VIII. 9. Impact of Serotonin Transporter Gene Polymorphism on Brain Activation by Colorectal Distention

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Visceral Perception and Cingulate Cortex

Our previous study using positron emission tomography (PET) demonstrated that colonic stimulation increases regional cerebral blood flow (rCBF) in the anterior cingulate cortex (ACC) and prefrontal cortex (PFC), showing correlation with increased anxiety[1)]. In a functional magnetic resonance imaging (fMRI) study, patients with irritable bowel syndrome (IBS) showed stronger activation of the ACC in response to intense rectal distention than control subjects[2)]. Imaging data on depressive disorders suggest that one of the common regions of brain activation is the ACC[3)]. 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. 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[4)]. 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[5)]. 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. The human serotonin transporter (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[6]. 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. Individuals with the *s* gene are at significantly greater risk for major depressive disorder following repeated adult stress or childhood trauma[7]. Hariri et al.[8] reported that individuals with the *s* allele show greater amygdala neuronal activity 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[9]. 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 the essential process of emotional formation, most previous studies used visual and cognitive tasks to demonstrate brain processing. 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[10].

Serotonin Transporter Gene Polymorphism and Positron Emission Tomography

Twenty-eight adult Japanese subjects without organic diseases or psychiatric disorders were enrolled in the study. 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[11]. This study was approved by the Tohoku University Ethics Committee and subjects provided written informed consent.

Peripheral blood was sampled with a heparinized syringe. Genotyping of 5-HTTLPR was performed using the same methods as in our previous report[12]. Colorectal stimulation was performed using the same methods as previously described[1,13]. 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 mmHg 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).

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 k.c.p.s.$kBq^{-1}ml^{-1}$ [1,13]. 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 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[14,15]. All rCBF images were stereotaxically normalized into the standard space defined by Talairach & Tournoux[16] using an rCBF template image supplied with SPM2. The normalized images were smoothed using a 12×12×12-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.

The brain image with 0 mmHg was subtracted from the brain image with 40 mmHg. 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)[10]. 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)[10]. The brain image with 0 mmHg was then subtracted from the brain image with 20 mmHg. 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)[10]. 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[10]. There were no other regions which differentiate the brain response to colorectal distention between the *s* group and the *l* group.

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.

Serotonin, Cingulate and Negative Emotion

Colorectal distention in individuals with the *s/s* genotype activated the ACC, hippocampus, and OFC more than in individuals with the *l* allele[10]. This study[10] are in line with those of Hariri et al.[8] and Pezawas et al.[9] Therefore, our study[10], 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 stem[17]. Serotonergic neurons originate from the dorsal raphe nucleus and innervate the limbic system (i.e., cingulate cortex, hippocampus, amygdala, OFC, and hypothalamus)[5]. 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[10]. This implies that our study[10] 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[6]. In our study[10], 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. 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[10], 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[3]. 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[9]. 5-HTTLPR *s* allele carriers show less coupling between the amygdala and the perigenual ACC than *l/l* individuals, particularly in the subgenual ACC[18]. 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[5]. 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[19]. 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[20]. 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.

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.

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Table 1. Subject characteristics of *s* group and *l* group[10]. *s* group: individuals with the *s/s* genotype; *l* group: individuals with the *l* allele (*l/s, l/l,* or *l/extra-l* genotype).

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

Table 2. Summary of differential brain activation between *s* group and *l* group[10]. Side: R: right, L: left; BA: Brodmann's area: regions with $p < 0.0001$ were shown.

Main Effect	Region	Side	BA	Cluster	p	x	y	z	T
s > *l* (40mmHg − 0 mmHg)									
	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* (20mmHg − 0 mmHg)									
	Orbitofrontal Cortex	L	47	215	0.012	-38	24	-20	4.32
l > *s*									
	No Suprathreshold Brain Regions								

