Am J Physiol Gastrointest Liver Physiol
281: G1512–G1523, 2001.

Gender differences in cortical representation of rectal distension in healthy humans

MARK K. KERN, SAFWAN JARADEH, RONALD C. ARNDORFER, ANDRZEJ JESMANOWICZ, JAMES HYDE, AND REZA SHAKER
Medical College of Wisconsin Dysphagia Institute, Division of Gastroenterology and Hepatology, Biophysics Research Institute, and Department of Neurology, Medical College of Wisconsin, Milwaukee, Wisconsin 53226

Received 27 March 2001; accepted in final form 1 September 2001

Kern, Mark K., Safwan Jaradeh, Ronald C. Arndorfer, Andrzej Jesmanowicz, James Hyde, and Reza Shaker. Gender differences in cortical representation of rectal distension in healthy humans. *Am J Physiol Gastrointest Liver Physiol* 281: G1512–G1523, 2001.—Cerebral cortical processing of information relayed via visceral afferents is poorly understood. We determined and compared cortical activity caused by various levels of rectal distension in healthy male and female subjects. Twenty-eight healthy, young (20–44 yr) volunteer subjects (13 male, 15 female) were studied with a paradigm-driven functional magnetic resonance imaging (fMRI) technique during barostat-controlled rectal distension at perception threshold and 10 mmHg below and above perception threshold. Male subjects showed localized clusters of fMRI activity primarily in the sensory and parietooccipital regions, whereas female subjects also showed activity in the anterior cingulate and insular regions. A progressive increase in maximum percent fMRI signal change and total volume of cortical activity was associated with the intensity of rectal distension pressure in both genders. Regions of cortical activity for below-threshold stimuli showed less substantial signal intensity and volume than responses for threshold and above-threshold stimuli. Volume of cortical activity during rectal distension in women was significantly higher than that for men for all distensions. We conclude that *1*) there are substantial differences in female cortical activation topography during rectal distension compared with males; *2*) intensity and volume of registered cortical activity due to rectal stimulation are directly related to stimulus strength; and *3*) rectal stimulation below perception level is registered in the cerebral cortex.

functional magnetic resonance imaging; visceral sensation; regional cortical activity

CEREBRAL CORTICAL REGISTRATION and processing of the information originating from the gastrointestinal tract is poorly understood. With the recent advancement in the development of modern functional brain imaging techniques such as functional magnetic resonance imaging (fMRI) and positron emission topography, a renewed interest has developed in the better understanding of cortical function during gut-brain interaction in health and disease. To date, a limited number of studies have addressed the cortical representation of intestinal visceral sensation. These studies have identified several cortical regions associated with stimulation of the lower gut in patients with functional bowel disorders and in healthy controls (3, 4, 5, 16, 22) as well as regions receiving input from the stomach and esophagus (13, 22). Cortical regions involved in colorectal visceral sensation include the anterior cingulate (2), anterior/posterior cingulate (3), insular (2, 3), prefrontal (2–4), and thalamic (2, 3) regions. These regions have generally been activated in response to various degrees of painful and nonpainful stimuli that by themselves potentially could have introduced ancillary activation of cortical regions related to the stimulus perception such as fear, anxiety, and memory recall.

A better understanding of the gut-brain interaction is critical in determining the pathophysiology of functional bowel disorders, a group of common clinical problems with a significantly higher prevalence in women than in men. The reason for this gender bias is not completely understood. Gender differences in cortical response to somatic stimulation such as noxious heat have been reported (5). Gender differences in cortical registration of rectal distension among patients with irritable bowel syndrome were recently described (3). Related information with regard to healthy individuals is not available, and it is not clear whether this difference is a result of the disease or simply reflects a condition normally present among individuals unaffected by the disorder. Delineation of possible gender differences in cortical representation of visceral stimulation originating from the gut in healthy individuals is important for interpretation of functional brain studies with respect to health and disease. The present study, therefore, was undertaken to compare the cortical fMRI response to various levels of rectal distension in healthy male and female subjects.

METHODS

Twenty-eight asymptomatic adult volunteers (age 20–44 yr; 13 male, 15 female) were studied. Study protocols were

Address for reprint requests and other correspondence: R. Shaker, Div. of Gastroenterology and Hepatology, Froedtert Memorial Lutheran Hospital, 9200 W. Wisconsin Ave., Milwaukee, WI 53226 (E-mail: rshaker@mcw.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

approved by the Human Research Review Committee of the Medical College of Wisconsin, and subjects gave written informed consent before the studies. All subjects completed a detailed health-related questionnaire before each study and did not have any present or previous history of gastrointestinal tract-related diseases.

To study the brain response to rectal distension, cerebral cortical activity was monitored in all subjects with a blood oxygenation level-dependent (BOLD) functional magnetic resonance imaging (fMRI) technique. MRI echo planar and spoiled gradient-recalled acquisition at steady state (GRASS) anatomic images were acquired in the sagittal plane for 13 contiguous slices, 10 mm in thickness, spanning the whole brain volume. MRI scanning was performed on a 1.5-T General Electric Signa Scanner (GE Medical Systems, Milwaukee, WI). The scanner was equipped with a custom three-axis head coil designed for rapid gradient field switching and a shielded transmit/receive "birdcage" radio frequency coil to acquire a time course of echo planar images across the entire brain volume with the desired slice specifications. Echo planar images resolved to 64 × 64 pixels/slice at TR (repetition time) of 1 s and echo time of 40 ms were obtained during six scanning sequences with a 1-min interval between scans.

Data Analysis

A nonbiased method of detecting cerebral cortical regions of stimulus-related changes in oxy/deoxyhemoglobin concentration was used to correlate an idealized wave representative of the stimulus paradigm to the actual MRI-generated magnetic signals. Regions of cortical signal changes associated with rectal distension are shown graphically as color overlaid images stereotaxically mapped on the anatomic images in the Talairach-Tournoux coordinate system (24). Correlation statistics, image registration, activity volume calculation, magnetic signal change graphics, and three-dimensional display were facilitated by the Analysis of Functional Neuroimages (AFNI) software package written by Robert Cox, Medical College of Wisconsin Biophysics Research Institute (7). The AFNI software runs on a Pentium III-based Linux workstation (Southwest Computers, Houston, TX). AFNI allows the user to display a three-dimensional "brick" of MRI data transformed from images captured as a sequence of two-dimensional images. The software was also used to detect regions of cortical activity with the correlation technique described above as well as to display and calculate cortical volumetric regions of correlated fMRI signal change. Regions of activity were displayed as color maps overlaid on the three-dimensional anatomic images.

