINS to AMYG, and greater negative

connectivity between the sgACC and the aMCC. Although similarities in strength and direction of effective connectivity between hypothesized nodes of the emotional-arousal circuit were present for both groups for drug versus PLA treatment, the most dramatic changes in effective connectivity were observed in patients (Table 4; Fig. 5). Strikingly, in IBS patients all paths to and from the AMYG showed dampening or qualitative changes in effective connectivity. For example, patients, but not HCs, showed significant drug-induced *increases* in positive effective connectivity for paths to the AMYG from OFC and HT, with levels approaching path estimates observed in HCs following PLA administration. Patients also showed significant drug-induced *reductions* in coupling between paths from aMCC and LCC to the AMYG, as well as from AMYG to HPC and ventral aINS. In HCs, only 50% of the AMYG afferents demonstrated drug-induced alterations in connectivity. Unlike the changes observed in IBS patients, the drug did not induce differences in connectivity in AMYG afferents to the HPC or aINS in HCs.

Moderating effects of anxiety on GW876008 induced BOLD signal changes in hypothalamus and locus coeruleus complex during expectation of pain

Hypothalamus—Baseline state anxiety (pre-treatment) moderated the observed drug effects on the HT. No significant main effects for Group [$F(1,22) = 1.86$, $p = 0.186$], Treatment [$F(2,37) = 0.34$, $p = 0.712$] or state anxiety [$F(1,61) = 0.21$, $p = 0.646$] were found. Significant Group × Treatment [$F(2,37) = 9.64$, $p < 0.001$], Group × state anxiety [$F(1,61) = 4.57$, $p = 0.037$] and Group × Treatment × state anxiety [$F(2, 39) = 6.36$, $p = 0.004$] interactions were observed for the L HT. At average and high levels of state anxiety, but not low levels of this construct, patients showed greater fMRI BOLD signal response activations in the L HT under PLA conditions compared to HCs (average: $t_{40} = 3.63$, $p = 0.001$; high: $t_{60} = 4.92$, $p < 0.001$). Additionally, patients at average and high levels of state anxiety showed greater reductions in BOLD signal responses for both 20 mg (average: $t_{41} = 2.06$, $p = 0.046$; high: $t_{61} = 3.30$, $p = 0.002$) and 200 mg dose of drug (average: $t_{37} = 2.17$, $p = 0.037$; high: $t_{53} = 2.16$, $p = 0.035$) compared to PLA.

Locus coeruleus complex region—For the L LCC region, no significant main effects or interactions for state anxiety were found, although a trend for a Group × Treatment interaction approached significance [$F(2, 43) = 2.53$, $p = 0.091$]. Due to *a priori* hypotheses, we examined Group × Treatment effects on BOLD signal responses for the L LCC at low, average and high levels of state anxiety in IBS and HCs. During PLA, patients showed significantly greater activation in the L LCC than HCs at both average ($t_{60} = 2.52$, $p = 0.015$) and high levels of state anxiety ($t_{66} = 2.72$, $p = 0.008$), but not low levels. At average and high levels of state anxiety, HCs, but not patients, showed drug-induced BOLD signal increases in the L LCC. For example, at average levels of state anxiety, HCs showed significant signal increases following administration of a 20 mg ($t_{40} = -2.01$, $p = 0.052$) dose of the antagonist compared to PLA treatment. At the 200 mg dose, HCs showed significant increases in L LCC activation at both average and high levels of state anxiety (average: $t_{68} = -2.35$, $p = 0.021$; high: $t_{68} = -2.28$, $p = 0.026$).

Discussion

Expectation of abdominal pain was associated with engagement of several cortical and limbic brain regions, a finding which parallels previous reports of somatic pain expectation (Phelps et al., 2001; Simpson et al., 2001; Straube et al., 2009). Following acute administration of the CRF_1 antagonist, patients with average and high state anxiety showed reductions in HT (but not LCC) activity, as well as a partial normalization of effective connectivity between key nodes of an emotional-arousal circuit, without detectable drug effects on HPA axis measures. The observed effects are consistent with a central role of

CRF/CRF_1 signaling during pain expectation, as well as the hypothesized attenuating effects of CRF_1 antagonism on regional activity and engagement of an emotional-arousal circuit in IBS patients.

