Brain Activation Associated 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 associated 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 associated with an increased LF/HF ratio were the bilateral insula, putamen, thalamus, midbrain, pons, and cerebellum ($p < .001$, uncorrected). Activated brain areas that were associated 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; **SPM** = statistical parametric mapping; **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; **MRI** = magnetic resonance imaging.

INTRODUCTION

Emotion has been conceptualized as having two components: the bodily state and the feeling (conscious sensation) (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 brain stem, whereas the feeling involves the cerebral cortex.

However, neuroscience and patient studies have also demonstrated that the bodily state is associated with cortical brain regions that are important in the feeling (2–5). Subjective mood changes occasionally accompany electrical stimulation of brain regions such as the insula and anterior cingulate cortex, or prefrontal cortex inducing changes in blood pressure and heart rate (2, 3). In studies assessing autonomic changes during the performance of mental tasks, patients with dysfunction of the prefrontal cortex or anterior cingulate cortex have not shown the type of changes associated with autonomic arousal that is apparent in healthy subjects (4, 5). The patient with a 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 techniques have noninvasively examined the relationship between autonomic arousal and brain activity. Hand gripping, mental arithmetic, mental tasks and the Valsalva maneuver have been shown to activate the anterior cingulate cortex, insula, prefrontal cortex, amygdala, hippocampus, cerebellum, and brain stem (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), and rectum (17, 18). These brain areas include the anterior cingulate cortex, insula, prefrontal cortex, cerebellum and brain stem (14–18). However, we are aware of no study that has examined the association between activation of brain regions and autonomic activity during gastrointestinal stimulation.

Recently, the processing of emotion has been conceptualized as involving hierarchal structures, visceral sensation, action tendencies, unidimensional and multidimensional processing, and the integration of multidimensional processing (6). Emotion and identified brain regions that were associated with autonomic changes in earlier studies are multidimensional because complex cognitive tasks were used (7–10). Therefore, identified brain regions that were associated 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 previously 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

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brain activity and serum catecholamine levels have not been studied yet in humans.

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 are associated with changes in heart rate, heart rate variability and serum catecholamine levels during rectal distention are the anterior cingulated cortex, insula, prefrontal cortex, amygdala, hippocampus, cerebellum and brain stem.

MATERIALS AND METHODS

Subjects

Twelve healthy male volunteers (22 ± 1 standard error of the mean) were recruited through local advertisement between July 2004 and June 2005. Each volunteer provided a basic medical history and underwent a medical interview and physical examination so that subjects with organic diseases could be excluded. All subjects were right handed and had no signs or symptoms of gastrointestinal, cardiovascular, or psychotic disorders. They had been free of medication for more than ten days and indicated that they had not been taking illegal drugs, smoking, drinking alcohol heavily, or ingesting excessive caffeine. All subjects gave 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: SF), was approved by the Ethics Committee of Tohoku University School of Medicine.

Experimental Design

On the day before examination, the subjects ingested 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 then fasted overnight. The experiment began the next day at 10:00 AM. First, the subjects lay quietly on a bed for a positron emission tomography (PET) scan at the Cyclotron Radioisotope Center, Tohoku University. Two polyethylene catheters were inserted into the bilateral cubital vein. A saline drip infusion was started at the rate of 1 ml/ min. 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. To ensure that radioactivity levels returned to baseline before each new scan, an approximate break of 10 minutes was taken between successive distentions. Throughout the experiment, patients were monitored by Holter electrocardiogram (ECG). 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 a 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 baseline stimulus was always without rectal distention. Subjects then received rectal distention with an intensity of 0 (sham stimulation), 20, or 40 mm Hg. The intensities of three rectal distentions were randomly ordered to avoid order effect. Average intensities of second, third and fourth stimulations were not significantly different among each other in one way analysis of variance (ANOVAs). 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 PET scan.

