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# Changes in salivary physiological stress markers induced by muscle stretching in patients with irritable bowel syndrome

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## Abstract

**Background:** Psychophysiological processing has been reported to play a crucial role in irritable bowel syndrome (IBS) but there has been no report on modulation of the stress marker chromogranin A (CgA) resulting from muscle stretching. We hypothesized that abdominal muscle stretching as a passive operation would have a beneficial effect on a biochemical index of the activity of the sympathetic/adrenomedullary system (salivary CgA) and anxiety.

**Methods:** Fifteen control and eighteen untreated IBS subjects underwent experimental abdominal muscle stretching for 4 min. Subjects relaxed in a supine position with their knees fully flexed while their pelvic and trunk rotation was passively and slowly moved from 0 degrees of abdominal rotation to about 90 degrees or the point where the subject reported feeling discomfort.

Changes in the Gastrointestinal Symptoms Rating Scale (GSRS), State Trait Anxiety Inventory (STAI), Self-rating Depression Scale (SDS), ordinate scale and salivary CgA levels were compared between controls and IBS subjects before and after stretching. A three-factor analysis of variance (ANOVA) with period (before vs. after) as the within-subject factor and group (IBS vs. Control), and sex (men vs. female) as the between-subject factors was carried out on salivary CgA.

**Results:** CgA showed significant interactions between period and groups ($F[1, 31] = 4.89$, $p = 0.03$), and between groups and sex ($F[1, 31] = 4.73$, $p = 0.03$). Interactions between period and sex of CgA secretion were not shown ($F[1, 3] = 2.60$, $p = 0.12$). At the baseline, salivary CgA in IBS subjects (36.7 ± 5.9 pmol/mg) was significantly higher than in controls (19.9 ± 5.5 pmol/mg, $p < 0.05$). After the stretching, salivary CgA significantly decreased in the IBS group (25.5 ± 4.5 pmol/mg), and this value did not differ from that in controls (18.6 ± 3.9 pmol/mg).

**Conclusion:** Our results suggest the possibility of improving IBS pathophysiology by passive abdominal muscle stretching as indicated by CgA, a biochemical index of the activity of the sympathetic/adrenomedullary system.

## Background

Paying attention to the gut may magnify perceptions of abdominal symptoms and symptom related emotion [1,2]. Irritable bowel syndrome (IBS) is associated with an increased incidence of psychological disorder in patient populations [3], and while the cause and nature of this association are a matter of discussion, several possible mechanisms, both psychological and physiological, have been proposed to account for the finding [4-6]. Although there are many treatment strategies [7], traditional IBS therapy is mainly symptom oriented and often unsatisfactory. Increasing knowledge of brain-gut physiology [8], mechanisms, and neurotransmitters and receptors [9] involved in gastrointestinal motor and sensory function have led to the development of several new therapeutic approaches [10,11].

No single medication has proven to be universally effective, and the multiple therapeutic approach of gastrointestinal neurophysiology has led to promising advances in medical and non-medical approaches to IBS. Most studies have examined the association between mood state and IBS symptom severity using between-subjects design. The mechanisms involved suggest an association between mood state and IBS symptom severity within the individual. For example, although self-report measures of symptom severity cannot distinguish between the effect of mood state on physiology and on symptom perception, both mechanisms would lead to a situation in which a worsening of mood would occur before a worsening of IBS symptoms when both are measured longitudinally.

Autonomic imbalance has been proposed as a pathophysiological factor of IBS. Adrenergic neural activity and rectal sensitivity are more pronounced in IBS patients than in normal controls [12]. The stress response system includes the sympathetic/adrenomedullary (S/A) system and the hypothalamic-pituitary-adrenal (HPA) axis. The activities of the HPA axis and the S/A system can be biochemically evaluated by measuring catecholamines and cortisol, and we can measure these hormones as objective markers of stress. Recently, as a result of investigating the derivatives of catecholamines that are detectable in saliva, chromogranin A (CgA) was determined to be a useful index of psychological stress. CgA is a member of a family of highly acidic proteins, chromogranins, which are co-stored in the adrenergic neurons and paraneurons and co-released with adrenaline and noradrenaline in response to stimulation [13,14]. The changes in salivary CgA secretion resulting from exposure to a cognitive task may indicate psychological stress in humans [15].

Colonic stimulation results in brain activation of the somatosensory, insular, anterior cingulate and prefrontal cortices [2]. The somatosensory cortex receives direct anatomical projections from the ventral posterior thalamic nucleus, it is generally assumed that the somatosensory cortex is involved in parallel processing of tactile sensory information derived from this thalamic source of input [16]. In contrast, psychological stress influences pain thresholds via activation of the prefrontal cortices. Corticotropin releasing hormone is released from the hypothalamus, binding to visceral muscles and causing abnormal movement of the colon [9,17]. A stress marker of the S/A system, CgA, is released in saliva due to negative feelings such as aversive stimuli and psychological stress [15,18]. Mental activity may modulate gut perception [18,19] and override the effect of somatic stimulation on gut perception. For example, afferent signals from muscle stretching might modulate visceral perception and emotion via the spinothalamic pathway.

Skeletal muscle stretching is a unique method for relaxation [20-22]. The effect of hypnotherapy on IBS has been well documented [23], but specific psychotherapy usually needs long-range training for therapists at much cost. On the other hand, skeletal muscle stretching is simple and applicable in daily practice. Skeletal muscle stretching improved subjective pain scores of the patients with low back pain, and salivary cortisol concentrations were also significantly decreased during exercise [24]. However, the effects of skeletal muscle stretching on IBS are still unknown.

We hypothesized that IBS subjects would show abnormal salivary CgA and that skeletal muscle stretching would have beneficial effects on the pathophysiology of IBS.

## Methods

### *Subjects*

This study was approved by the Ethics Committee of Niigata University of Health and Welfare. All subjects gave their written informed consent. The subjects were university students, including 15 healthy volunteers as controls (7 males, 8 females: university students at Niigata University of Health and Welfare) and 18 subjects with IBS (not receiving medical treatment for IBS, 7 males, 11 females) aged 20 to 23 years old. The IBS subjects were 20 subjects selected from 245 volunteers selected through a predesigned questionnaire based on the Rome III criteria [25] for functional gastrointestinal diseases. Two IBS subjects were excluded from the results because of incomplete examinations due to cold and headache.

As recommended by the Rome III committees [25], patients with IBS were classified by the predominant stool pattern: IBS with diarrhea (IBS-D) was defined as loose (mushy) or watery stool >25% and hard or lumpy stool <25% of bowel movements; and IBS with constipation (IBS-C) as hard or lumpy stool 25% and loose or watery

stool <25% of bowel movements. Based on questions about the proportion of bowel movements that were either loose or watery, or hard or like a ball (lumpy), IBS subjects were classified by Rome III criteria [25] as IBS-D (n = 4), IBS-C (n = 8), IBS-M (n = 2) and IBS-U (n = 4).

### *Stretching of the abdominal muscles*

Subjects attended a preliminary test session that included the measurement of psychological characteristics and maximal abdominal muscle stretch. Subjects relaxed in a supine position with their knees fully flexed while their pelvis and trunk were passively and slowly moved from 0 degrees of abdominal rotation to about 90 degrees or until the subject reported feeling discomfort.

Participants attended one of several 30-minute experimental stretch sessions that were conducted at the same time of day. Subjects were instructed not to begin a stretching program session and to reschedule their session, if symptoms of their IBS prevented the stretching. During the session, subjects engaged in a 1-minute cyclic stretching protocol, 2 times right and left side rotation of their pelvis and trunk, and a 4-minute static stretching protocol. For the 4-minute static stretching protocol, the subject's knees were moved at a rate of 30°/s from 90 degrees of trunk and pelvic rotation (neutral) to a static hold at 80% of the subject's maximal passive rotation angle for one minute [26]. Immediately following the static stretching, the knees were returned to neutral, then moved to 80% of maximal angle on the other side, and again returned back to neutral [27]. The last stretching sequence was necessary so that measurements of stiffness and abdominal discomfort before the stretching could be compared with measurements of sensation and emotion after the stretching.

### *Measurement of symptoms and psychological status*

Before the experiment, gastrointestinal (GI) symptoms and psychological status were evaluated using the Gastrointestinal Symptoms Rating Scale (GSRS) [28], Zung's Self-rating Depression Scale (SDS) [29], and the State-Trait Anxiety Inventory (STAI) [30]. In addition, the subjects were asked to report the following seven items of visceral sensation or emotion [2]: abdominal discomfort, abdominal distention, abdominal pain, urgency for defecation, perceived stress, sleepiness, and anxiety before and after perceived stretching. Each sensation was evaluated on a scale from 0 (no sensation) to 10 (maximal sensation) as previously described [2,31].

GSRS is a 15-item instrument designed to assess the symptoms associated with common GI disorders. It has five subscales (Reflux, Diarrhea, Constipation, Abdominal Pain, and Indigestion Syndrome). Subscale scores range from 1 to 7 and higher scores represent more discomfort. A total score is derived by summing the individual item scores, and ranges from 15 to 105 [32].

