# VIII. 5. Differential Activation of the Midbrain Periaqueductal Grey by Experience of Visceral Stimulation in Humans

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## Introduction

The midbrain periaqueductal grey (PAG) plays a pivotal role in integrating the analgesic and cardiovascular responses of an animal to threat, stress or pain[1)]. The PAG is also an important site for acute opioid actions [2] and the expression of some signs of opioid withdrawal[3)]. Experience of pain minimizes immediate harm by motivating escape[4-6)]. Anticipation of pain is mechanism to prevent future harm by learning signs of impending pain[7-10)], allowing avoidance of future painful events.

Hypersensitivity to visceral stimulation is major pathophysiology of IBS[11-13,14)]. Recent advances in the neurophysiology are providing a series of plausible mechanisms to explain the development of hyperalgesia of the human gastrointestinal tract[15-17)]. However, the brain area related to initial programming of sensitization provoked by the visceral perception is still unknown. Detailed information on brain activation in the initial programming of sensitization to distention of the descending colon in humans is needed.

This study clarified that brain activation due to the colonic distention is different between with initial intense stimulation and with intense stimulation after the mild stimulation. The brain behaves differently in response to the different order of visceral stimulation. This differential brain activation to colonic distention seems to be due to summation of central processing.

## Methods

*Subjects*

This study was approved by the Ethics Committee of Tohoku University School of

Medicine. All subjects gave written informed consent. Fifteen normal volunteers (4 female, 11 male, 20-26 years old, all right-handed) participated in this study. All subjects were free from gastrointestinal disorder symptoms or signs. All subjects scored Spielberger's State and Trait Anxiety Inventory (STAI)[18] and Zung's Self-rating Depression Scale (SDS)[19] before the experiment.

On the day before examination, the subjects took a low residue diet and at night (21:00), they ingested 17 g (13.6%) of magnesium citrate, 75 mg of sodium picosulfate, and 24 mg of sennoside A & B to cleanse the colon. By using a colonoscope, a plastic tube with a thin polyethylene bag (Synectics Medical, Stockholm) was inserted into the descending colon.

*PET scanning*

$^{15}$O-Labeled water was injected into the right arm vein at the beginning of colonic distention. rCBF in each subject was measured during 4 scans (70 seconds each) using a PET scanner in three-dimension sampling mode (HEADTOME V SET-2400W, Shimadzu, Japan).

The descending colon was distended with a computerized barostat pump (Medtronics Synectics, Shoreview, MN). To clarify the sensitization process to colonic distention, orders of stimuli was set with 2 different patterns as follows: group 1: 40 - 20 (n = 8), group 2: 0 - 20 (n = 7). No subject was informed of the order or intensity of stimuli.

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[20] from 0 (no sensation) to 10 (maximal sensation).

*Data Analyses*

PET images were analyzed using SPM2 (Wellcome, Department of Cognitive Neurology, London, UK) on a MATLAB platform (Mathworks Inc.). To estimate the neural sensitization to visceral stimulation by stimulus effect of 0, 20, and 40 mmHg distention, a conjunction analysis were made using 'multi-group, conditions and covariates' SPM model[21]. All brain images were analyzed in a conjunction analysis[21], rectified for gender difference and order effect on this analysis[22]. Ordinate visceral perception and emotion were compared between groups with Mann-Whitney U test.

## Results

*Visceral perception and emotional changes during equivalent stimulus in different order*

Visceral perception and emotion were found no difference in equivalent stimuli depending on stimulus order of the descending colon. Abdominal discomfort ($Z = -1.56$, $p = 0.12$) and abdominal pain ($Z = -0.90$, $p = 0.37$) during 20 mmHg distention after 40 mmHg were lower than during 20 mmHg distention only (not significant, Fig. 1).

*rCBF changes during equivalent colonic distention in different order*

Colonic distention with 20 mmHg after the experience of 40 mmHg significantly more activated the midbrain including PAG ($Z = 4.95$), left insula (BA13, $Z = 4.93$), right putamen ($Z = 3.96$), right middle temporal gyrus ($Z = 3.94$) and cerebellum than that without prior experience of visceral stimuli (group 3 and 4) (uncorrected $p < 0.001$, Fig. 2).