Heart Rate and Heart Rate Variability

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 heart rate variability (HRV) were then obtained at each of four stimulations. Overall spectral analysis was applied to compute the major frequency components of HRV signal, the low-frequency band (LF, 0.04–0.15 Hz), the high-frequency band (HF, 0.15–0.4 Hz), and LF/HF ratio. The LF is under the sympathetic and parasympathetic control, while 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 drawn from the left cubital vein immediately after each distention, mixed with disodium ethylenediamine tetraacetic 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 catecholamine levels were determined through the use of 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. Inter-assay 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) 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. The PET scanning room was darkened and the subjects were instructed to keep their eyes closed for the 70-second period of the scan. 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) and PET images were reconstructed using a three-dimensional filtered back projection algorithm (28–30). PET images were analyzed according to the method of Friston et al. (31–36) using 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 analyses 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 a "population main effect: two conditions, one scan/condition (paired t-test)" statistical parametric mapping (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 catecholamines levels as covariates of interest in the "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, additional analyses were performed using a significance level of ≤5% with correction for multiple comparisons. Significantly activated regions were identified on the basis of Talairach coordinates (37).

RESULTS

Changes in heart rate, heart rate variability and plasma catecholamines induced by rectal distention

Rectal distention with an intensity of 40 mm Hg produced significant increase in heart rate ($p < .001$), LF/HF ratio ($p < .001$), and plasma adrenaline ($p < .001$), compared with baseline (Table 1). Changes in LF, HF, and plasma noradrenaline were not significant. The sham (0 mm Hg) and 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, ie, the 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 a significant positive covariation between the increase in rCBF and that in heart rate during 40 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 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 correlated with heart rate.

Functional module of the brain in proportion to increase in LF/HF

Regions of the brain where the increase in rCBF was significantly and positively correlated 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 insula, right anterior insula, and bilateral putamen showed significant positive covariation with LF/HF ratio ($p < .001$, uncorrected, Figure 2). Additionally, significantly positive covariation 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.

TABLE 2. Activated Brain Areas That Were Significantly and Positively Correlated 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 $p < .05$*, .01**, and .001*** for multiple comparisons.

Functional module of the brain in proportion to increase in plasma adrenaline

Significant positive covariations between the increase in rCBF and that in plasma adrenaline during 40 mm Hg rectal 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 increase in rCBF in the right superior frontal gyrus, bilateral putamen, bilateral thalamus, periagueductal gray, pons and bilateral cerebellar hemisphere were significantly and positively correlated with the increase in plasma adrenaline.

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 ($n = 12$).
Values significantly different from the baseline are shown as follows: *$p < .001$.
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/min.