SDS is a 20-item self-report questionnaire. Each item is scored on a Likert scale ranging from 1 to 4. A total score ranges from 20 to 80. Most people with depression score between 50 and 69, while a score of 70 and above indicates severe depression [33].

Mean scores of STAI for normal subjects were substantially lower than those reported in the English STAI Manual (State 24.95 ± 11.36 vs. 36.54 ± 10.22 and the Trait score was 27.88 ± 11.43 vs. 35.55 ± 9.76). The reported scores for depressed patients were 56.22 ± 8.86 and 53.83 ± 10.87, The state score for healthy subjects was 34.30 ± 10.79 and the Trait score was 36.07 ± 10.47 [34].

### *Salivary CgA sampling*

Salivary samples were collected immediately before and after stretching. Saliva samples were extracted from cotton wads that subjects held in their mouths for 2 min by centrifuging at 3,000 rpm for 15 min. The tactile stimulation of the presence of the cotton wad in the oral cavity tends to stimulate a rather uniform salivary flow [35]. During collection, the cotton wad was rolled around like a hard candy in the oral cavity. The samples were stored at -20°C until the assay. Salivary CgA levels were determined using an enzyme-linked immunosorbent assay (EIA) kit (YK070 Human Chromogranin A EIA, Yanaihara Institute, Inc., Shizuoka, Japan), using the method of Yanaihara et al. [36] of Yanaihara Laboratories (Fujinomiya, Shizuoka, Japan). The corrected values of CgA (pmol/mg) were calculated by dividing by the raw results of EIA with the protein concentration of the saliva in the samples (pg/mg).

Levels of salivary CgA were evaluated, according to a previously described method [35,37,38]. Salivary CgA levels are reported as being within the range of 50.0 ± 40.0 pmol/mg (protein corrected) in healthy subjects [39]. Salivary CgA might be a sensitive and promising index for psychosomatic stress. Therefore, an understanding of the circadian rhythm of salivary CgA in normal humans is important. According to a recent report, CgA does not show any obvious circadian rhythm. Salivary CgA levels peak upon awakening, and then quickly decrease to the nadir after 1 hour and maintain a low level throughout the day [40]. The circadian variation of CgA is still not fully established.

### *Statistic analysis*

A three-factor analysis of variance (ANOVA) with period (before vs. after) as the within-subject factor and group (IBS vs. Normal) and sex (men vs. female) as the between-subject factors, was carried out on salivary CgA. Changes in revised salivary CgA levels between before and after

stretching sessions for each group were analyzed statistically by related-measures 3-factor ANOVA, followed by Bonferroni protected least significant difference for multiple comparisons; values of $p < 0.05$ were considered significant.

Values of visceral perception and emotion were compared between groups with the Mann-Whitney U test. Spearman's rank correlation was used for evaluating the intragroup correlation coefficient between CgA and psychological status and perceptional/emotional ratings; values of $p < 0.05$ were accepted as significant.

## Results

### *GI symptoms and psychological status*

Table 1 shows the scores for GI symptoms and psychological status in the Normal and IBS groups. The GSRS score of IBS subjects was significantly higher than that of Normal subjects (Mann-Whitney's U test, $p = 0.01$). State anxiety and trait anxiety scores of STAI of IBS subjects were significantly higher than those of normal subjects ($p < 0.01$). SDS scores did not significantly differ between Normal and IBS subjects.

Abdominal discomfort in IBS subjects before and after stretching was significantly higher than in Normal subjects ($p = 0.03$) (Table 2). After stretching, there was no difference in abdominal discomfort between the groups. Anxiety was significantly decreased by stretching in Normal subjects ($p = 0.04$).

Two-way ANOVA of abdominal discomfort showed that there were significant period × group interactions ($F [3, 62] = 6.82$, $p = 0.005$). Before stretching, abdominal discomfort in IBS subjects ($1.84 \pm 0.43$) was significantly higher than in Normal subjects ($0.27 \pm 0.12$, post-hoc, $p = 0.02$). After stretching, abdominal discomfort in IBS subjects ($1.74 \pm 0.37$) was significantly higher than in Normal subjects ($0.20 \pm 0.11$, post-hoc, $p = 0.01$). Abdominal discomfort changes between before and after stretching in Normal controls were not significant (post-hoc, $p = 0.89$). There were no changes in the other scales before and after stretching or between normal controls and IBS subjects.

**Table 1: Comparisons of GI symptoms and psychological status between IBS subjects and normal controls**

| | Controls (n = 15) | IBS (n = 18) | p |
|---|---|---|---|
| GSRS | 32.47 ± 5.21 | 51.16 ± 4.95* | <0.01 |
| STAI-S | 23.20 ± 3.23 | 41.90 ± 1.62* | <0.01 |
| STAI-T | 25.53 ± 3.22 | 42.53 ± 1.80* | <0.01 |
| SDS | 42.00 ± 1.60 | 45.21 ± 1.73 | 0.23 |

Mean ± Standard Error. *Comparison between normal group vs. IBS group by the Mann-Whitney's U test. GSRS: Gastrointestinal Symptoms Rating Scale, STAI-S: state anxiety, STAI-T: trait anxiety, SDS: Self-rating Depression Scale.

### *Changes of salivary CgA*

For multiple group comparisons, homogeneity of variance was assessed by the Levene test. Three-way repeated-measures ANOVA of CgA showed significant interactions between period and groups ($F[1, 31] = 4.89$, $p = 0.03$), and between groups and sex ($F[1, 31] = 4.73$, $p = 0.03$). Interactions between period and sex of CgA secretion were not significant ($F[1, 3] 2.60$, $p = 0.12$). Before stretching, salivary CgA in IBS subjects ($36.7 \pm 5.9$ pmol/mg) was significantly higher than in Normal subjects ($19.9 \pm 5.5$ pmol/mg, post-hoc, $p = 0.006$) (Fig. 1). CgA changes before and after stretching in Normal subjects were not significant (post-hoc, $p = 0.60$). In contrast, CgA was significantly decreased after stretching in IBS subjects ($22.5 \pm 4.5$ pmol/mg, post-hoc, $p = 0.02$). After stretching, there was no significant difference in CgA between Normal and IBS subjects (post-hoc, $p = 0.22$).

The Spearman rank correlation coefficient showed a significantly positive correlation between CgA secretion before stretching and SDS score in IBS ($r = 0.51$, $p = 0.03$) (Table 3). The change in CgA after stretching compared with before stretching was positively correlated to the SDS score in both groups (IBS: $r = 0.52$, $p = 0.03$. Normal: $r = 0.53$, $p = 0.04$). In ratings of perception and emotion, perceived stress to stretching was negatively correlated with CgA secretion in the Normal group ($r = -0.66$, $p = 0.007$).

## Discussion

This is the first study to demonstrate that the salivary CgA level of IBS subjects is higher than that of normal subjects. We also demonstrated that, after stretching, the CgA level of IBS subjects became comparable with that of normal subjects. CgA is a major soluble protein in adrenal medullary chromaffin granules and adrenergic neurons and is co-released with catecholamines, which are considered to be a good index of sympathetic activity [41,42]. In particular, salivary CgA is reported to be a sensitive and substantial marker of psychological stress, which does not respond well to physical stress [15]. The results of this study suggest that abdominal muscle stretching may improve sympathetic arousal in IBS subjects.

Patients with IBS show more psychiatric disorders and pathologic behavioral patterns than normal subjects [4,43]. A correlation between CgA secretion and the depression score was observed in this study. This result suggests a potential mechanism connecting events in the nervous system (central or enteric) with IBS symptoms. Noradrenaline in the brain plays a crucial role in anxiety, and colorectal distention induces noradrenaline release in the hippocampus [44]. Not only central but also periph-

**Table 2: Ratings of perception and emotion**

| Perception and emotion | Normal (n = 15) | | IBS (n = 18) | |
|---|---|---|---|---|
| | Before | After | Before | After |
| Abdominal discomfort | 0.27 ± 0.12 | 0.20 ± 0.11 | 1.84 ± 0.43*a | 1.74 ± 0.37*b |
| Abdominal distention | 0.40 ± 0.16 | 0.40 ± 0.16 | 1.32 ± 0.39 | 1.05 ± 0.35 |
| Abdominal pain | 0.20 ± 0.11 | 0.27 ± 0.15 | 0.74 ± 0.30 | 0.47 ± 0.25 |
| Urgency | 0.33 ± 0.16 | 0.47 ± 0.19 | 0.32 ± 0.23 | 0.26 ± 0.19 |
| Stress | 2.40 ± 0.54 | 2.27 ± 0.57 | 2.21 ± 0.41 | 2.37 ± 0.50 |
| Sleepiness | 3.00 ± 0.39 | 3.53 ± 0.45 | 4.42 ± 0.72 | 5.00 ± 0.64 |
| Anxiety | 2.20 ± 0.48 | 0.87 ± 0.35*c | 2.42 ± 0.47 | 1.90 ± 0.48 |

Mean ± Standard Error. *a: Comparison between Normal group vs. IBS group before stretching (p = 0.03), *b: Normal group vs. IBS group after stretching (p = 0.03), *c: before vs. after stretching of the Normal group (p = 0.04) by Mann-Whitney's U test.

eral adrenergic/noradrenergic functions may contribute to the pathophysiology of IBS. Elsenbruch et al. reported that IBS patients demonstrated significantly greater postprandial increases in plasma noradrenaline and systolic blood pressure [45]. In inflammatory bowel diseases, disturbed adrenergic regulation of interleukin-10 (IL-10) could be part of the mechanism underlying the modulation of disease activity due to psychological stress [46]. Disturbed autonomic or neuroendocrine modulation of cytokine production, may play a role in the pathogenesis of IBS [9]. Increased salivary CgA in IBS subjects suggests that IBS subjects have sympathetic arousal due to increased signaling to the gut afferent neurons.

