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CONFLICT OF INTEREST

Guarantor of the article: William E. Whitehead, Ph.D.
Specific author contributions: William E. Whitehead participated in study design, data collection, data analysis, and manuscript preparation. Motoyori Kanazawa, participated in study design, data analysis, and preparation of the manuscript. Olafur S. Palsson, participated in study design and data analysis. Syed I.M. Thiwan, Lisa M. Gangarosa, Marsha J. Turner, Denesh K. Chitkara and Douglas A. Drossman participated in data collection. Miranda A.L. van Tilburg participated in data collection and data analysis. Shin Fukudo participated in data analysis and manuscript preparation.
Financial support: This study was supported by RO1 DK31369, R24 DK67674, and MO1 RR00046.
Potential competing interests: None.

NeuroImage 47 (2009) 946–951

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Impact of serotonin transporter gene polymorphism on brain activation by colorectal distention

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ARTICLE INFO

Article history:
Received 5 January 2009
Revised 17 April 2009
Accepted 22 April 2009
Available online 6 May 2009

ABSTRACT

Background and aims: Determining the gene that plays a key role in brain–gut interactions is a crucial step for clarifying the pathophysiology of irritable bowel syndrome (IBS). We previously reported that the 5-hydroxytryptamine transporter gene-linked polymorphic region (5-HTTLPR) is related to anxiety in subjects with IBS. The amygdala is more activated during fearful face recognition in individuals with the s allele of 5-HTTLPR. Here, we tested our hypothesis that 5-HTTLPR differentially activates brain regions with colorectal distention in humans.

Methods: We enrolled 28 subjects without any organic disease. The study was approved by the Ethics Committee and all subjects gave written informed consent. DNA was extracted from the peripheral blood. The genotype of 5-HTTLPR was determined using polymerase chain reaction. Age, sex, diagnosis-matched individuals with the *s/s* genotype ($n=14$) and individuals with the *l* allele (genotypes *l/s*, *l/l*, *l/extra-l*, $n=14$) were compared. A barostat bag was inserted to the colorectum and was intermittently inflated with no (0 mm Hg), mild (20 mm Hg), or intense (40 mm Hg) stimulation on a random order. Radioactive H2 [^{15}O] saline was injected at bag inflation and then positron emission tomography was performed. Changes in rCBF were analyzed using statistical parametric mapping.

Results: Individuals with the *s/s* genotype showed a significantly larger increase in rCBF by colorectal distention from 0 mm Hg to 40 mm Hg than individuals with the *l* allele. The significantly more activated brain regions in individuals with the *s/s* genotype were the left anterior cingulate cortex and right parahippocampal gyrus ($p<0.0001$). The increase in rCBF by colorectal distention of 20 mm Hg compared with 0 mm Hg was significantly larger in the left orbitofrontal cortex of individuals with the *s/s* genotype than that of individuals with the *l* allele ($p<0.0001$).

Conclusion: These data suggest that individuals with a weak function of serotonin transporter respond to gut signals more in emotion-regulating brain regions. Functional gene polymorphism may partially predict the individual effect of a selective serotonin reuptake inhibitor on visceral pain.

Introduction

Recent concepts of brain science have started to propose that the formation of emotion initially depends on interoception (Craig, 2002). Among many modalities of interoception, visceral activation and visceral perception are key physiological events for clarifying the mechanism of irritable bowel syndrome (IBS), a prevalent prototype of functional gastrointestinal disorders (Mayer et al., 2006). Research on visceral perception is not only limited to gastroenterology but also has great impact on conceptualization of the role of the body as the origin of emotion, as indicated by the symbolic words "gut feeling" (Bechara et al., 2005). Furthermore, visceral perception is one of the demonstrable phenomena for exploring the origin of complex emotion and consciousness in humans (Lane, 2000, Bud Craig, 2009). Based on these studies, it is acceptable to point out that clarification of the brain processing of visceral perception has great impact on neuroscience.

Our previous study using positron emission tomography (PET) demonstrated that colonic stimulation increases regional cerebral

Abbreviations: 5-HT, 5-hydroxytryptamine; 5-HTTLPR, 5-hydroxytryptamine transporter linked polymorphic region; PET, positron emission tomography; IBS, irritable bowel syndrome.

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1053-8119/$ – see front matter © 2009 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2009.04.083

blood flow (rCBF) in the anterior cingulate cortex (ACC) and prefrontal cortex (PFC), showing correlation with increased anxiety (Hamaguchi et al., 2004). In a functional magnetic resonance imaging (fMRI) study, IBS subjects showed stronger activation of the ACC in response to intense rectal distention than control subjects (Mertz et al., 2000). Imaging data on depressive disorders suggest that one of the common regions of brain activation is the ACC (Ressler and Mayberg, 2007). Subjects with major depressive disorder compared with healthy controls have also shown increased activation of the ACC during anticipation of pain relative to nonpainful stimuli (Strigo et al., 2008). With regard to intrinsic functional connectivity, a significant difference for dorsal to rostral ACC connectivity between patients with depressive disorder and controls in terms of higher connectivity in patients has also been reported (Schlösser et al., 2008). Therefore, increased activity of the ACC is one of the key features of interoception-induced negative emotion.

Serotonin (5-hydroxytryptamine; 5-HT) plays a crucial role in multiple brain function including negative emotion (Kandel, 2000). Serotonin is released from serotonergic nerve terminals which distribute almost throughout the brain and mainly originate from the raphe nuclei in the brain stem. Among the brain regions, the limbic system (i.e., cingulate cortex, hippocampus, amygdala, orbitofrontal cortex (OFC), and hypothalamus) are densely innervated by serotonergic neurons. Serotonin has pathogenic roles in terms of the formation of negative mood typically characterized by depression and anxiety. Depressive disorders and anxiety disorders are thus treated with many agents that normalize serotonergic neurotransmission (Delgado et al., 1990). The serotonin transporter (5-HTT) regulates serotonergic activity and is the target of selective serotonin reuptake inhibitors (SSRIs), which are widely used antidepressants (Frazer, 2001). The human 5-HTT gene (SLC6A4) is located on chromosome 17q12, and a variant in the upstream promoter region of the 5-HTT gene has been identified (Lesch et al., 1996). The 5-HTT linked promoter region (5-HTTLPR) polymorphism with long (*l*, 528 bp) and short (*s*, 484 bp) forms affect the expression and function of 5-HTT. Those with the *s* allele of this polymorphism are associated with lower transcriptional efficiency of the promoter than the *l* allele, leading to a lower 5-HTT expression and a lower cellular uptake of serotonin in the presynaptic nerve terminals of serotonergic neurons. This results in a higher serotonin concentration in the synaptic cleft and increases susceptibility to negative mood in individuals with the *s* gene. In actuality, individuals with the *s* gene are at significantly greater risk for major depressive disorder following repeated adult stress or childhood trauma (Caspi et al., 2003). Hariri et al. (2002) reported that individuals with the *s* allele of 5-HTTLPR, which has been associated with reduced 5-HTT expression and function and increased fear and anxiety-related behaviors, show greater amygdala neuronal activity, as assessed by blood oxygen level-dependent fMRI, in response to fearful stimuli than individuals homozygous for the *l* allele. Functional analysis of the ACC and amygdala during perceptual processing of fearful stimuli demonstrated tight coupling as a feedback circuit implicated in the extinction of negative effect, and *s* allele carriers showed relative uncoupling of this circuit (Pezawas et al., 2005). The magnitude of coupling inversely predicted almost 30% of variation in temperamental anxiety. Taken together, these data suggest that 5-HTTLPR at least in part may predict the function of prefrontal–limbic circuits, especially of the ACC and amygdala, during emotional formation.

Although interoception is considered to be the essential process of emotional formation, most previous studies used visual and cognitive tasks to demonstrate brain processing. To date, no studies on the influence of 5-HTTLPR on brain processing of visceral perception have been reported. We therefore tested our hypothesis that 5-HTTLPR differentially activates brain regions with colorectal distention in humans.