In contrast to an extensive animal literature showing anxiolytic effects of acutely administered CRF_1 antagonists (Takahashi, 2001; Bale and Vale, 2004), acute administration of GW876008 in the current study had no significant effect on subjective measures of emotion, a finding compatible with results from clinical trials using the selective CRF_1 antagonist pexacerfont (Sweetser et al., 2009; Coric et al., 2010). Moreover, similar to other reports on HPA axis alterations in stress sensitive disorders, including IBS (Smith et al., 1989; Chang et al., 2009), patients showed significantly lower basal plasma ACTH, but not cortisol levels, compared to HCs. However, GW876008 administration did not affect plasma ACTH or cortisol levels. These data are consistent with findings from preclinical and early clinical studies demonstrating a lack of CRF receptor antagonist effects on HPA axis activity (Künzel et al., 2003; Sagami et al., 2004; Jutkiewicz et al., 2005).

Drug administration resulted in significant BOLD signal reductions within key regions of an emotional-arousal circuit during pain expectation in both patients and HCs. Significant BOLD signal reductions at both drug doses were observed in the AMYG, HPC, posterior INS, and OFC. These reductions were predominantly lateralized to the left hemisphere, although at high drug doses, significant BOLD signal reductions were also observed in the R AMYG and R HPC. These findings are in accord with immunohistochemical, *in situ* hybridization and autoradiographical studies conducted in rats and non-human primates demonstrating the presence of CRF_1 receptor mRNA and CRF_1 binding sites within these regions, and therefore fits well with the expected inhibitory effects of GW876008 (Millan et al., 1986; Radulovic et al., 1998; Sánchez et al., 1999; Chen et al., 2000).

The LCC supplies the major noradrenergic input to the forebrain, and mediates emotional arousal, autonomic and behavioral responses to stress, and attention-related processes (Aston-Jones and Cohen, 2005). In preclinical studies, CRF has been shown to modulate LCC neuronal activity, and CRF expressing neurons and CRF_1 mRNA in the LCC have been identified (Valentino et al., 1983, Dautzenberg and Hauger, 2002). CRF-induced increases in tonic LCC neuronal discharge patterns and inhibition of LCC phasic responses to somatosensory and auditory stimuli (Valentino and Foote, 1987, 1988) is thought to facilitate the rapid disengagement from focused, to labile attention (Aston-Jones and Cohen, 2005; Van Blockstaele et al., 2010). As expected, patients had greater threat-induced L LCC activation during PLA compared to HCs. In a previous study using a similar pain expectation paradigm, Berman et al. (2008) reported greater activation of the dorsal brainstem region (including the LCC) in IBS patients, and this activation was correlated with state anxiety, as well as with the BOLD responses observed during aversive visceral distension. Surprisingly, we observed no drug effect on LCC activity in patients, while HCs showed an unexpected drug-induced increase in BOLD response, which may be due to the differential effects of GW876008 on the phasic and tonic discharge patterns of LCC neurons (Aston-Jones and Cohen, 2005), or to partial agonist effects of the antagonist (Schulz et al., 1996; Kosoyan et al., 2005).

Several possible explanations for the apparent lack of drug effect on LCC activity in patients should be considered, including species differences in the molecular characteristics and binding affinity of the CRF_1 receptor in the LCC, and CRF_1 upregulation and/or sensitization in the LCC due to chronic stress exposure in IBS patients. However, activity did vary significantly during the PLA condition in IBS patients and GW876008 administration did reduce this variability (Fig. 6), bringing levels of activation in the LCC to that seen in HCs.

The HT, via the HPA axis and the autonomic nervous system, plays a critical role in the neuroendocrine control of a variety of homeostatic functions, including the rapid and acute response to physiological and psychological stress. For example, stress-induced release of CRF from the paraventricular nucleus of the HT initiates the HPA axis response, an effect blocked by centrally administered CRF_1 antagonists (Bale and Vale, 2004). Dysfunction in HPA axis regulation due to overactivation of CRF/CRF_1 signaling in response to chronic stress has been implicated in the pathophysiology of IBS symptoms (Chang et al., 2009). In the current study, patients showed significantly higher levels of trait anxiety than HCs which is consistent with an upregulation of central stress and emotional-arousal in this population (Spiller et al., 2007; Rapps et al., 2008). Patients also showed significant BOLD increases in the L HT during pain expectation following PLA compared to HCs, whereas in response to antagonist administration at either dose, patients but not HCs showed significant BOLD decreases in the L HT. The former finding that IBS patients showed enhanced activity in the HT during pain expectation under PLA conditions compared to HCs suggests that central stress circuits may be upregulated in these patients. This finding is interesting in light of previous studies demonstrating morphological alterations in gray matter density in corticolimbic pain modulatory systems and in the HT in patients with chronic pain syndromes, including IBS (Schweinhardt et al., 2008; Blankstein et al, 2010; Seminowicz et al., 2010). It has been suggested that such structural changes may be due to use-dependent hypertrophy, associated with upregulation of central stress response circuitry (Blankstein et al., 2010).

