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## VIII. 13. Brain Activity Following Esophageal Acid Infusion Using Positron Emission Tomography

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### **Introduction**

Gastroesophageal reflux disease (GERD) causes reflux symptoms such as heartburn and regurgitation due to abnormal acid reflux with or without mucosal damage. Although there is a correlation between the severity of macroscopic esophagitis and acid reflux, the severity of subjective symptoms is not necessarily correlated with that of acid reflux. Therefore, the possible involvement of esophageal hypersensitivity to acid in some GERD patients has attracted attention<sup>1</sup>). The susceptibility of afferent nerve terminals to luminal acid with the dilated intercellular space in the esophageal mucosa is one of the causative factors for acid hypersensitivity<sup>2</sup>). However, perceived acid reflux accounts for only a minority of reflux events, and the mechanism of symptom development remains to be elucidated. Recently, brain imaging analysis using PET and fMRI demonstrated that some key brain areas, such as the anterior cingulate cortex (ACC) or insula, are involved in the processing of visceral sensation and pain. Abnormality or modulation of those brain activation have been shown in patients with irritable bowel syndrome (IBS)<sup>3-5</sup>), suggesting the participation of abnormal symptom processing in visceral hypersensitivity. Until now, only a few studies have investigated brain activity after esophageal acid stimulation<sup>6-8</sup>). The aim of this study was to investigate induced symptoms and brain activity using PET in esophageal acid stimulation.

### **Materials and Methods**

#### *Subjects*

Fifteen right-handed healthy adult male volunteers (mean age: 26.7 years; range:

21–37 years), who had no typical reflux symptoms such as heartburn and regurgitation, were recruited for the study. A small-diameter catheter was transnasally indwelled in the mid esophagus. A wired pH glass electrode, pre-attached at its proximal side 3 cm from the infusion catheter, was connected to a pH meter. In order to obtain correction data for  $\gamma$ -ray absorption in the body, subjects initially underwent a transmission scan using a  $^{68}\text{Ge}/^{68}\text{Ga}$  radiation source.

### *Esophageal acid infusion*

The procedures for esophageal infusion and PET scan are schematically shown in Fig. 1. Infusions of 50 mL HCl (pH 1 and 2) or distilled water (pH 7) were provided by a catheter using an automatic syringe pump in the supine position. In order to counterbalance the effects of the infusion order, the order was randomly selected from pH 1-7-2-7-1, pH 2-1-7-7-1, and pH 7-1-1-7-2. Then  $^{15}\text{O}$ -labeled water was administered intravenously in synchronization with the completion of each 5-min infusion. After confirming that the brain activity could be detected, a PET emission scan of the head was performed for 60 s prior to the PET scan. Using a PET scanner in a 3D data acquisition mode, a total of 10 scans were taken before and after each of the five infusions, to measure the regional cerebral blood flow in each subject. Subjects were asked to rate the severity of heartburn symptoms on an analog scale of 0–10 after each infusion. Symptoms were statistically analyzed by Fisher's test and the Wilcoxon signed-ranks test. Differences were considered statistically significant when the  $P$  value was  $< 0.05$ .

### *PET data analysis*

The PET data were analyzed using statistical parametric mapping (SPM) software (SPM2), and significantly different changes in regional cerebral blood flow were mapped. First, brain images taken following infusion with hydrochloric acid (pH 1 and 2) or distilled water (pH 7), as well as images taken at baseline (prior to all infusions) were subjected to subtraction analysis to investigate the brain regions that were activated by each infusion. Next, the effects of repeated infusion of acid or distilled water were assessed by subtraction analysis of images obtained following the first and second infusions with pH 1 and pH 7 solutions. All statistical methods were evaluated using linear convolution and contrasts, and the voxel values for each image were constructed using a statistical parametric map of the t-statistic statistical parametric mapping. The location of statistical peaks was

determined in Talairach and Tournoux atlas.  $P$  (uncorrected)  $< 0.001$  was defined as statistically significant for increased cerebral blood flow.