Our criterion for including a volume element, or voxel, as a region of cortical activity required the calculated correlation between the actual MRI time-series magnetic signal and the idealized response waveform to be ≥0.70. Furthermore, we applied the additional clustering requirement that a displayed region of correlated activity must be represented by a cluster of three or more contiguous correlated voxels (4). In the present study, we used a 64 × 64-pixel matrix for each sagittal image covering a 240 × 240-mm field of view and a slice thickness of 10 mm so as to include the whole cerebral cortex. Thus one echo planar image voxel was 3.75 × 3.75 × 10 ≅ 141 mm^3. With this criterion, an activated cluster must be >423 mm^3 to be included in the analysis. This cluster

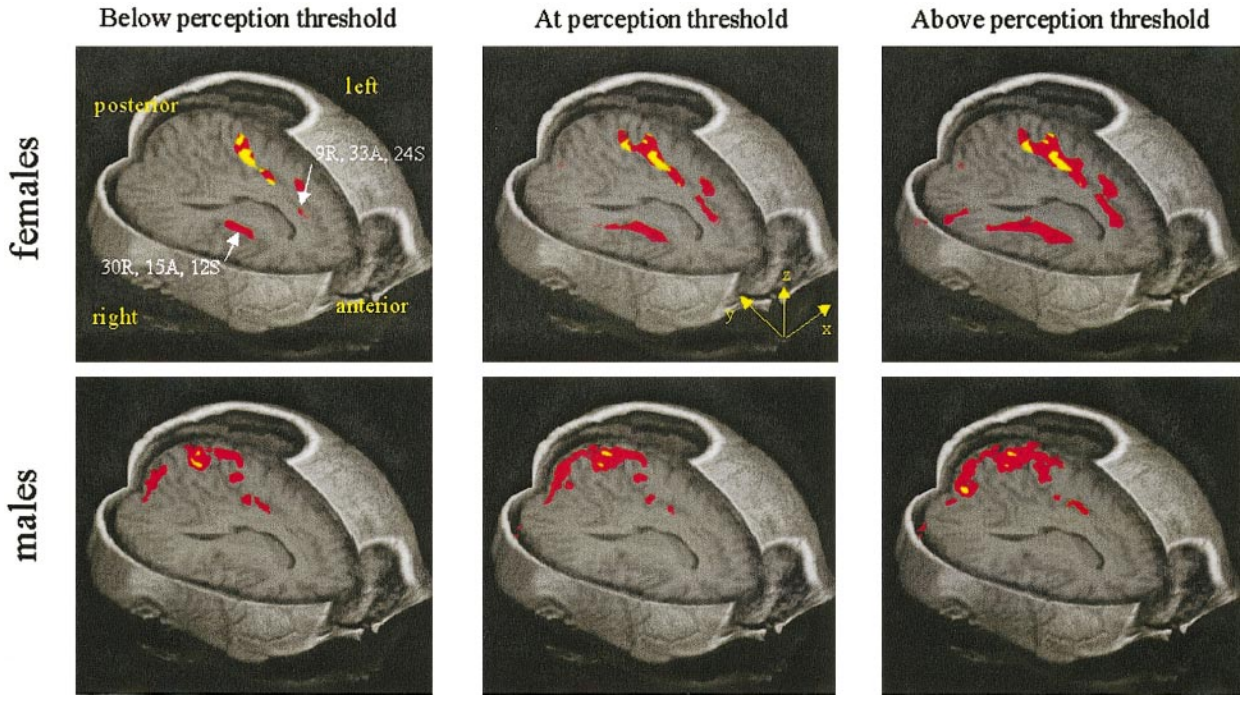

Fig. 1. Composite regions of cortical activity shown superimposed in color on a stereotaxic brain volume rendered in 3 dimensions. The right upper quadrant of the brain volume has been removed just above the midaxial plane (Talairach-Tournoux plane at z = 12 mm) and to the right of the midsagittal plane. As shown, the male subjects did not exhibit any activity in the insular or anterior cingulate/prefrontal cortices during all levels of distension. Also, the coordinates of the voxels with the largest average signal intensity change in the right insular cortex (30R, 15A, 12S) and anterior cingulate (9R, 33A, 24S) are shown on the below-perception threshold image. R, right; A, anterior; S, superior relative to the Talairach-Tournoux stereotaxic axes.

Table 1. *Below-perception threshold distension*

Subject No.	Brodmann's Areas														
	1	2	3	4	5	6	7	8	10	18	19	24	30	32	I
Male Subjects															
1	B	B			B						B		B		
2	B	B	B								B		B		
3	B	B	B		B					B	B		B		
4	B	B	B								B				
5	B	B			B					B	B		B		
6	B	B	B	B	B					B	B		B		
7	B	B								B	B		B		
8	B	B	B	B		B				B	B				
9	B	B	B		B					B	B		B		
10	B	B	B	B	B					B	B				
11	B	B			B		B			B	B		B		
12	B	B	B	B							B		B		
13							B			B	B		B		
Female Subjects															
1					B				B	B	B			B	R
2	B	B	B	B	B					B	B				
3	B		B				B		B		B	B		B	B
4	B								B			B		B	
5	B	B							B			B		B	B
6	B	B	B						B			B			R
7						B		B		B	B		B		B
8	B	B	B	B		B									L
9	B	B	B	B						B	B		B		
10						B			B						
11					B				B	B	B		B	B	B
12	B	B	B	B	B					B	B				
13	B	B							B			B		B	B
14	B	B	B						B			B			B
15	B								B			B	B	B	

L, left hemisphere activity; R, right hemisphere activity; B, bilateral activity; I, insular cortex; BA, Brodmann's area. Sensory/motor = BA 1, 2, 3, 4, 5, 6. Anterior cingulate/prefrontal = BA 8, 10, 24, 32. Parietal/occipital = BA 7, 18, 19, 30.

criterion was applied to avoid including single voxel activities that might represent artifact (9–11).

Perception Threshold and Rectal Distension Paradigms

All subjects participated in the same paradigm-driven rectal distension protocol. A catheter-affixed polyethylene bag was positioned in the rectum before MRI scanning. The polyethylene bag was roughly cylindrical with a length of 10 cm and a fully inflated diameter of 8 cm. Maximum bag volume was 500 ml, and the bag was infinitely compliant up to its distensible limit. The barostat device was kept outside of the scanner suite and was connected to the bag by a 30-ft polyethylene tube (3-mm OD, 1.8-mm ID). After the catheter-affixed bag was inserted within the rectum, the perception threshold for an individual subject was determined. Air was incrementally pumped into the rectal bag in 5-mmHg steps and sustained for 10 s by means of a computer-controlled barostat. After each pressure step, the subject was asked if he/she "felt anything." This stepwise procedure was continued until the subject reported feeling the inflated bag. The air in the bag was then evacuated, and the stepwise perception threshold procedure was repeated two more times to ascertain the threshold. The barostat pressure recorded at this level was deemed to reflect the perception threshold for rectal distension of that particular subject. All tested subjects reliably reproduced the same perception threshold pressure during the stepwise determination procedure.