The inhibitory effects of GW876008 in the HT were moderated by the presence of average to high levels (but not low) of state anxiety in IBS patients; patients with average to high state anxiety showed greater BOLD responses in the L HT following PLA, and greater BOLD signal reductions following drug than HCs. This finding parallels previous reports demonstrating that alterations in the central processing of visceral pain stimuli in IBS patients are moderated by anxiety symptoms (Elsenbruch et al., 2010a,b). Taken together, these findings support the hypothesis that the selective CRF_1 antagonist, GW876008, is capable of attenuating stress-induced hypothalamic activation during expectation of abdominal pain and that this effect is moderated, at least in part, by anxiety. The fact that patients were found to have lower plasma ACTH values prior to treatment, and that no drug effect was observed on ACTH or cortisol levels, suggests that GW876008 is not acting peripherally via the HPA axis, but rather having central effects.

Under PLA conditions, IBS patients showed strong positive coupling between aMCC and AMYG, consistent with absence of negative feedback inhibition from the AMYG (Pezawas et al., 2005; Labus et al., 2008). Also, IBS patients showed strong coupling between other nodes of the emotional-arousal circuit (LCC and AMYG, AMYG and ventral aINS), whereas HCs showed weak negative coupling for these paths. Both groups showed similar drug-induced changes in connectivity (including the path from aINS to AMYG), although drug effects on connectivity were more prominent in IBS patients with path coefficients approaching those of HCs following drug compared to PLA. Thus, it appears that high doses of GW876008 may have partially normalized the effective connectivity of brain circuits involved in mediating arousal and stress-related emotional responses in patients compared to HCs.

Limitations of the current study include the small sample size of female patients. For example, sexual dimorphism of the LCC and sex-related differences in CRF/CRF_1 signaling have recently been reported (Bangasser et al., 2010a, 2010b) and the majority of preclinical studies showing effectiveness of CRF_1 antagonism were performed in male rodents. Furthermore, IBS refers to a heterogeneous group of patients, with differences in bowel habits, a history of stress sensitivity of symptoms, and comorbid conditions (Schmulson et

al., 1999). These subgroups of patients may show differential responsiveness to a CRF_1 antagonist. Finally, due to limitations in spatial resolution of fMRI, the identification of specific nuclei within the LCC region was not possible, therefore we used previously published template maps to identify the LCC region (Keren et al., 2009).

Summary and clinical implications

This study provides the first evidence that acute oral dosing of GW876008 is sufficient to produce inhibitory effects on regional activity and connectivity within specific nodes of an emotional-arousal circuit in female IBS patients during pain expectation, confirming several hypotheses based on extensive preclinical data (Taché et al., 2009). However, early clinical trials with two different CRF_1 receptor antagonists, GW876008 and pexacerfont have not shown beneficial effects for IBS symptoms even though trends were observed in one study (Sweetser et al., 2009; Dukes et al., 2009; Thoua et al., 2009). The reason(s) for the apparent discrepancy between these findings and that of the current study, and the negative outcomes of several clinical trials, are unknown. However, it remains possible that these compounds only work in a subset of patients with clear stress sensitivity of their symptoms, high trait anxiety and underlying hyperresponsiveness of stress-related arousal circuits, including the HT.