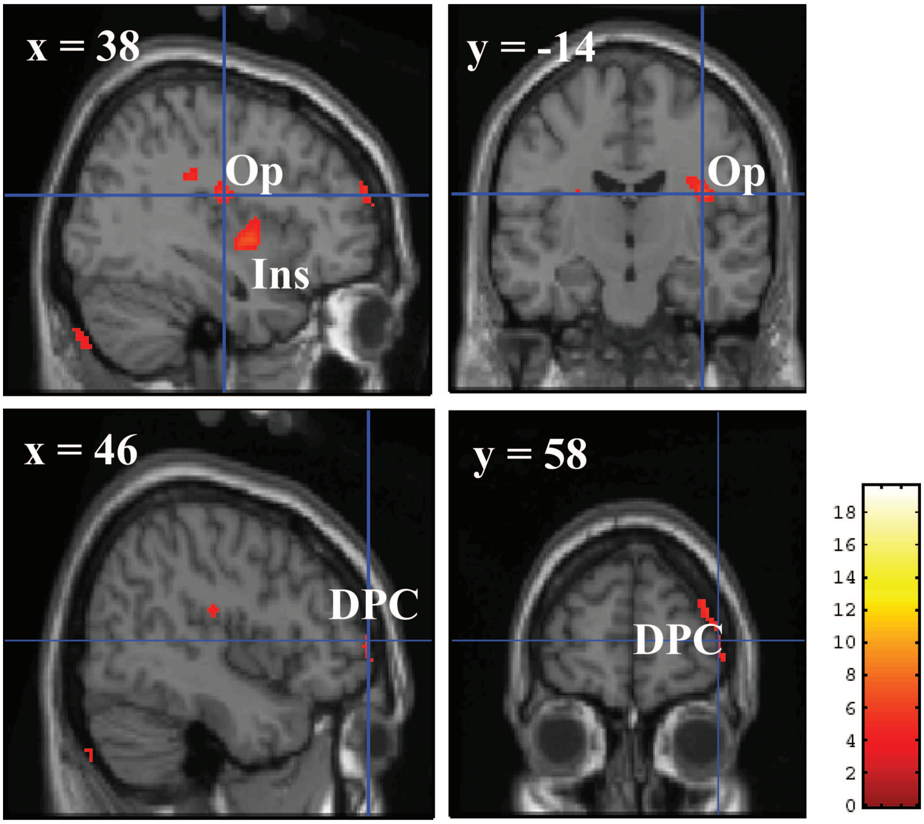

Figure 1. Activity in the middle portion of the right insula (Ins; 36, 0, −2), the right operculum (Op; 38, −14, 20), and the right dorsolateral prefrontal cortex (RPC; 46, 58, 6) positively correlated with increased heart rate during rectal distention with an intensity of 40 mm Hg. Results of covariation analysis were displayed on selected slices of the MRI 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 correlated areas with a threshold voxel alpha level of $p < .001$ (uncorrected).

TABLE 3. Activated Brain Areas That Were Significantly and Positively Correlated 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.

DISCUSSION

This study is the first to demonstrate that cortical and subcortical brain activation is correlated with increases in three different autonomic indices: heart rate, the LF/HF ratio, and plasma adrenaline during rectal distention. Regions of the brain that were significantly and positively correlated with changes in these three autonomic systems were the right insula, thalamus, putamen, periaqueductal gray, pons, and cerebellum. Regions of the brain that were correlated with heart rate only were the right operculum and the right dorsolateral prefrontal cortex, while those that were correlated with plasma adrenaline only were the right orbitofrontal cortex and the right parahippocampal gyrus. The only region that covaried with the LF/HF ratio was the left insula. These findings clearly show that activation of specific brain regions is associated with changes in a specific autonomic system.

Activation of the insula was associated with increases in heart rate, the 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). Research 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