IBS symptoms are generally worsened by stress and often improve with physical exercise and medications affecting serotonin function [47-49]. Sugano et al. reported that the skeletal muscle stretching program improved subjective pain scores of the patients with low back pain and that salivary cortisol concentrations were also significantly decreased up to 90 min after exercise [24]. Exercise may have beneficial effects on IBS symptoms [50].

CgA secretion before stretching was negatively correlated with the stress score of normal subjects. Additionally, the anxiety score was reduced after stretching in the normal subjects. Psychological factors influencing symptom reporting have been identified in the constructs of visceral perceptional amplification and alexithymia [51]. From a psychological viewpoint, IBS may be conceived as an abnormal cognitive processing of emotional stimuli, via verbal responses, and a tendency to perceive somatic stimuli as evidence of symptoms of disease.

Ghoncheh et al. examined the psychological and physical effect of passive muscle stretching and yoga stretching exercises for relaxation [22]. Muscle relaxation displayed higher levels of relaxation states, physical relaxation, disengagement and higher levels of joy as a post-training effect [52]. Muscle stretching provides sensation contrasts for learning relaxation in addition to fostering relaxation through the stretching of muscles [53]. Muscle stretching for patients with IBS may be of benefit to the patients and could be used as part of a multi-component approach to the treatment of IBS.

Evidence of a physiological component of IBS is based on gender differences in GI symptoms, central nervous system pain processing, and specific effects of estrogen and progesterone on gut function [54,55]. Additional factors may play a role, including gender-related differences in neuroendocrine, S/A system, and stress reactivity, which

**Table 3: Correlation between (r) CgA and GI symptoms and psychological status before and after stretching for the Normal and IBS groups.**

| | GSRS | SDS | STAI-S | STAI-T | Abdominal Discomfort | Abdominal Pain | Stress | Anxiety |
|---|---|---|---|---|---|---|---|---|
| **IBS** (n = 18) | | | | | | | | |
| before | -0.16 | 0.51* | -0.17 | -0.30 | 0.11 | -0.13 | -0.20 | 0.05 |
| after | -0.34 | 0.19 | -0.41 | -0.29 | 0.24 | -0.20 | -0.37 | -0.21 |
| ΔCgA | 0.08 | 0.52* | 0.04 | 0.09 | 0.10 | -0.05 | 0.18 | 0.36 |
| **Normal** (n = 15) | | | | | | | | |
| before | 0.07 | 0.24 | 0.16 | 0.44 | -0.23 | 0.13 | -0.53* | -0.01 |
| after | -0.20 | 0.08 | 0.15 | 0.07 | -0.36 | -0.04 | 0.09 | 0.18 |
| ΔCgA | 0.26 | 0.53* | -0.01 | 0.40 | 0.09 | 0.25 | -0.66* | 0.04 |

Significance, *p < 0.05. ΔCgA: CgA secretion before stretching – CgA after stretching.

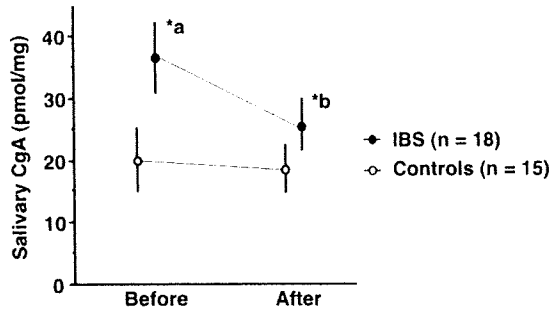


**Figure 1**
**Three-way repeated-measures ANOVA of CgA showed significant interactions between period and groups (F[1, 31] = 4.89, p = 0.03).** *a: Before stretching, salivary CgA in IBS subjects (36.7 ± 5.9 pmol/mg) were significantly higher than in Normal controls (19.9 ± 5.5 pmol/mg, post-hoc, p = 0.006). A significant CgA change between before and after stretching in Normal controls was not shown (post-hoc, p = 0.60). *b: CgA was significantly decreased after stretching in IBS subjects (22.5 ± 4.5 pmol/mg, post-hoc, p = 0.02). After stretching, there was no significant difference in CgA between Normal controls and IBS subjects (post-hoc, p = 0.22). Error bars are mean ± standard error.

are related to bowel function and pain. Although gender differences in the therapeutic benefits of serotonergic agents have been observed [56], less is known about potential differences in responsiveness to non-drug therapies for IBS. Multiple comparisons between CgA and gender related information suggest that stretch intervention may have gender dependent effects on IBS.

The following three points can be cited as limitations of this study. The first is that sample size was very small. The levels of CgA found in our subjects were somewhat different from the reported mean value [39], and our findings could not exclude the effect of the sample size. Additionally, we could not examine the effects on subtypes of IBS (i.e. constipation-predominant or diarrhea-predominant), because the sample sizes of the subtypes were too small to analyze them separately. However, a long follow-up study [57] proved the inconsistency of IBS subtypes, suggesting that whole IBS analysis is more important than subtype analysis. The second limitation is that the duration of muscle stretching might be too short. The duration of the effect of contraction-relaxation stretching on range of motion (ROM) in the lower extremities is 15 min and the increase in ROM usually remains for 90 min [58]. Proprioceptive neuromuscular facilitation (PNF) stretching techniques produced greater increases in ROM than static or dynamic stretching exercises. The stretching hold time at the hip is 3–10 sec in one hold-relax PNF stretch [59]. There is no study that clarifies the stretch duration required of the abdominal muscle for relaxation. Thus, it will be necessary to examine how long we should stretch the abdominal muscles for IBS treatment. Lastly, we could not analyze the effect of lifestyle and medical history on CgA in this study. Many stress-related biomarkers are affected by lifestyle or medical history [60]. Such relationships might contribute to increased knowledge about strategies to prevent progression of IBS.

## Conclusion

Our results suggested that it is possible to improve IBS pathophysiology by passive abdominal muscle stretching using a biochemical index of the activity of the S/A system (salivary CgA). In this study, we verified only the effects of stretching and presence of IBS on CgA levels. Further study of the S/A system and muscle stretching in IBS is warranted.

## Abbreviations

IBS: irritable bowel syndrome; CgA: chromogranin A; GSRS: Gastrointestinal Symptoms Rating Scale; STAI: State Trait AnxietyInventory; SDS: Self-rating Depression Scale; ANOVA: analysis ofvariance; S/A: sympathetic/adrenomedullary.

## Competing interests

The authors declare that they have no competing interests.

## Authors' contributions

TH was the main investigator and wrote the first draft of the manuscript. SF supervised the study, analyzed the data and wrote the final draft of the manuscript. MT and TT supervised the study. KS, MO and KS contributed to the study design. KS contributed to the data collection. All authors contributed to the preparation of the article and approved the final manuscript.

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# Brain Activation Covariated With Changes in Heart Rate, Heart Rate Variability, and Plasma Catecholamines During Rectal Distention

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**Objective:** To test the hypothesis that gut stimulation provokes autonomic arousal via activation of regional cerebral cortices. How the human brain processes interoceptive signals and forms initial autonomic arousal is one of the key questions to be answered in research on emotion. **Methods:** Twelve healthy males participated in this study. A barostat bag was inserted in the rectum and intermittently inflated with 0, 20, or 40 mm Hg at random for 80 seconds. $H_2{}^{15}O$ positron emission tomography (PET) of the brain, electrocardiography, and blood sampling for catecholamines were performed. Changes in regional cerebral blood flow were interpreted using statistical parametric mapping. **Results:** Rectal distention with 40 mm Hg induced a significant increase in heart rate, low-frequency (LF)/high-frequency (HF) ratio of heart rate variability, and plasma adrenaline. Activated brain areas that were covariated with increased heart rate during rectal distention were the right insula, right operculum, right dorsolateral prefrontal cortex, putamen, thalamus, periaqueductal gray, and cerebellum ($p < .001$, uncorrected), whereas those that were covariated with increased LF/HF ratio were the bilateral insula, putamen, thalamus, midbrain, pons, and cerebellum ($p < .001$, uncorrected). Activated brain areas that were covariated with increased plasma adrenaline were the right insula, right orbitofrontal cortex, right parahippocampal gyrus, putamen, thalamus, periaqueductal gray, pons, and cerebellum ($p < .001$, uncorrected). **Conclusion:** Our results suggest that the right insula and the related body mapping regions may form the functional module of sympathetic arousal in response to gut stimulation. **Key words:** positron emission tomography, heart rate, heart rate variability, catecholamine, visceral perception, rectal distention.

