Published in final edited form as:
Psychosom Med. 2014 ; 76(6): 404–412. doi:10.1097/PSY.0000000000000089.

Early Adverse Life Events and Resting State Neural Networks in Patients with Chronic Abdominal Pain: Evidence for Sex Differences

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Abstract

Background—Early adverse life (EAL) events and sex have been identified as vulnerability factors for the development of several stress-sensitive disorders, including irritable bowel syndrome (IBS). We aimed to identify disease and sex-based differences in resting state (RS) connectivity associated with EALs in individuals with IBS.

Method—A history of EALs before age 18 was assessed using the early trauma inventory. RS functional magnetic resonance imaging was used to identify patterns of intrinsic brain oscillations in the form of RS networks in 168 people (58 IBS, 28 females; and 110 healthy controls, 72 females). Partial Least Squares, a multivariate analysis technique was used to identify disease and sex differences and possible correlations between EALs and functional connectivity in six identified RS networks.

Results—Associations between EALs and RS networks were observed. While a history of EALs was associated with altered connectivity in the salience/executive control network to a similar extent in male and female IBS patients (Bootstrap ratio [BSR]=3.28-5.61; p=.046), male IBS patients demonstrated additional EAL-related alterations in the cerebellar network (BSR=3.92-6.79; p=.022).

Conclusion—This cross sectional study identified correlations between RS networks and EALs in individuals with IBS. These results suggest that exposure to EALs before age 18 can shape

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Disclosures: No conflicts of interest exist

adult RS in both male and female patients in the salience/executive control network, a brain network that has been implicated in the pathophysiology of central pain amplification.

Keywords

early adverse traumatic life events; irritable bowel syndrome (IBS); resting state networks; salience/executive control network; cerebellar network; sex differences

Introduction

Irritable bowel syndrome (IBS), is the most common functional pain disorder with a prevalence of up to 15% of US adults (1, 2). IBS patients are more likely to report histories of early adverse life events (EALs), such as loss or chronic illness of a parent, discordant relationships between parents or various types of abuse (3-6). A history of EALs is also associated with an increased risk to develop post-infectious IBS (7, 8), psychiatric and somatic co-morbidities (3, 9), and the likelihood of gastrointestinal related complications and outcomes such as lower quality of life, increased visceral pain, and poorer responses to treatment (3, 10). Converging evidence from clinical, epidemiological and neuroimaging studies are consistent with the hypothesis that EALs can modulate brain-gut interactions to increase IBS-related symptoms (11-13).

A large number of functional neuroimaging studies have characterized evoked brain responses during an active task condition in IBS compared to healthy controls (HCs) (14-18). The majority of these studies used controlled rectal distension as the stimulus and showed greater engagement of regions of the salience network (including thalamus [THAL], insula [INS], and anterior cingulate cortex [ACC]) in IBS patients, with some studies showing abnormal responsiveness of brain regions related to emotional arousal (such as amygdala [AMYG], subgenual and pregenual ACC, hippocampus, hypothalamus) and endogenous pain inhibition (such as AMYG, subregions of the ACC, THAL, anterior, middle and posterior INS) (19-22).”

Studies investigating structural (grey and white matter) brain differences between IBS and HCs have demonstrated disease related differences in regions involved in sensory processing, integration, and modulation (THAL, basal ganglia, somatosensory cortex), prefrontal cognitive modulation, and saliency (ACC, INS) (23-26). It remains to be determined if these structural changes reflect the brain's response to chronic nociceptive stimuli from the periphery, or represent preexisting genetic and epigenetic (such as EAL related) brain changes which increase the vulnerability of an individual to develop IBS.

Some evidence suggests that different brain mechanisms may contribute to IBS symptom generation in female and male patients (27, 28). For example, while female patients show evidence for an upregulation of emotional arousal circuits during an aversive rectal stimulus, male patients show greater engagement of prefrontal regions (21, 29). In females, the greater emotional arousal circuit activation was seen primarily during expectation (rather than actual delivery) of the rectal stimulus (2). Recently, marked sex related differences in regional alterations in the power of intrinsic oscillation frequency were reported in IBS, consistent with alterations in the salience network. Similar to the sex differences observed in evoked

brain responses, females showed evidence for higher activity in regions of an emotional arousal circuit (30).