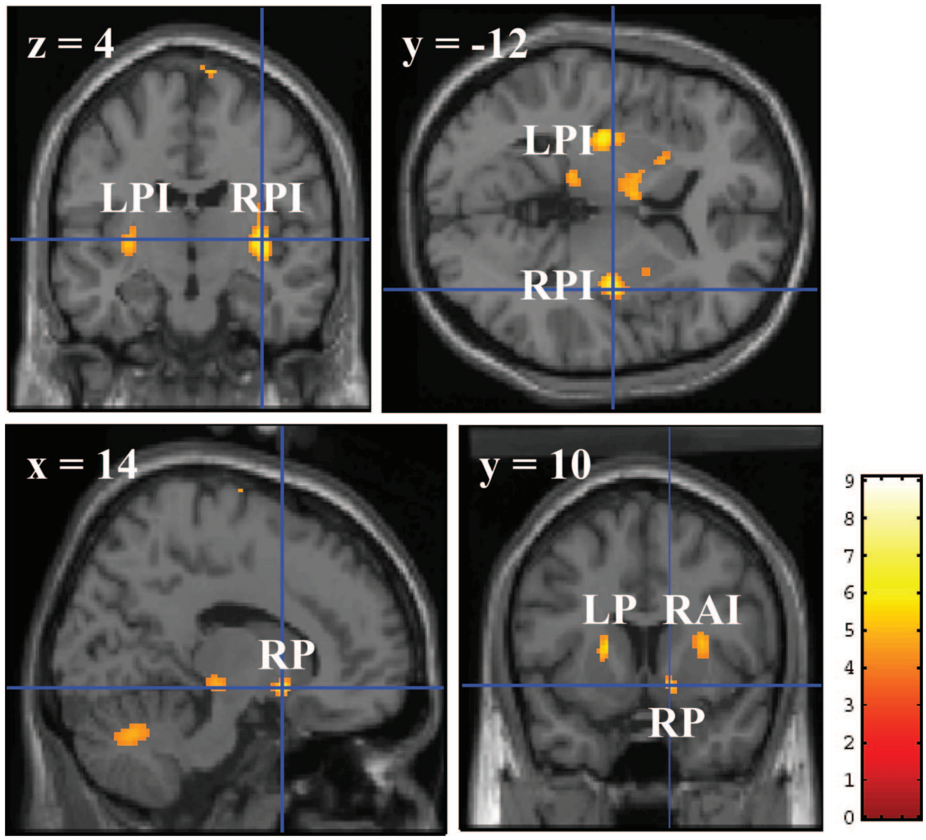

Figure 2. Activity in the right posterior insula (RPI; 36, 0, −2), the left posterior insula (LPI; −30, −16, 4), the right anterior insula (RAI; 30, 10, 12), the left putamen (LP; −20, 14, 8), and the right putamen (RP; 14, 10, −8) positively correlated with increased LF/HF ratio during rectal distention with an intensity of 40 mm Hg. The right putamen is in the caudal portion while the left putamen is more rostral. Results of covariation analysis were displayed on selected slices of the MRI 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 correlated areas with a threshold voxel alpha level of $p < .001$ (uncorrected).

TABLE 4. Activated Brain Areas That Were Significantly and Positively Associated 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
Superiorfrontal 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.

reported to to be associated with changes in heart period during mental tasks (10). 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.

Activated brain regions that were associated only with an increase in heart rate were the right operculum and the right dorsolateral prefrontal cortex. The frontoparietal operculum is activated by esophageal stimulation; it has been suggested that it is in involved in the control of facial, masticatory, lingual, and pharyngeal musculature (13). 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 correlated 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.