**PET** = positron emission tomography; **rCBF** = regional cerebral blood flow; **BA** = Brodmann's area; **ECG** = electrocardiogram; **HRV** = heart rate variability; **HF** = high-frequency component of HRV; **LF** = low-frequency component of HRV; **LF/HF** = ratio of LF to HF; **ANOVA** = analysis of variance.

## INTRODUCTION

Emotion was proposed to have two components, one as the bodily state and the other as the feeling (1). The bodily state, which is mediated by a family of peripheral, autonomic, endocrine, and skeletomotor responses, has been believed to involve subcortical structures: the amygdala, the hypothalamus, and the brainstem whereas the feeling involves cerebral cortex.

However, neuroscience and patient studies indicated that autonomic response, the bodily state, is associated with cortical brain regions which are important in the feeling (2–5). Electrical stimulation of brain regions like insula, anterior cingulate cortex, or prefrontal cortex induced changes in blood pressure and heart rate occasionally accompanied by subjective mood changes (2,3). Patients with dysfunction of prefrontal cortex or anterior cingulate cortex did not show autonomic changes in mental tasks, which elicit autonomic arousal in normal healthy subjects (4,5). Besides, the patient with damaged prefrontal cortex could not have emotional feelings (4). These cortical brain structures are thought to play a salient role in the processing of the autonomic response as well as the feelings (4,6).

Studies using functional neuroimaging technique have examined noninvasively the relationship between autonomic arousal and brain activity. Hand gripping, mental arithmetics, mental tasks, or Valsalva maneuver provoked activation of anterior cingulate cortex, insula, prefrontal cortex, amygdala, hippocampus, cerebellum, and brainstem that were associated with autonomic activity (7–10). Stimulation of the gastrointestinal tract also provokes autonomic changes (11–13) as well as visceral sensation. Functional imaging studies have identified brain areas activated during stimulation of the esophagus (14), stomach (15), descending colon (16), or rectum (17,18). These brain areas include the anterior cingulate cortex, insula, prefrontal cortex, cerebellum, and brainstem (14–18). However, no study has ever examined the association between activation of brain regions and autonomic activity during gastrointestinal stimulation.

Recently, the processing of emotion has been conceptualized as hierarchical structures, visceral sensation, action tendencies, unidimentional and multidimentional processing, and integration of multidimentional processing (6). Emotion and identified brain regions that were covariated with autonomic changes in earlier studies are multidimensional because complex cognitive tasks were used (7–10). Therefore, identified brain regions that were covariated with autonomic changes in earlier studies should be reexamined in the lower hierarchy of emotional processing, e.g., visceral sensation.

Heart rate variability (HRV) and galvanic skin conductance have already been used as indices of autonomic activity in the previous human study of rectal distention (13). Changes in heart rate during rectal distention and the association between brain activity and serum catecholamine levels have not been studied yet in humans.

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In this study, we tested the following hypotheses:

1. Rectal distention provokes changes in heart rate, HRV, and serum catecholamine levels in healthy young men.
2. Activated brain regions that were covariated with changes in heart rate, HRV, and serum catecholamine levels during rectal distention are the anterior cingulated cortex, insula, prefrontal cortex, amygdala, hippocampus, cerebellum, and brainstem.

## MATERIALS AND METHODS

### Subjects

Twelve healthy male volunteers (age = 22 ± 1 (standard error of the mean) years) were recruited to participate in this study through local advertisement between July 2004 and June 2005. Each volunteer underwent a basic medical history, medical interview, and physical examination to exclude subjects with organic diseases. All subjects were right handed and free from signs or symptoms of gastrointestinal, cardiovascular, or psychotic disorders. They were free of medication and no subject had been taking illegal drugs, smoking, drinking alcohol heavily, or taking excessive caffeine. All subjects gave their informed consent before starting the study. This study, which is a part of the Brain Imaging Project for Irritable Bowel Syndrome in Tohoku University (Principal Investigator, S.F.), was approved by the Ethics Committee of Tohoku University School of Medicine.

### Experimental Design

On the day before the examination, the subjects took a low residue diet. At night (9 PM), they ingested 17 (13.6%) g of magnesium citrate, 75 mg of sodium picosulfate, and 24 mg of sennoside A & B to cleanse the colon. The subjects were then fasted overnight. The experiment began the next day at 10 AM. First, the subjects lay quietly on a bed for positron emission tomography (PET) scan at the Cyclotron Radioisotope Center, Toholu University. Two polyethylene catheters were inserted into the bilateral cubital vein. A saline drip infusion was started at a rate of 1 ml/minute. A plastic catheter with a thin polyethylene bag (Synectics Medical, Stockholm, Sweden) was inserted into the rectum of each subject. After a radioactive tracer ($H_2{}^{15}O$) was injected through the right cubital vein, a PET scan of the brain was performed four times with or without rectal distention. Scanning time was set to 70 seconds. Approximately a 10-minute interval was given between successive distentions to ensure that radioactivity levels returned to baseline before starting a new scan. Holter electrocardiogram (ECG) was recorded throughout the experiment. Heart rate and HRV during rectal distention were analyzed later. Immediately after each distention of the rectum, blood was withdrawn via the left cubital vein for later analysis of plasma catecholamines.

### Rectal Distention

Rectal distentions were induced with a computerized barostat (Medtronics Synectics, Shoreview, Minnesota), which inflated the thin polyethylene bag at rate of 38 ml $s^{-1}$. The maximal volume of the barostat bag was 500 ml and the maximal diameter of the bag at full inflation was 10 cm. The first stimulus was always without rectal distention (namely, baseline). Subjects then received rectal distention with an intensity of 0 (sham stimulation), 20, or 40 mm Hg. The intensities of three rectal distentions were ordered randomly to avoid order effect. Average intensities of second, third, and fourth stimulations are not significantly different among each other in one-way analysis of variance (ANOVA). There was a lag time of 6 seconds before reaching peak pressure after initiation of the stimuli. The stimuli continued for 80 seconds, a period which matched the duration of the PET scan.

### Heart Rate and HRV

Data were analyzed from the recorded Holter ECG, and stimulation was marked with a specific key input. Premature ventricular or supraventricular contractions were reduced by a signal analyzer (SCM 6000, Fukuda Denshi, Tokyo). R-R intervals during stimulation were calculated by a computer software (R-R Interval Analyzing Program, HPS-RRA, Fukuda Denshi, Tokyo), which provided values for 64 seconds. Heart rate and HRV were then obtained at each four stimulation. Overall spectral analysis was applied to compute the major frequency components of HRV signal, the low-frequency (LF) band (0.04–0.15 Hz), the high-frequency (HF) band (0.15–0.4 Hz), and LF/HF ratio. The LF is under the sympathetic and parasympathetic control, whereas the HF is under the parasympathetic control (19–21). Increased LF/HF ratio reflects an increase in cardiac sympathetic tone (21,22).

### Plasma Catecholamines

Blood (16 ml) was withdrawn from the left cubital vein immediately after each distention, mixed with disodium ethylenediamine tetreaacetic acid, and centrifuged at 3000 rpm at 4°C. Separated plasma was then frozen and stored at −40°C. On the day of assay, the frozen plasma was defrosted, and plasma catecholamines were determined using high performance liquid chromatography with electrochemical detection after batch alumina extraction. Detection limits of adrenaline and noradrenaline were 2.56 pg/ml and 1.35 pg/ml, respectively. Intra-assay variances of adrenaline and noradrenaline were 0.50% and 0.55%, respectively. Interassay variances of adrenaline and noradrenaline were 1.77% and 2.27%, respectively.

### PET Scan

The method for brain imaging was essentially the same as that described in our previous studies (16,23). A plaster head support was set for each subject to minimize head movements during PET imaging. $H_2{}^{15}O$ (Tohoku University Cyclotron Radioisotope Center, Sendai, Japan) was injected into the right arm vein at the beginning of rectal distention. Ten seconds later, both radioactivity and peak pressure of the bag reached a plateau. As the radioactivity detected in the brain is proportional to the volume of regional cerebral blood flow (rCBF) (24), an increase in rCBF is seen as an index of neural activity evoked by stimulation (25,26). Using a $^{68}Ge/^{68}Ga$ radiation source, transmission scan for γ-ray absorption was corrected before PET scanning. PET scanning room was darkened and the subjects, while awake, were instructed to keep their eyes closed for the whole period of the scan (70 seconds). The rCBF in each subject was measured during four scans (70 seconds each), using a PET scanner in three-dimension sampling mode (HEADTOME V SET-2400W, Shimizu, Kyoto, Japan). The scanner produced 63 horizontal slices with a separation of 3.125 mm, an axial field of view of 200 mm, an in-plate resolution of 590 mm, a full width at half maximum (FWHM), and an axial resolution of 3.9 mm FWHM (27).