EAL-related alterations in the effective connectivity of the emotional arousal circuitry following visceral (2) and emotional (21) stimuli have been reported in IBS patients (2, 21, 31), suggesting that higher EALs are associated with reduced cortical inhibition of emotional arousal circuits (29). Based on this evidence supporting a role of EALs in IBS etiology, we aimed to test the general hypothesis that a history of EALs is related to brain activity in networks that are involved in determining the salience of somatic, visceral or environmental stimuli in IBS. To test this hypothesis, we used resting state fMRI to determine 1) IBS, and 2) EAL related alterations in the connectivity within six pain, emotion and cognition related RS networks.

Methods

Participants

185 participants were recruited through UCLA Digestive Diseases Clinic and advertisements in the Los Angeles community from April 2009 till March 2012. Advertisements were circulated through online social media websites, local newspapers, university and hospital community listservs and mailing lists, and flyers posted in the greater Los Angeles area and on the UCLA campus. 17 participants were dropped from the analyses, due to either abnormalities in scanning data acquisition or because behavioral data was not available (did not complete the early traumatic events questionnaire). 168 subjects consisting of 58 IBS (28 females) and 110 HC (72 females) were used in the analyses. All participants were right-handed. Participants were screened via medical exam for absence of significant medical conditions other than a diagnosis of IBS. A gastroenterologist or nurse practitioner made a diagnosis of IBS based on the ROME symptom criteria (32). IBS is defined as recurrent abdominal pain or discomfort for at least 3 days/month in the last 3 months and is associated with two or more of the following: 1) Improvement with defecation. 2) Onset associated with a change in frequency of stool. 3) Onset associated with a change in form (appearance) of stool. Exclusion criteria in both groups comprised pregnancy, substance abuse, abdominal surgery, tobacco dependence, excessive physical exercise (more than 8 hours/week of continuous exercise such as marathon runners or triathlon athletes), and ongoing psychiatric illness as determined by the Mini International Neuropsychiatric Interview (MINI) (33). Subjects taking medications that interfere with the central nervous system (full dose antidepressants including SSRI, NSRIs, sedatives or anxiolytics, and opioids) were also excluded. However, as most IBS patients take low dose tricyclic antidepressants, subjects on tricyclic antidepressants were allowed into the study provided that the dose of the drug was between 10-50mg, and that the subject had been on a stable dose for at least 3 months. Since female sex hormones such as estrogen are known to effect brain structure and function, in this study we used women who were premenopausal and who were during the follicular phase of their menstrual cycles. Study protocols were performed after approval by the review committee at UCLA's Office of Protection for Research Subjects, and written informed consent was obtained from all subjects. The demographic characteristics of the subjects are shown in Table 1.

Behavioral Measures

Questionnaires were completed before scanning.

The Early Traumatic Inventory (ETI) (34) was used to access histories of childhood traumatic and adverse life events in four domains: general trauma, physical, emotional, and sexual abuse. General traumatic events comprise a range of stressful and traumatic events that can be mostly secondary to chance events. Physical abuse involves physical contact, constraint, or confinement, with intent to hurt or injure. Emotional abuse is verbal communication with the intention of humiliating or degrading the victim. Sexual abuse is unwanted sexual contact performed solely for the gratification of the perpetrator or for the purposes of dominating or degrading the victim. The ETI has been found to have good internal consistency (Cronbach α=.70) (35).

The Neuroticism subscale on the NEO-Personality Inventory (36) was used to access personality traits associated with neuroticism. Internal reliability measures range between . 75 to .82 (37). Common clinical mental comorbidities associated with IBS such as anxiety and depression were measured using a self-report 14-item instrument (Hospital Anxiety and Depression Inventory; HAD) (38). Internal consistency measures have ranged between .78 and .90 (39).

fMRI Acquisition

Whole brain functional magnetic resonance imaging (fMRI) data was obtained by aggregating data from multiple studies, which has been documented to be a viable form of using data from multiple sources in order to describe comprehensive functional connectome maps in human studies (40). A high resolution structural image was acquired from each subject for registration purposes with a magnetization-prepared rapid gradient-echo (MP-RAGE) sequence, repetition time (TR) = 2200ms, echo time (TE) = 3.26ms, TA = 9m3s, slice thickness = 1 mm, 176 slices, 256 × 256 voxel matrices, and 1^3 mm voxel size. The duration of the resting scan varied from 5 - 10 minutes. Noise reducing headphones were used. Subjects rested with eyes closed while functional blood oxygen-level dependent (BOLD) images were acquired. We pooled resting state fMRI data using different scanning protocols and with different TRs (2000-3000ms) for the purposes of comparing patterns of BOLD oscillation intrinsic connectivity patterns in a large sample size. Scanning protocol details follow:

