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Sex differences in regional brain response to aversive pelvic visceral stimuli

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Berman, Steven M., Bruce D. Naliboff, Brandall Suyenobu, Jennifer S. Labus, Jean Stains, Joshua A. Bueller, Kim Ruby, and Emeran A. Mayer. Sex differences in regional brain response to aversive pelvic visceral stimuli. *Am J Physiol Regul Integr Comp Physiol* 291: R268–R276, 2006. First published April 13, 2006; doi:10.1152/ajpregu.00065.2006.—To explore sex differences in the response of seven brain regions to an aversive pelvic visceral stimulus, functional magnetic resonance images were acquired from 13 healthy adults (6 women) during 15 s of cued rectal distension at two pressures: 25 mmHg (uncomfortable), and 45 mmHg (mild pain), as well as during an expectation condition (no distension). Random-effects analyses combining subject data voxelwise found 45-mmHg pressure significantly activated the insular and anterior cingulate cortices in both sexes. In men only, the left thalamus and ventral striatum were also activated. Although all activations appeared more extensive in men, no sex difference attained significance. To explore the presence of deactivations, which are generally cancelled by more numerous activations when subjects are combined for each voxel, the number of activated voxels, number of deactivated voxels, and ratio of deactivated voxels to total voxels affected were assessed via random-effects, mixed-model analyses combining subject data at the region level. Greater insula activation in men compared with women was seen during the expectation condition and during the 25-mmHg distension. Greater deactivations in women were seen in the amygdala (25-mmHg distension) and midcingulate (45-mmHg distension). Women had a significantly higher proportion of deactivated voxels than men in all four subcortical structures during 25-mmHg distension. Greater familiarity of females with physiological pelvic visceral discomfort may have enhanced brain systems that dampen arousal networks during lower levels of discomfort.

functional magnetic resonance imaging; brain imaging; pain

CONSIDERABLE EPIDEMIOLOGICAL and experimental evidence suggests that men and women respond to pain differently in terms of perceptual and autonomic nervous system responses. Epidemiological data indicate that compared with men, women are overrepresented in many, but not all, chronic pain disorders, including fibromyalgia, migraine, interstitial cystitis, and functional gastrointestinal disorders, such as irritable bowel syndrome (IBS) (59). For example, women are more likely to suffer from IBS, develop the so-called postinfectious IBS, and develop comorbidities such as fibromyalgia or interstitial cystitis (36). A variety of mechanisms have been proposed to explain these sex differences, including differences in the response of the central nervous system to pelvic visceral stimuli.

Despite the similarity between sex differences in somatic and visceral pain prevalence in epidemiological and disease-related studies, the results of experimental pain studies with the two types of pain stimuli differ. Consistent with the epidemiological data, women have greater sensitivity and less tolerance to somatic pain stimuli (52). In contrast, the majority of studies using controlled rectal balloon distension (a frequently used pelvic visceral pain stimulus), to date, have not shown significant sex-related differences in pain or discomfort thresholds in either controls or IBS patients (26, 37, 42, 61). However, in a recent study, Chang et al. (11) found that healthy women had higher discomfort thresholds to rectal balloon distension than men (healthy or IBS), while female IBS patients had the lowest thresholds of any group. Consistent with prior demonstrations of enhanced peripheral sympathetic responses in men, compared with women, in regulation of resting heart-rate variability (29) and blood pressure reactivity to ischemic pain (21), male IBS patients have higher sympathetic and lower vagal activation as indexed by skin conductance and heart rate variability during balloon distension, compared with women with IBS and both male and female healthy control subjects (57). Thus, in response to an aversive pelvic visceral stimulus, healthy women show the lowest perceptual and sympathetic nervous system responses, whereas female IBS patients show greater perceptual sensitivity, and male patients show greater sympathetic nervous system responses.

A series of functional brain imaging studies have tried to identify possible brain correlates of the reported sex-related perceptual and autonomic nervous system responses to rectal stimulation. The initial focus in these analyses has been on the most commonly reported regions of central pain: the insula, the dorsal anterior cingulate cortex (dACC), and the thalamus. Insula activation by a painful somatic stimulus has been reported to show no sex-related difference (19), to be greater in women (43), and to be greater in men (3). Using functional magnetic resonance imaging (fMRI) during a visceral stimulus presented to healthy control subjects, Kern et al. (27) reported that women showed a progressive increase in the activation volume in the insular and ACC/prefrontal region with progressive intensity of rectal distension, while males showed virtually

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no response at any distension pressures. Using a similar visceral stimulus in male and female IBS patients, Berman et al. (4) reported that male but not female IBS patients show bilateral activation of the insula using ^{15}O-PET. A follow-up study by Naliboff et al. (41) confirmed the greater activation of the insular cortex in male patients and showed that female IBS patients had greater activation in limbic (amygdala) and paralimbic regions [ventromedial prefrontal cortex (PFC), infragenual cingulate cortex and dACC]. As the insula plays a major role in cortical modulation of central autonomic networks (1, 53), one may speculate that the sex-related differences in insula activation may be related to the reported sex-related differences in autonomic nervous system activation discussed earlier.

