Reduced Hippocampal Glutamate–Glutamine Levels in Irritable Bowel Syndrome: Preliminary Findings Using Magnetic Resonance Spectroscopy

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OBJECTIVES: **Enhanced stress responsiveness is an important pathophysiological factor in irritable bowel syndrome (IBS), suggesting the presence of a dysregulated hypothalamic-pituitary-adrenal (HPA) axis. A possible mechanism involves maladaption of the feedback mechanism of the HPA axis. We hypothesized that hippocampus, a key brain region providing inhibitory feedback to the HPA axis, would exhibit reduced excitatory glutamatergic neurotransmission and reduced N-acetyl-aspartate (NAA; a marker of neuronal integrity) levels in IBS patients.**

METHODS: **In this preliminary study, proton magnetic resonance spectroscopy was used to quantify absolute concentrations of metabolites in bilateral hippocampi of 15 IBS patients without significant psychiatric comorbidity and 15 age-matched controls.**

RESULTS: **The main finding was a reduction in hippocampal glutamate–glutamine (Glx) in IBS patients. Furthermore, Glx concentrations were inversely related to emotional stress indicators in patients only. No difference was found between subject groups for other metabolite concentrations, including NAA. However, an elevated myo-inositol (mI)/NAA ratio was found in IBS patients.**

CONCLUSIONS: **Our results provide preliminary evidence for the presence of abnormal hypofunction of hippocampal glutamatergic neurotransmission in IBS patients without psychiatric comorbidity, possibly as a result of the chronic pain. This supports the notion of an imbalance in regulatory brain regions in this subgroup of IBS patients. The inverse relationship between Glx and emotional stress indicators is in agreement with the inhibitory role of hippocampus on the stress system and suggests a sensitization of the mechanism to emotional arousal. The elevated mI/NAA ratio in IBS patients further suggests the presence of hippocampal glial proliferation and remodeling.**

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INTRODUCTION

Irritable bowel syndrome (IBS), a highly prevalent gastrointestinal disorder (1,2), is primarily characterized by continuous or recurrent abdominal cramping pain, bloating, and discomfort in absence of structural or tissue abnormality. The "brain-gut" axis is believed to have a crucial role in the pathogenesis of IBS (3), Hypersensitivity to visceral stimuli has been demonstrated both psychophysically, indicating the presence of hyperalgesia, and physiologically at peripheral (4,5), spinal (6), and supraspinal levels (7–10). In conjunction with visceral hypersensitivity, enhanced stress responsiveness is thought to be an important pathophysiological factor. IBS symptoms can be triggered or exacerbated by stress (11,12) and pain coping strategies such as catastrophizing have an important mediating role (13). Evidence suggests a dysregulated hypothalamic-pituitary-adrenal (HPA) axis in IBS patients, even in absence of psychiatric comorbidity, indicating altered central stress control mechanisms (14–16). However, a simple relationship between stress-related neuroendocrine function and IBS symptoms has not been found possibly reflecting the heterogeneous etiology of the syndrome, variations in psychiatric comorbidity and the effect of different phases of the chronic stress response.

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The hippocampus is a brain region not only involved in cognition but also in several aspects relevant to the IBS symptomatology, e.g., pain, anxiety, and stress (17–22). Evidence point to a functional dissociation along the septotemporal axis of the hippocampus with spatial learning and memory mainly localized to the posterior/dorsal portion and regulation of anxiety and stress responses localized to the anterior/ventral portion (17). The hippocampus, containing adrenal steroid receptors, is targeted directly by stress mediators and is among the most sensitive brain regions (20). Under prolonged stress, these mediators result in structural (loss of synaptic connectivity, replacement of neurons, and remodeling of dendrites) and functional (reduced synaptic functionality) changes (20). Such changes may initially be adaptive, e.g., to maintain homeostasis and enable coping with novel situations, but prolonged exposure may cause damage (e.g., glutamatergic excitotoxicity). Under normal acute stress, hippocampus exerts negative feedback upon the HPA axis to terminate the stress response. However, ongoing stress may impair the feedback mechanisms and result in prolonged or blunted HPA responses (20). It is at present unknown whether hippocampal mechanisms are implicated in the pathology of IBS. Possible functional hippocampal changes could be expressed as altered metabolism and/or glutamatergic neurotransmission.

Magnetic resonance spectroscopy (MRS) is a magnetic resonance imaging-based technique that allows to non-invasively identify, discriminate, and quantify several different neurochemical metabolites at the same time in a given brain region. MRS provides unique information that cannot be acquired by any other imaging modality. Proton MRS (^{1}H-MRS) can be used to detect a wide range of metabolites including choline (Cho) containing compounds, creatin (Cr), glutamate (Glu), myo-inositol (mI), and N-acetyl-aspartate (NAA). As Glu can be difficult to distinguish from glutamine (Gln), due to overlapping spectra, the combined (Glu–Gln:Glx) concentrations are often quantified. Metabolites in the ^{1}H-MRS spectrum subserve a diverse range of brain functions: Cho is involved in membrane metabolism and is a precursor of the neurotransmitter acetylcholine; Cre has an important role in cell energy metabolism; Glu is the most abundant excitatory neurotransmitter in the brain and Gln is a precursor of Glu; mI participates in phosphatidylcholine-based second messenger signaling and is predominantly located in glial cells and thus an index of glial cell integrity; NAA is predominantly localized to neurons and is considered a marker of neuronal density and integrity (23). In addition, the mI/NAA ratio has been suggested to be a marker of glial proliferation (24). MRS has previously been applied in patients with various types of pain including fibromyalgia (25–27), chronic low back pain (28,29), neuropathic pain (30–32), trigeminal neuralgia (33), headache (34,35), and migraine (36–38). Only two studies on fibromyalgia investigated metabolites in the hippocampus and both found decreases in NAA/Cre ratios indicating compromised neuronal integrity (25,27).