1. Siemens 3 Tesla Trio using the following parameters: 40-slice whole brain volumes, slice thickness = 4mm, Repetition time (TR) = 2000ms, Echo time (TE) = 28ms, Resting duration (TA) = 10m 6s, flip angle = 77°, FOV = 220. (23 female HCs, 24 male HCs, 20 male IBS subjects, 22 female IBS subjects) (21, 41).
2. Siemens 3 Tesla Trio using the following parameters: 38-slice whole brain volumes, slice thickness 3mm, TR = 2500ms, TE = 26ms, TA = 5m 8s, flip angle = 90°, FOV = 200. (41 female HCs) (42).
3. Siemens 3 Tesla Trio using the following parameters: 40-slice whole brain volumes, slice thickness 4mm, TR = 2000ms, TE = 28ms, TA = 8m 6s, flip angle = 77°, FOV = 220. (8 female HCs, 6 female IBS subjects) (43).

4. Siemens 3 Tesla Allegra using the following parameters: 38-slice whole brain volumes, slice thickness = 3mm, TR = 3000ms, TE = 28ms, TA = 6m 6s, flip angle = 90°, FOV = 200. (14 male HCs, 10 male IBS subjects) (21).

Following the resting scan, some subjects completed additional fMRI scans involving threat and pain related tasks; these data are not included in the current report, and have been reported elsewhere (25, 44).

fMRI Preprocessing

Functional imaging preprocessing and statistical analyses were carried out using SPM8 software (Welcome Department of Cognitive Neurology, London, UK). Images were realigned to the first image and were slice-time and motion corrected. The anatomical image was spatially normalized to the Montreal Neurological Institute (MNI) template, and the resulting parameter file was used to normalize the functional images (voxel size: 2×2×2 mm^3) and was spatially smoothed with a 5 mm^3 Gaussian kernel. The first two volumes were discarded to allow for stabilization of the magnetic field.

Statistical Analyses

Group independent component analysis (gICA) was performed to quantify resting state networks (RSN) in IBS and HCs and was implemented with GIFT 2.0c (http://www.icatb.sourceforge.com). The minimum description length (MDL) was used to objectively identify the number of independent components (IC) to be extracted (45). The RS data was decomposed into 15 IC maps per subject using infomax algorithm and ICASSO (46, 47). Spatial correlation was used to determine which ICs represented the RSNs of interest based on prior neuroimaging studies associated with IBS disease, sex differences or EALs (19, 21, 30). Specifically, we chose the ICs that correlated highest with canonical RSN templates (48), and where the majority of power spectral distribution was in the lower frequencies (< .08 Hz). RSNs of interest included: 1) Salience/Executive Control (referred to throughout the text as salience network) (associated with cognitive control, executive attention, emotion, perception-somesthesis-pain) 2) Left Frontal Parietal, (associated with cognitive modulation, somatosensory perception), 3) Right Frontal Parietal (associated with cognition, language), 4) Cognitive Control, 5) Cerebellar (associated with action-execution, perception-somesthesis-pain), and 6) Default Mode Network (associated with self-referential activity) (48). Individual subject maps for each IC were extracted. These maps contained z-score values that represented the degree of correlation between the voxel signal and the group averaged time-course of the component (i.e. network functional connectivity). Higher z values indicate greater connectivity strength or influence of that voxel on the network (49). As a final noise reduction step, all individual maps were entered into one-sample t-tests in SPM8 and thresholded at $p<0.05$ corrected for family-wise error (FWE).

Thresholded individual subject component maps were entered into a partial least squares (PLS) analysis. PLS (http://www.rotman- baycrest.on.ca) is a multivariate statistical technique similar to principle component analysis (PCA), where solutions are restricted to the part of the covariance structure that is attributable to group difference contrasts (non-rotated task PLS) or covariate measures (behavioral PLS) (50). PLS was our analysis of choice as it allows for inferences about networks (51). PLS essentially extracts common

information from two data sets (e.g., intrinsic network connectivity maps and EAL scores), which generalize to the population. PLS is considered a random effects model as it employs permutation tests that assess generalizability of the pattern and bootstrapping to identify the stable or most reliable elements of the pattern (For more detail on PLS methodology, see Krishnan et al., 2011) (50).