In recent years, the blood oxygenation level-dependent (BOLD) fMRI signal has become the tool of choice for functional brain mapping of blood flow. Increases in regional blood flow, taken as an index of activation, lead to an increase in blood oxygenation and a decrease in paramagnetic deoxygenated hemoglobin (22). This produces an increase in signal intensity at the site of activation. Even though reports have traditionally focused on increases in BOLD signals, corresponding to regional brain activation, a series of recent studies has reported decreases in BOLD signals as evidence for regional deactivations (25, 35).

The current study, evaluating brain responses to controlled rectal distension in male and female control subjects using fMRI, had two aims. The first was to identify possible sex differences in predominant activation or deactivation of brain regions of healthy men and women, associated with response to expected and delivered aversive pelvic visceral stimuli. Because examination of this aim using standard random-effects imaging analysis may obscure the presence of activations and deactivations occurring within the same region and perhaps differing in their proportion across conditions or sex, we also report an exploratory, hypothesis-generating analysis looking at individual subject level data on activations, deactivations, and their ratio within each region of interest. Parts of these results have been reported in abstract form (5).

MATERIALS AND METHODS

Participants

Thirteen healthy control adult subjects were recruited by advertisement (7 men, mean age 33.1, SD = 10.9, 6 women, mean age 34.8, SD = 7.9). One man and no women were left-handed. By subject report of menstrual history and the date of their last menstrual period, it was estimated that three of the women were imaged in the luteal phase and the other three in the follicular phase. On clinical examination by a gastroenterologist, subjects were clinically and endoscopically without inflammatory or structural intestinal disease. All subjects were evaluated for presence of depression and anxiety symptoms by the SCL-90R symptom checklist (20). Means for each scale fell within one standard deviation of the nonpsychiatric patient norm, and no individual subjects had *t*-scores >65 for the global symptom score. Written and verbal informed consents were obtained from all subjects. The protocol was approved by the UCLA Human Subject Protection Committee.

Visceral Distension Procedure

Distension of the rectum was accomplished using a computer-driven pump (barostat) programmed to deliver phasic pressure steps (38 ml/s) separated by interinflation intervals at the resting pressure. Methods for balloon insertion and inflation were as previously described (40). Briefly, a latex balloon was attached to a silastic elastomer tube (external diameter, 18 F) and tied at both proximal and distal ends (MAK-LA, Los Angeles, CA). The distance between the attachment sites was 11 cm. Distension to a maximal volume of 500 ml results in a cylindrical balloon shape. Before the procedure, each balloon was inflated repeatedly to rule out any leak and measure intrinsic compliance. The lubricated balloon was inserted into the rectum so that the distal attachment site was 4 cm from the anal verge. The tube was then secured with tape. In vitro and in vivo validations of this distension device have been published (31, 38). All studies were performed after an 8-h fast and application of 2 Fleet enemas (C. B. Fleet, Lynchburg, VA).

Magnetic Resonance Imaging Procedure

After placement of the balloon catheter, the subject was positioned in the MRI scanner on a cushioned headrest. Tape was put across the forehead to restrict movement. The subject was then given the following instructions: "The computer will now begin to inflate the balloon to various pressures in no particular order. You will probably feel sensations of varying intensity. At the beginning and end of the inflation there will be a visual cue. Remember, when you see the (green/white) rectangle, the inflation will soon begin and when you see the color change you will need to rate the inflation. Indicate the intensity of your sensation by pressing the button under your index finger associated with low or no intensity, by pressing the button under your middle finger for a middle intensity or by pressing the button under your ring finger for a high level of intensity. Please answer immediately when the visual cue comes on. In the case that you can no longer tolerate the test, tell us immediately, but please try to finish the test. Remember that during this part of the test the intensity of the inflations will not be in any particular order."