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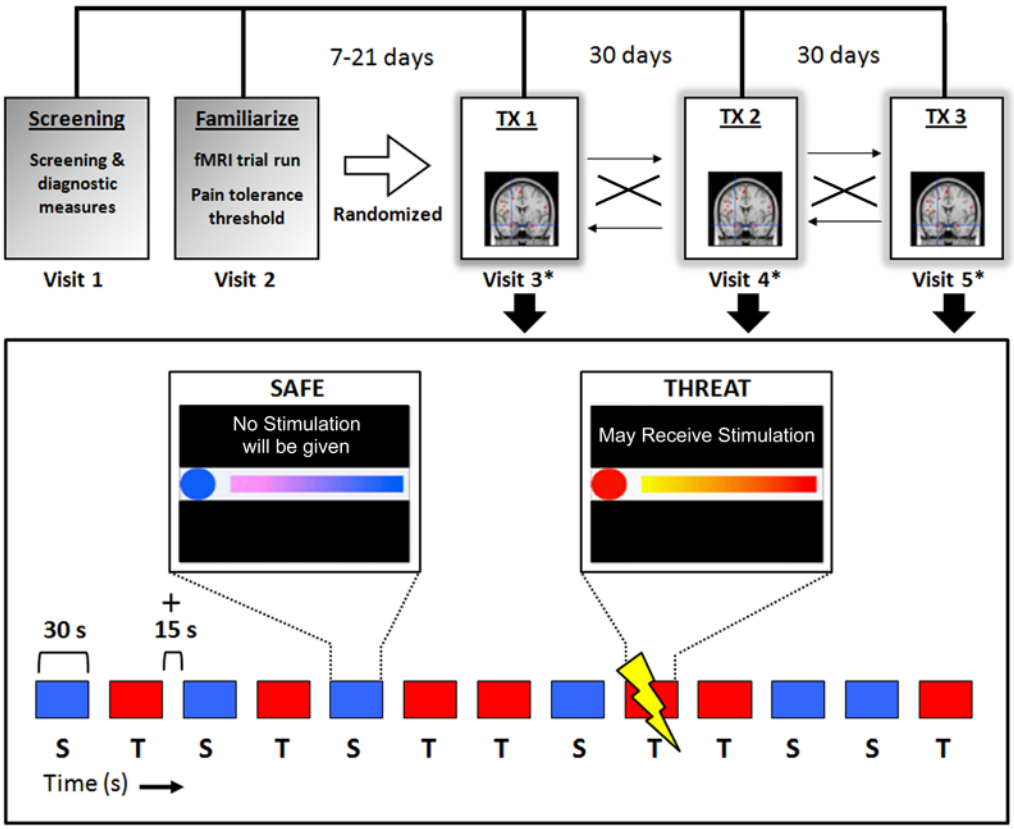

Figure 1.

Schematic illustrating experimental design (top panel) and the abdominal pain expectation protocol (bottom panel). Top panel: Each subject received a single acute oral dose of either placebo (PLA; 0 mg), 20 mg GW876008 or 200 mg GW876008 in a randomized, double-blind manner across three separate study treatment (TX) visits (visit day 3, 4, and 5) each separated by approximately one month. Bottom panel: shows the safe (S; blue) and threat (T; red) trials (30 s trials with 15 s intertrial intervals) for a single MRI run for the abdominal pain expectation paradigm.