To examine diagnosis differences in network connectivity, a non-rotated task PLS was performed specifying a main effect contrast: IBS vs. HC. This analysis produced patterns or spatial maps representing differences due to IBS diagnosis (52). To examine the relationship between EALs and RSN connectivity, a behavioral PLS was performed. In this analysis, threshholded individual resting state maps and EAL scores were used to compute group correlation maps (IBS females, IBS males, HC males, HC females). The behavioral PLS selects only the most robust patterns in these across-subject correlations maps, negating the need for specifying contrasts of interest (53). Significance of the patterns was assessed by permutation testing with 1000 permutations, and was applied to determine whether spatial maps could be differentiated from noise (52). Each voxel comprising the spatial map has an associated numerical weight called a “salience” and can be positive or negative, indicating the magnitude and direction in which each voxel correlates with the variable being tested (disease contrast, EAL score). The reliability of voxel saliences for non-rotated task and behavioral PLS analyses were assessed by bootstrap estimation (500 samples). Clusters with a peak voxel with a bootstrap ratio (BSR) exceeding ±2.81 ($p<.005$) with an extent of at least 60 voxels were considered reliable and reported.

Behavioral Analyses

Behavioral analyses were performed using Statistical Package for the Social Sciences (SPSS) software (version 19). Group differences in behavioral measure scores were evaluated by applying ANOVA and independent sample t-tests. Post-hoc false-discovery rate (FDR) correction tests (54) were conducted following one-way ANOVAs, whereby each significant main effect was identified by specifying 4 individual contrasts: Male IBS vs. Female IBS, Male IBS vs. Male HC, Female IBS vs. Female HC, and Male HC vs. Female HC. Clinical and behavioral variables tested included early adverse life trauma (ETI), neuroticism (NEO-PI), and anxiety and depression (HAD).

Results

Behavioral and Subject Data

Mean age, and EAL ratings are provided in Table 1. One-way ANOVAs indicated that EAL ($p=.002$), and Neuroticism ($p=.002$) scores were significantly higher for patients compared to HCs (Table 1). Although within normal clinical ranges, patients also had higher anxiety and depression symptom scores on the HAD compared to HCs, with male IBS having significantly higher scores than male HCs on anxiety ($p=.001$), and female IBS having significantly higher anxiety ($p=.003$) and depression ($p=.005$) symptoms than female HCs. A one-way ANOVA factoring sex, indicated that there were no significant differences on neuroticism ($F=.247$, $p=.620$), anxiety ($F=1.633$, $p=.203$), or depression ($F=3.392$, $p=.067$) between males and females.

IBS-related group differences in resting state network connectivity

Differences were found in the intrinsic connectivity of the salience, the left frontal parietal, and the default mode networks in IBS compared to HCs. IBS displayed greater within network connectivity of certain brain regions while no resting state networks displayed greater within network connectivity in HCs compared to IBS patients (Table 2). In the salience network of IBS patients, greater within-network connectivity was observed for the putamen, INS, ACC, mid cingulate cortex [MCC], and supramarginal gyrus (p=.010). In the left frontal parietal network increased connectivity was identified between numerous regions, including putamen, ACC, THAL, and frontal cortex (p=.009). In default mode network of IBS patients, increased connectivity was observed of parietal regions including the precuneus, inferior parietal gyrus and angular gyrus (p=.005).

Early adversity related differences in resting state network connectivity

Two of the identified resting state networks, the salience and cerebellar networks, displayed significant correlations between EAL measures and within-network intrinsic connectivity (Table 3). In the salience network, higher levels of EALs in IBS subjects (both males and females), but not in HCs were associated with increased connectivity with the left putamen, and decreased connectivity with the supplementary motor area (SMA), INS, ACC, parietal and frontal regions (p = .046) (Figure 1). In the cerebellar network, (Table 4) a disease and sex specific relationship between intrinsic connectivity and EAL were identified (p=.022). Higher levels of EALs were associated with increased connectivity of THAL, INS, ACC, pINS, cerebellum and middle temporal gyrus (MTG) with the cerebellar network, and decreased connectivity of right caudate with the cerebellar network in a greater extent in IBS males than in HC male subjects (Figure 2), while no correlations were seen in females. In addition, no significant correlations with anxiety, depression, neuroticism or age were identified for the salience and cerebellar networks (p>.05). None of the other resting state networks showed significant correlations with EAL scores.