## **Results**

### *Enhanced incidence and severity of symptoms following acid infusion*

As shown in Table 1, the incidence of heartburn symptoms following each infusion showed a step-wise increase with increasing acidity of the perfusate. The incidence of heartburn tended to be higher after the second pH 1 infusion than after the first, and these scores were significantly increased following the second pH 1 infusion. On the other hand, the heartburn incidence and scores in both pH 7 infusions were much lower compared to the pH 1 infusions. Heartburn incidence and scores following pH 2 infusion were higher than that of the pH 7 infusions and lower than that of the pH 1 infusions.

### *Activated brain areas following acid infusion Comparison of brain images following each infusion:*

The brain image obtained at rest prior to all infusions was defined as the baseline image. Differences between brain images at baseline and those taken after infusion with acid or distilled water were subjected to subtraction analysis. Brain regions with increased blood flow were defined as those neurologically activated by each infusion. Brain regions activated following each infusion are summarized in Table 2. In the insula, activation was observed at the second pH 1 and 2 and first pH 7 infusions. Activation in the cingulate cortex was observed in nearly all infusions, with no particular trend observed for the topography of the activated sub-regions. At pH 1 and 2, activation was observed in the more anterior (rostral) part of the ACC (BA 24a) and, at pH 7, in the more posterior (dorsal) part of the ACC (BA 24a'). After infusions at pH 1 and 2 but not pH 7, activation was observed in the temporal pole (BA 38). Activation was also observed in the cerebellum following infusions at pH 1 and 2, and in the parahippocampal gyrus after both pH 1 infusions. The frontal area, precentral gyrus, and thalamus were less activated after each infusion.

### *Comparison of brain imaging with first and second infusion at pH 1 and 7:*

As shown in Table 3, the second pH 1 infusion minus the first showed that cerebral blood flow was increased in the right orbitofrontal cortex (Fig. 3a), right cuneus, left cerebellum, right superior temporal gyrus, right middle frontal gyrus (Fig. 3b), right pons,

right lingual gyrus, left putamen, and right caudate nucleus. On the other hand, the result of the second pH 7 infusion minus the first showed an increase in cerebral blood flow in the right middle frontal gyrus, left cerebellum, right midbrain, left PCC, and right superior frontal gyrus.

## **Discussion**

In the present study, we found that brain activity was substantially increased in the cingulate cortex and frontal lobe following esophageal acid infusion, with little activity observed in the thalamus and somatosensory areas. The insula was not consistently activated by acidic or non-acidic stimulations in this study. Activation of the ACC predominantly occurred in the anterior part (BA 24) at pH 1 and 2 with severer heartburn symptom, consistent with the more anterior part of the ACC is involved in affective and emotional responses<sup>9</sup>.

The heartburn symptom scores following infusions at pH 1 and 2 were higher compared with those at pH 7. We found that the parahippocampal gyrus was activated only by pH 1 infusion. This area is an important part of the limbic system, which plays a major role in the processing of emotional reaction or memory<sup>10</sup>. Therefore, activation of the parahippocampal gyrus is also compatible with induction of uncomfortable heartburn by acid infusion. In addition, activation was observed in the temporal pole (BA 38) following infusions at pH 1 and 2, but not at pH 7. This area is activated by distention in the proximal stomach<sup>11</sup>, and another report has described activation of the temporal pole by distention of the descending colon, with a feeling of anxiety<sup>12</sup>. In a study using visual stimulation, the temporal pole was activated by emotions of comfort and discomfort, wakefulness, and attended stimulation<sup>13</sup>. Therefore, the activation of the temporal pole observed in our study could have been due to alterations in the level of arousal, attention and emotion following acid infusion.

In this study, the heartburn scores were significantly higher after the second pH 1 infusion compared to the first, which suggests that esophageal sensation was sensitized by repeated acid infusion. Visceral sensitization, which can occur at the primary afferent nerve level (peripheral sensitization) and/or the spinal cord level (central sensitization), is considered as a very important phenomenon in the development of visceral sensation<sup>14</sup>. Recent studies using cortical evoked potentials or fMRI have reported that esophageal sensitization induced by acid stimulation results in alterations in the neural activity of the ACC and insula<sup>15-17</sup>. We found that, by subtraction analysis of the second pH 1 minus the

first, that the orbitofrontal cortex was strongly activated with the highest Z-score and cluster level. The orbitofrontal cortex, which is frequently observed to be activated following stimulation of the lower gastrointestinal tract, was less activated following esophageal stimulation in previous studies<sup>18,19</sup>). As a higher center of sensory integration, this area is thought to participate in the assessment of reward, punishment, comfort, discomfort, and memory or its verification<sup>20</sup>). The orbitofrontal cortex might also play a role in symptom processing with esophageal acid sensitization.