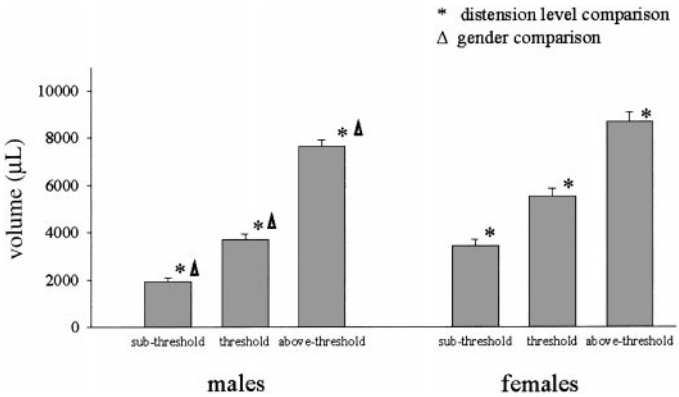

Fig. 2. Comparison of volume of cortical activity in response to unperceived (subthreshold), perceived (threshold), and above-perception-threshold rectal distension. Volume of cortical activity was directly related to intensity of stimulation in both genders. At subperception-threshold stimulation, the total volume of cortical activation in male subjects was significantly smaller than in female subjects (*, $\Delta P < 0.05$).

During the experimental scans, air was infused and evacuated from the rectal bag at the maximum possible flow rate of 60 ml/s to maintain the desired constant distension pressure or nondistension (zero) pressure. Before each MRI scan, air was infused into the rectal bag until a minimal static pressure of 3–5 mmHg was seen. A small quantity of air was then evacuated from the bag so that the pressure in the bag was zero relative to atmospheric pressure. Thus the rectal bag before

Table 2. *At-perception threshold distension*

Subject No.	Brodmann's Areas														
	1	2	3	4	5	6	7	8	10	18	19	24	30	32	I
Male Subjects															
1	B	B			B		B			B	B		B		
2	B	B	B	B	B					B	B				
3	B	B	B		B					B	B		B		
4	B	B	B		B		B			B	B				
5	B	B			B		B			B	B		B		
6	B	B	B	B	B					B	B		B		
7	B	B								B	B		B		
8	B	B	B	B		B				B	B		B		
9	B	B	B		B					B	B		B		
10	B	B	B	B	B					B	B		B		
11	B	B			B		B			B	B		B		
12	B	B	B	B							B		B		
13	B		B	B	B		B			B	B		B		
Female Subjects															
1					B				B	B	B		B	B	B
2	B	B	B	B	B					B	B				B
3	B		B				B		B		B	B		B	B
4	B	B							B			B		B	
5	B	B							B			B		B	B
6	B	B	B						B			B			B
7				B		B				B	B	B			B
8	B	B	B	B		B	B					B			L
9	B	B	B	B		B	B		B	B	B	B	B		B
10	B	B	B	B		B			B	B					B
11					B				B	B	B		B	B	B
12	B	B	B	B	B				B						B
13	B	B							B			B		B	B
14	B	B	B						B			B			B
15	B								B			B	B	B	

See Table 1 legend for definitions.

each scan was preloaded with air up to the volume at which a nominal pressure was measured. An example of typically registered volumes and pressures follows. For a perception level pressure reading of 30 mmHg, a bag air volume of 250 ml was registered. The subliminal distending pressure would then be 20 mmHg, requiring a bag volume of 220 ml of air, and the supraliminal pressure would be 40 mmHg, which requires 275 ml of air. A zero-pressure reading was established in the bag at a maximum volume of 175 ml of air. By starting each MRI scan with a preloaded volume of 175 ml of air in the rectal bag, the subliminal distending pressure could be achieved (or relieved) in <1 s, the perception-level pressure in 1.3 s, and the supraliminal pressure in 1.7 s.

We tested three barostat-controlled distension levels: *1*) 10 mmHg below perception threshold, *2*) at the perception threshold, and *3*) 10 mmHg above the perception threshold. There was a 1-min interval between each distension-scanning session. Subjects were asked whether or not they felt discomfort or pain after each distension scan. The subjects in our study were not cued as to the level of distension or the timing of the distension intervals. The instructions given before each scan were, ". . .You may or may not feel the bag inflating and deflating. . . ." MRI data were acquired during six 120-s scan sessions of 20-s intervals of sustained distension alternated with 20 s of no distension. The order of distension level sessions was randomized in each subject. Two scans were performed at each distension level.

In the correlation technique, a cross-correlation function between an idealized waveform that represents a theoretical approximation of the expected magnetic signal changes and the actual local cortical magnetic signal changes during a MR scanning sequence is calculated. The result of this calculation is a measure of the similarity between the shape of the idealized waveform and the experimental waveform. The probability of inappropriately identifying an experimental waveform as being significantly correlated can also be calculated from the correlation analysis. Similar to previous studies (4), we chose a minimum coefficient of 0.7 as a conservative measure of correlation. To reduce the magnetic signal artifact caused by subject motion, a gradient-descent least-squares method of image registration as described by Cox and Jesmanowicz (8) was used. Average data are expressed as means ± SE unless otherwise stated.

RESULTS

Cortical activity was detected for all levels of stimulation in all male and female subjects. The total volume of activated voxels in response to all three levels of distension in women was significantly higher than in men (Fig. 1). However, the maximum average percent signal intensity changes were similar between male and female subjects. The average distension pressure of the barostat bag at the perception threshold in healthy male volunteers (32 ± 1 mmHg) was similar to that of female volunteers (28 ± 2 mmHg). Neither of the two perceived distensions were reported to be associated with discomfort or pain.

Table 3. *Above-perception threshold distension*

Subject No.	Brodmann's Areas														
	1	2	3	4	5	6	7	8	10	18	19	24	30	32	I
Male Subjects															
1	B	B			B		B			B	B		B		
2	B	B	B	B	B					B	B				
3	B	B	B		B					B	B		B		
4	B	B	B		B		B			B	B				
5	B	B			B		B			B	B		B		
6	B	B	B	B	B					B	B		B		
7	B	B								B	B		B		
8	B	B	B	B		B				B	B		B		
9	B	B	B		B					B	B		B		
10	B	B	B	B	B					B	B		B		
11	B	B			B		B			B	B		B		
12	B	B	B	B							B		B		
13	B		B	B	B		B			B	B		B		
Female Subjects															
1					B				B	B	B		B	B	B
2	B	B	B	B	B					B	B				B
3	B		B				B		B		B	B		B	B
4	B	B							B			B	B	B	
5	B	B							B			B		B	B
6	B	B	B						B			B			B
7				B		B				B	B	B			B
8	B	B	B	B		B	B					B			L
9	B	B	B	B		B	B		B	B	B	B	B		B
10	B	B	B	B		B			B						B
11					B				B	B	B		B	B	B
12	B	B	B	B	B				B	B					B
13	B	B							B			B		B	B
14	B	B	B						B			B			B
15	B								B			B	B	B	

See Table 1 legend for definitions.