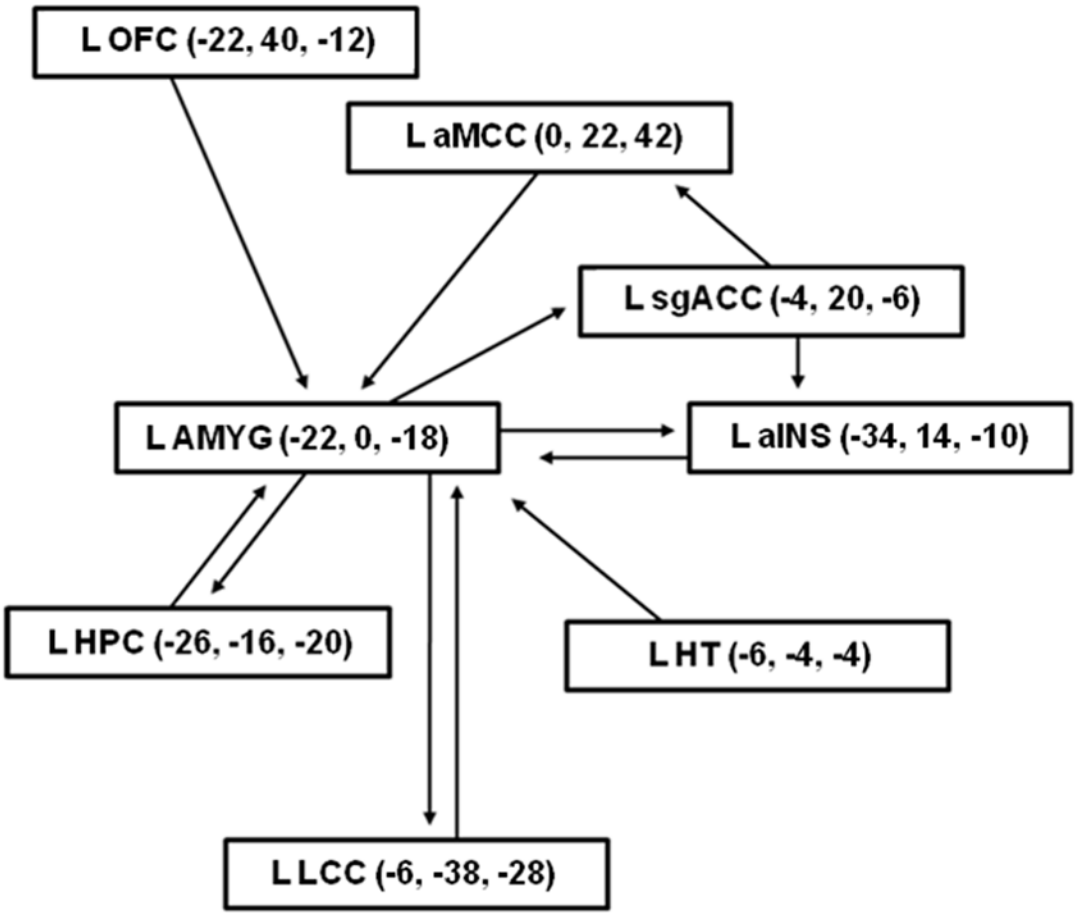

Figure 2.
Path diagram from structural equation modeling analysis used for testing effective connectivity of network nodes of an emotional-arousal circuit involving left hemispheric structures. Nodes of the circuit are illustrated along with MNI coordinates (x, y, z). Abbreviations: AMYG—amygdala, aINS—anterior insula, HPC—hippocampus, HT—hypothalamus, LCC—locus coeruleus complex, OFC—orbitomedial prefrontal cortex, aMCC—anterior midcingulate cortex, sgACC—subgenual anterior cingulate cortex.

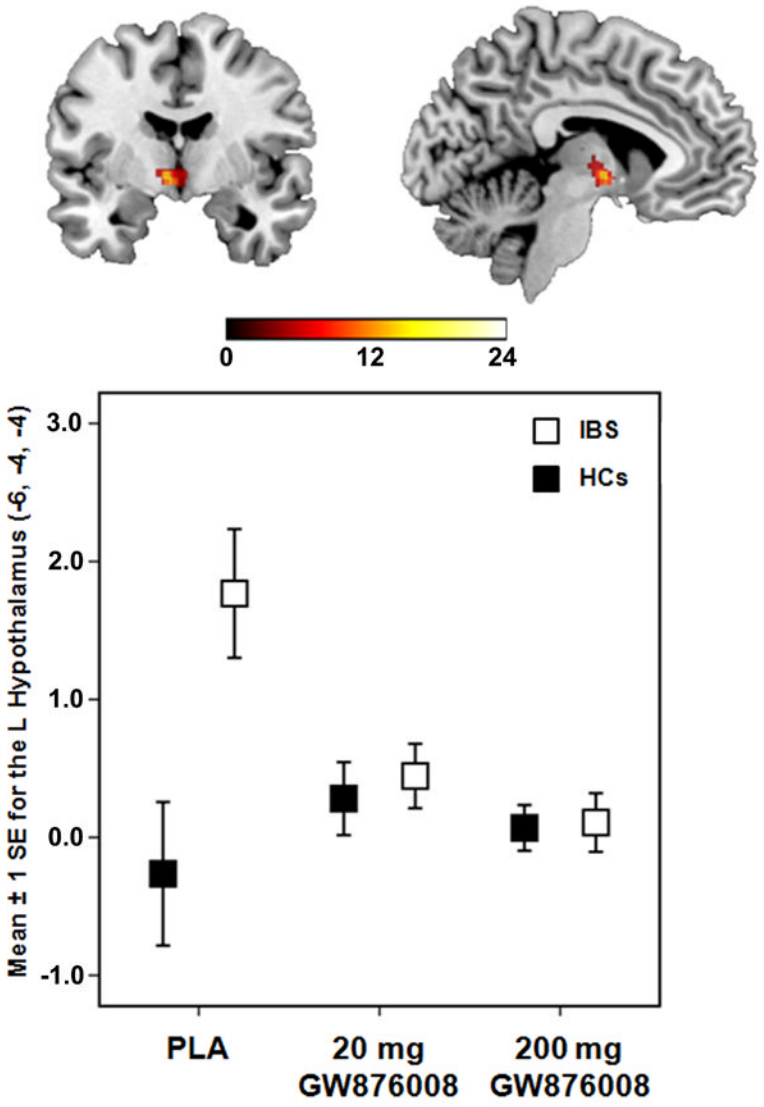

Figure 3.
Error plot showing standard mean errors (±1 SE) for beta contrasts (Threat – Safe) following placebo (PLA) versus a 20 mg GW876008 or 200 mg dose of GW876008 for the left (L) hypothalamus in IBS patients and healthy controls (HCs) during pain expectation.