Six 10-min runs of 16 inflation trials were then administered. The first inflation in each run was always at a pressure of 45 mmHg. This was followed by five additional 45-mmHg inflations, five 25-mmHg inflations, and five trials at the baseline pressure of 5-mmHg inflations (no actual inflation, but expectation of a known aversive stimulus) in pseudorandom order. Each trial consisted of 21 s before balloon inflation, followed by 15 s of inflation at the designated pressure (see Fig. 1). The visual cue preceded the first inflation by 2 s and the other 15 inflations by 3, 4, or 5 s (rectangular distribution). The cue was removed at the end of the inflation period, signaling the end of a trial and instructing subjects to rate the intensity of the stimulus. Ratings were made on a simple 3-point scale and were not intended as a psychophysical measure. The purpose of the rating instructions was to keep subjects focused on the stimuli and to provide a validity check of discrimination between stimulus levels. There was a brief rest (1–2 min) between runs. After the last run, subjects were removed from the MRI. They completed the Sensory Word Descriptor List and visual

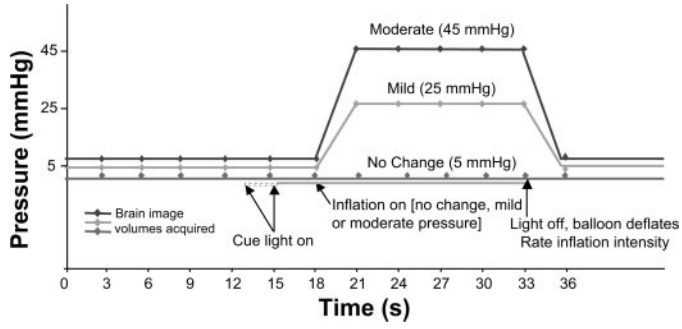

Fig. 1. Trial design. In each session, 30 moderate intensity rectal distensions (15 s at 45 mmHg) were randomly intermingled with an equal number of mild distensions (25 mmHg) and nondistensions (5 mmHg).

analog scales for intensity and unpleasantness basing their evaluation on the sensation from the highest inflation. The catheter was removed, and the subjects were excused.

Functional images were acquired on a 3T MRI scanner [GE, Signa with the echoplanar imaging (EPI) upgrade from Advanced NMR Systems; GE Medical Systems, Milwaukee, Wisconsin]. After a sagittal scout was used to position the head, functional T2*-weighted gradient-recalled EPI with BOLD contrast (repetition time [TR] = 3,000 ms, echo time [TE] = 42 ms, flip angle = 80°, slice thickness = 4 mm with a 1-mm interslice interval, matrix of 64 × 64, in-plane resolution = 3.12 mm^2) were recorded. Two hundred functional images were acquired for each of 19 axial slices through the brain in each 10-min acquisition.

Data Analysis

BOLD fMRI images from each session were first examined by a software program that processes a time series of images and identifies those likely to be artifacts based on outliers in motion, signal intensity, and other sources of change between successive images [Outlier provided by M. Cohen, UCLA]. The signal spike threshold used by the Outlier software program was 30% above background noise levels determined on a per-slice basis. The motion threshold was 2%. Images tagged as outliers were visually inspected and unacceptable images, generally because of intensity spikes in a single slice, were dropped. The following preprocessing procedures were then implemented using Statistical Parametric Mapping 99 (SPM99; Wellcome Trust Centre for the Study of Cognitive Neurology, London, UK). Correction for acceptable head movement between the images in each session was carried out by alignment with the central image (image 100 of run 3). Each realigned set of scans from every subject was normalized into the standardized anatomical space of the average MRI image provided by the Montreal Neurological Institute (MNI).

SPM. The images were assigned to six conditions (see Fig. 1): rest (5 images per trial modeled as an implicit baseline), cue (1 image per trial), mild distension (5 images on 1/3 of the trials), moderate distension (5 images on 1/3 of the trials), expectation of distension (5 images on 1/3 of the trials), and rate (1 image per trial). The other conditions were compared with the implicit baseline in individual fixed-effects contrasts for each subject.

For each condition, activated and deactivated voxels were identified at the 0.01 (uncorrected) alpha level and used to construct individual statistical parametric maps. We then used a region-of-interest (ROI) approach to assess BOLD signal increases and decreases, specifically in seven structures previously associated with pain or functional response to visceral distension (anterior insula, midcingulate, anterior cingulate, thalamus, amygdala, ventral striatum, and dorsal brainstem), using the SPM99 small-volume correction tool. Within each structure, hemisphere, and condition, the spatial extent (number of voxels) of each contiguous cluster of affected voxels was corrected for the volume of the ROI. If the corrected probability value was <0.05, the ROI was considered to contain a significant activation/deactivation. By this method, the same ROI can contain both activation and deactivation (although not at the same location). This method was used to test the hypothesis that the seven a priori regions showed sex differences in the BOLD response to the experimental stimuli.