Subjects and methods

Subjects

Twenty-eight adult Japanese subjects without organic diseases or psychiatric disorders were enrolled in the study. Psychiatric disease was excluded through an unstructured clinical interview conducted by a board-certified specialist of the Japanese Society of Psychosomatic Medicine and via a structured interview using the Structured Clinical Interview (First et al., 1996, 2001) for DSM-IV (American Psychiatric Association, 2000). Subjects were genotyped as described below. Individuals with the *s/s* genotype ($n=14$, *s* group) and those with the *l* allele (genotype *l/s*, $n=10$; genotype *l/l*, $n=2$; genotype *l/extra-l*, $n=2$; total $n=14$, *l* group) were compared. All subjects were right-handed. Age, sex, gastrointestinal symptoms, and the stimulated site did not differ among groups (Table 1). Each group was composed of 11 healthy subjects and 3 IBS subjects who fulfilled the Rome III criteria (Longstreth et al., 2006). This study was approved by the Tohoku University Ethics Committee and subjects provided written informed consent.

Genotyping

A plastic catheter was inserted into the left forearm vein of each subject and saline was infused at a speed of 1.6 ml/min. Peripheral blood was sampled with a heparinized syringe. Genotyping was performed using the same methods as in our previous report (Mizuno et al., 2006). In brief, DNA was extracted from lymphocytes. The polymorphism in the regulatory region of the 5-HTT gene was genotyped by polymerase chain reaction (PCR). PCR-amplification was carried out using primer pairs reported by Lesch et al. (1996) (5′-GGC GTT GCC GCT CTG AAT GC-3′ and 5′-GAG GGA CTG AGC TGG ACA ACC AC-3′). A 25 μl PCR reaction consisted of a 0.2 μM concentration of each primer, 1.5 mM $MgSO_4$, 0.2 mM each of deoxynucleotide triphosphate, 1× PCR_X Amplification Buffer, 2.5 U of Platinum Taq DNA Polymerase, and 1× PCR_x Enhancer Solution (GIBCO BRL, Life Technologies Inc., Rockville, MD, USA). After initial denaturation at 95°°C for 2 min, amplification was performed using 35 cycles at 95 °C for 30 s, 60 °C for 30 s (annealing), and 68 °C for 1 min, followed by a final elongation at 68 °C for 3 min. The amplification products were separated on 2% agarose gel by electrophoresis and classified as long and short alleles.

To ensure genotype accuracy, sequence analysis of 5-HTTLPR genes was performed on PCR fragments which were amplified according to the previously described protocol. PCR products were purified from agarose gel using a QIAquick Gel Extraction kit (QIAGEN, Hilden, Germany). Amplimers were sequenced directly using the ABI PRISM dRodamine™ Terminator Cycle Sequencing Ready Reaction kit (PE Applied Biosystems, Foster City, CA, USA), and excess dye terminators were removed using CENTRI-SEP Columns (PRINCETON SEPARATIONS, Adelphia, NJ, USA). Automated sequencing was performed on an ABI 310 Genetic Analyzer (PE Applied Biosystems). All procedures

Table 1
Subject characteristics of *s* group and *l* group.

Group		*s*	*l*
Number		14	14
Age (mean ± SD)		23.9 ± 3.5	22.1 ± 1.4
Sex (male/female)		11/3	10/4
Protocol (colon/rectum)		8/6	8/6
Diagnosis (normal/IBS)		11/3	11/3
5-HTTLPR Genotype	*s/s*	14	0
	l/s	0	10
	l/l	0	2
	l/extra-l	0	2

s group: individuals with the *s/s* genotype; *l* group: individuals with the *l* allele (*l/s*, *l/l*, or *l/extra-l* genotype).

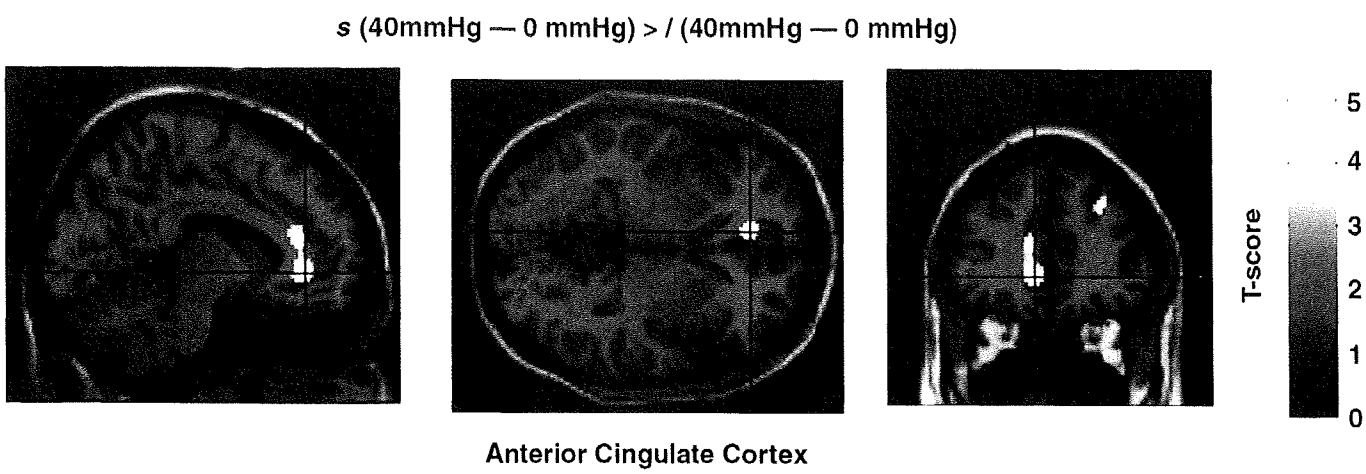

Fig. 1. Moderate colorectal distention in the *s* group significantly activated more the left anterior cingulate cortex than that in the *l* group. The image with 40 mm Hg was subtracted by that with 0 mm Hg . BA 32, *x*, *y*, *z* = −8, 40, −2, $p<0.0001$.

were performed according to the manufacturer's instructions. Forward and reverse primers were used to sequence the PCR products.

Visceral stimulation

Colorectal stimulation was performed using the same methods as previously described (Hamaguchi et al., 2004, Suzuki et al., in press). On the day before the experiment, subjects were given low-residue meals and their colorectum was cleansed. On the experimental day, a catheter with a barostat bag (700 ml in volume) was inserted into the rectum or the upper part of the descending colon by colonoscopy. Colorectal distention stimuli were provided with a computerized barostat equipment (Medtronics Synectics, Shoreview, MN, USA), which inflated the bag at a rate of 38 ml/s. First, each subject underwent a baseline PET scan without bag stimulation. Thereafter, the colorectum was stimulated with bag pressures of 0, 20 and 40 mm Hg for 80 s. The intensity of each stimulus was randomly chosen to avoid stimulation order effect, and the time interval between two stimuli was 15 min. After each stimulation, the subjects were asked to report the following 7 items of visceral perception or emotion: abdominal discomfort, abdominal distention, abdominal pain, urgency for defecation, perceived stress, sleepiness, and anxiety. Each sensation was evaluated on an ordinate scale from 0 (no sensation) to 10 (maximal sensation) (Hamaguchi et al., 2004, Kano et al., 2007).

PET scan

Scans of the distribution of $H_2^{15}O$ were obtained using a SET-2400W PET scanner (Shimadzu, Japan) operated on a high sensitivity three-dimensional mode with an average axial resolution of 4.5 mm at maximum strength and sensitivity for a 20-cm cylindrical phantom of 48.6 kcps kBq^{-1} ml^{-1} (Fujiwara et al., 1997, Kano et al., 2007). For each scan, a subject received approximately 5 mCi (185 MBq) of $H_2^{15}O$ intravenously through the forearm vein and underwent colorectal distention during rCBF measurement. The radioactivity peak to the scan onset was about 10 s after the start of colorectal distention at which both the radioactivity peak and peak pressure of the bag simultaneously reached a plateau. The PET scanning room was darkened and the subjects, while awake, were instructed to keep their eyes closed for the whole period of scanning (70 s).