In the present study, we hypothesized that IBS is associated with abnormal hippocampal function. More specific, we hypothesized that reduced hippocampal feedback to the HPA axis would be expressed as lower Glu and Gln levels in IBS patients possibly concomitant with reduced NAA concentrations. Because of the inhibitory role of hippocampus on the HPA axis, we further hypothesized an inverse relationship between these functional markers and clinical symptoms, e.g., pain and emotional stress indicators. To test this hypothesis, absolute metabolite concentrations were determined by means of single-voxel quantitative ^{1}H-MRS within bilateral hippocampi and correlated with clinical parameters. To our knowledge, this is the first study employing MRS to IBS patients.

Table 1. Clinical and demographic information for IBS patients and healthy controls

	IBS	Controls	*P* value
Gender (F/M)	8/7	10/5	0.71
Age (years)	36.6±11.6	33.0±9.0	0.35
Pain duration (years)	7.2±6.8	—	—
MPQ total (0–78)	38.9±6.07	—	—
PCS (0–52)	19.0±12.5	16.1±6.2	0.44
Depression (0–63)	7.8±6.3	6.8±6.1	0.66
State anxiety (20–80)	40.7±11.3	41.3±8.3	0.88
Trait anxiety (20–80)	47.6±11.2	44.9±7.92	0.45

F, females; IBS, irritable bowel syndrome; M, males; MPQ, McGill pain questionnaire; PCS, pain catastrophizing scale.
Note: score ranges are given in parentheses. Values are given as mean±s.d. See text for further details.

METHODS

Participants

Sixteen IBS patients and seventeen healthy control subjects were recruited in the study. One patient and two controls were excluded from the study due to poor MRS signal leaving 15 participants in each group (see **Table 1** for details). Selection of IBS patients were based on a positive diagnosis according to the ROME III criteria (39). The diagnosis was made by an experienced gastroenterologist (CLL) and excluded organic disease and other chronic pain conditions. Before inclusion in the study, all participants were interviewed about their medical history including psychiatric conditions, which had required hospitalization and the use of drugs. In addition, anxiety and depression inventories were applied (see below). Exclusion criteria for all participants encompassed major surgery, metal implants incompatible with magnetic resonance imaging scanning, and major psychiatric disorders, including clinical anxiety or depression and previous use of anxiolytics and antidepressants. In addition, controls were free of acute or chronic somatic and abdominal pain at the scan time. Patients received treatment with fiber supplements and antispasmodic medication according to their symptoms. Before the study, participants gave their informed consent to the protocol, which had been approved by the Institutional Review Board of Taipei Veterans General Hospital. The study was conducted in accordance with the Declaration of Helsinki.

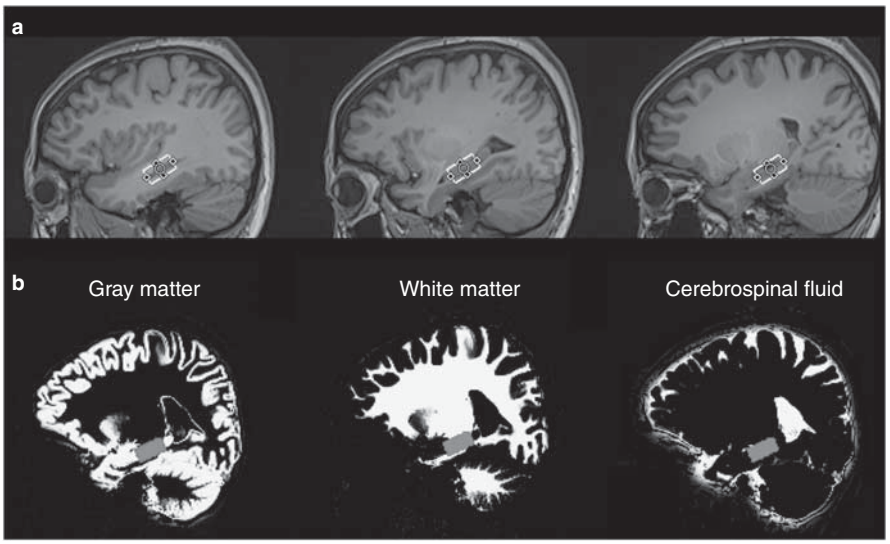

Figure 1. Voxel location and tissue composition. (**a**) An example of voxel location in a single subject. Lateral (left), middle (mid), and medial (right) sagittal voxel borders are shown. (**b**) Segmentation and extraction of mean densities of gray matter, white matter, and cerebrospinal fluid within the hippocampal voxel.

Assessment of clinical outcomes

Psychological assessment was performed immediately before magnetic resonance imaging scanning. All participants completed the Spielberger state-trait anxiety inventory and the Beck's depression inventory (40). To further evaluate emotional coping strategies, the pain catastrophizing scale was administered (41). The pain experience of each patient was assessed by the McGill pain questionnaire.

Image acquisition

Data were acquired on a 3-T Magnetom Trio MR scanner (Siemens AG, Erlangen, Germany) with a 32 channel phased array coil. For each participant, single-voxel ^{1}H-MRS was obtained from left (L) and right (R) hippocampi in addition to a three-dimensional high-resolution MPRAGE (Magnetization-Prepared Rapid Acquisition with Gradient Echo) anatomical scan (repetition time/echo time/flip angle: 2,530 ms/3.03 ms/7°; field of view: 224×256×192; voxel size: 1×1×1 mm^3) used for localization and offline partial volume correction. The hippocampal voxels (size: 15×20×10 mm^3) were placed on sagittal anatomical images and adjusted based on identifiable anatomical landmarks, e.g., one side of the voxel extending to the lateral border of the hippocampal formation and the long axis of the voxel tilted at an angle to match the septotemporal axis of the individual hippocampi (**Figure 1a**). The voxel included most of the ventral portion and excluded the most dorsal portion. Single-voxel ^{1}H-MRS was conducted using a PRESS (Point RESolved Spectroscopy) sequence (echo time: 30 ms; TR: 2,000 ms; bandwidth: 2 kHz; water suppressed spectra: 128 acquisitions; non-water suppressed spectra: four acquisitions) with short echo time for enhancement of the glutamate peak. Acquisitions from left and right hippocampi were counter balanced across subjects. Scanning was performed in the morning at the same time interval (9–11 AM) for all participants.