Second-level random-effects data analyses were first computed from the individual subject contrast images for each condition for the males, females, and a combined analysis across all subjects. We used a traditional approach for assessing brain images that have been normalized into the same atlas space (see preprocessing above), combining data across subjects at each voxel to evaluate evidence of activation and/or deactivation within each predefined ROI.

Mixed-effects analyses. The traditional random effects SPM analysis described above can obscure the presence of deactivations in structures that also include activations (or vice versa). The ability of current methods to normalize the images of different brains into the same image space does not have a resolution on the order of one voxel. Errors in superimposition can superimpose activations over neighboring deactivations, which would then cancel or reduce both effects. Preserving the number of voxels and the proportion of each structure that is activated or deactivated in each individual subject allows comparison of these values across conditions and groups with no such cancellation effects. Therefore, because presence of both strongly activated and strongly deactivated voxels within each ROI was a strong feature of this data (and indeed, every fMRI data set of individual subjects we have seen), additional exploratory analyses were performed by combining the results from the individual subject SPMs for each ROI instead of each voxel.

We extracted a data set containing information on the proportion of activated (# voxels/size of ROI) or deactivated brain voxels at $P < 0.01$ (uncorrected) in each bilateral ROI for all 13 subjects. We also computed the ratio of deactivated-to-total affected voxels, a measure that combined information about activations and deactivations. We analyzed each of these three variables with mixed-effects models in statistical analysis software (33). Specifically, three mixed-effects analyses specifying subject and condition as random effects were applied to determine the influence of sex, condition (5 mmHg, 25 mmHg, and 45 mmHg) and region (amygdala, dorsal brainstem, anterior cingulate, midcingulate, anterior insula, thalamus, and ventral striatum) on the proportion of activated or deactivated brain voxels and the ratio of deactivated to total affected voxels. Planned comparisons were used to examine potential sex differences within each ROI and condition. Significant results were defined as P value < 0.05, and marginal or borderline significance was defined as $P < 0.10$. Because the purpose of this analysis was exploratory and not confirmatory and our emphasis was on hypothesis generation rather than on hypothesis testing, we did not adjust for multiple comparisons in seven a priori regions, and the results of significance tests should be interpreted cautiously.

It is well established that the brain regions under investigation differ in their response to painful stimuli and that higher levels of pressure increase these responses. Although our results strongly support these expectations both visually and statistically, we will report formally only on the sex differences that are the main focus of this paper and their interaction with the other factors.

RESULTS

SPM Random Effects ROI Analyses (Subjects Pooled at the Voxel Level)

Figure 2 colors the voxels featuring significant change during 45-mmHg distension for all subjects (green), men (blue), and women (yellow) superimposed on a structural MRI representative of MNI-space for a midsaggital slice, a coronal slice 4 mm behind the anterior commissure, and a transaxial slice through the anterior commissure. The results of the ROI analyses are presented in Table 1. Combined analysis of all 13 subjects indicated significant (volume-corrected $P < 0.05$) activations within the insula (seen in the coronal and transaxial slices of Fig. 2) and both middle and anterior portions of the cingulate gyrus (midsagittal slice of Fig. 2). For each significant cluster, the cluster size is given (k), followed by the volume-corrected spatial extent P value and the total percentage of the ROI that was activated at $P < 0.05$ (uncorrected). To the right of this column, Table 1 presents the maximum t-score and the location of the peak voxel. The x, y, z coordinates represent approximately millimeter distances from the anterior commissure, with positive values to the right, anterior, and superior directions, respectively.

Table 1. *Significant results (cluster spatial extent corrected $P < 0.05$) from SPM random effects ROI analyses*

		All ($n = 13$)							Men ($n = 7$)							Women ($n = 6$)						
		Cluster			Voxel				Cluster			Voxel				Cluster			Voxel			
ROI		k	*P* corr	% ROI	*t*	x	y	z	k	*P* corr	% ROI	*t*	x	y	z	k	*P* corr	% ROI	*t*	x	y	z
Insula	L	716	0.000	50%	5.57	−42	16	−8	560	0.000	40%	5.63	−36	6	0	279	0.004	20%	12.07	−40	14	6
	R	621	0.000	38%	7.39	34	22	4	679	0.000	41%	5.42	42	6	−2							
Midcingulate	L	164	0.043	14%	6.31	−8	20	28	219	0.001	20%	2.16	−4	2	38							
	R	307	0.004	26%	6.73	10	6	38	291	0.000	25%	5.27	8	12	32							
Anterior cingulate	L	214	0.024	13%	3.73	−8	24	24	235	0.001	16%	4.50	−10	32	28							
	R								206	0.002	14%	4.39	6	34	22							
Ventral striatum	L								108	0.033	12%	4.44	−26	−8	0							
	R																					
Thalamus	L								184	.004	11%	5.64	−12	−2	12							
	R																					