Discussion

We examined relationships between a history of EALs and resting state networks of regional intrinsic BOLD fluctuations in IBS subjects and in HCs, with a special emphasis on identifying sex related differences in these networks. We used a data-driven multivariate approach in order to investigate the relationship between EAL scores and resting state brain connectivity within six networks. The main findings of the study were: 1. Disease related differences were present in the salience, left frontal parietal, and default mode networks. 2. Both male and female IBS subjects showed significant EAL related correlations with intrinsic connectivity within the salience network. 3. The intrinsic connectivity within the cerebellar network was related to EALs in a sex specific manner, with male IBS subjects showing EAL-related alterations compared to male HCs. Despite the fact that EALs are common environmental factors associated with IBS and other stress sensitive disorders, to our knowledge, this is the first report that demonstrates sex and disease related differences in resting state network connectivity that are related to a history of EALs in a large patient population.

IBS and EAL related alterations in the salience network

IBS patients (males and females) compared to HCs displayed greater within-network connectivity of the putamen, INS, ACC, MCC, and supramarginal gyrus within the salience network. With increasing levels of EALs, increased connectivity of the left putamen with the salience network was observed in IBS patients, while the connectivity of several other regions of the salience network (SMA, INS, ACC, parietal and frontal regions) was reduced. The salience network is thought to play a key role in selecting from multiple internal (including cognitive, emotional, interoceptive) and extrapersonal (including somatosensory) stimuli those with the greatest personal relevance, in order to optimally guide behavior and adjust autonomic nervous system output (55-57). Patients with chronic pain conditions such as fibromyalgia, chronic back pain, migraines and IBS show abnormal activation in core structures of the salience network in response to stimuli, and such alterations in the engagement of the salience network have been implicated in the pathophysiology of these conditions (58, 59).

The putamen-salience network connectivity is increased in the presence of salient nociceptive stimuli (60). This increased connectivity not only guides motor behavior, and motivation towards salient stimuli, but also is associated with reduced mental processing speed (61). In the current study, the putamen-salience connectivity was increased in IBS compared to HCs, and was further enhanced in the presence of EALs, suggesting that a history of EALs is related to increased impairments in the ability to detect, process and modulate sensory information, and decreased cognitive modulation and flexibility functions (58, 59) beyond that seen in IBS alone.

Two additional regions with increased salience network connectivity in IBS subjects, the INS and ACC, also demonstrated a relationship with EALs in IBS. However, unlike with the putamen, increased EALs were associated with reduced INS and ACC connectivity with the salience network. Thus, IBS with lower EALs appeared to have mainly INS/ACC-salience alterations while IBS with higher EALs appeared to have mainly putamen-salience alterations. The INS is the integral hub of the salience network which interfaces with other regions within the salience network (e.g. ACC, MCC, SMA, prefrontal cortex [PFC]), and with regions within other networks such as the default mode network and cognitive control network (62). As a result, the INS-salience network connectivity plays a role in cognitive, attention, affective and homeostatic processes which link the external stimulus driven environment and the internal milieu of the body. The current study demonstrated similar results to those reported in trauma patients (63), in that decreased connectivity between certain limbic and paralimbic regions within the salience network (anterior INS and subgenual ACC) were observed. These results associated with the salience network in IBS patients suggest that experiences of adversity or trauma early in life are associated with intrinsic network alterations, which may lead to an impaired ability to detect, process and modulate sensory information and to generate appropriate autonomic behavioral responses (56).

IBS, EALs and sex related alterations in the cerebellar network

Although no significant IBS-related differences in the connectivity of the cerebellar network were identified, significant sex and IBS-related differences in the relationship between EALs and cerebellar network connectivity were demonstrated. Higher EAL scores were associated with increased connectivity of the THAL, aINS, pINS, ACC, cerebellum, and MTG with the cerebellar network and decreased connectivity of caudate with the cerebellar network in male IBS but not HC males with no relationship demonstrated in female subjects. The cerebellar network is involved in fear perception, motor function and visual-motor learning (64, 65). More recently the cerebellar network has been identified as being central to both physical pain (66) and psychological pain (67), and abnormalities in cerebellar network connectivity have been reported in various disorders, including substance abuse (68, 69), and major depression (70, 71).

A recent meta-analysis has shown that trauma related alterations in the connectivity between the THAL, cerebellum, INS and cingulate regions within the cerebellar network may play an important role in sensorimotor integration (64, 65, 72). Thus, the observation of greater connectivity within the cerebellar network with increasing EALs in IBS males may suggest a role of EALs in altering sensorimotor integration in IBS males. Since the caudate is known to modulate pain intensity through expectancy and attentional mechanisms, the decreased connectivity between the caudate-cerebellar network with increasing EALs in male patients compared to male HCs is consistent with an impaired pain modulation system which is influenced by the interaction of disease status and level of EALs. The reasons and the functional consequences of the sex specific alterations in cerebellar network alterations remain to be determined.