Data processing

Spectroscopic data were analyzed using the fully automatic LCModel (42) in the range 4.0–0.2 p.p.m. The water scaling method was applied for estimation of absolute metabolite concentrations (43,44). With this method the spectrum from the unsuppressed water signal is used as a concentration reference. However, water scaling is based on the assumption that the water concentration in the voxel is accurately measured. As the water signal arises from gray matter (GM), WM (white matter), and CSF (cerebrospinal fluid), this method is sensitive to partial volume effects. To correct for this, high-resolution anatomical images were segmented into GM, WM, CSF (**Figure 1b**) using statistical parametric mapping (SPM8 software; Wellcome Trust Centre for Neuroimaging, University College London) and mean probability values were extracted from each hippocampal region using a mask generated in MRIcro (http://cnl.web.arizona.edu/mricro.htm). The water concentration in the hippocampal voxels was then calculated according to the formula: [water] = (GM%)×43.30 + (WM%)×35.88 + (CSF%) ×55.55. Absolute concentrations of the following metabolites were obtained: Cho (glycerophosphocholine + phosphocholine), Cr, Glu, Glx, mI, and NAA. Only individual metabolites with a standard deviation of the fitting error (% s.d.) < 20% were included in further analysis. Evaluation of spectral quality was based on the full-width at half maximum (in parts per million) and signal-to-noise ratio of the NAA peak as provided by LCModel. To estimate the quantification precision (45) of Glx, the mean standard deviation of the fitting error was calculated across subjects as an estimate of the total relative error of [Glx]. In addition, the total absolute error estimate of [Glx] was calculated as %s.d.×[Glx].

Validation of absolute concentrations

To validate the absolute concentrations found in IBS patients and healthy controls using water scaling and phased array data

acquisition, a 50-mM NAA phantom underwent eight separate scans (parameters as above) with new shimming for each scan to simulate the same conditions the participants underwent. NAA concentrations were calculated as above but with [water] = 55.55 M.

Statistics

Student's two-sample *t*-test (two-tailed) was used to test for significant differences between patients and controls for age and clinical outcomes (pain catastrophizing, depression rating, and state and trait anxiety scores). To examine differences in gender distribution between the two subject groups a χ^2 test incorporating Yate's correction for continuity was used. Differences in spectral quality parameters, absolute concentrations of metabolites, and tissue densities within the hippocampal volumes were tested by two-way analysis of variance with the factors hemisphere (left and right) and subject groups (IBS patients and controls). In addition, for metabolite concentrations exhibiting significant differences between subject groups a correlation analysis was performed in the patient group with pain indices (duration and total McGill pain questionnaire scores) and in both subject groups with indices of emotional stress (state and trait anxiety, catastrophizing). For metabolites of interest (Glu, Glx, and NAA) with *a priori* hypothesis about the correlational relationship one-tailed tests were used if the analysis of variance yielded significant differences between the subject groups. In all cases, a threshold value of $P < 0.05$ was considered significant.

RESULTS

Demographic and clinical information

Of the 15 IBS patients, 7 patients were diagnosed with diarrhea predominant IBS, 6 with constipation predominant IBS, and 2 patients with mixed-type IBS. Additional demographic and clinical information for IBS patients and healthy control subjects is provided in **Table 1**. The two groups were matched in age and no difference in gender distribution was found between IBS patients and controls. Although mean "trait" anxiety and pain catastrophizing scores were slightly elevated in IBS patients, none of the clinical parameters differed significantly between the two subject groups. In accord with our exclusion criteria, anxiety and depression scores were within the normal range for both groups showing that all subjects were without significant mood disturbance or psychiatric comorbidity at the scan time.

Hippocampal volume and metabolites

No differences were found between IBS patients and healthy controls or between hemispheres when comparing hippocampal densities of GM (IBS-R (mean±s.d.), 0.594±0.054; IBS-L, 0.585±0.086; Controls-R, 0.597±0.050; Controls-L, 0.599±0.054), WM (IBS-R, 0.359±0.063; IBS-L, 0.359±0.096; Controls-R, 0.360±0.056; Controls-L, 0.350±0.062) and CSF (IBS-R, 0.046±0.026; IBS-L, 0.053±0.022; Controls-R, 0.052±0.043; Controls-L, 0.049±0.017).

An example of measured and fitted spectra from bilateral hippocampi in IBS patients and healthy controls is shown in **Figure 2a** and **b**. Hippocampal metabolite concentrations of Cho, Cr, Glu, Glx, mI, and NAA for left and right hemispheres in the two subject groups are listed in **Table 2**. A satisfactory fit for Glu concentrations was only obtained for the left hemisphere that showed a subsignificant decrease in concentrations for IBS patients (one-tailed two-sample *t*-test, $P = 0.058$). The two-way analysis of variance revealed significant differences in Glx concentrations for factors subject group ($N = 13$, $F = 4.27$, $P = 0.044$) and hemisphere ($F = 6.55$, $P = 0.014$). For Cr concentrations, significance was found for the factor hemisphere ($N = 15$, $F = 4.12$, $P = 0.047$). None of the other metabolites showed any significant differences. However, a subsignificant decrease in NAA concentrations ($N = 14$, $F = 2.32$, $P = 0.134$) combined with a subsignificant increase in mI concentrations ($N = 15$, $F = 1.95$, $P = 0.168$) resulted in a significant change of the mI/NAA ratio for subject group ($N = 14$, $F = 6.30$, $P = 0.015$). The spectral quality in IBS patients was comparable to that in healthy controls. Spectral quality also did not differ between hemispheres (**Table 2**). The relative error estimate of Glx (% s.d. = 12.86±0.04%) was well within the 20% standard threshold and the absolute error estimate of Glx was 0.99±0.36 mM.