L, left; R, right; k, cluster size in voxels; SPM, statistical parametric mapping; Pcorr, corrected P value; ROI, region of interest.

Men assessed alone showed significant bilateral activation in all three regions, and also demonstrated activation in the left thalamus and left ventral striatum, with the latter focus mainly located in the ventral putamen. These structures are best seen in the transaxial slice of Fig. 2 medial to the insula. Although activation can be seen in men in both structures bilaterally, it was only in the left hemisphere where they attained significance after volume correction.

Women showed significant activation in the left insula, as well as trends toward activation (both $P < 0.08$) in the right insula and right midcingulate. There were no areas of statistically significant deactivation in either group or in the combined analysis. Although Fig. 2 suggests more extensive activations in men than women, these effects did not attain statistical significance in the voxelwise analysis.

Mixed-Effects Analyses (Subjects Pooled at the ROI Level)

As can be seen in Fig. 3, there were many more voxels, which showed both increases and decreases in the BOLD signal during 45-mmHg distension than during the expectation condition (5 mmHg).

BOLD signal increases. As can be seen in Fig. 3, there was a clear increase in the number of voxels that showed activation as distension pressure increased from 5 to 25 to 45 mmHg. This was particularly true of men, who showed greater activa-

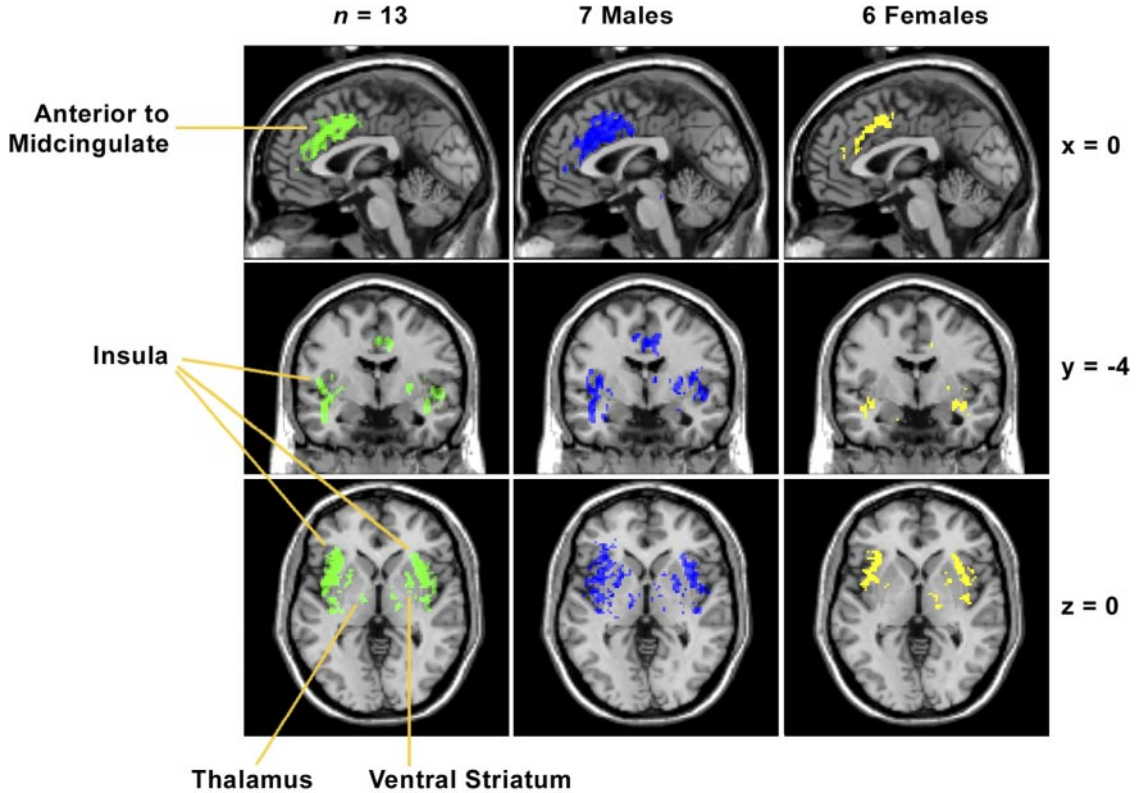

Fig. 2. Response to 45-mmHg distension (statistical parametric mapping random effects voxelwise analyses).