*rCBF changes by colonic distention*

Colonic distention with 20 mmHg and 40 mmHg induced significant activation of the regional brain; right orbitofrontal gyrus (BA 11), bilateral inferior parietal gyrus (BA 40), right putamen, bilateral caudate, bilateral thalamus, and cerebellum (data not shown). These area are essentially the same brain regions of the first report in our laboratory[23]. Sham distention with 0 mmHg also induced significant activation of the right inferior parietal gyrus (BA 40) (data not shown), replicating the previous analysis with smaller sample size[23].

*Basic Characteristics of Subjects*

There were no difference in age and sex ratio among groups. Moreover, STAI and SDS did not differ among groups.

## Discussion

By establishing a direct comparison to equivalently visceral stimulus in the different order, these data show the possibility that the modulation of visceral perception and emotion by experience of stimulation dependent on neural activity in a specific area of midbrain, insula, and cerebellum.

The PAG is an important component of the descending noxious inhibitory system[24], which contains a high concentration of opiate neurons with descending spinal afferents[25]. One mechanism by which the PAG modulates visceral perception may

involve the release of endogenous opioids. The PAG contains significant quantities of all families of endogenous opioid peptides, and it is known that μ opioids act by releasing PAG projection cells from GABAergic inhivition[26]. Specific projections of the medial network to the PAG, hypothalamus, and amygdala presumably mediate integrated autonomic and antinociceptive responses to acute aversive stimuli[24, 27]. Activation in the PAG is significantly increased during attentional modulation of pain intensity[25], and changes in pain responses attributable to changes in arousal or attention result from the action of modulatory networks that control the transmission of nociceptive signals of the brain[28]. Therefore, this study suggest that mild (20 mmHg) distention after the experience of 40 mmHg distention to the descending colon might be enough to reduce visceral sensation with activating descending pain inhibitory system in normal subjects.

In the previous study has shown in humans that a conditioning stimulus is able to modulate the perceptual and reflex response to intestinal distention[29]. In healthy human subjects and patients with IBS, repetitive distention of a balloon in the rectum or colon to noxious pressures altered the perception of colonic distention consistent with the development of hyperalgesia[14, 30]. Analogous short-term stimulation paradigms have been shown to induce central sensitization in animal models[31]. Because the development of central sensitization is modulated by endogenous pain modulation systems, including bulbospinal descending inhibitory system[32], a short-term sensitization paradigm may detect differences in the activation of these systems. Our findings indicated an adequate activation of central perceptional systems in response to preceding visceral stimuli as a possible mechanism in the etiology of altered visceral perception in functional bowel disorders.

**References**


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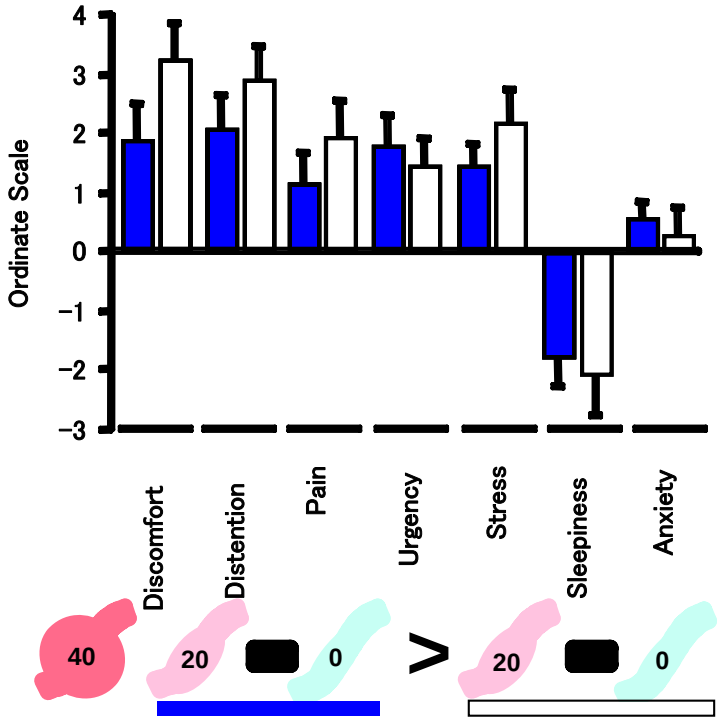


Figure 1. Comparisons of visceral perception and emotion during stimulation minus baseline. Mild (20 mmHg) stimulation after intense stimuli v.s. 20 mmHg without prior stimulation. Solid bars indicated the group 1. Open bars were group 2 (mean and standard error). Vertical axis indicated the visceral perception and emotion changes from baseline of the ordinate scale. There were no significantly differences in the ordinate scale during mild stimulation between with intense stimuli and without prior stimuli. Statistical analyses was used by Mann-Whitney U-test.