In summary, this present study showed that the insula, cingulate gyrus, temporal gyrus, and cerebellum were activated in esophageal acid perception in healthy volunteers, and that involvement of the somatosensory and prefrontal areas was minimal. In particular, emotion-related brain regions such as the anterior part of ACC, the parahippocampal gyrus and the temporal pole were activated under acidic conditions in the esophagus. It is also suggested that activation of the orbitofrontal area is involved in esophageal sensitization to repeated acid stimulation at the cerebral level. Dysfunction of these brain areas may be associated with the pathogenesis of functional heartburn or non-erosive reflux disease. Further studies are warranted to elucidate the mechanism of esophageal acid perception and sensitization

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Table 1. Incidence of heartburn symptoms and heartburn scores induced by each infusion.

	Heartburn incidence	Mean heartburn scores (range)
pH 7 (first infusion)	5/15 (33.3%)	1.4 (0-7)
pH 7 (second infusion)	5/15 (33.3%)	1.0 (0-6)
pH 2	7/15 (46.7%)	1.9 (0-9) <sup>b</sup>
pH 1 (first infusion)	10/15 (66.7%)	3.2 (0-10) <sup>c</sup>
pH 1 (second infusion)	12/15 (80.0%) <sup>a</sup>	5.0 (0-10) <sup>d</sup>

a:  $P = 0.0253$  vs. pH 7 (first infusion) and pH 7 (second infusion)

b:  $P = 0.0269$  vs. pH 7 (second infusion)

c:  $P = 0.0464$  vs. pH 7 (first infusion),  $P = 0.0253$  vs. pH 7 (second infusion)

d:  $P = 0.0040$  vs. pH 1 (first infusion),  $P = 0.0075$  vs. pH 2,  $P = 0.0041$  vs. pH 7 (first infusion),  $P = 0.0071$  vs. pH 7 (second infusion)

Table 2. Summary of brain activated regions by each infusion (comparison with baseline).

Major brain regions	Subregions	BA	First pH7	Second pH7	pH2	First pH1	Second pH1
Frontal lobe	Superior frontal gyrus	10		R	L		
	Middle frontal gyrus	10		R			
	Inferior frontal gyrus	47		L			
Temporal lobe	Superior temporal gyrus	38			L	R	L
	Superior temporal gyrus	42	L				
	Middle temporal gyrus	21		L		L	
	Middle temporal gyrus	42	L		L		
	Inferior temporal gyrus	45			R		
PMA	Precentral gyrus	4	R				
	Precentral gyrus	6				R	
PSA	Postcentral gyrus	1,2,3		R			
ACC	Anterior part	24			R	L+R	
	Mid/posterior part	24'	L+R	R			
PCC				R	L		L
Insula	Anterior part		R		R		R
cerebellum					C+L		L
thalamus				R+L			R+L
PHG						L	R

PMA, primary motor area; PSA, primary somatosensory area; ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; PHG, parahippocampal gyrus; BA, Brodmann area; R, right; L, left; C, center

Table 3. Results of subtraction analysis of brain images after the first and second infusions at pH 1 and 7.

Condition	Region	Side	BA	x	y	z	Z-score	Voxels in cluster
Second pH 1 – first	Orbitofrontal cortex	R		38	36	-24	4.44	167
	Cuneus	R	19	2	-82	36	3.79	51
	Cerebellum	L		-22	-40	-50	3.74	23
	Superior temporal gyrus	R	8	26	36	40	3.69	37
	Middle frontal gyrus	R	32	22	50	8	3.69	23
	Pons	R		10	-36	-12	3.47	31
	Lingual gyrus	R	19	4	-60	-2	3.37	12
	Putamen	L		-24	-8	2	3.32	16
	Caudate nucleus	R		18	-34	16	3.30	16
Second pH 7 – first	Middle frontal gyrus	R	10	36	46	0	4.03	47
	Cerebellum	L		-18	-98	-18	3.98	40
	Midbrain	R		2	-40	2	3.65	27
	Posterior cingulate cortex	L	23	-14	-16	30	3.61	11
	Superior frontal gyrus	R	8	14	22	42	3.57	33

