VIII. 3.   Does Colonic Motility Really become Conditioned in Humans? A PET Study Using Transcutaneus Electrical Nerve Stimulation (TENS).

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INTRODUCTION

In classical or Pavlovian conditioning, the conditional stimulus (CS), which is a neutral stimulus paired with an uncomfortable unconditional stimulus (US) previously, comes to elicit behavioral and physiological responses as well as the US alone[1-3].   This learning process provides a model to understand anticipatory reports of pain and anticipatory gastrointestinal symptoms in situations that are not objectively threatening or painful[4]. However, little is known about the process of anticipatory response in gastrointestinal motility in humans.

Classical conditioning is considered to be a model to understand anticipatory responses to aversive events, which is an essential component of how the brain-gut interaction develops in functional gastrointestinal disorders. Previously, we have reported the following observations in humans[5]: (1) The colonic motility becomes conditioned with increasing smooth muscle tone and increasing number of phasic contractions; and (2) Characteristic brain areas become activated during anticipation regardless of the stimulus intensity.   In this report, anticipatory responses in the brain and the colon in humans were reviewed.

METHODS

Subjects

Nine right-handed healthy male subjects (mean age 24 ± 1 years; 19 to 29 years)

were recruited from Tohoku University Campus in Sendai, Japan. All participants were free of gastrointestinal complaints and had not taken any medications within 4 weeks prior to testing. Each participant in this study underwent a medical history evaluation and was given a physical examination. Written informed consent was obtained from all participants, and this study was approved by the Tohoku University Ethics Committee.

Measurement of Rectosigmoid Function

The experiment was performed after a fasting period of at least 9 hours. The subjects were placed in supine position and were instructed not to move during each session because of positron emission tomography (PET) scanning at the same time. A computer-driven barostat (Synectics Visceral Stimulator; Synectics, Stockholm, Sweden) was used to assess the rectosigmoid function[6-8]. A polyethylene bag (diameter; 9 cm, length; 9 cm, volume; 0-500 ml), which was tightly fixed at both ends to a catheter, was inserted into the rectosigmoid colon of each subject and placed with distal end of the bag 10 cm from the anal verge 30 min before the study.

Measurement of Brain Activation

Using a similar technique which we have described in the previous report[9], regional cerebral blood flow (rCBF) was measured. Subjects were instructed to lie on their back in the positron emission tomography (PET) scanner and to minimize head movement and keep their eyes closed during the scanning (for 70 sec). Using a $^{68}Ge/^{68}Ga$ radiation source, transmission scans were carried out prior to PET scanning. ^{15}O-Labelled water (Tohoku University Cyclotron Radioisotope Center) was injected into the right arm vein 10 sec before the beginning of each stimulus session. Ten seconds later, the radioactivity in the brain reached a plateau and an increase in rCBF was detected by the PET scanning as an index of neural activity evoked by the stimulus. As shown in Figure 1, five scans of rCBF in each subject were measured using PET scanner in 3-dimensional sampling mode (HEADTOME V SET-2400W, Shimadzu, Kyoto, Japan)[10]. The scanner produced 63 horizontal slices with a separation of 3.125 mm, an axial field of view of 200 mm, an in-plane resolution of 590 mm, a full width at half maximum (FWHM), and an axial resolution of 3.9 mm FWHM. 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.

Protocol

There were 3 sessions; pre-conditioning, conditional and post-conditioning trials. Subjects were exposed 7 times to a loud buzzer (500 Hz with an intensity of 87dB) lasting 1 second and being followed by a 9 seconds break. This sequence served as the conditional stimulus (CS). For the first sequence, only the CS tones were administrated as a pre-conditioning trial.

The unconditional stimulus (US), which followed the conditional stimulus during the conditional trials and a part of the post-conditioning trials, was composed of transcutaneous electrical nerve stimulations (TENS; OG GIKEN AUDIO TREATER EF-501, Okayama, Japan) delivered to the back of the right hand at a frequency of 15 Hz with 2 different levels of intensity (7 or 4 mA). The US started just after each tone was finished and the stimulus period lasted 70 sec. After three sets of the conditional stimulus or the post-conditioning conditional stimulus sequence, high-mA TENS was applied as the unconditional stimulus. After the post-conditioning conditional stimulus sequence, low-mA TENS was applied as weak unconditional stimulus. After the pre- or post-conditioning CS-alone sequence, the unconditional stimulus was not applied. In the post-conditioning session, stimulus intensities of 0 (sham), 4 and 7 mA were given in random order.

PET scanning was performed at the resting period as a background, and the pre- and post-conditioning trials for each subject (5 injections/scans). Each combination of the stimulus (the conditional stimulus with/without the unconditional stimulus) with break (10-second duration) was repeated 7 times because the PET technique requires a 70-sec recording window for each scan. The intra-bag pressure of barostat was kept at 10 mmHg to measure changes in the bag volume in the rectosigmoid colon.