Comparison of Regional Cerebral Cortical fMRI Activity

Evaluation of the regions of the cortical signal changes stereotaxically mapped on the anatomic images in the Talairach-Tournoux coordinate system revealed that activated clustered voxels during rectal distensions were concentrated in distinct Brodmann areas, encompassing parietooccipital, sensory motor, and anterior cingulate/prefrontal areas as well as the insula. These evaluations also revealed that there were distinct differences in the regions of cortical activity between male and female subjects for all levels of perceived and unperceived rectal distensions. These differences are presented below for each level of rectal distension.

Regional comparison for distension at subperception level. In both male and female subjects, subperception rectal distension was associated with a detectable increase in cerebral cortical fMRI activity. The cortical activity regions in female subjects were different from those in male subjects (Fig. 1). Whereas activity in the sensory motor cortex and parietooccipital area was common in both genders, 9 of 15 female subjects, but none of the male subjects, exhibited activity in the insular cortex. Similarly, cortical activity was detected in the anterior cingulate/prefrontal area in 11 of 15 female subjects and none of the male subjects (Table 1).

Comparison of the total cortical volume of activated voxels between male and female subjects showed that the stimulus at subthreshold for perception of distension induced a significantly larger volume of activity in female compared with male subjects ($P = 0.013$; Fig. 2).

Regional comparison for distension at perception level. Balloon distension of the rectum to threshold for perceiving the stimulus induced cerebral cortical activity in areas similar to that of subperception stimulus in both male and female subjects (Fig. 1). Insular activity was found in 13 of 15 female subjects and in none of the male subjects. Similarly, anterior cingulate/prefrontal activity was found in 14 of 15 female subjects and in none of the male subjects (Table 2). The total volume of activated voxels in female subjects was significantly larger than that of male subjects (Fig. 2).

Regional comparison for rectal distension at above-perception level. A representative example of cerebral cortical activity induced by rectal distension at 10 mmHg above the perception threshold is shown in Fig. 1. Similar to the lower stimulation intensities, although both genders exhibited activity in the sensory motor and parietooccipital cortices, female subjects showed fMRI activity in the insula and anterior cingulate/prefrontal areas, whereas male subjects did not (Table 3).

Table 4. *Liminal rectal distension*

Subject No.	SM			ACPF			PO			Insula		
	L or ***R***	P or ***A***	S or ***I***	L or ***R***	P or ***A***	S or ***I***	L or ***R***	P or ***A***	S or ***I***	L or ***R***	P or ***A***	S or ***I***
						Males						
1	***24***	30	60				***2***	88	19			
2	9	15	72				4	86	24			
3	7	34	71				***5***	89	19			
4	11	14	69				5	55	51			
5	***14***	27	67				3	87	21			
6	19	14	65				13	86	19			
7	12	30	65				***2***	92	21			
8	9	16	68				***6***	79	26			
9	***14***	7	70				2	56	9			
10	12	7	71				***12***	87	20			
11	23	14	68				***7***	88	20			
12	***31***	25	58				***12***	89	18			
13	***28***	21	62				7	49	62			
						Females						
14	1	33	67	5	***31***	38	***4***	76	28	***34***	***27***	***2***
15	***1***	20	62				6	72	21	37	***5***	***4***
16	2	29	66	***5***	***37***	***4***	1	41	51	45	***18***	3
17	6	25	58	10	***63***	9						
18	***7***	26	61	2	***44***	***1***				***36***	***10***	10
19	***1***	30	66	0	***42***	***1***				40	***13***	***5***
20	40	9	45	3	***44***	***2***	7	85	24	***29***	***22***	1
21	4	17	63	***5***	***39***	***4***				42	***27***	***1***
22	***22***	***26***	45	27	***55***	16	***5***	42	54	46	7	9
23	2	11	62	8	***60***	18	8	75	21	***42***	***25***	1
24	***1***	36	63	***4***	***32***	36	***11***	71	25	***34***	***29***	***4***
25	3	16	64	2	***44***	***2***				43	***29***	***5***
26	2	32	66	0	***63***	9				***37***	***28***	***4***
27	0	30	67	***3***	***38***	***5***				34	***27***	***1***
28	2	29	66	***5***	***35***	***4***	***3***	52	18			

Table values are in millimeters relative to the Talairach-Tournoux stereotaxic coordinates. SM, sensory/motor; ACPF, anterior cingulate/prefrontal; PO, parietal/occipital; L, left; R, right; P, posterior; A, anterior; S, superior; I, inferior.

Regional comparison for rectal distension at three perception levels—Talairach-Tournoux coordinates. Tables 4-6 show the Talairach-Tournoux stereotaxic coordinates of the voxel with the greatest fMRI magnetic signal change in the four previously described cortical regions.

Relationship of Cerebral Cortical Activity With Intensity of Rectal Stimulation

Comparison of the total volume of bilateral cerebral cortical activity associated with various levels of rectal distension demonstrated significant differences between the volume of activated voxels in response to the three levels of distension ($P < 0.05$; Fig. 2) in both male and female subjects. Multiple pairwise comparisons (Tukey test) demonstrated that higher levels of distension resulted in larger volumes of cortical activity ($P < 0.05$). In addition, comparisons of the volumes of various regions of cortical activity (Fig. 3) showed significantly larger volumes represented in the sensory/motor and parietal/occipital regions in male compared with female subjects (multiple unpaired *t*-test with Bonferroni correction for multiple comparison, $P < 0.01$).

The average maximum fMRI signal change also showed distension level dependence (Fig. 4). Analysis of variance showed significant differences among the average maximum percent fMRI signal increase in response to rectal distension below threshold, at threshold, and above threshold of perception. Multiple pairwise comparison with Tukey correction showed that the higher the stimulus intensity the higher the percent maximum fMRI signal change ($P < 0.01$). Gender-related differences were not detected for the average maximum percent fMRI signal increases in response to rectal distension.