R, right; L, left

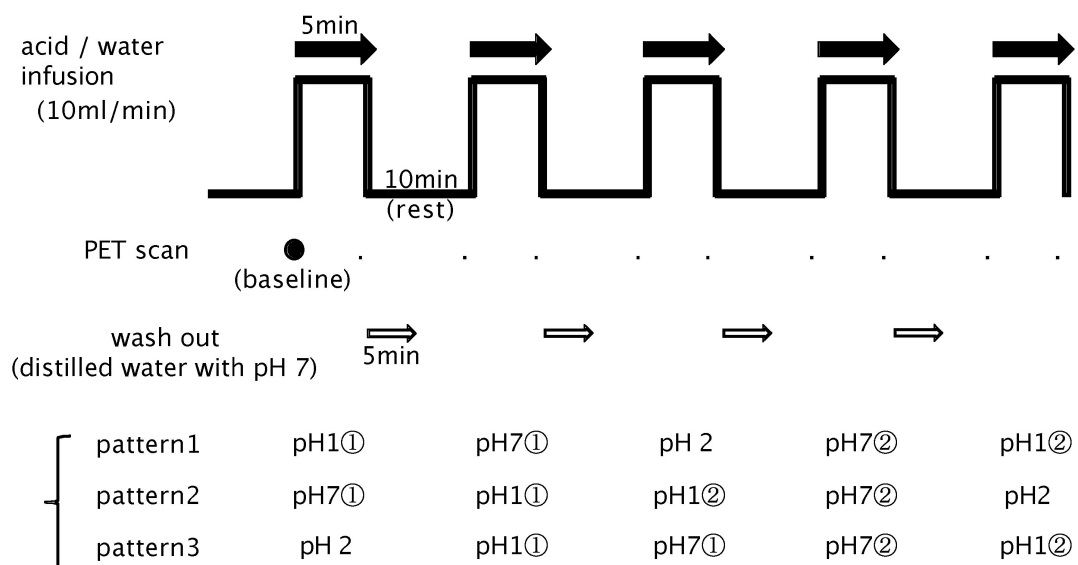


Figure 1. This schema illustrates the procedure of esophageal infusion and brain PET scanning. The infusions were performed twice for pH 1 and 7 solutions (distilled water) and once for the pH 2 solution. In order to counterbalance the effects of the infusion order, the order was randomly selected per each subject from pH 1-7-2-7-1, pH 7-1-1-7-2 and pH 2-1-7-7-1 as shown. □First infusion, □Second infusion, PET: Positron emission tomography.