Analysis

The intrabag volume in the rectosigmoid colon was measured continuously and its variations were visually analyzed. Mean bag volume over each two-minute interval served as a measure of muscle tone, and number of phasic volume events (PVEs), served as a measure of phasic contractions. In the present study, 2-minute interval for the analysis of barostat measurement was selected not to fail to observe changes in the rapid volume waves[6]. To control for occasional, minor changes in colorectal tone, the volume had to differ more than 10 % from the baseline tone occurring at a frequency of 1-4 min^{-1} to be characterized as a change[6]. Movement artifacts were defined as sudden changes in bag volume that did not continue for more than 15 sec and/or did not differ more than 10 %

from baseline[6]; these artifacts were excluded from data analysis. Changes in the bag volume or number of phasic volume events from each two-minute baseline interval just before the stimulus (baseline interval) to each two-minute interval just after the beginning of the stimulus (stimulus interval), and each following two-minute interval (post-stimulus interval), were considered to represent the colorectal wall reactivity to the conditional stimulus with/without the unconditional stimulus. The paired Student t-test or Wilcoxon's rank-sum test was used for comparing the rectosigmoid function in the two-minute baseline, stimulus, and post-stimulus intervals of each trial. Alpha level was set at 5% for these statistical analyses.

PET data were transferred to a super computer (NEC SX-4/128H4, Tokyo, Japan) at the computer center of Tohoku University through the optical network. The image reconstruction of all brain area was carried out using the Three Dimensional Filtered Back Projection Algorithm[11]. The PET image data were analyzed using standard software (Statistical Parametric Mapping; SPM99, The Welcome Department of Cognitive Neurology, London) according to the method of Friston, et al[12]. All brain slices were analyzed. The PET images were realigned, spatially normalized, and transformed into an approximate Talairach-Tournoux stereotactic space, 3-D Gaussian filtered (FWHM; 13 mm), and proportionally scaled to account for global confounders. The size of each voxel was set at 2 x 2 x 2 mm. A t-test was used to compare rCBF differences between the pre- and post-conditioning CS-alone trials as a primal analysis for the effect of the conditioning. We set alpha equal to 0.1% (uncorrected for multiple comparisons) as the region of significant differences. The region which showed the significant activity correlations was identified on the basis of Talairach coordinates.

RESULTS

All the subjects reported pain to the right hand and different given stimulus intensities during the post-conditioning buzzer (the conditional stimulus; CS) with high- or low-mA stimulus (the unconditional stimulus; US) trials. They did not report any pain or discomfort to the right hand in the buzzer alone test trials. The buzzer with TENS or the buzzer alone did not induce any gastrointestinal symptoms.

Assessment of Rectosigmoid Function

The mean bag volume during two-minute baseline interval was not significantly

different among the sessions before and after the conditioning. In the post-conditioning CS + high-mA US trial, the mean bag volume during two-minute post-stimulus interval was significantly smaller than that during two-minute baseline interval (65 ± 29 ml vs 47 ± 18 ml, $p<0.05$).   In the pre-conditioning trial and the post-conditioning CS-alone (baseline; 36± 11 ml vs post-stimulus; 34 ± 13 ml) and CS + low-mA US trials (48 ± 20 ml vs 38 ± 11 ml), the mean bag volume during post-stimulus intervals did not show significant difference compared to that during each baseline interval.   Thus, no conditioned effect was demonstrated for rectosigmoid muscle tone.

In the post-conditioning CS-alone trial, the number of phasic volume events (PVEs) during the two-minute post-stimulus interval was significantly greater than that during the immediately preceding two-minute baseline interval (0 [0-2] /min vs 1 [0-2.5] /min, $p<0.05$).   Also, the number of PVEs during the post-stimulus intervals were significantly greater than those during the baseline intervals in the post-conditioning CS + low-mA US (0.5 [0-1.5] /min vs 1 [0.5-2] /min, $p<0.05$) and CS + high-mA US (0 [0-1.5] /min vs 1 [0-2.5] /min, $p<0.05$) trials, respectively.   There were no significant differences in the number of PVEs in the pre-conditioning trial (0 [0-1.5] /min vs 0.5 [0-1.5] /min).   These data support a conditioning effect for colonic phasic contractions.

Assessment of Central Activation

The average PET data from all the subjects showed the conditioning elicited significant activation of the left lateral prefrontal, right anterior cingulate, bilateral parietal cortices, right insula, right pons and left cerebellum ($p \leq 0.001$, uncorrected, Fig. 1) when comparing rCBF differences between pre- and post-conditioning CS-alone trials of PET images.

DISCUSSION

In the present study, the loud buzzer used prior to conditioning as a conditioned stimulus (CS) did not cause any alteration in rectosigmoid motility.   However, following a series of conditional trials in which the buzzer was paired with painful electrical stimulation to the right hand, the buzzer alone elicited increases in the phasic contractions of the rectosigmoid colon, which were similar to those seen following the conditioned stimulus plus the unconditional stimulus.   This provides evidence for Pavlovian conditioning of phasic motor responses.   However, we did not find evidence for conditioning of the tonic

motor response (barostat volume) or subjective pain; following conditional trials, the CS-alone did not elicit changes in barostat volumes or reports of any gastrointestinal symptoms in the healthy subjects.