To further examine the abnormal change in Glx concentrations in IBS patients a correlation analysis was performed between both bilateral Glx and left Glu concentrations and the subjective pain scores and pain duration. Left Glu concentrations showed a negative correlation with pain duration that did not reach significance ($r = -0.395$, $P = 0.081$, one-tailed). None of the other tests had a significant outcome. Finally, to examine the influence of emotional stress on bilateral Glx and left Glu levels, correlations were performed with catastrophizing and "state" and "trait" anxiety in both IBS patients and healthy controls. In IBS patients but not in healthy controls both "state" anxiety ($N = 15$, $r = -0.751$, $P = 0.0006$, one-tailed) and catastrophizing ($N = 15$, $r = -0.633$, $P = 0.006$, one-tailed) exhibited a highly significant negative correlation with left Glx concentrations (**Figure 2c** and **d**).

Validation of the water scaling method

A phantom containing a solution of 50 mM NAA was used for validation of the water scaling method using phased array MRS data acquisition for quantification of absolute concentrations. Results yielded a concentration of 52.86±1.17 mM corresponding to a mean deviation of 5.4% assuming the phantom concentration to be exact. The variability in NAA concentration across phantom measurements corresponded to a coefficient of variation of 2.2%. Thus, the absolute concentrations obtained in the present study using water scaling and LCModel can be considered reasonable estimates with sufficient sensitivity.

DISCUSSION

In the present study, ^{1}H-MRS was used to investigate changes in the absolute concentrations of hippocampal neurochemical metabolites in IBS patients relative to healthy age-matched

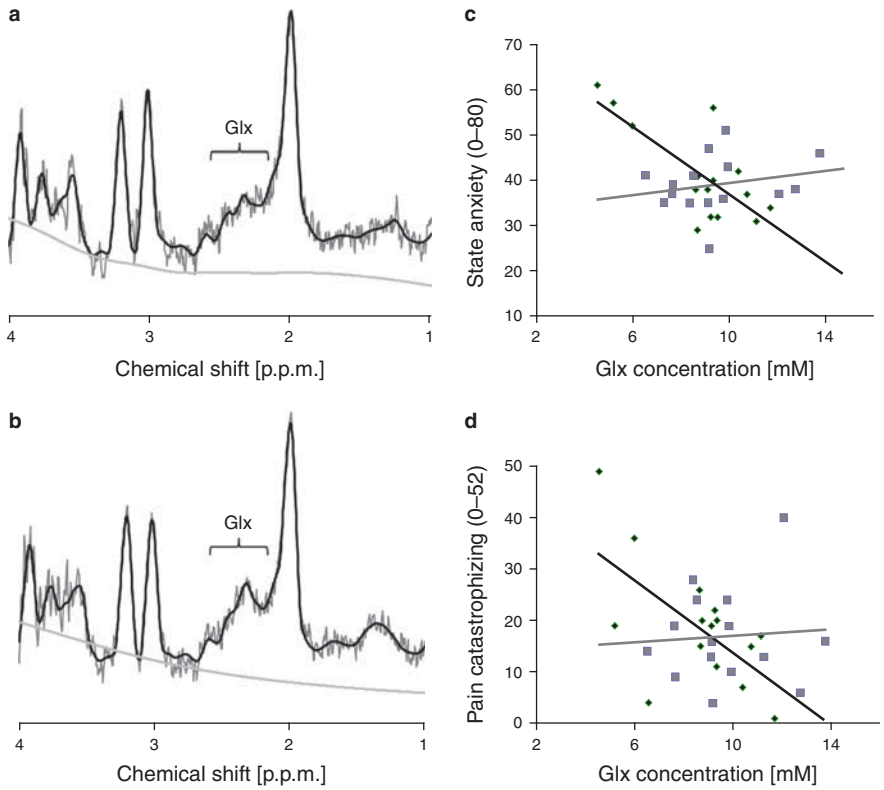

Figure 2. Representative hippocampal spectra (baseline subtracted) from (**a**) an irritable bowel syndrome (IBS) patient and (**b**) a control subject including the averaged data (dark gray), the fitted spectra from LCModel (black) and the estimated baseline (light gray). The location of the main glutamate–glutamine (Glx) signal is marked in each spectrum. Association between left hippocampal Glx concentrations and (**c**) state anxiety and (**d**) pain catastrophizing for IBS patients (black) and control subjects (gray). Both scatterplots with regression lines demonstrate a significant negative correlation for IBS patients only.

Table 2. Absolute metabolite concentrations (mM) and spectral quality parameters for IBS patients and healthy control subjects

Metabolite	*N*	IBS (R)	IBS (L)	Controls (R)	Controls (L)
Cho	15	1.84±0.33	2.03±0.44	1.89±0.37	1.87±0.45
Cre	15	4.96±1.04	6.07±1.36	5.51±1.15	5.61±1.04
Glu	14	—	5.65±1.10	—	6.35±1.17
Glx	13	7.24±1.69	8.50±2.22	8.24±1.50	9.72±2.20
mI	15	4.26±1.90	5.02±1.30	3.93±1.03	4.35±1.15
NAA	14	4.86±1.39	5.60±1.20	5.63±1.13	5.84±1.20
mI/NAA	14	0.94±0.37	0.92±0.27	0.72±0.24	0.77±0.16
FWHM	15	0.103±0.038	0.090±0.027	0.093±0.029	0.079±0.029
SNR	15	5.86±1.51	6.47±1.60	6.40±1.50	6.93±1.71