DISCUSSION

In this study we compared the cerebral cortical fMRI response for three levels of rectal distension between healthy male and female volunteers. Study findings demonstrated substantial gender differences in the cerebral cortical regions activated in response to rectal distension among these healthy volunteers. Whereas activity of the sensory/motor and parietooccipital area was common between genders, insular and anterior cingulate/prefrontal cortices were activated overwhelmingly in female subjects. In addition, at all levels of perception the total volume of cortical activity was significantly smaller in men compared with women. These findings clearly suggest significant differences in cortical processing of visceral sensation from the distal intestine between genders. These gender differences cannot be explained by variability in peripheral re-

Table 5. *Subliminal rectal distension*

Subject No.	SM			ACPF			PO			Insula		
	L or ***R***	P or ***A***	S or ***I***	L or ***R***	P or ***A***	S or ***I***	L or ***R***	P or ***A***	S or ***I***	L or ***R***	P or ***A***	S or ***I***
						Males						
1	***27***	31	64				***5***	84	19			
2	14	12	75				4	86	22			
3	7	34	69				***5***	81	19			
4	9	12	69				5	50	57			
5	***11***	27	67				3	79	21			
6	16	12	62				13	86	19			
7	12	30	65				***3***	93	21			
8	9	16	68				***6***	75	26			
9	***14***	7	70				2	55	9			
10	12	7	71				***17***	85	20			
11	23	14	68				***6***	88	20			
12	***31***	25	58				***14***	90	18			
13							7	49	62			
						Females						
14	3	33	67	5	***31***	38	***4***	76	28	***34***	27	***2***
15	***1***	20	62				6	72	21			
16	6	29	66	***5***	***37***	***4***	4	40	55	45	***18***	3
17	6	25	58	10	***63***	9						
18	***7***	26	61	2	***39***	***1***				***31***	***14***	10
19	***1***	30	66	0	***42***	4				***42***	***13***	5
20	44	11	43	3	***44***	***2***	7	85	24	***29***	***22***	1
21	4	17	64	***5***	***35***	***4***				40	***27***	***1***
22	***22***	***26***	45	27	***58***	16	***5***	42	54			
23	2	11	61	8	***60***	18	8	75	21			
24	***1***	36	67	***4***	***30***	36	***11***	71	25	***34***	***29***	***4***
25	3	16	61	2	***44***	***2***		73	27			
26	2	32	66	0	***68***	9				***37***	***28***	***4***
27	***4***	30	61	***3***	***38***	0				34	***27***	1
28	2	29	66	***5***	***33***	***1***	***3***	52	18			

Table values are in millimeters relative to the Talairach-Tournoux stereotaxic coordinates.

sponses to stimulus intensity, because only the magnitude of cortical involvement was altered by higher stimulus intensity, for the male subjects, with no recruitment of functionally different cortical regions such as the insular and anterior cingulate/prefrontal cortices.

One of the two gender-related regional differences in cortical registration of rectal distension involves the insular cortex. The insular cortex consists of an anterior and a posterior region and has connections to many cerebral structures such as the entorhinal area, hippocampus, amygdala, prefrontal cortex, and the motor/premotor cortex and basal ganglia (6). The insula is also reported to have connections with the sensory association areas and is considered to be the cortical visceral sensory area (6). The insular cortex is also involved in diverse cortical functions such as gustation, cardiac control (7), appreciation of the emotional aspects of pain (9), processing of verbal material (18), and recalled sadness (15). Previous studies documented blood flow increase in the anterior insula during anticipatory anxiety and panic symptoms induced by lactate infusion, suggesting that the anterior insular cortex participates in the emotional response to distressing cognitive or interoceptive sensory stimuli (20, 21). Activation of the insular cortex caused by experimental stimulation of the upper (1, 4, 11) and lower (3–5, 16, 22) gut was reported previously. The posterior insula has been identified as a cardiac control center as well as playing a role in the appreciation of the emotional aspects of pain (17, 18). The greatest part of the insular activity detected in our study was seen in the anterior insula; however, activity in the mid- and posterior portion of the insula was also noted. Bearing in mind the relatively large voxels employed in our fMRI scanning sequences, further discussion of delineating which particular insular subregions are activated in female subjects must wait until functional scanning sequences can be performed at greater special resolution.

The pain-related processes involving the insular cortex cannot play a role in our female subjects because pain was not induced in any of our volunteers. Anticipation anxiety due to the experimental circumstances of the study could be postulated to have played a role in female subjects, but this supposition would not necessarily explain the increase in insular activity during unperceived rectal distension that was not preceded by perceived stimuli. On the other hand, subjects could have experienced anticipatory anxiety during unperceived rectal distension if they had experienced perceived distension before the unperceived-distension scan. Because our distensions were randomized without cues, some of the subjects did receive perceived distensions before the unperceived distensions. Even in this circumstance, it seems unlikely that anticipa-

Table 6. *Supraliminal rectal distension*

Subject No.	SM			ACPF			PO			Insula		
	L or ***R***	P or ***A***	S or ***I***	L or ***R***	P or ***A***	S or ***I***	L or ***R***	P or ***A***	S or ***I***	L or ***R***	P or ***A***	S or ***I***
						Males						
1	***24***	30	60				***2***	88	19			
2	9	15	72				4	86	24			
3	7	34	71				***5***	89	19			
4	11	14	69				5	55	51			
5	***14***	27	67				3	87	21			
6	19	14	65				13	86	19			
7	12	30	65				***2***	92	21			
8	9	16	68				***6***	79	26			
9	***14***	7	70				2	56	9			
10	12	7	71				***12***	87	20			
11	23	14	68				***7***	88	20			
12	***31***	25	58				***12***	89	18			
13	***28***	21	62				7	49	62			
						Females						
14	1	33	67	5	***31***	38	***4***	76	28	***34***	***27***	***2***
15	***1***	20	62				6	72	21	37	***5***	***4***
16	2	29	66	***5***	***37***	***4***	1	41	51	45	***18***	3
17	6	25	58	10	***63***	9						
18	***7***	26	61	2	***44***	***1***				***36***	***10***	**10**
19	***1***	30	66	0	***42***	***1***				40	***13***	***5***
20	40	9	45	3	***44***	***2***	7	85	24	***29***	***22***	1
21	4	17	63	***5***	***39***	***4***				42	***27***	***1***
22	***22***	***26***	45	27	***55***	16	***5***	42	54	46	7	9
23	2	11	62	8	***60***	18	8	75	21	***42***	***25***	1
24	***1***	36	63	***4***	***32***	36	***11***	71	25	***34***	***29***	***4***
25	3	16	64	2	***44***	***2***				43	***29***	***5***
26	2	32	66	0	***63***	9				***37***	***28***	***4***
27	0	30	67	***3***	***38***	***5***				34	***27***	***1***
28	2	29	66	***5***	***35***	***4***	***3***	52	18			

Table values are in millimeters relative to the Talairach-Tournoux stereotaxic coordinates.

tory anxiety would result in a rising and falling fMRI signal in delayed synchrony with a stimulus of which the subject is unaware. This remote possibility, however, cannot be completely excluded from consideration.