PET data were transferred to a super computer (NEC, SX-4/128H4, Tohoku University Computer Center, Sendai, Japan) and PET images were reconstructed, using three-dimensional filtered back projection algorithm (28–30). PET images were analyzed according to the method of Friston et al. (31–36), using a statistical parametric mapping software (SPM2, Wellcome Department of Cognitive Neurology, London, UK). PET images were realigned, spatially normalized, and transformed into approximates in Talairach-Tournoux stereotactic space (37). Finally, the images were smoothed by a 3D Gaussian filter (FWHM = 13 mm) and proportionally scaled to account for global confounders.

### Analysis

Values of changes in heart rate, LF, HF, LF/HF ratio, and plasma levels of catecholamines were analyzed by one-way ANOVA. In cases where significant interactions were found in the ANOVAs, post hoc analysis using Ryan's method ($p < .05$) were conducted to examine which combinations of rectal distention intensities differed significantly. To estimate rCBF differences between baseline and each rectal distention, an intragroup comparison was made using "population main effect: two conditions, one scan/condition (paired $t$ test)" SPM model. To evaluate the covariation between heart rate, LF/HF ratio, or catecholamine levels and rCBF during two conditions (baseline and intensity of rectal distention), regression with all ratings was performed by entering the values of heart rate, LF/HF ratio, and catecholamine levels as covariates of interest in "multi subjects, covariate only" SPM model (38). First, a level of significance was set at ≤0.1% (uncorrected for multiple comparisons) as the region of significant correction. Second, much more significant analysis were performed with a level of significance of ≤5% with

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**TABLE 1. Changes in Heart Rate, Heart Rate Variability, and Plasma Catecholamines Induced by Rectal Distention**

| Parameters/Bag Pressure | Baseline (0 mm Hg) | Sham (0 mm Hg) | Mild (20 mm Hg) | Intense (40 mm Hg) |
|---|---|---|---|---|
| Heart rate (bpm) | 58.5 ± 3.8 | 60.3 ± 3.9 | 62.2 ± 3.5 | 72.0 ± 4.8* |
| LF ($bpm^2$) | 1723 ± 801 | 2429 ± 889 | 3043 ± 1825 | 1293 ± 491 |
| HF ($bpm^2$) | 2705 ± 1259 | 2070 ± 846 | 2129 ± 816 | 489 ± 134 |
| LF/HF | 0.98 ± 0.30 | 1.68 ± 0.40 | 1.42 ± 0.35 | 2.85 ± 0.54* |
| adrenaline (pg/ml) | 29 ± 5 | 27 ± 5 | 30 ± 7 | 44 ± 9* |
| noradrenaline (pg/ml) | 196 ± 24 | 185 ± 21 | 212 ± 25 | 216 ± 21 |

Values are mean ± standard error of the mean ($n$ = 12).
HR = heart rate; LF = low-frequency power expressed as an integrated area; HF = high-frequency power expressed as an integrated area; LF/HF = area power ratio; bpm = beats/minute.
Values significantly different from the baseline are shown as follows: *$p < .001$.

**TABLE 2. Activated Brain Areas That Were Significantly and Positively Covariated With Increased Heart Rate During Rectal Distention With 40 mm Hg**

| Region (Brodmann Area) | Side | Coordinates (x, y, z) | T Score | Voxels in Cluster |
|---|---|---|---|---|
| Cerebellum*** (vermis) | — | 0, −54, −22 | 19.50 | 2162 |
| Cerebellum* | R | 28, −44, −50 | 6.45 | 126 |
| Cerebellum* | R | 8, −54, −50 | 5.18 | 36 |
| Middle insula** | R | 36, 0, −2 | 7.95 | 612 |
| Periaqueductal grey** | — | −2, −30, 0 | 7.87 | 229 |
| Primary motor cortex** ($^{4}$) | L | −12, −30, 70 | 7.78 | 49 |
| Operculum* ($^{42}$) | R | 38, −14, 20 | 7.06 | 168 |
| Dorsolateral prefrontal cortex* ($^{10,46}$) | R | 46, 58, 6 | 5.65 | 50 |
| Putamen* | L | −20, 6, 18 | 5.57 | 79 |
| Supplementary motor cortex* ($^{8}$) | L | −58, 12, 38 | 5.08 | 21 |
| Thalamus* | L | −16, −32, 16 | 5.24 | 157 |
| Thalamus* | L | −10, −6, 6 | 4.57 | 28 |

Coordinates refer to location in stereotaxic space. The table shows maxima of search values. Height threshold: T = 4.02, $p < .001$. Extent threshold k = 20 voxels, $p < .234$ (uncorrected). Corrected $p < .05$*, 0.01**, and 0.001*** for multiple comparisons.

correction for multiple comparisons. Significantly activated regions were identified on the basis of Talairach coordinates (37).

## RESULTS

### Changes in Heart Rate, HRV, and Plasma Catecholamines Levels Induced by Rectal Distention

Rectal distention with an intensity of 40 mm Hg produced significant increases in heart rate ($p < .001$), LF/HF ratio ($p < .001$), and plasma adrenaline ($p < .001$), compared with T1 baseline (Table 1). Changes in LF, HF, and plasma noradrenaline were not significant. The sham (0 mm Hg) or 20 mm Hg stimulation did not evoke any significant autonomic response.

### Functional Module of the Brain in Proportion to Increase in Heart Rate

Intense rectal distention (40 mm Hg) significantly increased rCBF in the previously reported visceral pain circuit, i.e., left thalamus, middle portion of the right insula, right operculum, bilateral putamen, periaqueductal gray, cerebellar vermis, and bilateral cerebellum ($p < .001$, uncorrected, data not shown).

Brain regions that showed significant positive covariation between the increase in rCBF and that in heart rate during 40 T2 mm Hg rectal distention are shown in Table 2. Activity in the middle portion of the right insula, right operculum, and right dorsolateral prefrontal cortex showed significant positive covariation with heart rate ($p < .001$, uncorrected) (Figure 1). In F1 addition to these regions, rCBF in the left thalamus, periaqueductal gray, left primary motor cortex, left supplementary motor cortex, left putamen, cerebellar vermis, and right cerebellum were significantly covariated with heart rate.

### Functional Module of the Brain in Proportion to Increase in LF/HF

Regions of the brain where the increase in rCBF significantly and positively were covariated with that in LF/HF ratio during rectal distention with 40 mm Hg are shown in Table 3. Activity in the posterior portion of the bilateral T3 insula, right anterior insula, and bilateral putamen showed significant positive covariation with LF/HF ratio ($p < .001$, uncorrected) (Figure 2). Besides, significantly positive co- F2 variation between the increase in rCBF and that in LF/HF ratio was found in the right superior frontal gyrus, left thalamus, midbrain, pons, bilateral cerebellar hemisphere, and cerebellar vermis.

### Functional Module of the Brain in Proportion to Increase in Plasma Adrenaline

Significant positive covariation between the increase in rCBF and that in plasma adrenaline during 40 mm Hg rectal

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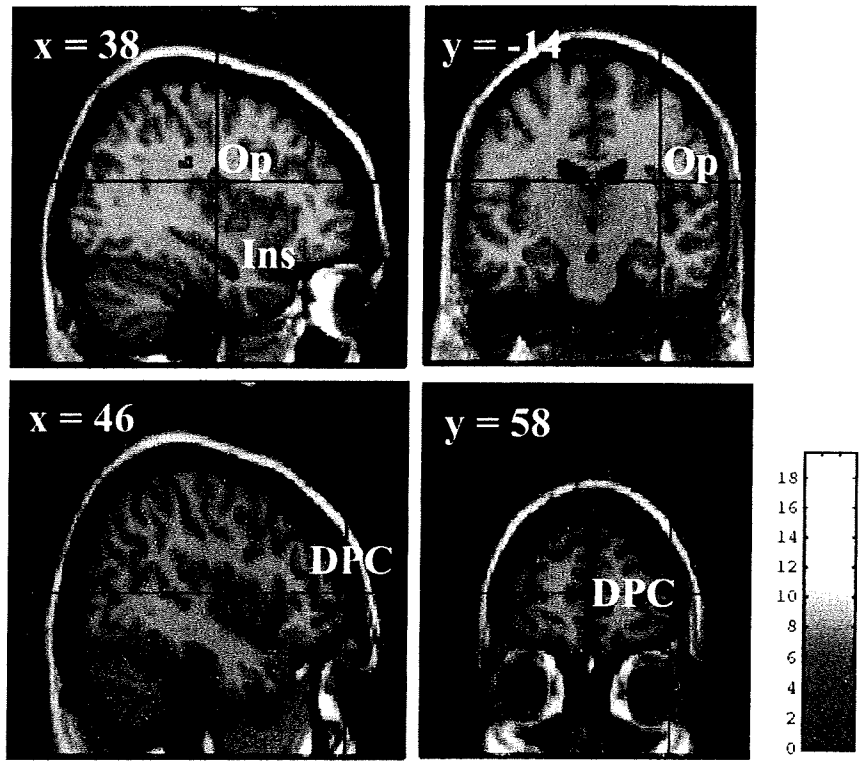


Figure 1. Activity in the middle portion of the right insula (Ins) (36, 0, −2), the right operculum (Op) (38, −14, 20), and the right dorsolateral prefrontal cortex (RPC) (46, 58, 6) positively covariated with increased heart rate during rectal distention with an intensity of 40 mm Hg. Results of covariation analysis were displayed on selected slices of the magnetic resonance imaging template available in SPM2 system. Coordinates are relative to anterior commeasure in the interaural (x), anterior-posterior (y), and superior-inferior (z) directions. Color calibration bars that apply to each image represent critical T-score magnitude of the covariated areas with a threshold voxel $\alpha$ level of $p < .001$ (uncorrected).