Considering the conditioning effect in the brain, our findings of the brain imaging (Fig. 1) were in accordance with previous studies showing cerebral activation in the frontal and parietal cortices following Pavlovian conditioning[13-15]. Activation of the prefrontal cortex was seen during somatic stimulus, and has been implicated in cognitive appraisal of the stimulus[16]. In addition, significant cortical activation in the anterior cingulate cortex (ACC) which is believed to play a role in mediating the affective qualities of the pain experience[17,18] and expectation of pain[19], and in the insula which serves as limbic integration cortex[20] was also seen as anticipatory responses in this study. Therefore, our results support that activation of the cognitive- and affective-related brain regions may contribute to the learned anticipatory responses and that this learned process was confirmed after the conditional trials in this experimental model. However, the direct relationships between the brain activation and the gastrointestinal response during anticipation have not been clarified with this model.

In summary, the Pavlovian conditioning study is significant because of positive findings that the conditioned phenomenon in this model is a first step to understand the anticipatory colonic motility responses. Significant increases in colonic phasic contractions and significant increases in cerebral blood flow in the cognitive- and affective-related cortical regions were observed in this study. This conditioning paradigm could be a model to investigate anticipatory responses in gastrointestinal motility and brain function which may contribute to development of functional gastrointestinal disorders. We concluded that the colonic motility can become conditioned by pairing a painful somatosensory stimulus with a neutral stimulus in humans.

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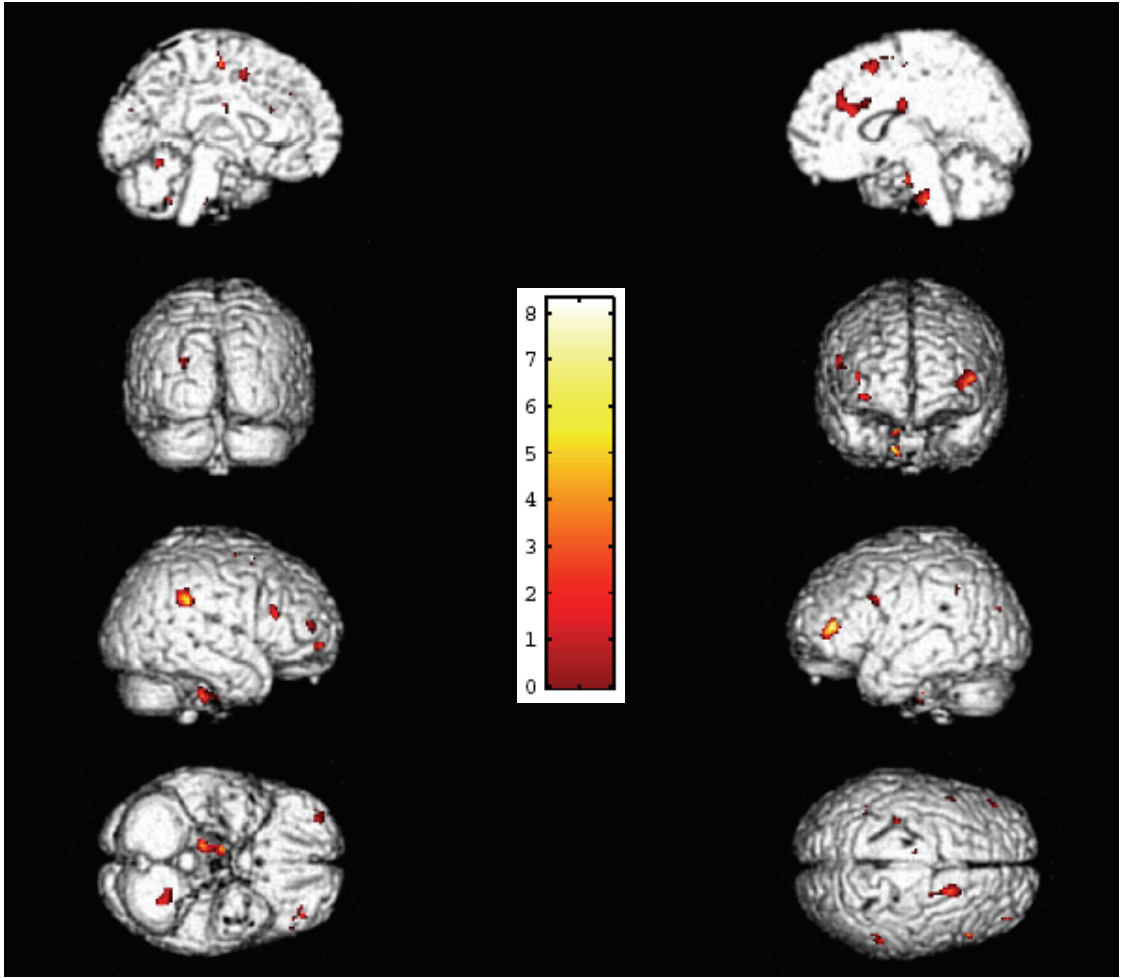
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Figure 1. Conditioning effects on regional cerebral blood flow.