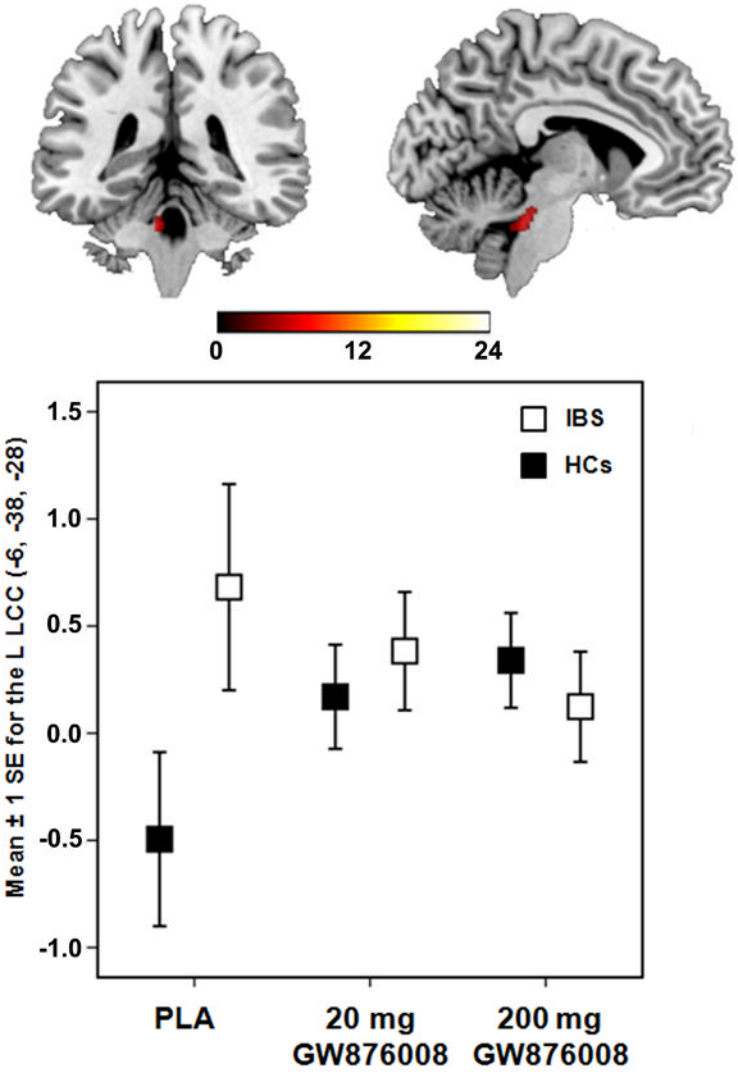

Figure 4.

Error plot showing standard mean errors (±1 SE) for beta contrasts (Threat – Safe) following placebo (PLA) versus a 20 mg GW876008 or 200 mg dose of GW876008 for left locus coeruleus complex (L LCC) in IBS patients and healthy controls (HCs) during pain expectation.