Statistical analysis

Statistical parametric mapping software (SPM2, Wellcome Department of Cognitive Neurology, London, UK) was used for PET image realignment, normalization, smoothing, and to create statistical maps of significant rCBF changes (Friston et al., 1995a,b). All rCBF images were stereotaxically normalized into the standard space defined by Talairach and Tournoux (1988) using an rCBF template image supplied with SPM2. The normalized images were smoothed using a

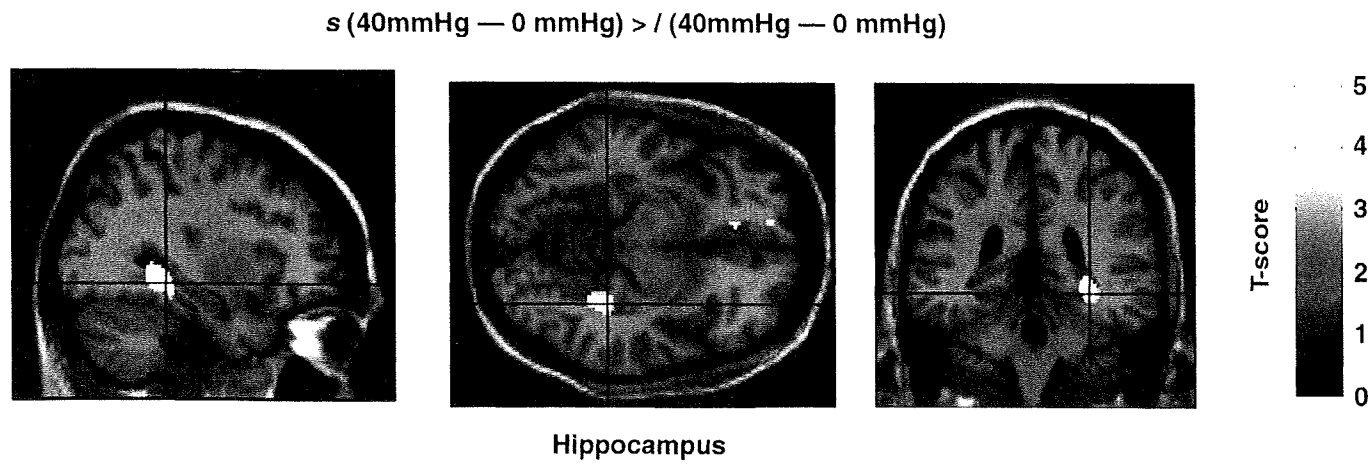

Fig. 2. Moderate colorectal distention in the *s* group significantly activated more the right hippocampus than that in the *l* group. The image with 40 mm Hg was subtracted by that with 0 mm Hg . *x*, *y*, *z* = 32, −42, −4, $p<0.0001$.

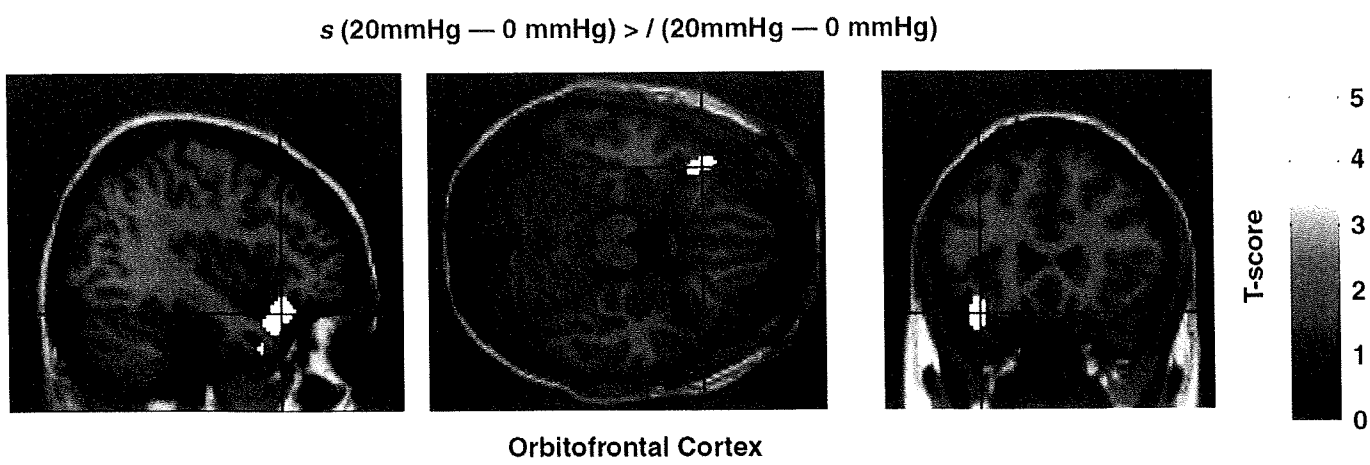

Fig. 3. Mild colorectal distention in the *s* group significantly activated more the left hippocampus than that in the *l* group. The image with 20 mm Hg was subtracted by that with 0 mm Hg . BA 47, *x*, *y*, *z* = −38, 24, −20, $p<0.0001$.

$12\times12\times12$-mm Gaussian filter, and the rCBF values were expressed in ml dl^{-1} min^{-1}, adjusted for individual global CBF values using ANCOVA, and scaled to a mean of 50. The contribution of each parameter of interest to changes in rCBF was estimated by SPM2 according to the general linear model at the voxel level. Estimates were made using linear compounds of contrasts, and the resulting set of voxel values constituted a parametric map for each contrast. To examine whether specific brain regions differ between the *s* group and the *l* group, we performed subtraction analysis between rCBF changes at stimulation. Brain regions with significant cluster level ($p<0.05$) and significant voxel level (T>4.0 and $p<0.0001$) were demonstrated.

Results

The brain image with 0 mm Hg was subtracted from the brain image with 40 mm Hg . This subtraction implies analysis of the brain area specific to the moderate colorectal stimulation. The *s* group showed a significantly larger increase in rCBF in the left ACC (BA 32, *x*, *y*, *z* = −8, 40, −2) by moderate colorectal distention than the *l* group ($p<0.0001$) (Fig. 1). The spatial distribution of the more activated area in the *s* group than in the *l* group was mainly the perigenual ACC including the supragenual ACC and subgenual ACC. The *s* group also showed a significantly larger increase in rCBF in the right hippocampus (*x*, *y*, *z* = 32, −42, −4) by mild colorectal distention than the *l* group ($p<0.0001$) (Fig. 2). The spatial distribution of the more activated area in the *s* group than in the *l* group included the parahippocampal cortex.

The brain image with 0 mm Hg was then subtracted from the brain image with 20 mm Hg . This subtraction implies analysis of the brain area specific to the mild colorectal stimulation. The increase in rCBF by mild colorectal distention in the *s* group was significantly larger in the left OFC (BA 47, *x*, *y*, *z* = −38, 24, −20) than that in the *l* group ($p<0.0001$) (Fig. 3). The more activated area in the *s* group than in the *l* group was located in the lateral margin of the OFC adjacent to the left temporal pole.

Table 2 shows a summary of the significantly more activated brain regions in response to colorectal stimulation in the *s* group than in the *l* group. There were no other regions which differentiate the brain response to colorectal distention between the *s* group and the *l* group.

We also analyzed the data after eliminating the 3 subjects with IBS in each group. The comparisons of the *s* and *l* groups remained statistically significant after excluding these subjects. Colorectal distention significantly and intensity dependently increased the ordinate scale of abdominal discomfort, abdominal distention, abdominal pain, urgency for defecation, perceived stress, and anxiety, and significantly reduced sleepiness in both groups (data not shown). However, the effect of 5-HTTLPR genotype on the changes in the ordinate scale was not significant.

Discussion

This is the first study to clarify that colorectal distention in individuals with the *s*/*s* genotype activates the ACC, hippocampus, and OFC more than in individuals with the *l* allele. The results of this study are in line with those of Hariri et al. (2002) and Pezawas et al. (2005). Hariri et al. (2002) reported that individuals with the *s* allele of 5-HTTLPR exhibit greater amygdala neuronal activity in response to fearful stimuli than individuals homozygous for the *l* allele. Pezawas et al. (2005) demonstrated tight coupling as a feedback circuit implicated in the extinction of negative affect by functional analysis of the ACC and amygdala during perceptual processing of fearful stimuli and relative uncoupling of this circuit in *s* allele carriers. Therefore, our present study, together with earlier studies, suggests that the *s* allele and *l* allele of 5-HTTLPR exhibit dysfunction of the prefrontal–limbic circuits in response to stimuli that usually evoke negative emotion.