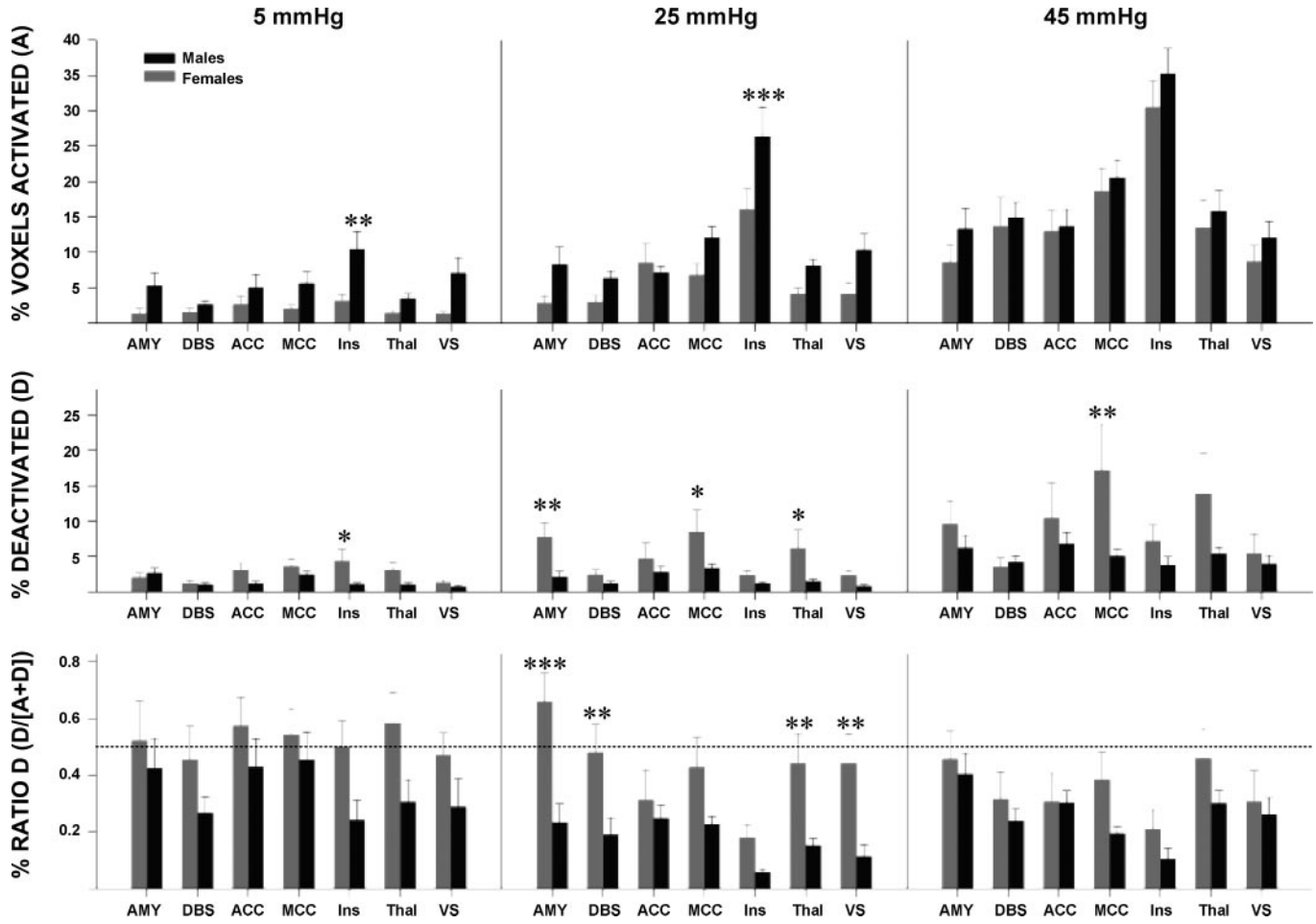

Fig. 3. Sex differences in the seven regions of interest (ROIs; mixed-model random effects ROI-wise analyses; means ± SE). In almost all of the ROIs for each of three levels of inflation, men activate more and women deactivate more. During the 25-mmHg (nonpainful) distension, a significantly larger proportion of affected voxels in all four subcortical ROIs were deactivations in women, compared with men ($P < 0.05$ for all). In the bottom row, the horizontal line at 0.5 represents equal amounts of activation and deactivation. AMY, amygdala; DBS, dorsal brainstem; ACC, anterior cingulate; MCC, midcingulate; Ins, insula; Thal, thalamus; VS, ventral striatum. *$P < 0.1$; ** $P < 0.05$; ***$P < 0.01$.