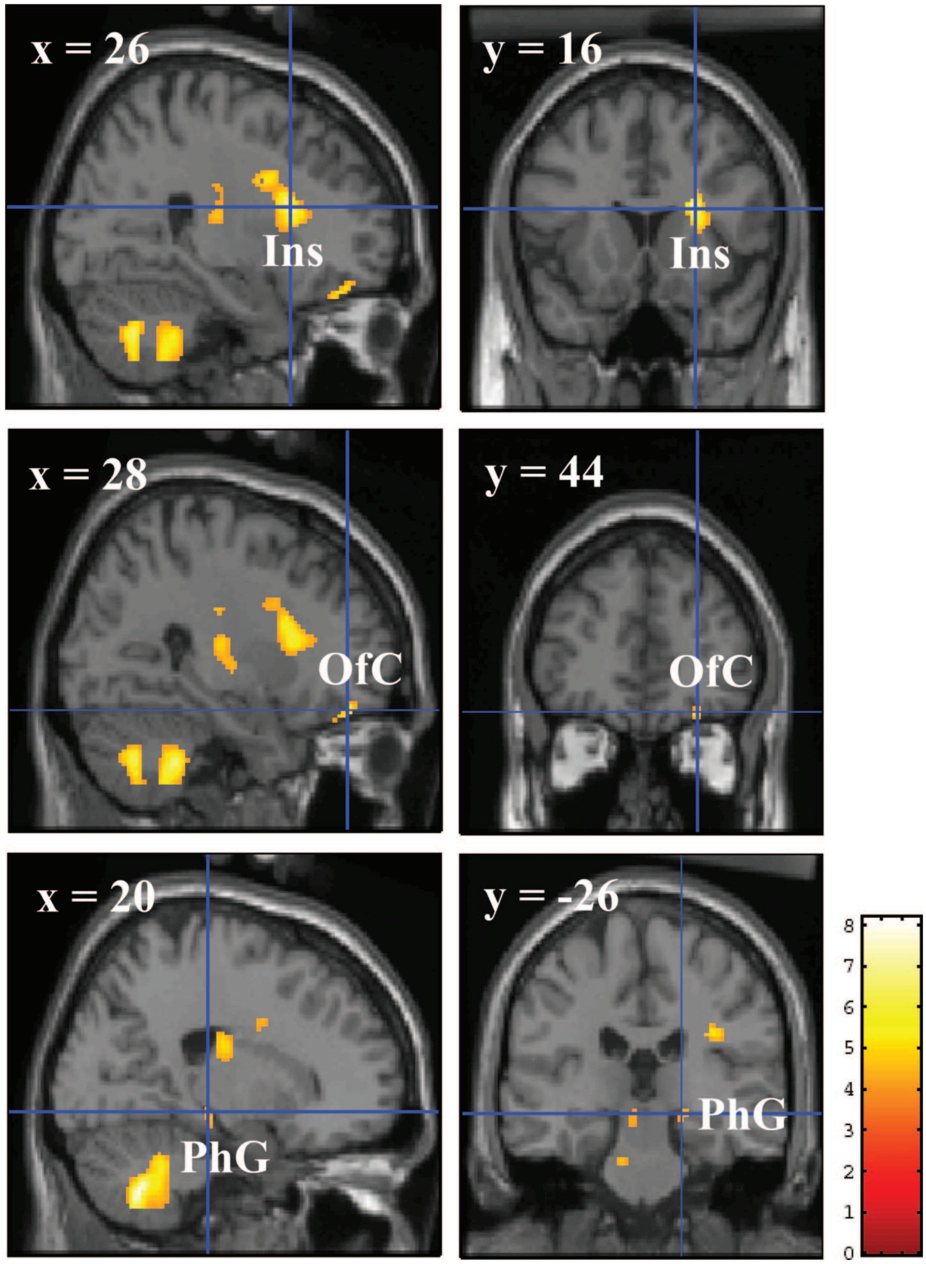

Figure 3. Activity in the anterior portion of the right insula (Ins; 26, 16, 18), the right orbitofrontal cortex (Orf; 28, 44, −18), and the right parahippocampal gyrus (PhG; 20, −26, −8) positively correlated with increased plasma adrenaline during rectal distention with an intensity of 40 mm Hg. Results of covariation analysis were displayed on selected slices of the MRI 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 correlated areas with a threshold voxel alpha level of $p < .001$ (uncorrected).

The only brain region with increased rCBF that was correlated 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). The LF/HF ratio is commonly believed to be associated with a decrease in parasympathetic activity as well as in sympathetic arousal (21). Stimulation of the left insula decreased heart rate, indicating an association between the left insula and parasympathetic activity (2). Therefore, the correlation between activity in the left insula and the increase in the LF/HF ratio in this experiment might reflect a decrease in parasympathetic activity of HF components and/or an increase in sympathetic activity of LF components.

The only brain regions that showed increased rCBF associated 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 it 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 such as 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 an increase in plasma adrenaline during rectal distention.

In our experiments, there were activations in brain regions that were correlated 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 the solitary tract, the parabrachial nucleus in the pons and the periaqueductal gray in the midbrain are well-established components of the brain stem autonomic center (40, 47). The periaqueductal gray regulates coordinated behavioral and autonomic responses (48), which can explain the 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 study, patients with medial cerebellar lesions were shown to have lost fear-conditioned changes in heart rate (49). Co-occurrence of emotional flattening and autonomic reactions have also been seen in a patient after a left cerebellar infarction (50). Brain regions with increased rCBF that were correlated 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 correlated 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 have shown 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 the 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). One explanation for the lack of detectable covariation of activity in the anterior cingulate cortex is 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). A second explanation relates to the intensity of stimulation. Vague stimulation can barely activate the anterior cingulate cortex whereas discrete stimulation can easily fire the anterior cingulate cortex (15–17). It has been reported that activity of the anterior cingulate cortex is associated with intensity of urgency during rectal distention with 40 mm Hg in healthy male subjects (16). Thus, the anterior cingulate cortex is activated to process a part of the feeling but it 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 our two hypotheses, i.e., (1) rectal distention provokes changes in heart rate, HRV, and serum catecholamine levels; (2) brain regions that show activity that is correlated 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.

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