The advantage of this study is that the stimulus we used (visceral stimulation) is known to directly activate the raphe nuclei in the brain

Table 2
Summary of differential brain activation between *s* group and *l* group.

Main effect	Region	Side	BA	Cluster	*p*	*x*	*y*	*z*	*t*
s>*l* (40 mm Hg–0 mm Hg)	Hippocampus	R		215	0.012	32	−42	−4	5.05
	Anterior cingulate cortex	L	32	183	0.019	−8	40	−2	4.93
s>*l* (20 mm Hg–0 mm Hg)	Orbitofrontal cortex	L	47	215	0.012	−38	24	−20	4.32
l>*s*	No suprathreshold brain regions								

Side: R: right, L: left; BA: Brodmann's area: regions with $p<0.0001$ were shown.

stem (Brink and Mason, 2004, Rodella et al., 1998, Gioia et al., 2003). Neurons of the nucleus raphe magnus respond to colorectal distention with a variety of patterns, each of which may contribute to the sculpting of colorectal distention reactions in different ways (Brink and Mason, 2004). Visceral noxious stimulation increases Fos-positive cells (Rodella et al., 1998) and positive cells to phosphorylated extracellular signal-regulated kinases 1 and 2 (Gioia et al., 2003) in the dorsal raphe nucleus. Serotonergic neurons originate from the dorsal raphe nucleus and innervate the limbic system (i.e., cingulate cortex, hippocampus, amygdala, OFC, and hypothalamus) (Kandel, 2000). In our study of these brain regions, the differentially activated areas in individuals with the *s/s* genotype from those with the *l* allele were the ACC, hippocampus, and OFC. This implies that our study presents more reliable neuroanatomical evidence than earlier studies.

Individuals with the *s* allele of 5-HTTLPR are associated with lower transcriptional efficiency of the promoter than those with the *l* allele, leading to a lower 5-HTT expression and a lower cellular uptake of serotonin to presynaptic nerve terminals in serotonergic neurons (Lesch et al., 1996). This phenomenon results in a higher serotonin concentration in the synaptic cleft and increased susceptibility to negative mood in individuals with the *s* gene (Caspi et al., 2003). Therefore, in our study, serotonin neurons in the dorsal raphe nucleus stimulated by colorectal distention would release serotonin from nerve terminals and consequently a higher serotonin concentration may remain in the synaptic cleft of the ACC, hippocampus, and OFC in individuals with the *s/s* genotype. It has been shown that serotonin released from presynaptic nerve terminals excite postsynaptic neurons (Colino and Halliwell, 1987). Serotonin produces a more slowly developing and long-lasting suppression of intrinsic voltage-dependent K-conductance, leading to neuronal depolarization and excitation. It also simultaneously suppresses slow Ca-dependent K-conductance largely responsible for the accommodation of cell firing in hippocampal CA1 neurons, and this in turn produces a paradoxical increase in neuronal discharge in response to a depolarizing input. However, serotonin often produces complex and variable responses. It also activates a Ca-independent K-current responsible for neuronal hyperpolarization and is also shown to be inhibitory. The hyperpolarizing response is mediated by 5-HT1A receptors, which differentially distributes in different brain regions. Endogenously released serotonin could therefore change the probability or duration (or both) of neuronal firing in human brain regions in different ways to produce excitatory, inhibitory, or mixed effects. The ACC, hippocampus, and OFC are more activated in individuals with the *s/s* genotype than in those with the *l* allele in our study, which could therefore be attributed to the local serotonin action on conductance and the receptors.

The ACC, hippocampus, and OFC are key areas of the emotional circuit as well as serotonergic neurotransmission. The perigenual part of the ACC is related to negative emotion and conflict monitoring (Ressler and Mayberg, 2007). The perigenual ACC is divided into two parts, namely, the supragenual ACC and subgenual ACC. The function of the supragenual ACC negatively correlates with amygdala activity, while that of the subgenual ACC positively correlates with amygdala function (Pezawas et al., 2005). 5-HTTLPR *s* allele carriers show less coupling between the amygdala and the perigenual ACC than *l/l* individuals, particularly in the subgenual ACC (Shah et al., 2009). The influence of 5-HTTLPR on coupling between the ACC and amygdala during visceral perception processing warrants future study. The hippocampus is the key region for explicit and implicit memory (Kandel, 2000). In this case, the hippocampus may work to recall possible noxious visceral stimuli as a negative somatic marker. On the other hand, the OFC evaluates reward, punishment and unavoidable sensation (O'Doherty et al., 2001). It is also implicated in emotion and emotion-related learning. Distinct areas of the OFC were shown to be activated by monetary rewards and punishments. Moreover, these areas are reported to be correlated with the magnitude of brain activation and the magnitude of rewards and punishments received. Further, medial OFC activity is related to monitoring the reward value of many different reinforcers, whereas lateral OFC activity is related to the evaluation of punishers which may lead to a change in ongoing behavior (Kringelbach and Rolls, 2004). A posterior–anterior distinction exists with more complex or abstract reinforcers (such as monetary gain and loss) represented more anteriorly in the OFC than simpler reinforcers such as taste or pain. Our data showing more activation of the lateral and posterior OFCs suggest that individuals with the *s/s* genotype tend to evaluate mild visceral activation as a punishment marker.

There was no significant association between 5-HTTLPR genotype and self-reported distress and discomfort. We believe this finding to be reasonable because quantification of subjective feeling is relatively insensitive compared with neural or chemical changes (Hamaguchi et al., 2004, Kano et al., 2007, Suzuki et al., in press). The brain processes unconscious signals on a moment-to-moment basis and only a part of them is processed consciously. This finding also suggests that 5-HTTLPR genotype first influences rCBF as the endophenotype. We assume that either a larger number of subjects or long-term analysis of subjective feeling in response to colorectal distention is necessary to detect the effect of 5-HTTLPR genotype on phenotype (negative emotion).

The limitations of the present study are as follows. First, there is a difference in treating heterozygosity of 5-HTTLPR between our study and earlier well-known studies. Earlier studies compared the *l/l* genotype versus the *s/l* and *s/s* genotypes based on the hypothesis that the *s* allele is dominant (Hariri et al., 2002, Pezawas et al., 2005). We compared the *s/s* genotype versus the *l* allele because the *s* allele is more frequent in the Asian population (Mizuno et al., 2006). In a Caucasian sample, the *l* allele is reported to be dominant (Hanna et al., 1998, Du et al., 1999, Williams et al., 2001). If 5-HTTLPR truly affects emotional sensitivity to stimuli, the *l* or *s* allele will be responsible for emotion independent of race. There are some reports showing that patterns of linkage disequilibrium between 5-HTTLPR and other sites on the 5-HTT gene vary considerably across racial (including European, African, and Japanese) groups (Gelernter et al., 1997) and that there are race and sex differences in the association between 5-HTTLPR genotype and personality traits associated with negative emotion (Gelernter et al., 1998). It is possible that race differences exist with respect to the transcriptional efficiency of the *s* versus *l* alleles. Because our sample was composed mainly of men, it is impossible to evaluate the sex modulation of 5-HTTLPR effects on brain activation. In previous larger sample studies, however, it should be noted that Caucasian (Lesch et al., 1996, Gelernter et al., 1998) and Japanese (Mizuno et al., 2006) men with more *s* alleles consistently showed increased anxiety levels. Further studies will be needed to determine whether the effects of 5-HTTLPR on brain activation relate to other racial groups. Second, we did not assess the single nucleotide polymorphism of an A → G substitution which is located at nucleotide 6 within the first of two extra 22-base pair repeats that characterize the *l* allele located 1629 nt 5′ of exon 1 of 5-HTT (Hu et al., 2006). The l_A allele has greater transcriptional activity than the l_G allele which has transcriptional activity similar to the *s* allele. We cannot completely exclude the possibility that this 5-HTTLPR triallele may have influenced our results to some extent. This possibility should be explored in future studies. Third, our sample contained 3 subjects with IBS among the 14 subjects in each group, which is similar to the prevalence of IBS in the general population, and analysis eliminating the IBS subjects resulted in the same results. Moreover, the aim of the present study was to ascertain the genetic effects on the brain processing of visceral activation. Possible differential influence of 5-HTTLPR between controls and IBS should be examined in future studies with a greater number of subjects. Fourth, the descending colon and rectum were stimulated. However, the numbers of stimulated site were not statistically different between the *s* group

and the *l* group. Moreover, the activated brain regions are not discriminatory parts along the somatotopic order of the descending colon and rectum. More samples of the *l*/*l* genotype and the same site of stimulation should be used in future studies. Despite these limitations, the present study clearly demonstrated the effect of 5-HTTLPR on interoceptive emotional processing.