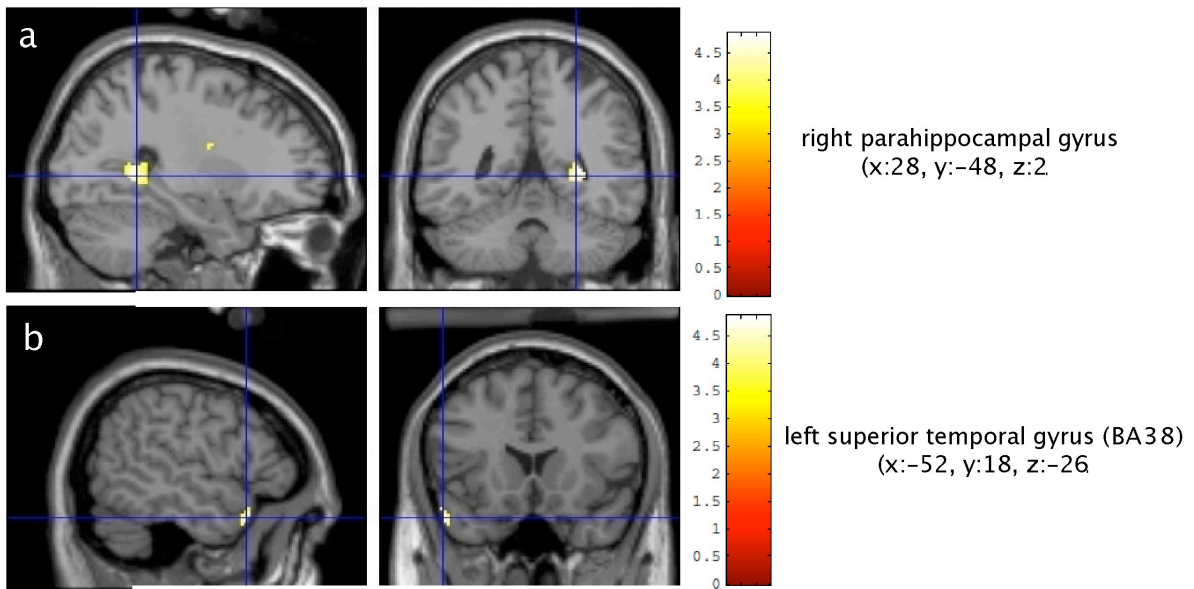


Figure 2. Representative images from the subtraction analysis of the second pH 1 infusion minus the baseline. Left, sagittal view; right, cranial view. a. Right parahippocampal gyrus (x: 28, y: -48, z: 2). b. Left superior temporal gyrus (temporal pole, BA38) (x: -52, y: 18, z: -26)

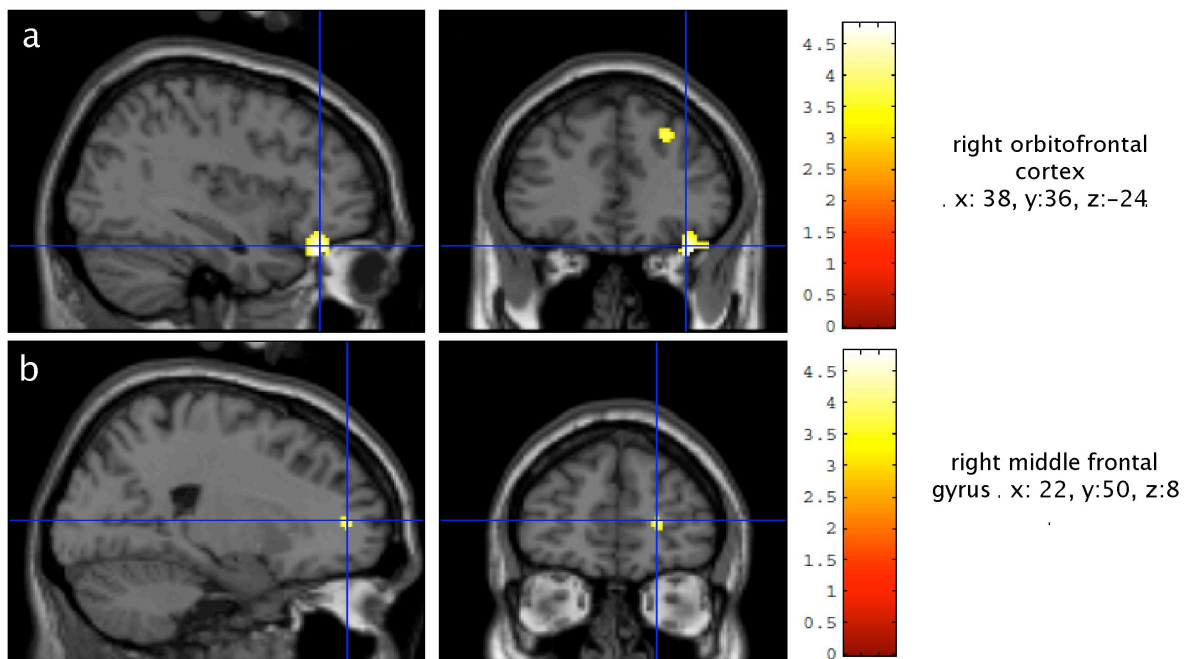


Figure 3. Representative images from the subtraction analysis of the second pH 1 infusion minus the first. Left, sagittal view; right, cranial view. a. Right orbitofrontal cortex (x: 38, y: 36, z: -24). b. Right middle frontal gyrus (x: 22, y: 50, z: 8)