Study Limitations

Limitations of the study include the fact that data was pooled from multiple studies, possibly contributing heterogeneity to the sample. There were also unbalanced sample sizes for disease groups and for sex, and there were significant differences between groups in age. However, we were still able to detect significant differences in the groups with smaller sample sizes (e.g. IBS). Future studies that would potentially match patients and healthy control subjects on various demographic indices such as age, demographics, socioeconomic status (SES), and body mass index (BMI) would require greater samples, and would be possible through the availability of mechanisms such as large data pain repositories. Even though this study used predominantly premenopausal women during the follicular phase of the menstrual cycle, we did not measure female sex hormones and therefore could not address a possible influence of sex hormones on the current findings. Since a psychiatric diagnosis was an exclusion criterion, patients with severe IBS and comorbid pathological levels of anxiety were excluded from the study sample, resulting in a less severe study population. It is true that some of the measures including the ETI are based on subjective recall, and could therefore be influenced by recall and reporting bias. However, previous studies have demonstrated that the ETI has acceptable psychometric properties and it has been validated against more extensive interview measures of EALs. Lastly, some of the observed differences could be influenced by the intake of centrally acting medications, in particular tricyclic antidepressants, a factor that could not be addressed in the current study.

Conclusions and Clinical Implications

These results are consistent with previous reports that have shown long lasting effects of EALs on brain responses in patients with chronic pain disorders. The results indicate that EALS are correlated with resting state network alterations, which play a role in anxiety and altered sensorimotor integration. The fact that certain resting state network patterns were not seen in HCs with a history of EALs, implies that it is not the experience of EAL per se, but that the interaction of EALs with other factors involved in IBS pathophysiology must be involved. Interestingly, some of the observed changes were only seen in male patients, highlighting the importance in the need to consider sex differences in these interactions. The functional and behavioral consequences this sex difference remain to be determined but the results underscore the importance of assessment of EAL in both male and female patients

Acknowledgments

Acknowledgements/Funding Support: Supported by NIH grants R01 DK048351, P50 DK064539, P30 DK041301, R03 DK084169, K01 DK085133. Pilot scanning provided by UCLA Ahmanson-Lovelace Brain Mapping Center

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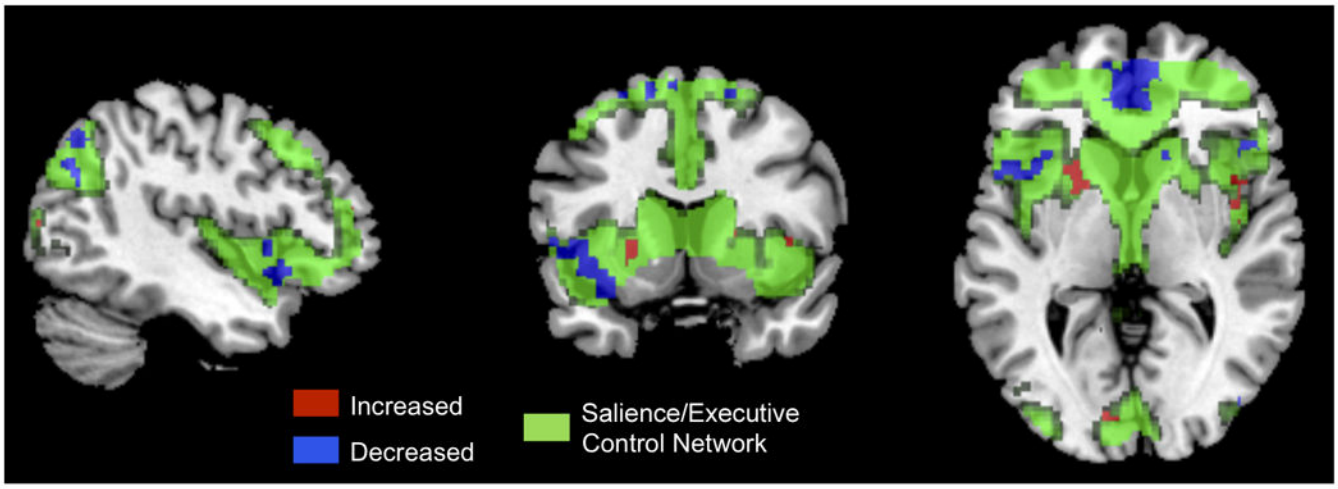