Cho, choline; Cre, creatin; FWHM, full-width at half maximum; Glu, glutamate; Glx, glutamate–glutamine; IBS, irritable bowel syndrome; L, left hemisphere; mI, myo-inositol; NAA, N-acetyl-aspartate, SNR, signal-to-noise ratio; R, right hemisphere.
Note: values are given as mean±s.d. See text for further details.

controls. According to the inception interview and assessment with inventories, the patient group was without psychiatric comorbidity. Psychiatric factors are potentially confounding since they may affect regulation of the HPA axis. No differences in tissue composition of the sampled volumes were observed between subject groups and hemispheres, suggesting comparable hippocampal GM densities in the two groups. The main finding of this study was the presence of abnormal hippocampal glutamatergic

neurotransmission in IBS patients as shown by the reduction in Glx. Furthermore, in patients Glx concentrations were inversely related to emotional stress indicators. This coupling was not observed in healthy individuals.

Hippocampal excitability is affected by a variety of neuromodulators. Perhaps, the most well-known example is the stress-dependent secretion of cortisol from the adrenal cortex into circulation via a cascade response initiated in the hypothalamus. Cortisol stimulates excitatory Glu release from hippocampal neurons (20,46). Once released, Glu is removed from the synaptic cleft and recycled via glial cells by converting it into Gln, which is transported back into the neurons and then converted back into Glu (47). Hence, the Glu–Gln cycle between glial cells and neurons is considered a marker for regulation of synaptic activity. The Gln and Glu signal measured by ^{1}H-MRS arises from neuronal and non-neuronal cells and from intracellular and extracellular spaces. A decrease in Glx (Gln + Glu) as observed in this study may therefore not only represent reduced synaptic activity/hippocampal excitation but also a general perturbation/downregulation of the Glu–Gln cycle between neurons and glial cells.

Our finding of reduced Glx in IBS patients on the one hand and comparable levels in the two subject groups of anxiety, depression and pain catastrophizing on the other hand suggest that this reduction could be a result of the chronic pain. This is further supported by the indicated inverse relationship between Glu and pain duration. Two related mechanisms may account for reduced glutamatergic neurotransmission (1): hippocampal remodeling and (2) altered modulatory feedback to hippocampus. Prolonged exposure to stress may result in remodeling of hippocampal structure and function (20,46). Excitatory amino acids such as glutamate are known to have an important role in this remodeling (46). It is conceivable that the reduced hippocampal Glx observed in this study is a response to excessive excitatory input and serves to avoid potential excitotoxicity in order to maintain homeostasis. There are indications in animal models that repeated stress increases the efficiency of glutamate release. To avoid excitotoxic damage dendritic atrophy occurs as a compensatory response resulting in the loss of glutamatergic synapses (46,48). Such a loss may be accompanied by downregulation in the Glu–Gln cycle. Glial proliferation, as suggested by the elevated mI/NAA ratio in IBS patients, further indicate abnormal hippocampal remodeling/damage. Interestingly, Glx was inversely related to emotional stress indicators in patients, only. Together with the comparable levels of anxiety, depression and pain catastrophizing in the two subject groups, this suggests that hippocampal glutamatergic neurotransmission in our patient group is influenced by emotional stress rather than causing altered mood. Such a coupling may provide the substrate for enhanced sensitivity to stress or emotional arousal as observed in IBS patients. As the emotional stress indices used in this study are related to the concurrent state, the influence of early-life stressors and chronic stress remains to be elucidated.

A causal relationship between hippocampal glutamatergic neurotransmission and modulatory feedback to the hippocampus cannot be established in the present study due to the absence of neuroendocrine measures. Interpretation of existing reports is made difficult by conflicting results possibly due to methodological differences and mixed symptomatology. However, even in IBS patients without psychiatric comorbidity results remain inconclusive (15,16,49,50). It, therefore, remains possible that the hippocampal glutamatergic hypofunction observed in this study could result from a generally impaired HPA axis tone or it could represent compensatory mechanisms of adaption to enhanced glucocorticoid feedback. In addition, reduced hippocampal glucocorticoid sensitivity may be a contributing mechanism.

Alterations in hippocampus and HPA axis dysfunctions are not unique to IBS. Early-life trauma and post-traumatic stress disorder are associated with elevated central levels of corticotrophin-releasing factor, reduced basal cortisol levels, and elevated cortisol levels in response to the dexamethasone suppression test (51–53). These findings are indicative of elevated hypothalamic corticotrophin-releasing factor levels possibly as a result of reduced negative cortisol feedback to the hippocampus. The dexamethasone suppression test further suggests enhanced responsiveness to negative cortisol feedback at the pituitary level (54). Also, reduced hippocampal glucocorticoid receptor expression was found in victims of childhood abuse which further supports impaired negative feedback to the HPA axis (55). Other congruent findings include reduced hippocampal volume (56–58) and reduced levels of hippocampal NAA (59). Although early-life trauma is highly prevalent in IBS (60), findings in IBS patients without psychiatric comorbidity do not match those described above possibly due to the absence of early-life stressors. For example, plasma cortisol was found to be normal in response to the dexamethasone suppression test in IBS patients with (14) and without (49) psychiatric comorbidity. Moreover, we did not find any difference in NAA concentrations and a change in hippocampal GM densities is neither supported by a recent voxel-based morphometry study (61) nor our results. This is congruent with early-life trauma resulting in a distinct HPA profile that separates it from major depression without early-life trauma and other chronic stress types (51–53).

Our data may not only be interpreted in the context of general stress mechanisms. Hippocampal glutamatergic neurotransmission also contributes to cognition, anxiety, and pain (17,21,62). However, a reduction in glutamate, as observed in this study, would likely be associated with an analgesic (21,22) or anxiolytic (62) effect in conjunction with a positive relationship with pain duration and subjective pain and anxiety scores. This is incongruent with our findings and the presence of substantial abdominal pain in the patient group. Although it is not possible to exclude subtle cognitive impairment from the interpretation, as chronic stress and blocking of glutamate receptors have a negative impact (20,62), evidence does not support this in the majority of IBS patients (63). Also metabolites were obtained from voxels excluding the upper most dorsal portion of the hippocampus, which has a preferential role in cognition (17).