Studies of central processing of rectal pain in healthy individuals reported a similarity to the processing of somatic pain and involvement of multiple regions such as anterior cingulate gyrus, prefrontal, insular and sensory/motor cortices, as well as the inferior parietal lobule, posterior cingulate gyrus, and visual cortex (2). Gender-related differences in somatic pain perception and patterns of cerebral activation during noxious heat stimulation have been reported in healthy humans

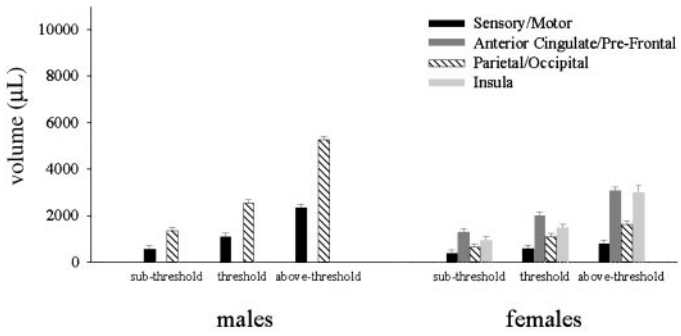

Fig. 3. Comparison of the volume of regions of cerebral cortical activity. A significantly larger volume of activity was detected in sensory/motor and parietal/occipital cortices in male compared with female subjects. However, although female subjects exhibited progressively larger volumes of activity in the insular and anterior cingulate/prefrontal cortices in response to increasing degrees of rectal distension ($P < 0.05$), male subjects did not exhibit clustered activity in these regions.

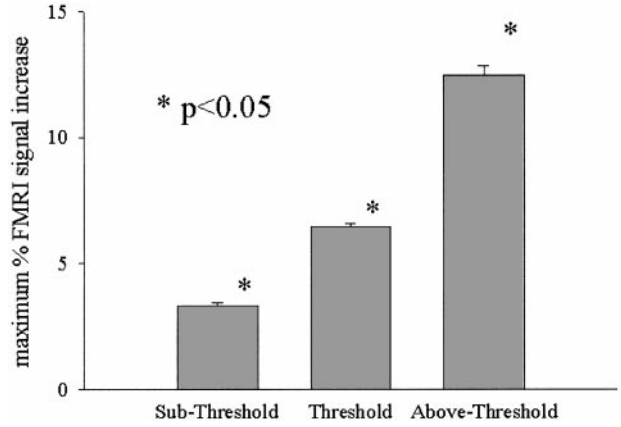

Fig. 4. Comparison of maximum percent functional magnetic resonance imaging (fMRI) signal increase in response to subthreshold, threshold, and above-threshold stimulus. Because the findings were similar for both genders, pooled data from male and female subjects are shown. As seen, there was a direct relationship between the intensity of the stimulus and magnitude of fMRI signal change.

Table 7. *Activated voxels in insular region in male subjects (without cluster criterion)*

Perception Level	Subjects																									
	1		*2*		*3*		*4*		*5*		*6*		*7*		*8*		*9*		*10*		*11*		*12*		*13*	
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Below	0	0	1	2	0	0	0	0	0	0	0	0	2	1	0	0	1	1	0	0	0	0	0	0	0	0
At	0	0	0	0	0	0	0	0	0	0	0	0	5	1	0	0	0	0	0	0	0	0	0	0	0	0
Above	0	0	0	0	1	0	0	0	0	0	0	0	3	3	0	0	0	0	0	0	0	0	0	0	0	0

(19). These differences include greater activation of the contralateral prefrontal cortex and greater volume of activation of the contralateral insula and thalamus in female compared with male subjects. Absence of insular, cingulate/prefrontal cortex activation in males, however, was not reported in these studies.

Studies of gender differences in regional brain response to a mix of painful and nonpainful visceral pressure in patients with irritable bowel syndrome (3) report bilateral insular activation during rectal pressure in male but not female patients. These findings are opposite to the findings of the present study of normal healthy volunteers that showed negligible or absence of insular activation in male subjects during unperceived as well as perceived, but not painful, rectal distensions. Other studies of female irritable bowel syndrome patients have shown insular activation during perceived nonpainful and painful rectal distension. These discrepant findings among irritable bowel syndrome patients and their differences from the findings of the present study of normal individuals support the idea of nonuniform alteration of cerebral cortical response to rectal balloon distension among patients suffering from irritable bowel syndrome.

The second of the two gender-related regional differences in cortical registration of rectal distension involved the anterior cingulate cortex. The anterior cingulate cortex filters and controls the relationship between the emotional limbic system and the skeletomotor and autonomic part of the nervous system (6). Different subdivisions of the anterior cingulate cortex are involved in various aspects of emotionally related behavior (6). The affect division of the cingulate cortex regulates autonomic and endocrine functions and assesses the emotional and motivational content of internal and external stimuli. The cognitive division of the anterior cingulate cortex includes the nociceptive and skeletomotor regions and participates in response selection. The activity of the anterior cingulate cortex precedes the execution of the behavior, suggesting its executive and planning role (6). The anterior cingulate cortex is a major component of the medial pain system and is activated by noxious stimuli applied anywhere on the body (26). It has been speculated that the anterior cingulate cortex is responsible for organizing an appropriate response to pain (6). Previous studies have generally shown activation of the anterior cingulate cortex in response to painful and nonpainful upper (1, 4, 11) and lower (2, 3, 5, 16) gut stimulation in healthy controls and patients with irritable bowel syndrome. Findings of the available studies, however, have not been uniform and are not always in agreement.

In the present study, activity of the anterior cingulate/prefrontal area was observed in 10, 14, and 14 of 15 female subjects below, at, and above perception threshold, respectively. The male subjects, however, exhibited meager or no activity in this region in response to any of the stimuli. This finding is in contrast with previous reports (2, 3). Differences in study populations in terms of gender and age may have contributed to this discrepancy. The findings of the present study are similar to those findings of gender differences in cortical activity during noxious heat stimulation, in which the distributed pain network of the anterior cingulate, prefrontal, and insular regions are more robustly activated in female compared with male subjects. Unfortunately, the physiological basis for these gender differences cannot be uniquely identified with the brain-imaging techniques used in these studies. Without this physiological basis, one can only speculate on several possibilities that may account for the observed gender differences in cortical activity during the visceral stimulation presented in our study. Hormonal contributions may play a role in the transduction of visceral afferents in female subjects. Reports of menstrual cycle-related variability in response to ischemic pain (8b, 19a) are indicators of the possible influence of hormonal factors in the registration of stimulus. Recent studies reported that fMRI cortical activity in women varies during two phases of the menstrual cycle (12a).

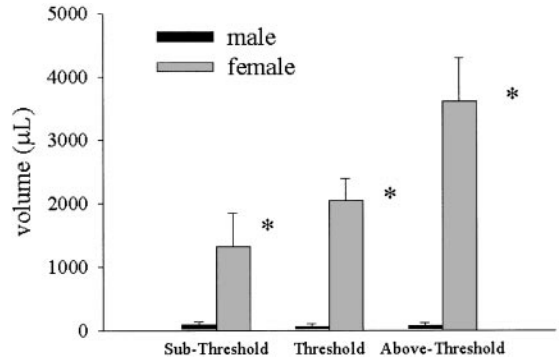

Fig. 5. Comparison of the fMRI activity volume in the insular region between male and female subjects. The clustering criterion has been eliminated, and all voxels, including single isolated voxels, have been included in the regional activity volume calculation. As seen, the volume of cortical activity in female subjects was significantly larger for all 3 levels of distension compared with male subjects (*$P < 0.01$).