Figure 1. Influence of Early Adverse Life Events in the Salience/Executive Control Network

Green: Salience/Executive Control Network

Red: Positive correlations between brain regions and the salience/executive control network with increasing EALs

Blue: Negative correlations between brain regions and the salience/executive control network with increasing EAL

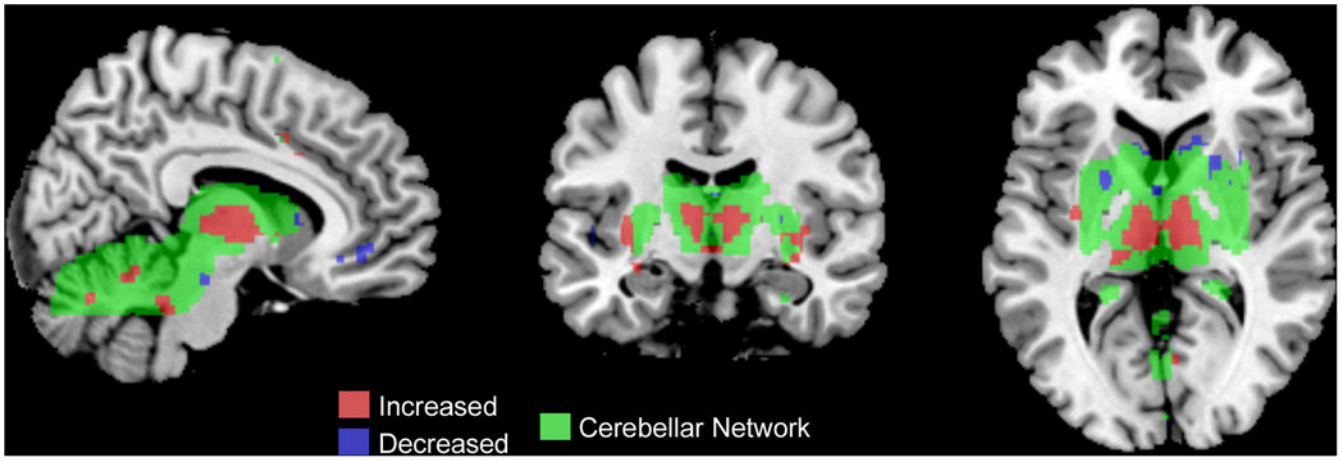

Figure 2. Influence of Early Adverse Life Events in the Cerebellar Network

Green: Cerebellar Network

Red: Positive correlations between brain regions and the cerebellar network with increasing EALs

Blue: Negative correlations between brain regions and the cerebellar network with increasing EALs

Table 1

Study Demographics and Clinical Behavioral Measures

	Male HC		Female HC		Male IBS		Female IBS		F	Sig
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**		
N	38		72		30		28		-	-
Age (yrs)	35.95	12.97	29.39	9.93	37.28	10.75	30.65	10.71	5.53	.001
ETI	4.17	3.97	3.27	3.45	6.48	4.77	5.33	4.97	5.01	.002
NEO_Neuroticism	47.03	10.40	43.60	10.14	52.72	14.78	49.06	9.52	5.26	.002
HAD Anxiety	3.26	2.60	3.04	2.44	5.69	4.45	5.03	3.67	7.12	.000
HAD Depression	1.64	1.56	1.09	1.73	2.79	3.40	2.42	2.50	5.48	.001

Questionnaires: Early Traumatic Inventory (ETI), NEO_Neuroticism; Hospital Anxiety and Depression (Anxiety), Hospital Anxiety and Depression (Depression).

Subject Number (N), Standard Deviation (SD)

Significant = $p<0.05$*, $p<0.005$**

Table 2

Regions within the Intrinsic Connectivity Networks Associated with Disease

COMPONENT: Salience/Executive Control								
Positive correlation in IBS; no correlation in HC								
Region	**BA**	**Hemisphere**	**X**	**Y**	**Z**	**Cluster k**	**Bootstrap Ratio**	**p-value**
Insula		Left	-40	14	-2	86	4.69	<.001
		Left	-32	16	-10	117	3.59	<.001
	13	Right	32	18	-14	238	3.78	<.001
Supramarginal Gyrus		Right	58	-44	34	148	4.06	<.001
MCC		Right	6	24	34	133	3.83	<.001
ACC		Left	-10	42	14	288	3.54	<.001
		Right	4	40	0	323	3.44	<.001
Putamen		Left	-20	10	2	67	3.09	<.001