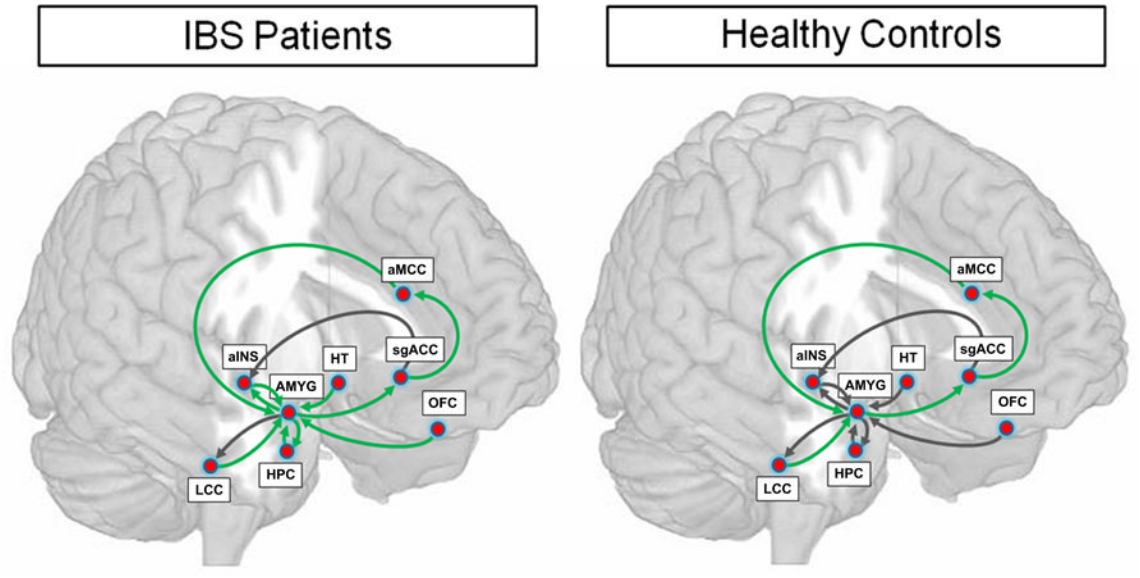

Figure 5.

Path coefficients for the effective connectivity analysis of an 'emotional-arousal circuit' during expectation of abdominal pain following placebo (PLA) versus high dose of the CRF_1 antagonist (200 mg GW876008) in healthy controls (HCs) and IBS patients. Parameter estimates that were significantly different are represented by green arrows (light gray arrows in print version) whereas those that were not significantly different are represented by dark gray arrows. Abbreviations: AMYG—amygdala, aINS—anterior insula, HPC—hippocampus, HT—hypothalamus, LCC—locus coeruleus complex, OFC—orbitomedial prefrontal cortex, aMCC—anterior midcingulate cortex, sgACC—subgenual anterior cingulate cortex.

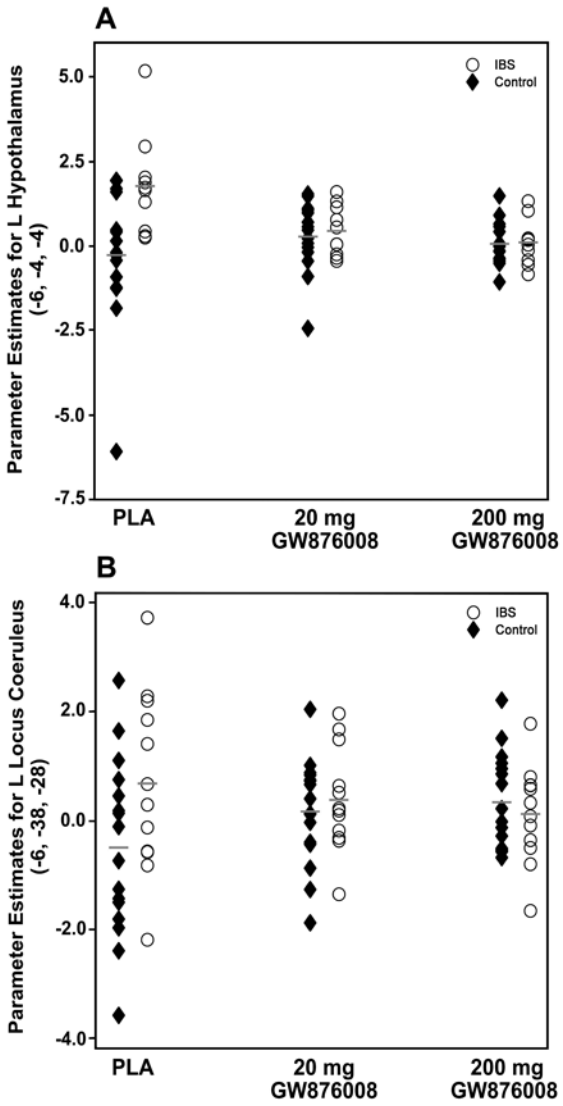

Figure 6.

Scatterplots illustrating the distribution of parameter estimates for BOLD signal activity in the left (A) hypothalamus and (B) locus coeruleus complex in IBS patients and healthy controls (HCs) across the three different treatment conditions (placebo, PLA; 20 mg GW876008; 200 mg GW876008). Gray lines indicate parameter estimate means within group for each treatment condition.

Table 1

Clinical characteristics

	IBS Patients		Healthy Controls		Test statistics and p-values	
	Mean	**SD**	**Mean**	**SD**	**F**	***p***
Age	35.67	12.46	34.56	15.92	0.039	0.844
Current Intensity	4.50	1.65	4.50	2.55	< 0.001	1.000
Body Mass Index	25.88	7.67	25.74	5.15	0.004	0.952
Unpleasantness Ratings	9.08	2.87	8.71	2.35	0.103	0.751
Intensity Ratings	11.58	2.50	11.94	3.15	0.136	0.715
State Anxiety	31.76	9.17	27.15	6.90	2.317	0.140
Trait Anxiety	35.33	7.95	27.34	8.28	6.432	0.018

Means (SD) and one-way analysis of variances (F test statistics and p-values) for clinical characteristics in female IBS patients and healthy female controls.