tion than women in 20 of 21 combinations of region and stimulus (top row, Fig. 3). Mixed-effects analysis of the percentage of activations indicated a significant effect for the interaction of sex and region, $F_{6,66} = 2.92$, $P < 0.01$. Planned contrasts revealed that compared with females, males showed a greater percentage of activated voxels in the anterior insula during the expectation condition ($t_{132} = 2.29$, $P < 0.05$) and during the 25-mmHg inflation ($t_{132} = 2.88$, $P < 0.01$), but not during the 45-mmHg inflation ($t_{132} = 0.95$, NS). Across all conditions, males, compared with females, showed a significantly greater percentage of activated voxels in the anterior insula ($t_{66} = 2.60$, $P < 0.01$)

BOLD signal decreases. As can be seen in Fig. 3, during the expectation condition, there were no structures where 5% or more of the voxels were deactivated in either group. However, deactivations increased in response to the 25-mmHg distension overall, especially in women. Even where deactivation remained small during the 25-mmHg inflation, there was a clear increase over the level induced by expected but undelivered inflation in response to the 45-mmHg inflation. Over 15% of the midcingulate was deactivated in women. Women deactivated more voxels than men in 19 of the 21 combinations of region and stimulus (center row, Fig. 3).

Analysis of the percentage of deactivated voxels revealed significant effects for the interaction of sex and region, $F_{6,66} = 4.88$, $P = < 0.001$, as well as sex, condition, and region, $F_{12,132} = 2.14$, $P < 0.05$. During the expectation condition, there was a trend for women to show a greater percentage of deactivation in the anterior insula compared with men ($t_{132} = 1.70$, $P < 0.10$). During the 25-mmHg inflation, women, compared with men, showed a greater percentage of deactivated voxels in the amygdala ($t_{132} = 2.10$, $P < 0.05$), with trends in the midcingulate ($t_{132} = 1.90$, $P < 0.10$) and the thalamus ($t_{132} = 1.75$, $P < 0.10$). During the 45-mmHg inflation, females showed a greater percentage of deactivated voxels in the midcingulate than men ($t_{132} = 2.38$, $P < 0.05$). Across all conditions and compared with men, women showed a significantly greater percentage of deactivated voxels in the midcingulate ($t_{66} = 2.45$, $P < 0.05$) and thalamus ($t_{66} = 2.03$, $P < 0.05$).

BOLD signal ratio. The ratio measure showed a greater proportion of deactivations, relative to activations, in women compared with men, across all regions and conditions (bottom row, Fig. 3). Analysis of the ratio of deactivated to total affected voxels revealed significant effects for the interaction of sex and condition ($F_{6,66} = 3.83$, $P < 0.05$), as well as a

trend for the interaction between sex, condition and region ($F_{12,132} = 1.64$, $P < 0.10$). Compared with men, women had a higher proportion of deactivated voxels during the 25-mmHg distension in all four subcortical structures: amygdala ($t_{132} = 3.09$, $P < 0.01$), dorsal brainstem ($t_{132} = 2.09$, $P < 0.05$), thalamus ($t_{132} = 2.10$, $P < 0.05$), and ventral striatum ($t_{132} = 2.06$, $P < 0.05$). No sex differences were observed in any ROI during the 5- or 45-mmHg conditions. Across all ROIs in the 25-mmHg condition, women compared with men had a higher proportion of the affected voxels being deactivated ($t_{22} = 1.98$, $P < 0.10$).

Behavioral Stimulus Responses

Both sexes discriminated well between the three levels of visceral pressure. Subjects rated the expectation condition (no change from resting pressure of 5 mmHg) reliably the lowest on the 3-point scale (1.01 ± 0.03; means ± SD). The ratings for the 45-mmHg pressure ranged from 1.63 to 2.96 with a mean rating of 2.74 ± 0.23. All mean ratings for the 25-mmHg pressure (1.74 ± 0.51) were between the mean rating for the 5- and 45-mmHg pressure in that subject.