In conclusion, the present data suggest that individuals with a weak function of serotonin transporter respond to gut signals more in emotion-regulating brain regions. Functional gene polymorphism may partially predict the individual effects of long-lasting neural processing from visceral organs.

Acknowledgments

This work was supported by Grants-in-Aid from the Ministry of Education, Culture, Sports, Science and Technology and Grants-in-Aid from the Ministry of Health, Welfare, and Labor, Japan. The authors have no conflict of interest and have nothing to disclose financially regarding the study.

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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 re-examined 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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Received for publication March 31, 2008; revision received September 2, 2008.

Supported by Grant-in-Aid for Scientific Research from the Ministry of Education, Science, and Culture and from the Ministry of Health, Welfare, and Labor of Japan (S.F.).

DOI: 10.1097/PSY.0b013e31819b69ca

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.

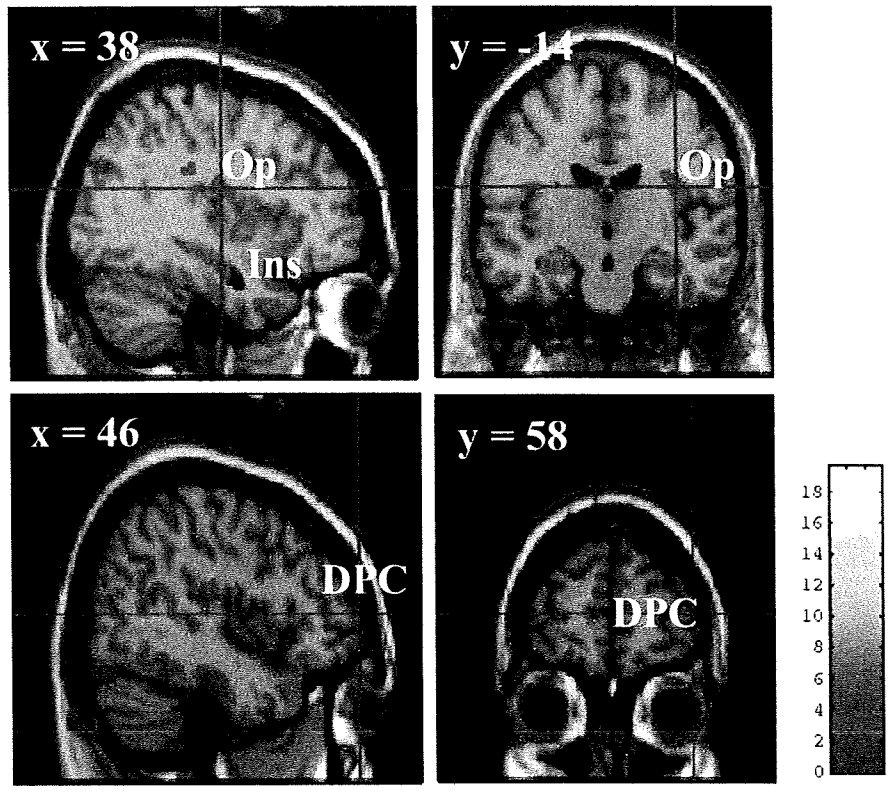

Figure 1. Activity in the middle portion of the right insula (Ins; 36, 0, −2), the right operculum (Op; 38, −14, 20), and the right dorsolateral prefrontal cortex (RPC; 46, 58, 6) positively correlated with increased heart rate during rectal distention with an intensity of 40 mm Hg. Results of covariation 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

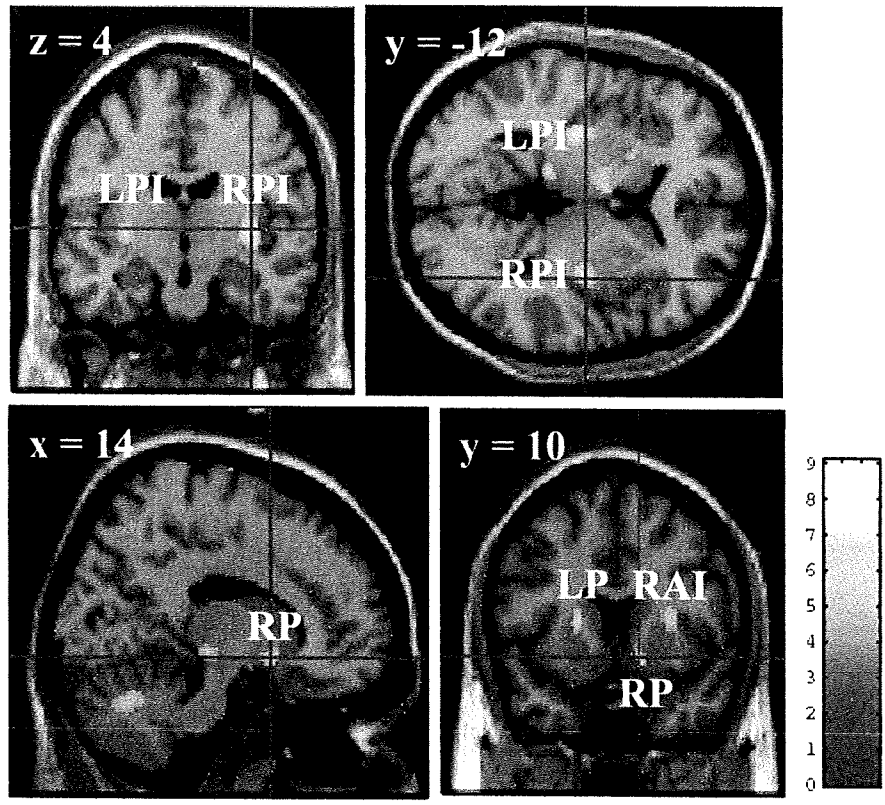

Figure 2. Activity in the right posterior insula (RPI; 36, 0, −2), the left posterior insula (LPI; −30, −16, 4), the right anterior insula (RAI; 30, 10, 12), the left putamen (LP; −20, 14, 8), and the right putamen (RP; 14, 10, −8) positively correlated with increased LF/HF ratio during rectal distention 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.