**TABLE 3. Activated Brain Areas That Were Significantly and Positively Covariated With Increased LF/HF Ratio During Rectal Distention With 40 mm Hg**

| Region (Brodmann Area) | Side | Coordinates (x, y, z) | T Score | Voxels in Cluster |
|---|---|---|---|---|
| Cerebellum* | L | −18, −62, −26 | 9.04 | 180 |
| Cerebellum* | R | 32, −74, −50 | 8.83 | 434 |
| Cerebellum (vermis) | — | 10, −64, −28 | 5.50 | 346 |
| Cerebellum | L | −14, −53, −44 | 5.08 | 53 |
| Cerebellum | L | −38, −40, −46 | 4.63 | 27 |
| Cerebellum | L | −46, −76, −34 | 4.52 | 42 |
| Superior frontal gyrus* ([6]) | R | 6, −18, 84 | 8.47 | 30 |
| Pons* | — | −4, −32, −30 | 7.35 | 592 |
| Putamen* | L | −20, 14, 8 | 7.23 | 310 |
| Putamen* | R | 14, 10, −8 | 6.42 | 36 |
| Anterior insula* | R | 36, −12, 4 | 7.07 | 251 |
| Posterior insula* | L | −30, −16, 4 | 5.98 | 179 |
| Posterior insula | R | 30, 10, 12 | 5.03 | 88 |
| Thalamus | L | −14, −32, 6 | 5.00 | 34 |
| Midbrain region | — | 16, −22, −6 | 4.87 | 52 |

Coordinates refer to location in stereotaxic space. The table shows maxima of search values. Height threshold: T = 4.02, $p < .001$. Extent threshold k = 20 voxels, $p < .264$ (uncorrected). Corrected $p < .05$* for multiple comparisons.

T4 distention are shown in Table 4. A significant positive covariation between the increase in rCBF and that in plasma adrenaline was detected in the anterior portion of the right insula, right orbitofrontal cortex, and right parahippocampal gyrus ($p < .001$, uncorrected) (Figure 3). Moreover, the

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increase in rCBF in the right superior frontal gyrus, bilateral putamen, bilateral thalamus, periagueductal gray, pons, and bilateral cerebellar hemisphere were significantly and positively covariation with the increase in plasma adrenaline.

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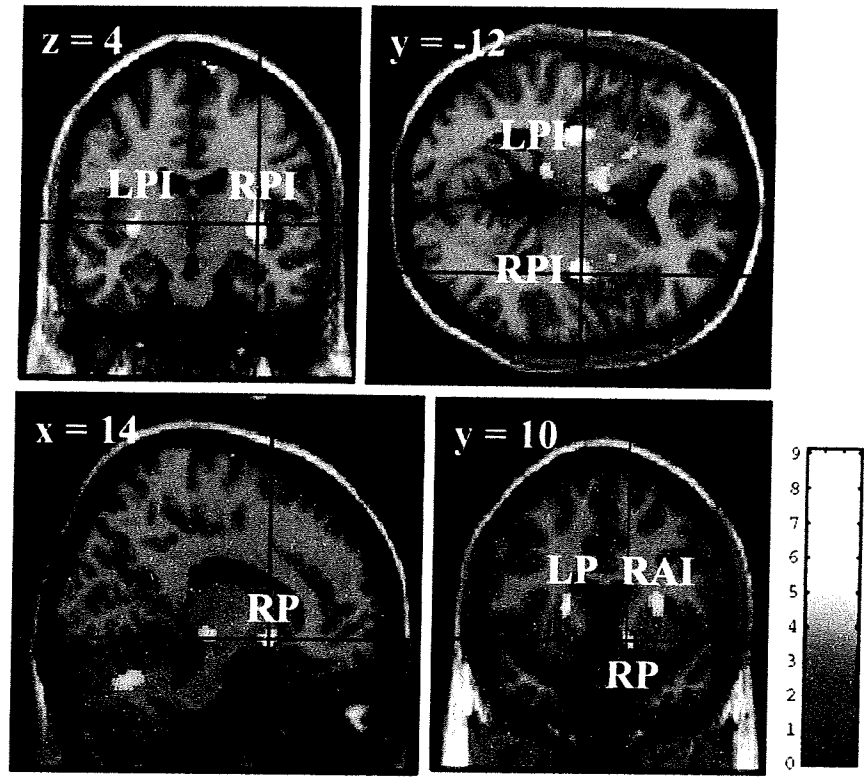


Figure 2. Activity in the right posterior insula (RPI) (36, 0, −2), the left posterior insula (LPI) (−30, −16, 4), the right anterior insula (RAI) (30, 10, 12), the left putamen (LP) (−20, 14, 8), and the right putamen (RP) (14, 10, −8) positively covariated with increased LF/HF ratio during rectal distention with an intensity of 40 mm Hg. The RP is in the caudal portion whereas the LP is more rostral. Results of covariation analysis were displayed on selected slices of the magnetic resonance imaging template available in SPM2 system. Coordinates are relative to anterior commeasure in the interaural (x), anterior-posterior (y), and superior-inferior (z) directions. Color calibration bars that apply to each image represent critical T-score magnitude of the covariated areas with a threshold voxel $\alpha$ level of $p < .001$ (uncorrected).

**TABLE 4. Activated Brain Areas That Were Significantly and Positively Covariated With Increased Plasma Adrenaline During Rectal Distention With 40 mm Hg**

| Region (Brodmann Area) | Side | Coordinates (x, y, z) | T Score | Voxels in Cluster |
|---|---|---|---|---|
| Cerebellum* | R | 14, −50, −34 | 8.14 | 1442 |
| Cerebellum* | L | −26, −68, −50 | 6.13 | 324 |
| Putamen* | L | −20, −2, 18 | 8.10 | 674 |
| Putamen | R | 18, −2, 10 | 4.36 | 47 |
| Anterior insula* | R | 26, 16, 18 | 7.24 | 324 |
| Periaqueductal grey* | — | −8, −20, −10 | 6.30 | 109 |
| Orbitofrontal cortex* ([11]) | R | 28, 44, −18 | 6.00 | 25 |
| Superior frontal gyrus* ([6]) | R | 14, −12, 80 | 5.78 | 22 |
| Thalamus* | R | 16, −16, 24 | 5.50 | 332 |
| Thalamus* | L | −18, −36, 8 | 4.70 | 52 |
| Pons* | L | −14, −32, −28 | 5.43 | 23 |
| Parahippocampal gyrus ([28]) | R | 20, −26, −8 | 4.51 | 26 |

Coordinates refer to location in stereotaxic space. The table shows maxima of search values. Height threshold: T = 4.02, $p < .001$. Extent threshold k = 20 voxels, $p < .265$ (uncorrected). Corrected $p < .05$* for multiple comparisons.