COMPONENT: Left Frontal Parietal								
Positive correlation in IBS; no correlation in HC								
Region	**BA**	**Hemisphere**	**X**	**Y**	**Z**	**Cluster k**	**Bootstrap Ratio**	**p-value**
Putamen		Left	-16	14	-2	162	4.23	<.001
ACC		Left	-6	28	32	99	3.04	.002
IPG		Left	-24	-66	44	95	4.10	<.001
		Left	-40	-40	42	388	3.75	<.001
SFG	8	Left	-24	4	66	62	3.96	<.001
MFG		Right	30	4	54	63	3.92	<.001
IFG		Left	-46	42	8	238	3.92	<.001
ITG		Left	-46	-64	-6	260	3.70	<.001
MOG		Left	-42	-70	30	189	3.07	<.001

COMPONENT: Default Mode Network								
Positive correlation in IBS; no correlation in HC								
Region	**BA**	**Hemisphere**	**X**	**Y**	**Z**	**Cluster k**	**Bootstrap Ratio**	**p-value**
Precuneus		Right	12	-56	24	929	4.70	<.001
Angular Gyrus		Right	50	-56	28	130	3.88	<.001
IPG		Left	-34	-60	50	98	3.63	<.001
Calcarine		Left	-4	-58	6	100	3.48	<.001

BSR=2.81 ($p<0.005$); (voxel>60)

HC: Healthy Control, IBS: Irritable Bowel Syndrome, BA: Broadmann's Area

All regions represented in Montreal Neurological Institute (MNI) space with X, Y, Z coordinates

Regions: MCC, mid cingulate cortex; ACC, anterior cingulate cortex; IPG, inferior parietal gyrus; SFG, superior frontal gyrus; MFG, middle frontal gyrus; IFG, inferior frontal gyrus; ITG, inferior temporal gyrus; MOG, middle occipital gyrus

Table 3

Regions of the Salience/Executive Control Network Associated with Early Adverse Life Events

Region	BA	Hemisphere	X	Y	Z	Cluster k	Bootstrap Ratio	p-value
Positive correlation in IBS; no correlation in HC								
Lentiform Nucleus/Putamen		Left	-20	8	2	75	3.32	<.001
Negative correlation in IBS; no correlation in HC								
SMA		Left	-4	12	66	72	3.28	.001
ACC	32	Right	2	44	2	515	5.61	<.001
	32	Bilateral	0	36	34	293	3.68	<.001
Cingulate Gyrus	24	Right	4	-10	44	219	4.16	<.001
IFG/Insula		Left	-48	12	0	486	5.17	<.001
	13	Right	28	16	-12	196	4.06	<.001
MFG		Right	30	54	18	171	4.13	<.001
	9	Left	-30	38	40	118	3.94	<.001
SFG	6	Right	12	16	64	68	3.73	<.001
Parietal	39	Left	-46	-68	32	123	3.45	<.001

BSR=2.81 ($p<0.005$); (voxel>60)

HC: Healthy Control, IBS: Irritable Bowel Syndrome, BA: Broadmann's Area

All regions represented in Montreal Neurological Institute (MNI) space with X, Y, Z coordinates

Regions: SMA, supplementary motor area; ACC, anterior cingulate cortex; IFG, inferior frontal gyrus; MFG, middle frontal gyrus; SFG, superior frontal gyrus

Table 4

Regions of the Cerebellar Network Associated With Early Adverse Life Events

Region	BA	Hemisphere	X	Y	Z	Cluster k	Bootstrap Ratio	p-value
More positive correlation in IBS males than in HC males; no correlation in females								
Thalamus		Left	-12	-16	6	667	6.79	<.001
Insula		Left	-34	-10	0	66	5.37	<.001
ACC	32	Bilateral	0	14	38	126	5.55	<.001
Posterior Insula		Right	36	0	-10	103	5.63	<.001
Cerebellum		Right	18	-60	-20	121	3.99	<.001
		Right	2	-58	-20	216	3.92	<.001
MTG	39	Left	-40	-72	26	109	4.19	<.001
More negative correlation in IBS males than in HC males, no correlation in females								
Caudate		Right	12	18	6	74	5.12	<.001

BSR=2.81 ($p<0.005$); (voxel>60)

HC: Healthy Control, IBS: Irritable Bowel Syndrome, BA: Broadmann's Area

All regions represented in Montreal Neurological Institute (MNI) space with X, Y, Z coordinates

Regions: ACC, anterior cingulate cortex; MTG, middle temporal gyrus