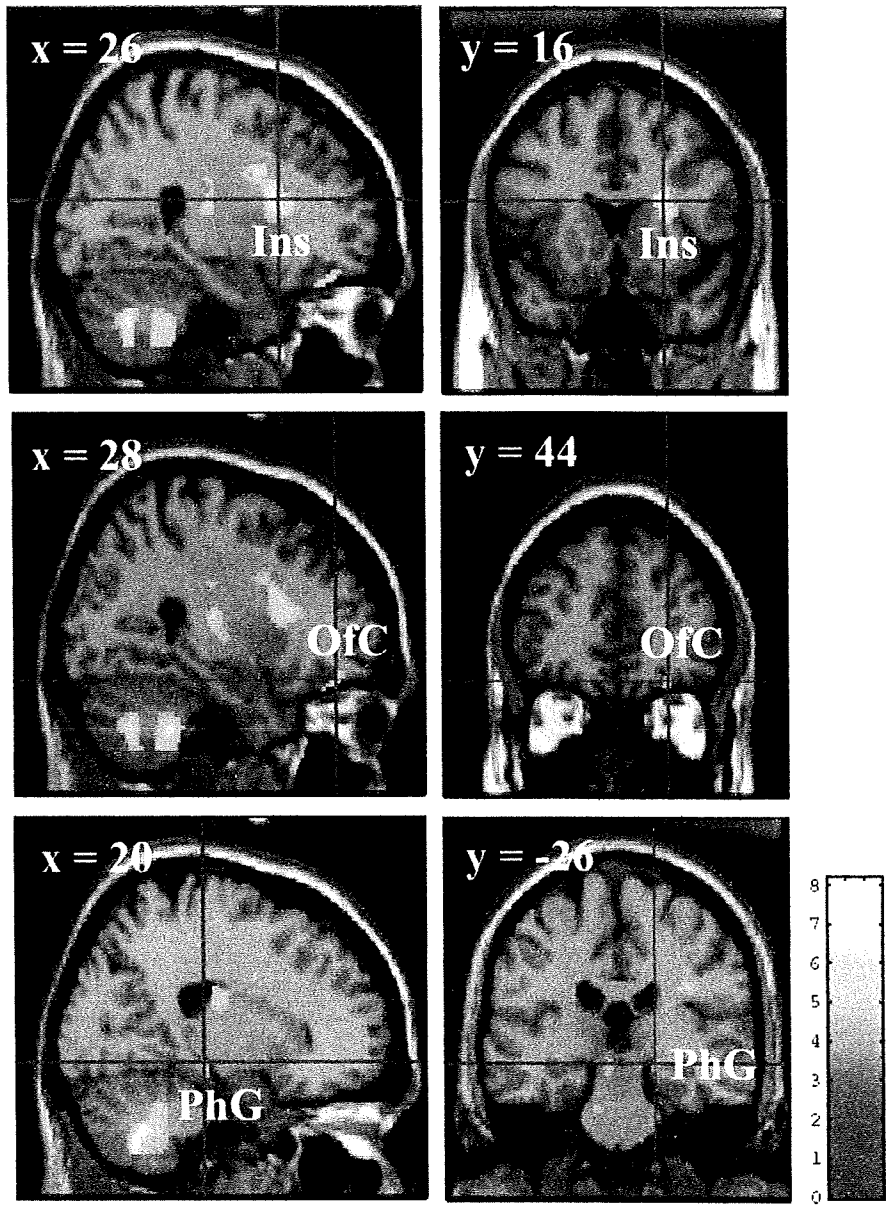

Figure 3. Activity in the anterior portion of the right insula (Ins; 26, 16, 18), the right orbitofrontal cortex (Orf; 28, 44, −18), and the right parahippocampal gyrus (PhG; 20, −26, −8) positively correlated with increased plasma adrenaline during rectal distention with an intensity of 40 mm Hg. Results 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.

This study was presented in 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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neurogastroenterology & motility

Neurogastroenterol Motil (2010) **22**, 173–e53 doi: 10.1111/j.1365-2982.2009.01417.x

Site-specific differences in central processing of visceral stimuli from the rectum and the descending colon in men

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Abstract

Background *It has been reported that different brain activation areas are demonstrated during somatosensory and visceral stimulation. However, no study thus far has investigated how activated patterns in the human brain differ during visceral stimulation of different sites of the digestive tracts. The aim of this study was to determine possible site-specific differences in brain responses and perceptions during visceral stimulation of two different sites, the intraluminal distentions of the rectum and descending colon.* ***Methods*** *Regional cerebral blood flow was assessed in 32 healthy right-handed male subjects using* $H_2{}^{15}O$ *positron emission tomography during distention of the rectum (R group, n = 16) or descending colon (DC group, n = 16) at 40 or 20 mmHg.* ***Key Results*** *R group reported significantly higher scores of abdominal pain (P < 0.05) and urge to defecate (P < 0.001) during the application of stimulus at 40 mmHg compared with DC group but not of abdominal bloating or anxiety. In comparisons of response to the 40-mmHg stimulus, R group showed significantly greater activation in posterior midcingulate cortex (MCC) and right anterior and posterior insula, whereas DC group showed greater activation in subgenual anterior cingulate cortex (ACC), perigenual ACC and left orbitofrontal and superior temporal cortices.* ***Conclusions & Inferences*** *These findings suggest that central projections of painful visceral stimulation from the rectum and descending colon differ in affective, cognitive and nociceptive processing in the brain, which may result in different perceptions of visceral stimulation from different sites.*

Keywords *brain activation, colonic distention, descending colon, positron emission tomography, rectum.*

Abbreviations: R, rectum; DC, descending colon; IBS, irritable bowel syndrome; PET, positron emission tomography; BA, Brodmann area; (s/p) ACC, (subgenual/perigenual) anterior cingulate cortex; (p) MCC, (posterior) midcingulate cortex; (d/v) PCC, (dorsal/ventral) posterior cingulate cortex.

INTRODUCTION

Differentiation of cerebral registration of sensory signals originating from different sites in the digestive tract remains unclear. Subjective perception and responses in smooth muscle tone during intraluminal stimulation between the rectum and the more proximal part of the colon are not always comparable.[1,2] The innervation of the descending colon is considered to be purely visceral (involving the pelvic splanchnic nerves), while the most distal part of the rectum is somatically co-innervated.[3] Thus, a distinct population of low-threshold, slow-adapting mechanoreceptors may be identified within terminal endings in the rectum but not in the descending colon.[3]

Visceral hypersensitivity in the distal colon has been demonstrated in patients with functional gastrointestinal (GI) disorders. In previous studies using a barostat device, pain thresholds to intraluminal distention in the rectum were lower in patients with irritable bowel syndrome (IBS) than in healthy subjects.[4–6] Recently, it has been revealed that the lower pain threshold

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Received: 8 October 2008
Accepted for publication: 13 September 2009

observed in IBS patients was associated with increased abdominal pain severity not only in the rectum[7,8] but also in the descending colon.[9]

The development of brain imaging techniques [e.g. positron emission tomography (PET) and functional magnetic resonance imaging (fMRI)] in recent years has helped us assess central processing of emotional, cognitive, somatosensory and visceral inputs.[10,11] Previous brain imaging studies confirmed that brain responses to intraluminal distention of the rectum are observed in certain areas such as the anterior cingulate cortex (ACC), prefrontal cortex, insula and thalamus in healthy subjects, which is summarized in a systematic review of 10 studies.[12] On the other hand, there have been only a few studies that investigated brain activations during distention of the descending colon.[13,14] Thus, little is known how visceral perceptions and brain responses to visceral stimulation are different between the rectum and descending colon.

It has been reported that different brain activation patterns in the ACC were demonstrated during somatosensory and visceral stimulation of the upper (proximal and distal oesophagus)[15] or lower digestive tracts (anus and rectum).[16,17] These studies revealed that visceral stimulation (e.g. distal oesophageal or rectal distention) elicited a response in the more rostral part of the cingulate cortex compared with somatic stimulation (e.g. proximal oesophageal or anal distension), suggesting that perceptions of visceral and somatic sensations are differentiated in the limbic system. Therefore, central processing and cortical representation of visceral stimulation may differ even in close but different sites of the colon. However, no study has so far investigated similarities or differences in brain activated or deactivated areas during visceral stimulation between the rectum and descending colon.

The aim of this study was to determine possible site-specific differences in brain responses and subjective perception during visceral stimulation of two different sites by intraluminal distention of the rectum and the descending colon. We tested the following hypotheses: (i) that there are differences in cerebral activations during both painful and non-painful stimulation of the rectum and descending colon and (ii) that subjective perception induced by intraluminal distention of the rectum is different from that of the descending colon in healthy male subjects.

METHODS

Subjects

Thirty-two healthy right-handed male subjects (mean age, 22 ± 2 years; 19–29 years) were recruited from Tohoku University Campus in Sendai, Japan. All participants had no GI complaints and had not taken any medications within 4 weeks prior to testing. Each participant underwent a medical history evaluation and was given a physical examination. Subjects were randomly allocated into the rectum group (R group; $n = 16$; mean age, 21 ± 1 years) and descending colon group (DC group; $n = 16$; mean age, 22 ± 2 years). Written informed consent was obtained from all participants, and this study was approved by the Ethics Committee of Tohoku University School of Medicine.

Distention protocol

The distention protocol performed in this study followed previously reported methods.[13] In brief, on the day before examination, the subjects were required to follow a low residue diet and ingest 17 g (13.6%) of magnesium citrate, 75 mg of sodium picosulphate and 24 mg of sennosides A & B to cleanse the colon. On the day of the experiment, a colonoscope was inserted into the splenic flexure in DC group and a splinting device was inserted along the scope. After removal of the scope, a catheter with a thin polyethylene bag (Synectics Medical, Stockholm, Sweden) was inserted into the descending colon. The maximal volume of the bag was 600 mL and the maximal diameter at full inflation was 10 cm. The location of the bag was confirmed by X-ray fluoroscopy. In the R group, the same catheter was inserted into the rectum without X-ray fluoroscopy and placed with the distal end of the bag 5 cm from the anal verge.