Several issues pertaining to the reproducibility and limitations of our findings need to be discussed. Since the actual *in vivo* concentrations are unknown, phantom experiments can be used

to assess the measurement accuracy albeit without accounting for complex *in vivo* factors. According to our phantom measurements, the methodology employed had sufficient sensitivity. Also, the hippocampal metabolite concentrations measured in this study are comparable to those found in healthy individuals of other studies despite the use of different parameters (45,64,65). Reproducibility also depends on the quantification precision and the intra-subject variability of repeated measurements. These factors are influenced by the spectral quality, the quantification method, voxel placement, physiological variation, and instrumental stability (45). Several of these factors were optimized in this study including using a user independent fitting routine and partial volume correction for quantification. The latter not only eliminates intra- and inter-subject variations due to differences in tissue composition of the MRS voxel, but it has also been shown that absolute concentrations are more reliable than concentration ratios (66). Also, voxel placement along the septotemporal axis of the hippocampus is facilitated by its clear boundaries. The hippocampal spectral quality is in general poorer than that from cortex resulting in lower quantification precision and larger intra-subject variability (45). This may be attributed to the lower signal-to-noise ratio due to differences in cytoarchitecture and susceptibility effects, among others. In addition, it has been shown that the reproducibility of overlapping multiplet resonances such as Glu and Gln is lower than for singlet resonances such as NAA due to larger error estimates (45). However, the signal-to-noise ratio and spectral resolution can be improved by using higher field-strength scanners and short echo times (67) as in this study. Regarding the quantification precision of the present study, the total relative error estimate for Glx is well within the accepted criteria of 20%. Although the total absolute error estimate for Glx was sufficiently small to detect changes between subject groups it was in the lower range relative to the difference between subject groups. Our findings must therefore be interpreted with caution.

Our preliminary results indicate the presence of abnormal hypofunction of hippocampal glutamatergic neurotransmission and an elevated mI/NAA ratio in IBS patients without psychiatric comorbidity. These changes suggest perturbed hippocampal excitability in conjunction with structural and functional remodeling possibly as result of the chronic pain. The inverse relationship between Glx and emotional stress indicators further suggests a sensitization of the mechanism to emotional arousal. Overall, our findings support the notion of an imbalance in stress regulatory brain regions in IBS patients. Further research is needed to establish whether our finding is an adaptive epiphenomenon, as suggested by absence of a relationship between Glx, Glu and pain scores, or predisposes the patients to enhanced pain perception, e.g., through glucocorticoid-related increase of proinflammatory cytokines or functional changes in cortico-hippocampal projections. It is noteworthy to mention that neurochemical pathology in the hippocampus most likely is not specific to IBS. Also, our findings may not be extrapolated to IBS patients with psychiatric comorbidity or early-life trauma. Further studies of larger patient populations separating the different subtypes of IBS patients are warranted.

CONFLICT OF INTEREST

Guarantor of the article: David M. Niddam, PhD.
Specific author contributions: Planning, conducting the study, collecting and interpreting the data, and drafting the manuscript: David M. Niddam; planning, conducting the study, and collecting data: Shang-Yueh Tsai; planning, patient recruitment and selection: Ching-Liang Lu; planning and interpreting the data: Cheng-Wen Ko; planning and interpreting the data: Jen-Chuen Hsieh; each of the authors have approved the final draft submitted.
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Potential competing interests: None.

Study Highlights

WHAT IS CURRENT KNOWLEDGE

- ✓ Hypersensitivity to visceral stimuli and enhanced stress responsiveness are important pathophysiological factors in irritable bowel syndrome (IBS).
- ✓ The hippocampus is a key brain region in the control of responsiveness to pain, anxiety, and stress.
- ✓ The hippocampus exerts negative feedback upon the hypothalamic-pituitary-adrenal axis.
- ✓ The hippocampus is functionally and structurally among the most sensitive brain regions affected by prolonged stress.

WHAT IS NEW HERE

- ✓ Irritable bowel syndrome (IBS) patients had abnormal hypofunction of excitatory hippocampal glutamatergic neurotransmission.
- ✓ IBS patients had an elevated hippocampal myo-inositol/N-acetyl-aspartate ratio, indicating glial cell proliferation and hippocampal damage.
- ✓ The inverse relationship between [Glx] and emotional stress indicators in IBS patients is in agreement with the inhibitory role of hippocampus on the stress system and suggests a sensitization of the mechanism to emotional arousal.
- ✓ Evidence is provided for perturbed hippocampal excitability and structural and functional remodeling.
- ✓ Findings support an imbalance in stress regulatory brain regions in IBS patients.

REFERENCES

1. Grundmann O, Yoon SL. Irritable bowel syndrome: epidemiology, diagnosis and treatment: an update for health-care practitioners. J Gastroenterol Hepatol 2010;25:691–9.
2. Saito YA, Schoenfeld P, Locke GR 3rd. The epidemiology of irritable bowel syndrome in North America: a systematic review. Am J Gastroenterol 2002;97:1910–5.
3. Mayer EA, Naliboff BD, Chang L. Evolving pathophysiological model of functional gastrointestinal disorders: implications for treatment. Eur J Surg Suppl 2002;587:3–9.