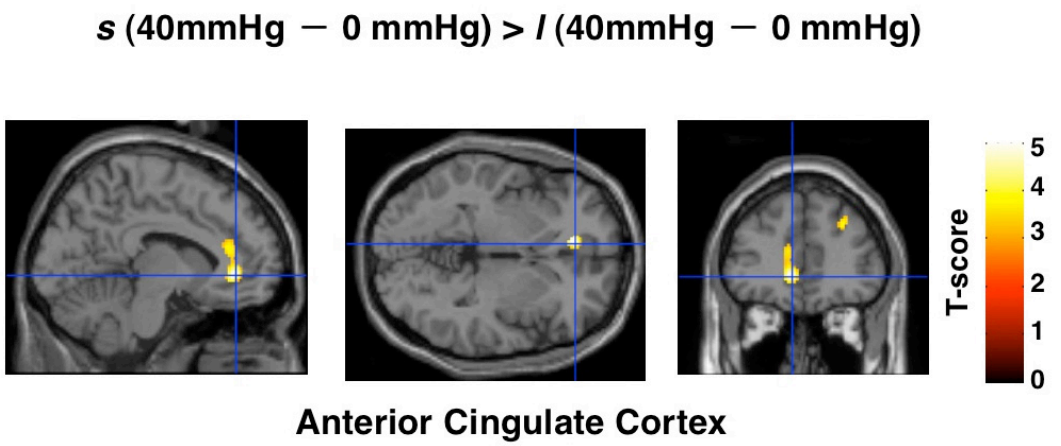

Figure 1. Moderate colorectal distention in the *s* group significantly activated more the left anterior cingulate cortex than that in the *l* group[10]. The image with 40 mmHg was subtracted by that with 0 mmHg. BA 32, x, y, z = -8, 40, -2, $p < 0.0001$.

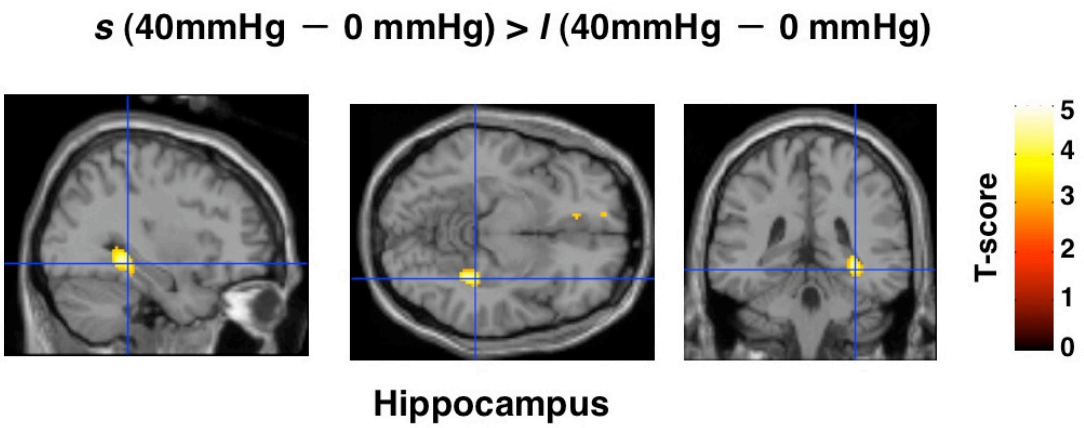

Figure 2. Moderate colorectal distention in the *s* group significantly activated more the right hippocampus than that in the *l* group[10]. The image with 40 mmHg was subtracted by that with 0 mmHg. x, y, z = 32, -42, -4, $p < 0.0001$.

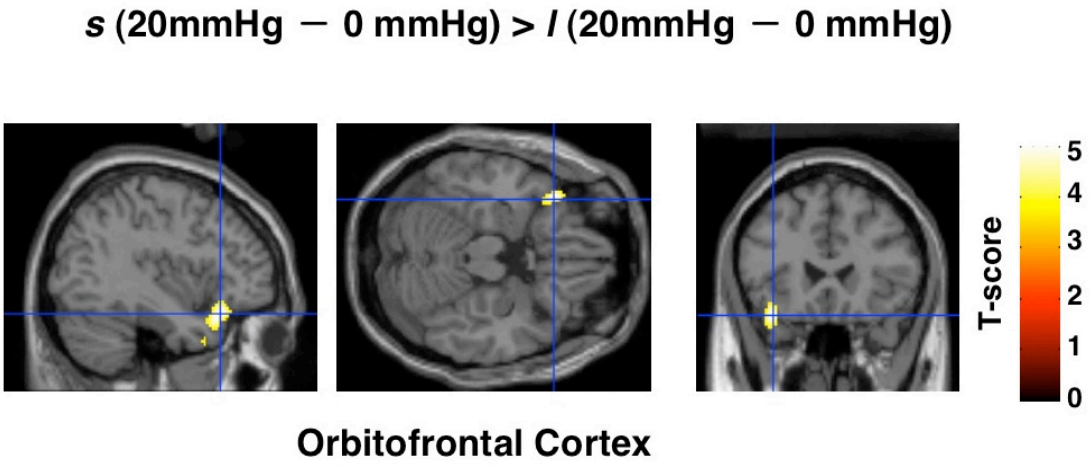

Figure 3. Mild colorectal distention in the *s* group significantly activated more the left hippocampus than that in the *l* group[10]. The image with 20 mmHg was subtracted by that with 0 mmHg. BA 47, x, y, z = -38, 24, -20, $p < 0.0001$.