Colonic distention stimuli were induced with a computerized barostat pump (Medtronics Synectics, Shoreview, MN, USA), which inflated the taped bag at a rate of 38 mL s^{-1}. Firstly, the subjects underwent no stimulation with a bag pressure of 0 mmHg (baseline). Thereafter, the colon was stimulated with bag pressures of 20 and 40 mmHg for 80 s. The intensity of each stimulus was pseudorandomized to avoid an order effect. There was a time lag of 6 s before reaching peak pressures after initiation of the highest intensity stimulus. After each stimulus, the subjects were asked to report the following items of visceral perception: abdominal pain, urge to defecate, abdominal bloating and anxiety. Each sensation was verbally evaluated on an ordinate scale from 0 (no sensation) to 10 (maximal sensation), namely, an 11-point Likert scale as previously described.[13]

Positron emission tomography scanning

A plaster head support was set for each subject to minimize head movements during PET imaging. [^{15}O]-labelled water (Tohoku University Cyclotron Radioisotope Center) was injected into a vein in the right arm at the beginning of colonic distention. Scans were started about 10 s after the beginning of colonic balloon distensions, at which both radioactivity peak and peak pressure of the bag reached simultaneously a plateau. As the radioactivity detected in the brain is proportional to the volume of cerebral blood flow, an increase in regional cerebral blood flow (rCBF) is seen as an index of neural activity evoked by stimulation. Using a ^{68}Ge/^{68}Ga radiation source, the 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 but remain awake for the whole period of the scan (70 s). rCBF was measured during scans (70 s each) using a PET scanner in the three-dimension sampling mode (HEADTOME V SET-2400W; Shimadzu, Kyoto, Japan).[18] The scanner operated on high-sensitivity three-dimensional (3D) mode with an axial resolution of 3.9 mm. To ensure that radioactivity levels in each subject returned to baseline before starting a new scan, a 10-min interval was given between successive scans.

Analysis

The PET data were transferred to a super computer (NEC, SX-4/128H4; Tohoku University Computer Center) and PET images were reconstructed using a 3D filtered back projection algorithm. PET images were analysed according to the method of Friston *et al.*[19] 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. Finally, the images were smoothed using a 7 × 7 × 7 mm Gaussian filter and proportionally scaled to account for global confounders.

To estimate rCBF differences between 20- and 40-mmHg distention periods and the baseline period, an intra-group comparison (distention minus baseline images, or baseline minus distention images) was conducted using 'population main effect: two conditions, one scan/condition (paired *t*-test)' in the SPM model. To compare regional brain activities in response to each stimulus between groups, a between-group comparison of the contrasts was applied. The level of significance was set at voxel level of $P_{uncorrected} < 0.001$, with an extent threshold of 20 voxels as the region of significant correction. Talairach Daemon database[20] was used to complement transformation of the coordinates in Talairach space[21] and to determine precise cortical activated regions.

Subjective perception score was analysed using with one-way analysis of variance (ANOVA) for comparisons between groups. Data were expressed as mean with standard deviation (SD). For the analyses, a *P*-value of 0.05 defined statistical significance.

RESULTS

All subjects in both the R and DC groups completed the study protocol. No aversive effects or complications were observed throughout the study.

Subjective visceral perception

There was no difference in symptom score of abdominal pain [0.3 ± 0.8 *vs* 0.1 ± 0.5, $F(1, 30) = 0.29$], urge to defecate [0.8 ± 1.3 *vs* 0.8 ± 1.2, $F(1, 30) = 0.00$], abdominal bloating [0.3 ± 0.8 *vs* 0.7 ± 1.1, $F(1, 30) = 1.62$], or even anxiety [0.4 ± 0.7 *vs* 1.1 ± 1.4, $F(1, 30) = 3.09$] between the R and DC groups during the baseline non-stimulation period (Table 1). During the 20-mmHg distention period, scores for abdominal pain [2.8 ± 2.3 *vs* 0.9 ± 1.1, $F(1, 30) = 9.18$, $P < 0.01$] and urge to defecate [5.1 ± 2.3 *vs* 2.6 ± 1.9, $F(1, 30) = 10.43$, $P < 0.01$] were significantly higher in the R group than those in the DC group (Table 1). There was no difference in score of abdominal bloating [3.6 ± 1.9 *vs* 2.8 ± 1.9, $F(1, 30) = 1.44$] or anxiety [1.8 ± 1.8 *vs* 1.6 ± 1.4, $F(1, 30) = 0.11$] to the 20-mmHg stimulus between the R and DC groups. During the distention period at 40 mmHg, the R group reported significantly higher scores of abdominal pain [5.3 ± 3.3 *vs* 3.2 ± 2.2, $F(1, 30) = 4.56$, $P < 0.05$] and urgency [9.3 ± 0.9 *vs* 4.4 ± 3.5, $F(1, 30) = 29.67$, $P < 0.001$] but not of abdominal bloating [6.0 ± 2.9 *vs* 5.2 ± 2.3, $F(1, 30) = 0.76$] or anxiety [4.5 ± 2.7 *vs* 3.1 ± 2.7, $F(1, 30) = 2.12$] compared with those in the DC group (Table 1).

Table 1 Differences in subjective symptoms induced by intraluminal distention

Symptom score (0–10)	Stimulus intensity	Rectum Mean (SD) (n = 16)	Descending colon Mean (SD) (n = 16)	*P*-value
Abdominal pain	Baseline	0.3 (0.8)	0.1 (0.5)	0.592
	20 mmHg	2.8 (2.3)	0.9 (1.1)	0.005
	40 mmHg	5.3 (3.3)	3.2 (2.2)	0.041
Urge to defecate	Baseline	0.8 (1.3)	0.8 (1.2)	1.00
	20 mmHg	5.1 (2.3)	2.6 (1.9)	0.003
	40 mmHg	9.3 (0.9)	4.4 (3.5)	<0.001
Abdominal bloating	Baseline	0.3 (0.8)	0.7 (1.1)	0.214
	20 mmHg	3.6 (1.9)	2.8 (1.9)	0.240
	40 mmHg	6.0 (2.9)	5.2 (2.3)	0.390
Anxiety	Baseline	0.4 (0.7)	1.1 (1.4)	0.089
	20 mmHg	1.8 (1.8)	1.6 (1.4)	0.748
	40 mmHg	4.5 (2.7)	3.1 (2.7)	0.156

Abdominal pain, urge to defecate, abdominal bloating and anxiety were assessed using an 11-point ordinate scale (0, no sensation; 10, maximal sensation). SD, standard deviation.

Brain regions activated during intraluminal distention

Rectal distention at a pressure of 40 mmHg significantly activated the right anterior insula and the bilateral thalamus (Fig. 1 and Table 2). Brain regions comprising the right inferior parietal lobule and thalamus were significantly activated during rectal distention at 20 mmHg (Table 2). During the 40-mmHg stimulus of the descending colon, significant brain activations of the posterior midcingulate cortex (pMCC), perigenual ACC (pACC), subgenual ACC (sACC) and right supramarginal gyrus were observed (Fig. 2A–D and Table 3). The 20-mmHg stimulus of the descending colon significantly induced the pACC, sACC, pMCC and right supramarginal gyrus (Table 3).