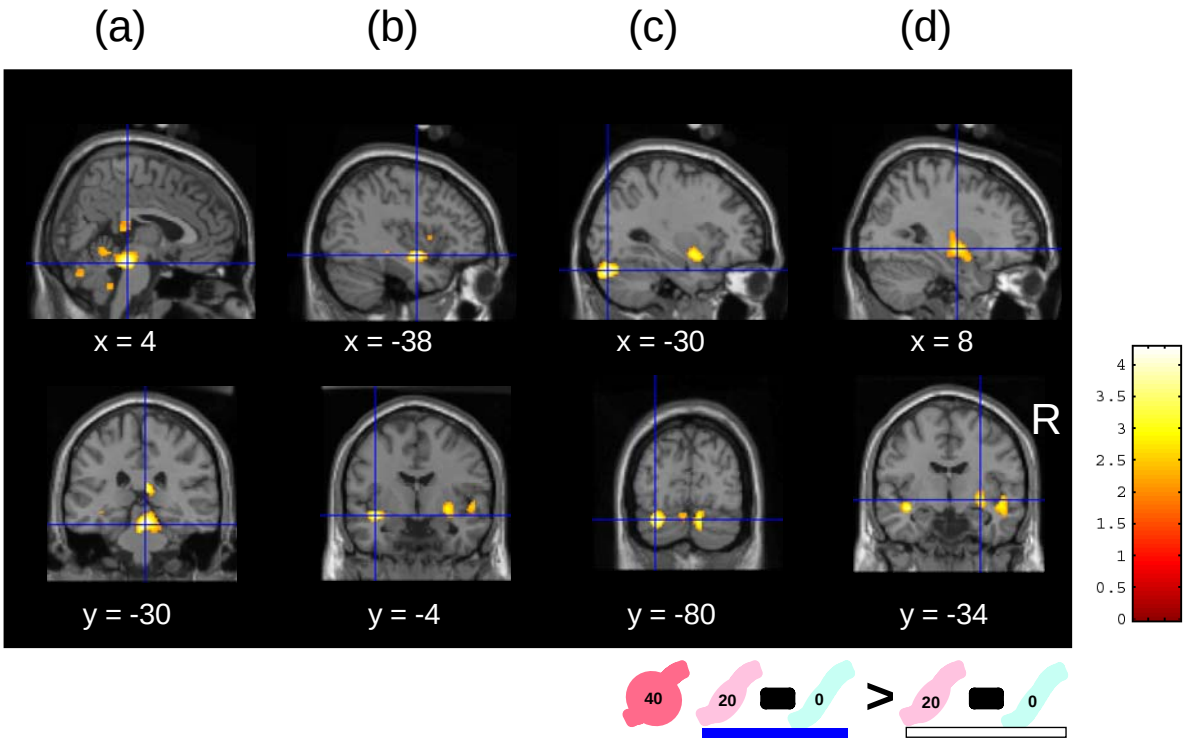


Figure 2. Statistical (Z) maps of brain during 20 mmHg stimulation compared to 20 mmHg distention after the experience of 40 mmHg distention (group 1) and 20 mmHg distention without the experience of prior stimuli (group 2). (a) Note the significant higher activation of the midbrain including PAG (x, y, z = 4, -30, -20, Z = 4.95), (b) left insula (BA13, x, y, z = -38, -4, -12, Z = 4.93), (c) left cerebellum (x, y, z = -30, -80, -26, Z = 4.74), (d) right putamen (x, y, z = 30, -8, -6, Z = 3.96). Color calibration bars that apply to each image represent critical Z-value magnitude of the activation areas. 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 Z-value magnitude of the activation areas with a threshold voxel alpha level of $p < 0.001$ (uncorrected). R indicated right side of the brain image.