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Table 2

MNI coordinates for peak voxels showing significant treatment effects for each contrast (Threat > Safe) across all subjects

Region		Voxels		MNI coordinates		
		t	*p*	x	Y	z
PLA > 20 mg GW876008						
AMYG	L	3.03	0.045	-26	2	-22
	R	3.10	0.068	24	-2	-20
HPC	L	3.27	0.053	-32	-14	-14
INS	L	4.88	0.011	-42	-28	14
sgACC	L	3.31	0.015	-2 0	20	-6
	R	3.27	0.037		18	-6
OFC	L	1.94	0.044	-22	38	-12
PLA > 200 mg GW876008						
AMYG	L	2.92	0.049	-22	0	-18
	R	3.26	0.046	24	-2	-22
HPC	L	4.11	0.018	-26	-16	-20
	R	4.49	0.003	28	-16	-22
INS	L	4.79	0.019	-42	-28	14
sgACC	L	3.77	0.010	-4	20	-6
	R	3.65	0.034	0	18	-6
OFC	L	2.56	0.014	-22	40	-12

MNI coordinates (x, y, z), *t* test statistics and p-values for each contrast of interest.

Abbreviations: AMYG—amygdala, HPC—hippocampus, INS—insula (posterior), OFC—orbitomedial prefrontal cortex, sgACC—subgenual anterior cingulate cortex (FDR corrected $p > 0.05$).

Table 3

MNI coordinates for peak voxels showing significant group differences between treatment conditions for each contrast (Threat > Safe)

		IBS > HCs				
		Voxels		MNI coordinates		
		t	*p*	x	y	z
PLA > 20 mg GW876008						
HT	L	4.19	0.002	-4	-4	-6
LCC	L	2.27	0.030	-6	-38	-30
PLA > 200 mg GW876008						
HT	L	3.98	0.002	-6	-4	-4
LCC	L	2.62	0.021	-6	-38	-30

MNI coordinates (x, y, z), *t* test statistics and p-values for each contrast of interest.

Abbreviations: HT—hypothalamus, LCC—locus coeruleus complex (FDR corrected $p > 0.05$).

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Table 4

Path coefficients and chi-square differences for placebo versus GW876008 in IBS patients and healthy controls

			IBS Patients			Healthy Controls		
			Placebo	200 mg GW876008		Placebo	200 mg GW876008	
Paths			**Beta**	**Beta**	**χ^2 Δ**	**Beta**	**Beta**	**χ^2 Δ**
AMYG	→	sgACC	-0.284	0.604**	**9.5**	0.654**	0.255	**4.5**
AMYG	→	LCC	0.118	-0.363**	2.8	-0.003	0.051	0.1
AMYG	→	HPC	0.449**	-0.380	**11.6**	0.086	0.352	2.9
AMYG	→	aINS	1.019**	0.264**	**5.1**	-0.066	-0.426	2.4
LCC	→	AMYG	1.878**	-0.266	**7.5**	-0.867	0.859	**13.9**
HPC	→	AMYG	-2.242*	0.161	**8.0**	0.190	0.254	0.0
aINS	→	AMYG	-0.646**	0.534	**5.6**	-0.456	0.925	**8.6**
aMCC	→	AMYG	3.328**	0.100	**18.5**	-0.089	-0.639	**4.0**
HT	→	AMYG	-4.409**	1.051	**18.6**	0.864	0.751	0.0
OFC	→	AMYG	-0.725**	1.715**	**8.4**	-0.041	-0.118	0.0
sgACC	→	aMCC	0.623**	-0.759**	**18.1**	0.245	-0.288	**5.2**
sgACC	→	aINS	-0.370**	-0.718**	1.0	0.197	0.247	0.0

Critical values for the chi-square difference tests (χ^2 Δ) are 2.71, $p < 0.10$; 3.84, $p < 0.05$; 6.64, $p < .01$ and 10.83, $p < 0.001$.

Bolded values indicate chi-square differences that reached significance.

Significant beta path coefficients are designated by

* $p < 0.05$ and

** $p < 0.01$.

Abbreviations: AMYG—amygdala, HPC—hippocampus, HT—hypothalamus, LCC—locus coeruleus complex, aINS—anterior insula (ventral subregion), aMCC—anterior midcingulate cortex, OFC—orbitomedial prefrontal cortex, sgACC—subgenual anterior cingulate cortex.