## DISCUSSION

This study is the first to demonstrate that cortical and subcortical brain activation was covariation with increase in three different autonomic indices, heart rate, LF/HF ratio, and plasma adrenaline during rectal distention. Regions of the brain that were significantly and positively covariated with changes in these three autonomic systems were the right insula, thalamus, putamen, periaqueductal gray, pons, and cerebellum. Regions of the brain that were covariated with heart rate only were the right operculum and the right dorsolateral prefrontal cortex, whereas those that were covariated with plasma adrenaline only were the right orbitofrontal cor-

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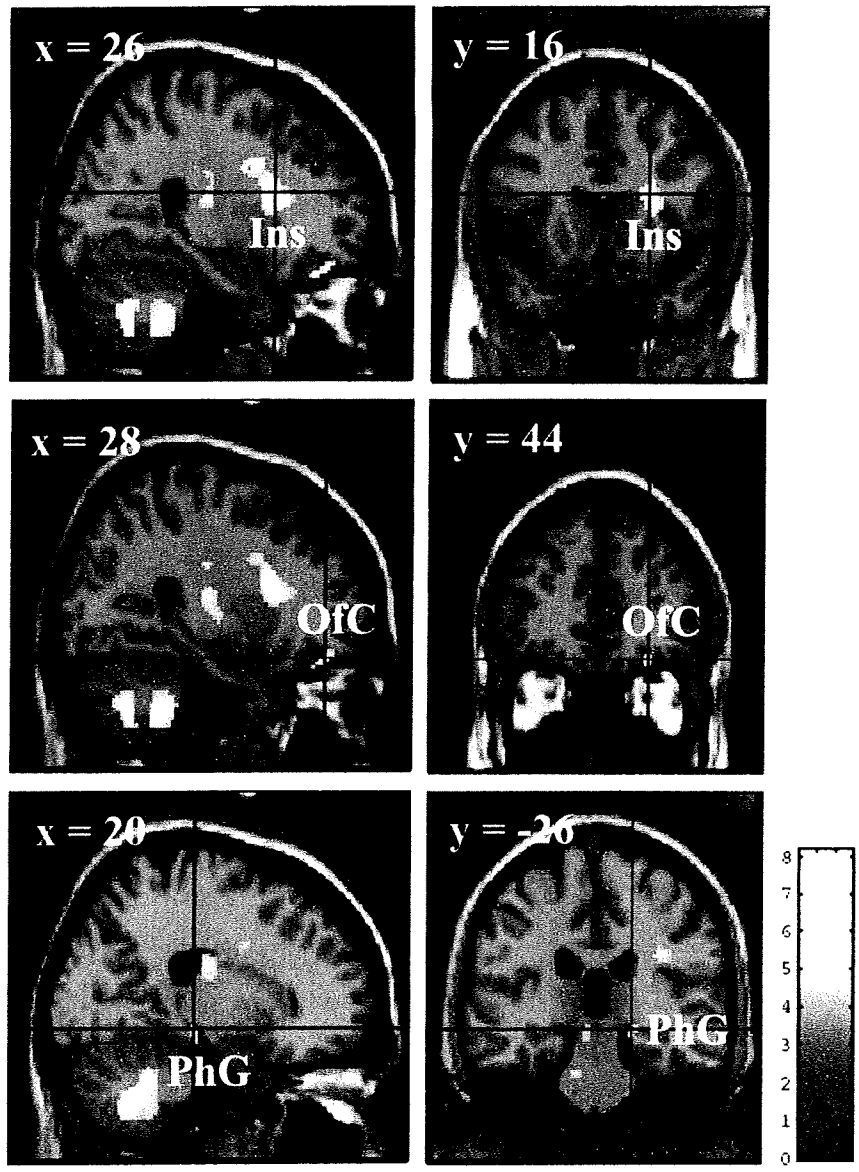


Figure 3. Activity in the anterior portion of the right insula (Ins) (26, 16, 18), the right orbitofrontal cortex (Orf) (28, 44, −18), and the right parahippocampal gyrus (PhG) (20, −26, −8) positively covariated with increased plasma adrenaline during rectal distention with an intensity of 40 mm Hg. Results of covariation analysis were displayed on selected slices of the magnetic resonance imaging template available in SPM2 system. Coordinates are relative to anterior commeasure in the interaural (x), anterior-posterior (y), and superior-inferior (z) directions. Color calibration bars that apply to each image represent critical T-score magnitude of the covariated areas with a threshold voxel $\alpha$ level of $p < .001$ (uncorrected).

tex and the right parahippocampal gyrus. The only region that was covariated with LF/HF ratio was the left insula. These findings show that activation of specific brain regions is associated with changes in a specific autonomic system.

Activation of the insula was covariated with increases in heart rate, LF/HF ratio, and plasma adrenaline. The insula has been reported to be involved in the processing of emotion via mapping and/or regulation of internal body states (39). In addition, the anterior portion of the insula has been shown to be involved in interoception, a sensation of body physiological conditions (40). Evidence has shown that the insula is activated during stimulation of the rectum (18) and the descending colon (16). Changes in the activity of the insula have been reported to correlate with changes in heart period during mental tasks (10). Besides, subjects' accuracy in heart beat detection task can be predicted by neural activity in the right insular/opercular cortex (41). In our previous study, activation of the insula was associated with discrimination between mild (20 mm Hg) and intense (40 mm Hg) colonic distention (16). Therefore, the insula may be activated by unusual internal signals that stimulate the sympathetic nervous system for homeostatic regulation.

## rCBF COVARIATED WITH AUTONOMIC AROUSAL

Activated brain regions that were covariated with an increase in heart rate only were the right operculum and the right dorsolateral prefrontal cortex. The frontoparietal operculum is activated by esophageal stimulation and is suggested to be involved in the control of facial, masticatory, lingual, and pharyngeal musculature (13). Besides, the right opercular region is associated with interoception of heartbeats (41). The dorsolateral prefrontal cortex, on the other hand, has reciprocal connections with other brain regions including the higher-order sensory cortices (42). In our previous study, activation of the Brodmann's area (BA)10 was covariated with feelings in the gut (16). From these findings, it is suggested that the right dorsolateral prefrontal cortex and the right operculum, working in collaboration with the insula, participate in interoception-induced acceleration of heart rate. The only brain region with increased rCBF that was covariated with increased LF/HF ratio was the left insula. Increased LF/HF ratio reflects sympathetic arousal (21,22). It has been reported that sympathetic arousal is predominantly controlled by the right hemisphere (4,43). However, there are reports that indicate activity in the bilateral insula covaries with sympathetic nervous activity (9,10). LF/HF ratio is commonly believed to be associated with decrease in parasympathetic activity as well as sympathetic arousal (21). Stimulation of left insula decreased in heart rate, indicating an association with left insula and parasympathetic activity (2). Therefore, the covariation between activity in left insula and increase in LF/HF ratio in this experiment might reflect both or either decrease in parasympathetic activity of HF components or even increase in parasympathetic activity of LF components.

The only brain regions that showed increased rCBF covariated with increased plasma adrenaline were the right orbitofrontal cortex and the right parahippocampal gyrus. The orbitofrontal cortex has direct reciprocal connections with brain structures, such as the insula/operculum, the dorsolateral prefrontal cortex, the amygdala, and the hippocampus, and participates in multiple functions including the processing of emotion and sensory integration (44). The parahippocampal gyrus, on the other hand, conducts memory encoding and retrieval in cooperation with other medial temporal regions like the hippocampus and the amygdala (45). Memory encoding is strengthened by emotion, and adrenaline promotes emotional memory formation (46). Therefore, the right orbitofrontal cortex and the right parahippocampal gyrus may work together to induce arousal of emotion (gut feeling) and memory formation (unpleasant memory) accompanied by increase in plasma adrenaline during rectal distention.

In our experiments, there were activated brain regions that were covariated with increases in heart rate, LF/HF ratio, and plasma adrenaline. Among them, the thalamus, which is the gate of sensory information to the brain, is well known to be activated by visceral stimulation (16). In addition to the nucleus of solitary tract, the parabrachial nucleus in pons and the periaqueductal gray in midbrain are well-established components of the brainstem autonomic center (40,47). The periaqueductal gray regulates coordinated behavioral and autonomic responses (48), which can explain activation of motor-related brain areas accompanied by sympathetic arousal in this study. The cerebellum is also important in autonomic regulation (7). In a recent human study, patients with medial cerebellar lesions have been shown to lose fear-conditioned changes in heart rate (49). Co-occurrence of emotional flattening and autonomic reactions was also seen in a patient after left cerebellar infarction (50). Brain regions with increased rCBF that were covariated with autonomic arousal were the bilateral putamen but the right one was located more caudally than the left one. The caudal ventromedial striatum receives inputs from several limbic brain areas like the amygdala and the anterior insula, whereas the rostral striatum primarily regulates motor function (51). However, the majority of patients with pure autonomic failure and multiple system atrophy have an intact striatum (52), and electrical stimulation of the putamen does not induce remarkable changes in blood pressure or heart rate (53). Therefore, activation of the right putamen in our experiments does not directly control sympathetic regulation but may be responsible for other actions accompanied by sympathetic activity. The superior frontal gyrus (BA6) receives inputs from the insula (54), explaining the covariation of BA6 with LF/HF ratio and plasma adrenaline. Therefore, the activated brain regions except for the putamen were in plausible association with autonomic regulation and emotion during the interoception.

RCBF in the amygdala, an important component of autonomic arousal accompanied by emotion, was not covariated with changes in the three autonomic variables. There are two possible explanations for this result. The first is that activation of the amygdala might be transient in our experiments. In a fear conditioning study, firing of the amygdala was limited in the earlier phase of the experiment (55). Because PET brain image needs 70 seconds, the methodology may limit the detection. The second explanation is that the amygdala is not necessary for autonomic and emotional arousal during interoception. Although the amygdala is easily activated by fearful visual stimuli (56), its vulnerability to interoception is unknown. Most functional neuroimaging studies in gastrointestinal stimulation showed no activation of the amygdala (14–18). Therefore, the amygdala may not play as important a role in sympathetic arousal by visceral sensation as the other activated brain regions.