4. Lembo T, Munakata J, Mertz H *et al.* Evidence for the hypersensitivity of lumbar splanchnic afferents in irritable bowel syndrome. Gastroenterology 1994;107:1686–96.
5. Price DD, Zhou Q, Moshiree B *et al.* Peripheral and central contributions to hyperalgesia in irritable bowel syndrome. J Pain 2006;7:529–35.
6. Coffin B, Bouhassira D, Sabate JM *et al.* Alteration of the spinal modulation of nociceptive processing in patients with irritable bowel syndrome. Gut 2004;53:1465–70.
7. Hall GB, Kamath MV, Collins S *et al.* Heightened central affective response to visceral sensations of pain and discomfort in IBS. Neurogastroenterol Motil 2009;22:11.
8. Mertz H, Morgan V, Tanner G *et al.* Regional cerebral activation in irritable bowel syndrome and control subjects with painful and nonpainful rectal distention. Gastroenterology 2000;118:842–8.
9. Naliboff BD, Derbyshire SW, Munakata J *et al.* Cerebral activation in patients with irritable bowel syndrome and control subjects during recto-sigmoid stimulation. Psychosom Med 2001;63:365–75.
10. Verne GN, Himes NC, Robinson ME *et al.* Central representation of visceral and cutaneous hypersensitivity in the irritable bowel syndrome. Pain 2003;103:99–110.
11. Blanchard EB, Lackner JM, Jaccard J *et al.* The role of stress in symptom exacerbation among IBS patients. J Psychosom Res 2008;64:119–28.
12. Whitehead WE, Crowell MD, Robinson JC *et al.* Effects of stressful life events on bowel symptoms: subjects with irritable bowel syndrome compared with subjects without bowel dysfunction. Gut 1992;33: 825–30.
13. Lackner JM, Quigley BM, Blanchard EB. Depression and abdominal pain in IBS patients: the mediating role of catastrophizing. Psychosom Med 2004;66:435–41.
14. Bohmelt AH, Nater UM, Franke S *et al.* Basal and stimulated hypothalamic-pituitary-adrenal axis activity in patients with functional gastrointestinal disorders and healthy controls. Psychosom Med 2005;67:288–94.
15. Chang L, Sundaresh S, Elliott J *et al.* Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis in irritable bowel syndrome. Neurogastroenterol Motil 2009;21:149–59.
16. FitzGerald LZ, Kehoe P, Sinha K. Hypothalamic-pituitary-adrenal axis dysregulation in women with irritable bowel syndrome in response to acute physical stress. West J Nurs Res 2009;31:818–36.
17. Bannerman DM, Rawlins JN, McHugh SB *et al.* Regional dissociations within the hippocampus—memory and anxiety. Neurosci Biobehav Rev 2004;28:273–83.
18. Gol A, Faibisch GM. Hippocampectomy for relief of intractable pain. Tex Med 1966;62:76–9.
19. Halgren E, Walter RD, Cherlow DG *et al.* Mental phenomena evoked by electrical stimulation of the human hippocampal formation and amygdala. Brain 1978;101:83–117.
20. McEwen BS. Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol Rev 2007;87:873–904.
21. McKenna JE, Melzack R. Blocking NMDA receptors in the hippocampal dentate gyrus with AP5 produces analgesia in the formalin pain test. Exp Neurol 2001;172:92–9.
22. Prado WA, Roberts MH. An assessment of the antinociceptive and aversive effects of stimulating identified sites in the rat brain. Brain Res 1985;340:219–28.
23. Soares DP, Law M. Magnetic resonance spectroscopy of the brain: review of metabolites and clinical applications. Clin Radiol 2009;64:12–21.
24. Huang W, Alexander GE, Daly EM *et al.* High brain myo-inositol levels in the predementia phase of Alzheimer's disease in adults with Down's syndrome: a 1H MRS study. Am J Psychiatry 1999;156:1879–86.
25. Emad Y, Ragab Y, Zeinhom F *et al.* Hippocampus dysfunction may explain symptoms of fibromyalgia syndrome. A study with single-voxel magnetic resonance spectroscopy. J Rheumatol 2008;35:1371–7.
26. Harris RE, Sundgren PC, Craig AD *et al.* Elevated insular glutamate in fibromyalgia is associated with experimental pain. Arthritis Rheum 2009;60:3146–52.
27. Wood PB, Ledbetter CR, Glabus MF *et al.* Hippocampal metabolite abnormalities in fibromyalgia: correlation with clinical features. J Pain 2009;10:47–52.
28. Grachev ID, Fredrickson BE, Apkarian AV. Abnormal brain chemistry in chronic back pain: an *in vivo* proton magnetic resonance spectroscopy study. Pain 2000;89:7–18.
29. Grachev ID, Fredrickson BE, Apkarian AV. Brain chemistry reflects dual states of pain and anxiety in chronic low back pain. J Neural Transm 2002;109:1309–34.
30. Fukui S, Matsuno M, Inubushi T *et al.* N-Acetylaspartate concentrations in the thalami of neuropathic pain patients and healthy comparison subjects measured with (1)H-MRS. Magn Reson Imaging 2006;24:75–9.
31. Pattany PM, Yezierski RP, Widerstrom-Noga EG *et al.* Proton magnetic resonance spectroscopy of the thalamus in patients with chronic neuropathic pain after spinal cord injury. Am J Neuroradiol 2002;23:901–5.
32. Sorensen L, Siddall PJ, Trenell MI *et al.* Differences in metabolites in pain-processing brain regions in patients with diabetes and painful neuropathy. Diabetes Care 2008;31:980–1.
33. Gu T, Ma XX, Xu YH *et al.* Metabolite concentration ratios in thalami of patients with migraine and trigeminal neuralgia measured with 1H-MRS. Neurol Res 2008;30:229–33.
34. Sarmento E, Moreira P, Brito C *et al.* Proton spectroscopy in patients with post-traumatic headache attributed to mild head injury. Headache 2009;49:1345–52.
35. Wang SJ, Lirng JF, Fuh JL *et al.* Reduction in hypothalamic 1H-MRS metabolite ratios in patients with cluster headache. J Neurol Neurosurg Psychiatry 2006;77:622–5.
36. Dichgans M, Herzog J, Freilinger T *et al.* 1H-MRS alterations in the cerebellum of patients with familial hemiplegic migraine type 1. Neurology 2005;64:608–13.
37. Prescot A, Becerra L, Pendse G *et al.* Excitatory neurotransmitters in brain regions in interictal migraine patients. Mol Pain 2009;5:34.
38. Sarchielli P, Tarducci R, Presciutti O *et al.* Functional 1H-MRS findings in migraine patients with and without aura assessed interictally. Neuroimage 2005;24:1025–31.
39. Longstreth GF, Thompson WG, Chey WD *et al.* Functional bowel disorders. Gastroenterology 2006;130:1480–91.
40. Beck AT, Rial WY, Rickels K. Short form of depression inventory: cross-validation. Psychol Rep 1974;34:1184–6.
41. Sullivan MJ, Bishop SR, Pivik J. The pain catastrophizing scale: development and validation. Psychol Assess 1995;7:524–32.
42. Provencher SW. Estimation of metabolite concentrations from localized *in vivo* proton NMR spectra. Magn Reson Med 1993;30:672–9.
43. Barker PB, Soher BJ, Blackband SJ *et al.* Quantitation of proton NMR spectra of the human brain using tissue water as an internal concentration reference. NMR Biomed 1993;6:89–94.
44. Christiansen P, Henriksen O, Stubgaard M *et al. In vivo* quantification of brain metabolites by 1H-MRS using water as an internal standard. Magn Reson Imaging 1993;11:107–18.
45. Geurts JJ, Barkhof F, Castelijns JA *et al.* Quantitative 1H-MRS of healthy human cortex, hippocampus, and thalamus: metabolite concentrations, quantification precision, and reproducibility. J Magn Reson Imaging 2004;20:366–71.
46. Sandi C. Stress, cognitive impairment and cell adhesion molecules. Nat Rev Neurosci 2004;5:917–30.
47. Hertz L, Zielke HR. Astrocytic control of glutamatergic activity: astrocytes as stars of the show. Trends Neurosci 2004;27:735–43.
48. McKinney RA. Excitatory amino acid involvement in dendritic spine formation, maintenance and remodelling. J Physiol 2009;588:107–16.
49. Dinan TG, Quigley EM, Ahmed SM *et al.* Hypothalamic-pituitary-gut axis dysregulation in irritable bowel syndrome: plasma cytokines as a potential biomarker? Gastroenterology 2006;130:304–11.
50. Ehlert U, Nater UM, Bohmelt A. High and low unstimulated salivary cortisol levels correspond to different symptoms of functional gastrointestinal disorders. J Psychosom Res 2005;59:7–10.
51. Heim C, Nemeroff CB. Neurobiology of posttraumatic stress disorder. CNS Spectr 2009;14:13–24.
52. Heim C, Shugart M, Craighead WE *et al.* Neurobiological and psychiatric consequences of child abuse and neglect. Dev Psychobiol 2010;52:671–90.
53. Yehuda R. Advances in understanding neuroendocrine alterations in PTSD and their therapeutic implications. Ann N Y Acad Sci 2006;1071:137–66.
54. Cole MA, Kim PJ, Kalman BA *et al.* Dexamethasone suppression of corticosteroid secretion: evaluation of the site of action by receptor measures and functional studies. Psychoneuroendocrinology 2000;25:151–67.
55. McGowan PO, Sasaki A, D'Alessio AC *et al.* Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat Neurosci 2009;12:342–8.
56. Apfel BA, Ross J, Hlavin J *et al.* Hippocampal volume differences in gulf war veterans with current versus lifetime posttraumatic stress disorder symptoms. Biol Psychiatry 2010;2010:20.
57. Bremner JD, Randall P, Scott TM *et al.* MRI-based measurement of hippocampal volume in patients with combat-related posttraumatic stress disorder. Am J Psychiatry 1995;152:973–81.