Table 8. *Activated voxels in anterior cingulate/prefrontal region in male subjects (without cluster criterion)*

Perception Level	Subjects																									
	1		*2*		*3*		*4*		*5*		*6*		*7*		*8*		*9*		*10*		*11*		*12*		*13*	
	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L	R	L
Below	3	3	2	0	0	0	0	0	0	0	0	0	0	0	5	4	2	4	0	0	0	0	0	0	0	0
At	3	4	1	1	0	0	0	0	0	0	0	0	0	0	4	5	1	3	0	0	0	0	0	0	0	0
Above	1	0	1	3	3	2	0	0	0	2	0	0	0	0	6	5	0	0	0	0	0	0	0	0	0	0

Activation of prefrontal regions and the associated links between the prefrontal region with limbic and parietooccipital structures may not only reflect activation of the cortex by external sensory input but also represent the affective components of cortical processing. Because there are reports of gender differences in the affective neural processing associated with cognitive and auditory stimulation (8a, 12b, 21a), one might consider the possibility that a portion of the cortical response to visceral rectal stimulation might include an affective component. Reported gender-related differences in the cortical response to rectal distension are due, in part, to the gender-related differences in affective neural processing.

Gender differences found in the present study in regard to lack of participation of the insula and anterior cingulate in perceived and unperceived rectal distension in males is striking, and no previous studies that have addressed this issue among healthy individuals could be found. For this reason, we entertained the notion that perhaps the clustering criterion that was applied in this study eliminated the insular and anterior cingulate/prefrontal activated voxels that could have been present but did not reach the "at least three contiguous voxels" criterion. To address this issue we reanalyzed the insular and anterior cingulate/prefrontal activity maps post hoc without applying the clustering criterion in both male and female subjects.

For the insular cortex at below-perception-threshold rectal stimulation, 3 of 13 male subjects showed one or two isolated, noncontiguous voxels in either hemisphere. Similarly, at perception-threshold distension, 1 of 13 subjects showed one to five isolated, noncontiguous voxels of activity during various scans. Above perception threshold, 2 of 13 male subjects showed one to three noncontiguous voxels of correlated activity. These results are shown for male subjects in Table 7, in which the number of activated voxels is shown for each subject when the cluster criterion is not enforced.

On the other hand, for the female insular regions, cortical activity was found to be registered in 14 of 15 subjects for all perceived and unperceived distension levels. Relaxing the cluster criterion for the female insular region also resulted in a further increase in registered cortical volume compared with the volumes calculated with the cluster criterion. As seen in Fig. 5, the volume of cortical activity in the insular region for female subjects is strikingly larger than the volume registered for male subjects ($P < 0.01$) even after eliminating the cluster criterion from the analysis of cortical activity volume.

For the anterior cingulate/prefrontal region at below-perception-threshold rectal stimulation, 4 of 13 male subjects showed two to five isolated, noncontiguous voxels in either hemisphere. Similarly, at perception-threshold distension, 4 of 13 subjects showed one to five isolated, noncontiguous voxels of activity. At above-perception threshold stimulation, 5 of 13 subjects showed one to six noncontiguous voxels of correlated activity. These results are shown for male subjects in Table 8, in which the number of activated voxels is shown for each subject when the cluster criterion is not enforced.

For the female subjects' anterior cingulate/prefrontal region volume data, cortical activity was found to be registered in 14 of 15 subjects for all subthreshold, threshold, and above-perception-threshold distension levels. Relaxing the cluster criterion for the female anterior cingulate/prefrontal region also resulted in a further increase in registered cortical volume compared with the volumes calculated with the cluster criterion. As seen in Fig. 6, the volume of cortical activity in the anterior cingulate/prefrontal region for female subjects is strikingly larger than the volume registered for males ($P < 0.01$) even after eliminating the cluster criterion from the analysis of cortical activity volume.

The distending pressure that was associated with perception in the current study is higher than that in some previously reported studies (14, 16, 22, 23). This discrepancy is possibly caused by differences in the length of the connecting tube (in our case ~30 ft) that

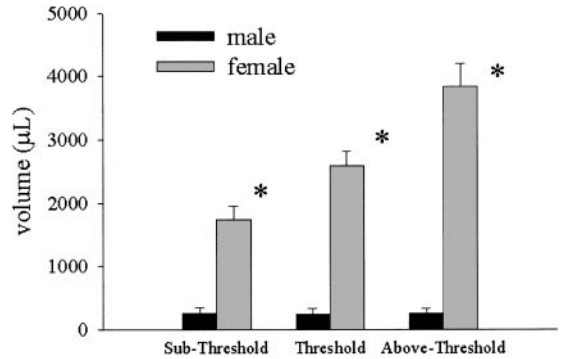

Fig. 6. Comparison of the fMRI activity volume in the anterior cingulate/prefrontal region between male and female subjects. The clustering criterion has been eliminated, and all voxels, including single isolated voxels, have been included in the regional activity volume calculation. As seen, the volume of cortical activity in female subjects was significantly larger for all 3 levels of distension compared with male subjects (*$P < 0.01$)

was necessitated by the physical limitations of the scanner suite used in our study. Therefore, the pressure registered at the perception threshold level reported in the current study may not accurately reflect the distension pressure for perception measured clinically, but it provided a quantifiable measure for inducing uniform subthreshold and above-threshold distension in all study subjects.

Our findings also indicate that afferent signals originating from the rectum before reaching the perception threshold are registered in the cerebral cortex and can be detected by fMRI. The volume of activated cerebral cortical areas and maximum fMRI signal intensity change in these areas caused by subperception afferent signals are significantly smaller compared with sensory signals that reach the perception threshold and above. This finding of cortical regurgitation of subliminal stimulations corroborates previous reports of esophageal acidification in healthy subjects in which cortical activity was induced even though the tested subjects perceived no heartburn (11). These findings indicate a more expanded involvement of the cerebral cortex in normal gastrointestinal function than generally recognized and provide a unique design for studying a variety of physiological and pathological conditions at subperception level devoid of ancillary cortical activation of regions related to the perception of stimuli such as pain, fear, anxiety, and memory recall.

In summary, *1*) there are substantial differences in female cortical activation topography during rectal distension compared with males, in that the insula and anterior cingulate/prefrontal cortices are predominantly activated in females; *2*) the intensity and volume of registered cortical activity due to rectal stimulation are directly related to stimulus strength; and *3*) rectal stimulation below the perception level is registered in the cerebral cortex.

This work was supported in part by National Institute of Diabetes and Digestive and Kidney Diseases Grant R01-DK-25731.