The important point of our study is lack of covariation between increased rCBF in the anterior cingulate cortex and changes in the three autonomic variables. The anterior cingulate cortex is known to be a motor center of the limbic system and is responsible for emotional and autonomic arousal (40). The first explanation of no detectable covariation of activity in the anterior cingulate cortex in this study is due to the fact that only male subjects participated in this study. Males show less activation of the anterior cingulate cortex in response to rectal distention than females (57). The second explanation is based on the intensity of stimulation. Vague stimulation can hardly activate the anterior cingulate cortex whereas discrete stimulation can easily fire the anterior cingulate cortex (15–17). It

was reported that activity of anterior cingulate cortex was covariated with intensity of urgency during rectal distention with 40 mm Hg in healthy male subjects (16). Thus, anterior cingulate cortex is activated to process a part of the feeling but is not associated with autonomic arousal, the bodily state, in healthy male subjects in visceral sensation, the lower hierarchy of emotional processing.

In conclusion, the results of this study support the two hypotheses we set as aims of the study: a) rectal distention provokes changes in heart rate, HRV, and serum catecholamine levels and b) brain regions that show activity, which is covariated with autonomic changes during rectal distention, are identifiable. These brain regions are: the right insula, thalamus, putamen, periaqueductal gray, pons, and cerebellum as well as the right operculum, the right dorsolateral prefrontal cortex, left insula, right orbitofrontal cortex, and right parahippocampal gyrus.

*This study was presented at the 64th Annual Meeting of the American Psychosomatic Society in 2006 and was awarded the Scholar Award of the American Psychosomatic Society.*

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# The roles of histamine $H_1$ receptors on cognition

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## Introduction

Histamine neurons are located exclusively in the posterior hypothalamus, from where they project diffusely to all regions of brain. Neuronal histamine has been implicated in a variety of brain functions including wakefulness, learning and memory [1]. Although it is well known that sedative antihistamines induce cognitive decline in humans through blockage of $H_1$ receptor [2], both facilitatory and inhibitory effects of neuronal histamine on learning and memory have been described in animal behavioral studies [3, 4]. Histaminergic neurotransmission has been also implicated in pathophysiology of stress-related psychiatric diseases [5]. Although several atypical antipsychotics are potent $H_1$ antagonists [6], the clinical significance of interaction between atypical antipsychotics and $H_1$ receptors is still unknown. The aim of this study was to investigate the role of histamine $H_1$ receptors on cognition in normal conditions using $H_1$ receptor gene knockout mice ($H_1$KO). We also investigated the effects of $H_1$ receptor blockade on social isolation-induced behavioral and neurochemical changes in $H_1$KO mice and their wild-type (WT) mice.

## Materials and methods

Experiment 1: Under socially-reared normal conditions, learning and memory were evaluated in $H_1$KO mice by object recognition, Barnes maze and fear conditioning tests. These behavioral tasks are dependent on the function of prefrontal cortex, hippocampus or amygdala. Furthermore, we also examined long-term potentiation (LTP) in CA1 area of hippocampus in $H_1$KO mice and their WT mice.

Experiment 2: $H_1$KO and WT mice were subjected to social isolation immediately after weaning. After 4-week social isolation, locomotion, pre-pulse inhibition (PPI) of startle response and Morris water maze were evaluated. After the experiments, contents of monoamines were measured by HPLC.

All experimental protocols were approved by the Animal Care Committee of Tohoku University, and all experiments were performed in compliance with relevant laws and institutional guidelines.

## Results and discussions

Results are summarized in Table 1 and Figure 1. In normal socially-reared conditions, object recognition and Barnes maze performance were significantly impaired in $H_1$KO mice when compared to the wild-type (WT) mice. Conversely, $H_1$KO mice showed better auditory and contextual freezing acquisition than their respective WT mice. LTP in CA1 area of hippocampus was significantly reduced in $H_1$KO mice when compared with their respective WT mice. Auditory fear conditioning tests are appropriate behavioral tasks for assessing emotional memory. Auditory fear conditioning depends on activation of the amygdala. The prefrontal cortex, where densities of $H_1$ receptors are high, has the potential to regulate affective processes by inhibition of lateral nucleus of amygdala. Therefore potentiation of conditioned freezing behavior was described in rats subjected to lesions of medial prefrontal cortex [7]. This work suggests that histaminergic neuron system exerts a negative influence on freezing behavior through $H_1$ receptors. Our results of Experiment 1 demonstrate that $H_1$ receptors are involved in learning and

**Table 1.** Effects of $H_1$ receptor deficiency on cognition in socially-reared normal conditions.

| | Object recognition index | Latency of Barnes maze (4th Day) | Freezing time in fear conditioning | LTP |
|---|---|---|---|---|
| $H_1$KO | 25 % decrease * | 122 % increase * | 48 % increase * | 30 % decrease * |

Data of $H_1$KO mice were expressed by the % change when compared to those in WT mice. * Significant changes

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**Table 2.** Effect of social isolation on cognition in $H_1$KO and their WT mice.

| | Locomotion | PPI (71 bB prepulse) | Latency of Morris water maze (4th Day) | Dopamine turnover rate |
|---|---|---|---|---|
| $H_1$KO mice | 9 % decrease | 3 % decrease | 15 % increase | 21 % decrease |
| WT mice | 31 % decrease * | 55 % decrease * | 59 % increase * | 47 % increase * |

Data were expressed by the % change by social isolation when compared to group housing. * Significant changes

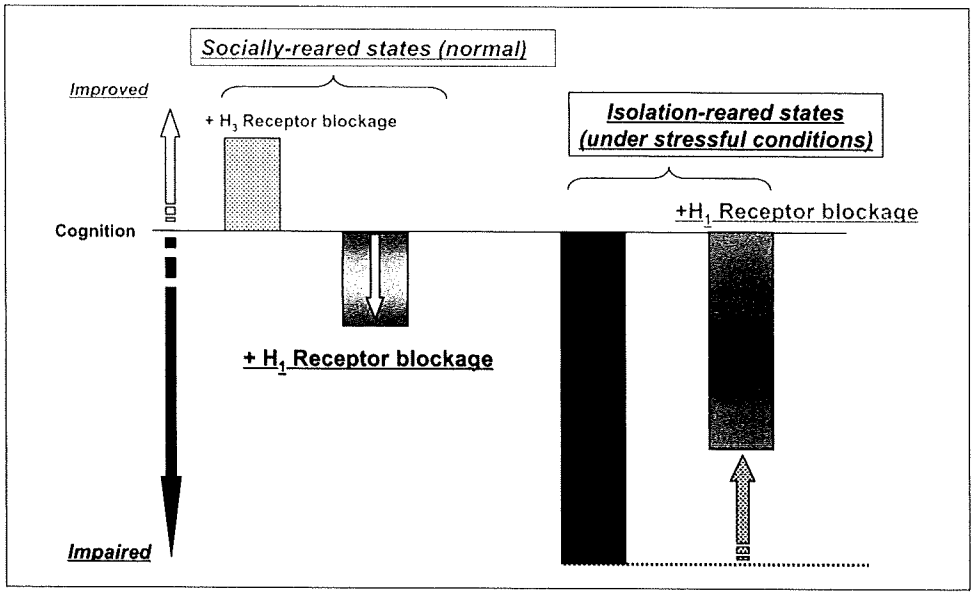


**Fig. 1.** Effects of histamine $H_1$ receptor blockage on cognition are states-dependent: A hypothesis.

memory processes for which the frontal cortex, amygdala and hippocampus interact.

In Experiment 2, locomotor activity in home cages was significantly lower in isolation-reared WT mice than in socially reared WT mice. However, no significant change in locomotor activity was observed between socially and isolation-reared $H_1$KO mice. Social isolation significantly impaired PPI of startle response in WT mice but not in $H_1$KO mice. Additionally, social isolation significantly impaired spatial learning and memory in WT mice but not in $H_1$KO mice. A neurochemical study revealed that isolation-reared WT mice had significantly lower dopamine (DA) levels and slightly increased DA turnover in cortex than socially reared WT mice. Conversely, isolation-reared $H_1$KO mice showed significantly higher DA contents as compared with socially reared $H_1$KO mice. Experiment 2 results indicate that blockage of $H_1$ receptor-mediated neurotransmission attenuates social isolation-induced behavioral and neurochemical changes and that therapeutic effects of atypical antipsychotics are mediated, at least in part, by interaction with $H_1$ receptors in the brain.

The conceptual hypothesis from this study is that the effects of histamine $H_1$, receptor blockage could be states-dependent (Fig. 1). In socially-reared normal states, blocking $H_1$ receptors impairs cognition. Sedative antihistamines induce cognitive decline in humans, and $H_3$ receptor blocking can stimulate activity of histamine neurons, and also improve cognition [8]. However, in isolation-reared states, this is under stressful condition, blockage of $H_1$ receptors attenuates impaired cognition induced by social isolation. This explains why both facilitatory and inhibitory effects of neuronal histamine on learning and memory have been described in animal behavioral studies.

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