58. Wang Z, Neylan TC, Mueller SG *et al.* Magnetic resonance imaging of hippocampal subfields in posttraumatic stress disorder. Arch Gen Psychiatry 2010;67:296–303.
59. Neylan TC, Schuff N, Lenoci M *et al.* Cortisol levels are positively correlated with hippocampal N-acetylaspartate. Biol Psychiatry 2003;54:1118–21.
60. Drossman DA, Leserman J, Hu JB. Gastrointestinal diagnosis, abuse history, and effects on health status. Gastroenterology 1996;111:1159–61.
61. Blankstein U, Chen J, Diamant NE *et al.* Altered brain structure in irritable bowel syndrome: potential contributions of pre-existing and disease-driven factors. Gastroenterology 2010;138:1783–9.
62. Barkus C, McHugh SB, Sprengel R *et al.* Hippocampal NMDA receptors and anxiety: at the interface between cognition and emotion. Eur J Pharmacol 2009;626:49–56.
63. Rey E, Moreno Ortega M, Garcia Alonso MO *et al.* Constructive thinking, rational intelligence and irritable bowel syndrome. World J Gastroenterol 2009;15:3106–13.
64. Geurts JJ, Reuling IE, Vrenken H *et al.* MR spectroscopic evidence for thalamic and hippocampal, but not cortical, damage in multiple sclerosis. Magn Reson Med 2006;55:478–83.
65. Milne A, MacQueen GM, Yucel K *et al.* Hippocampal metabolic abnormalities at first onset and with recurrent episodes of a major depressive disorder: a proton magnetic resonance spectroscopy study. Neuroimage 2009;47:36–41.
66. Schirmer T, Auer DP. On the reliability of quantitative clinical magnetic resonance spectroscopy of the human brain. NMR Biomed 2000;13:28–36.
67. Brooks WM, Friedman SD, Stidley CA. Reproducibility of 1H-MRS *in vivo*. Magn Reson Med 1999;41:193–7.