Brain regions deactivated during intraluminal distention

Visceral stimulation with an intensity of 40 mmHg in the rectum caused a significant and broad deactivation of regions of the occipital, middle temporal [including the posterior cingulate cortex (PCC)] and medial prefrontal cortices (Fig. 3A and Table S1). During the 20-mmHg stimulus of the rectum, significant brain deactivations were also observed in the occipital, middle temporal and medial prefrontal cortices (Table S1). During the 40- or 20-mmHg distentions of the

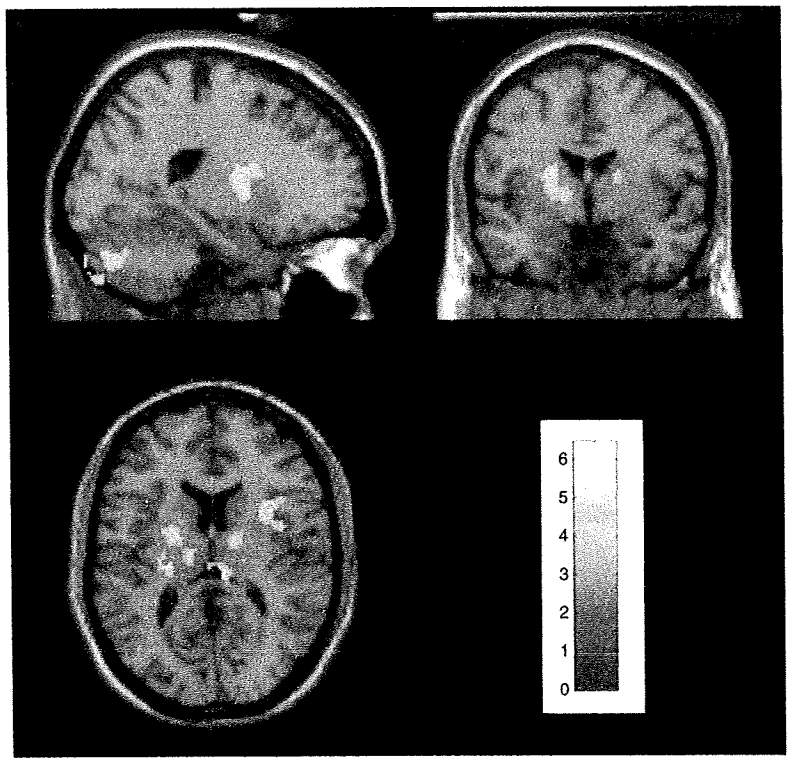

Figure 1 Regions of cerebral activation induced by intraluminal distention in the rectum with a bag pressure of 40 mmHg. Brain regions activated during intraluminal distentions of the rectum were superimposed on the Talairach–Tournoux stereotaxic atlas of the human brain. Significant activations during 40-mmHg stimulus in the rectum (x,y,z = 32,8,8) are shown. The level of significance was set at 0.1% or less (voxel level).

Table 2 Brain regions activated during intraluminal distention in the rectum

Cordinate of local maximum x,y,z (mm)	Tentative anatomical localization	t-Value (voxel level)	$P_{FWE\text{-}corrected}$ (voxel level)	$P_{FDR\text{-}corrected}$ (voxel level)	No. voxels	$P_{corrected}$ (cluster level)
40-mmHg stimulus						
8,−26,8	Right thalamus	6.02	0.029	0.003	306	<0.001
32,8,8	Right anterior insula (BA13)	5.40	0.165	0.004	193	<0.001
−26,−20,10	Left thalamus	5.39	0.167	0.004	57	0.181
−20,−2,12	Left thalamus	4.78	0.636	0.009	369	<0.001
20-mmHg stimulus						
48,−56,48	Right inferior parietal lobule (BA40)	4.87	0.547	0.038	75	0.068
8,−26,12	Right thalamus	4.63	0.778	0.058	37	0.519
52,−30,30	Right inferior parietal lobule (BA40)	4.36	0.952	0.089	26	0.809

Significance threshold was set at $P_{uncorrected} < 0.001$ (voxel level). Degree of freedom = [1, 45]. BA, Brodmann area.

descending colon, significant deactivation occurred in brain regions similar to those deactivated during the rectal stimulus (Fig. 3B and Table S2).

Comparisons of contrasts in activated brain regions

In response to the intraluminal distention at 40 mmHg, the R group showed greater activation in the pMCC and right anterior and posterior insula (Fig. 4A,B and Table 4), whereas the DC group showed greater activation in the sACC, pACC, and left orbitofrontal and superior temporal cortices (Fig. 4C,D and Table 4). There was little significant difference in regional contrasts during the application of stimulus at 20 mmHg between the groups (Table 4).

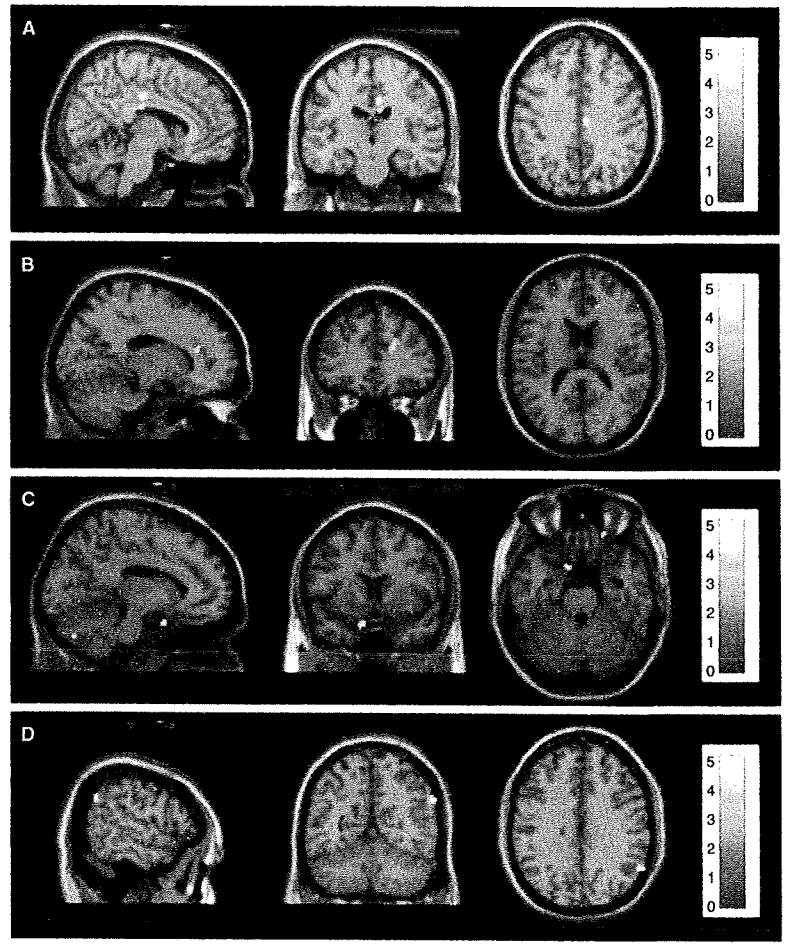

Figure 2 Regions of cerebral activation induced by intraluminal distention in the descending colon with a bag pressure of 40 mmHg. Brain regions activated during intraluminal distentions of the descending colon were superimposed on the Talairach–Tournoux stereotaxic atlas of the human brain. Significant activations during 40-mmHg stimulus in the descending colon (*x,y,z* = 8,−18,38: A; 16,32,16: B; −12,10,−24: C; and 60,56,32: D) are shown. The level of significance was set at 0.1% or less (voxel level).

Table 3 Brain regions activated during intraluminal distention in the descending colon

Cordinate of local maximum *x,y,z* (mm)	Tentative anatomical localization	*t*-Value (voxel level)	$P_{FWE-corrected}$ (voxel level)	$P_{FDR-corrected}$ (voxel level)	No. voxels	$P_{corrected}$ (cluster level)
40-mmHg stimulus						
8,−18,38	Right pMCC (BA24)	5.18	0.263	0.250	32	0.635
22,32,28	Right pACC (BA32)	4.65	0.736	0.250	26	0.796
60,−56,32	Right supramarginal gyrus (BA40)	4.42	0.910	0.250	36	0.531
6,−22,26	Right pMCC (BA23)	4.35	0.943	0.250	37	0.506
−12,10,−24	Left sACC (BA25)	4.22	0.982	0.250	27	0.770
20-mmHg stimulus						
−10,30,38	Left pACC (BA32)	5.28	0.210	0.212	38	0.483
64,−56,32	Right supramarginal gyrus (BA40)	4.62	0.757	0.397	27	0.770
4,−20,40	Right pMCC (BA24/23)	4.25	0.975	0.397	27	0.770
−12,10,−24	Left sACC (BA25)	4.07	0.997	0.397	34	0.582

Significance threshold was set at $P_{uncorrected} < 0.001$ (voxel level). Degree of freedom = [1, 45]. BA, Brodmann area; ACC, anterior cingulate cortex (s, subgenual; p, perigenual); MCC, midcingulate cortex (p, posterior).