REFERENCES

1. **Aziz Q, Rothwell JC, Hamdy S, Barlow J, and Thompson DG.** The topographic representation of esophageal motor function on the human cerebral cortex. *Gastroenterology* 111: 855–862, 1996.
2. **Baciu MV, Bonaz BL, Papillon E, Bost RA, Le Bas J-F, Fournet J, and Segebarth CM.** Central processing of rectal pain: a functional MR imaging study. *Am J Neuroradiol* 20: 1920–1924, 1999.
3. **Berman S, Munakata J, Naliboff BD, Chang L, Mandelkern M, Silverman D, Kovalik E, and Mayer EA.** Gender differences in regional brain response to visceral pressure in IBS patients. *Eur J Pain* 4: 157–172, 2000.
4. **Binkofske F, Schnitzler A, Enck P, Frieling T, Posse S, Seitz RJ, and Freund H-J.** Somatic and limbic cortex activation in esophageal distention: a functional magnetic resonance imaging study. *Ann Neurol* 44: 811–815, 1998.
5. **Bouras EP, O'Brien TJ, Camilleri M, O'Connor MK, and Mullan BP.** Cerebral topography of rectal stimulation using single photon emission computed tomography. *Am J Physiol Gastrointest Liver Physiol* 277: G687–G694, 1999.
6. **Clark DL and Boutros NN.** *The Brain and Behavior: An Introduction to Behavioral Neuroanatomy*. Malden, MA: Blackwell Science, 1999.
7. **Cox RW and Hyde JS.** Software tools for analysis and visualization of fMRI data. *NMR Biomed* 10: 171–178, 1997.
8. **Cox RW and Jesmanowicz A.** Real-time 3-D image registration for functional MRI. *Magn Reson Med* 42: 1014–1018, 1999.

8a. **Esposita G, Van Horn JD, Weinberger DR, and Berman KF.** Gender differences in cerebral blood flow as a function of cognitive state with PET. *J Nucl Med* 37: 559–564, 1996.

8b. **Filingim RB, Maixner W, Girdler SS, Light KC, Harris MB, Sheps DS, and Mason GA.** Ischemic but not thermal pain sensitivity varies across the menstrual cycle.. *Psychosom Med* 59: 512–520, 1997.

9. **Geschwind N.** Disconnexion syndromes in animals and man. *Brain* 88: 269–272, 1965.
10. **Kern M, Birn R, Jaradeh S, Jesmanowicz A, Cox R, Hyde J, and Shaker R.** Swallow-related cerebral cortical activity maps are not specific to deglutition. *Am J Physiol Gastrointest Liver Physiol* 280: G531–G538, 2001.
11. **Kern MK, Birn RM, Jaradeh S, Jesmanowicz A, Cox RW, Hyde JS, and Shaker R.** Identification and characterization of cerebral cortical response to esophageal mucosal acid exposure and distention. *Gastroenterology* 115: 1353–1362, 1998.
12. **Kern MK, Jaradeh S, Arndorfer RC, and Shaker R.** Cerebral cortical representation of reflexive and volitional swallowing in humans. *Am J Physiol Gastrointest Liver Physiol* 280: G354–G360, 2001.

12a. **Kern MK, Arndorfer RC, Cox R, Hyde J, and Shaker R.** Cerebral cortical response to rectal distension varies with phases of the menstrual cycle (Abstract). *Gastroenterology* 120: A397, 2001.

12b. **Kulynych J, Vladar K, Jones D, and Weinberger D.** Gender differences in the normal lateralization of the supratemporal cortex: MRI surface-rendering morphology of Heschl's gyrus and plenum temporale. *Cereb Cortex* 4: 107–118, 1994.

13. **Ladabaum U, Minoshima S, Hasler WL, Cross D, Chey WD, and Owyang C.** Gastric distention correlates with activation of multiple cortical and subcortical regions. *Gastroenterology* 120: 369–376, 2001.
14. **Lagier E, Delvaux M, Vellas B, Fioramonti J, Bueno L, Albarede JL, and Frexinos J.** Influence of age on rectal tone and sensitivity to distension in healthy subjects. *Neurogastroenterol Motil* 11: 101–107, 1999.
15. **Lane R, Reiman EM, Ahern GL, Schwartz GE, and Davidson RJ.** Neuroanatomical correlates of happiness, sadness and disgust. *Am J Psychiatry* 154: 926–933, 1997.
16. **Mertz H, Morgan V, Tanner G, Pickens D, Price R, Shyr Y, and Kessler R.** Regional cerebral activation in irritable bowel syndrome and control subjects with painful and nonpainful rectal distention. *Gastroenterology* 118: 842–848, 2000.
17. **Oppenheimer S.** The anatomy and physiology of cortical mechanisms of cardiac control. *Stroke* 24, *Suppl* 12: 13–15, 1993.
18. **Paulesu E, Frith CD, and Frackowiak RSJ.** The neural correlates of the verbal component of working memory. *Nature* 362: 342–344, 1993.
19. **Paulson PE, Minoshima S, Morrow TJ, and Casey KL.** Gender differences in pain perception and patterns of cerebral activation during noxious heat stimulation in humans. *Pain* 76: 223–229, 1998.

19a. **Pfeeger M, Stravena PA, Fillingim RB, Maixner W, and Girdler SS.** Menstrual cycle, blood pressure and ischemic pain sensitivity in women. *Int J Psychophysiol* 27: 161–166, 1997.

20. **Reiman EM, Lane RD, Ahern GL, Schwartz GE, Davidson RJ, Friston KJ, Yun L-S, and Chen K.** Neuroanatomical correlates of external and internal generated human emotion. *Am J Psychiatry* 154: 918–925, 1997.
21. **Reiman EM.** The application of positron emission tomography to the study of normal and pathologic emotions. *J Clin Psychiatry* 58, *Suppl* 16: 4–12, 1997.

21a. **Reite M, Cullum CM, Stocker J, Teale P, and Kozora E.** Neuropsychological test performance and MEG-based brain lateralization: sex differences. *Brain Res Bull* 32: 325–328, 1993.

22. **Silverman DHS, Munakata JA, Ennes H, Mandelkern MA, Hoh CK, and Mayer EA.** Regional cerebral activity in normal and pathological perception of visceral pain. *Gastroenterology* 112: 64–72, 1977.

23. **Sloots CE, Felt-Bersma RJ, Cuesta MA, and Meuwissen SG.** Rectal visceral sensitivity in healthy volunteers: influences of gender, age and methods. *Neurogastroenterol Motil* 12: 361–368, 2000.
24. **Talairach J and Tournoux P.** *Co-Planar Stereotaxic Atlas of the Human Brain.* New York: Thieme Medical, 1988.
25. **Vogt BA, Sikes RW, and Vogt LJ.** Anterior cingulate and medial pain system. In: *Neurobiology of Cingulate Cortex and Limbic Thalamus: A Comprehensive Handbook*, edited by Vogt BA and Gabriel M. Boston: Birkhauser, 1993, p. 313–344.
26. **Vogt BA.** Structural organization of the cingulate cortex. In: *Neurobiology of Cingulate Cortex and Limbic Thalamus: A Comprehensive Handbook*, edited by Vogt BA and Gabriel M. Boston: Birkhauser, 1993, p. 19–70.