DISCUSSION

The results of this exploratory study demonstrate that in healthy subjects, simultaneous increases and decreases in the BOLD signal in multiple regions concerned with the processing and modulation of visceral afferent signals are the most common brain response to rectal distension in both sexes. Although men tend to activate relatively more voxels than women, particularly in the insula, women tend to deactivate relatively more voxels than men in all of the regions assessed, particularly in the dorsal midcingulate and amygdala. When activations and deactivations were both considered by assessing the proportion of affected voxels that showed BOLD decreases, these sex-related differences were strongest for the uncomfortable, but not painful, 25-mmHg distension in subcortical structures.

In the current study, two types of analyses were performed: a conventional SPM random-effects analysis, which uses a voxel-based comparison, and an exploratory, mixed-effects analysis, aimed at assessing the relative number of activated and deactivated voxels within a specific brain region. The latter exploratory analysis was derived to test the hypothesis that the results seen in the traditional SPM random-effects analysis are the net effect of activations and deactivations within the same region. Specifically, we were interested in exploring whether previously reported sex differences in the activation of brain regions, such as the insula, thalamus, or amygdala, result from sex-related differences in the number of activated and deactivated voxels in these regions. For example, in a structure with substantially more activation than deactivation, signs of activation are likely to remain, whereas even highly significant deactivations will disappear entirely, along with the ability to contrast the number or amplitude of these effects between groups. This is particularly problematic if there are similar magnitudes of activity in both directions, such as in the amygdala and thalamus ROIs of the current study.

Central Nervous System Activation During Visceral Distension

Analysis of all 13 subjects indicated significant activations within anterior and midcingulate, and anterior insula, all regions of a central "pain matrix", consistent with a growing literature of visceral pain-imaging studies (16). These activations were seen in both types of analyses (SPM random-effects ROI analysis and mixed-effects analysis) and were seen in both men and women. The brain network that processes visceral afferent signals during rectal distension overlaps to a large extent with what has come to be referred to as the pain matrix. Both types of stimuli produce consistent activation in the dACC and the insula, generally along with adjacent operculum and posterior secondary somatosensory cortex (S2). Less reliable activations are reported in primary somatosensory cortex (S1), (more consistently during somatic pain), thalamus, basal ganglia, cerebellum, and dorsal brainstem, including the periaqueductal gray matter (for recent reviews, see Refs. 16 and 58). Insula activations are clustered more anteriorly during visceral distension, consistent with this being the part of the insula maximally associated with internal feeling states, emotions, and associated autonomic responses (13–15).

Deactivation of Brain Regions During Aversive Stimuli and Their Expectation

Although some voxels were activated during expectation and delivery of rectal distention, compared with baseline activity, there were other voxels in all assessed regions of most individual subjects where the BOLD signal was decreased, particularly during the 45-mmHg distension. However, these effects were seen across subjects only in the mixed-effects regional analysis of spatial extent, not in the voxelwise comparison of the SPM random-effects analysis.

Localized brain activity decreases below resting levels have also been noted in response to pain and other stimuli (22). Most of the pain studies have used the ^{15}O-PET method, in which decreased regional cerebral blood flow (rCBF) in a region is well linked to decreased local firing rates. Deactivations have been reported in inferior parietal cortex during noxious heat (60) and nitroglycerine-induced cluster headache (23). A study of laser heat pain noted deactivations in several structures, including parahippocampal gyrus, amygdala, occipital, and medial frontal cortex (17). These areas, along with areas at the juncture of orbitofrontal with temporal cortex and a dorsomedial frontal region, provided additional evidence of functional deactivation by being negatively correlated with subjective pain ratings. In addition to heat pain, reduced amygdala activity was also elicited by cold-pressor pain and mechanical pain (46, 47). During rectal distension in IBS patients, we have also noted prominent amygdala deactivations in several PET studies (9).

Anticipation of pain, which is confounded with perception of pain in most studies, can also produce decreased brain activity. Anticipating an unpredictable and unlearned pain stimulus activated the right ACC and ventromedial PFC, whereas anticipating a learned pain-stimulus resulted in decreased activity in the same structures (24). Anticipation of a longer pain stimulus resulted in decreased amygdala activity relative to anticipation of a shorter stimulus (45). Anticipation of painful finger shock decreased blood flow in medial PFC

and infragenual cingulate (56). Moreover, subjects with the least anxiety had the greatest decreases. On the basis of the most commonly reported deactivations in such brain regions as the ventral cingulate, ventromedial PFC, and amygdala, Petrovic and Ingvar (46) concluded that actively decreasing limbic activity during pain may be a cognitive coping mechanism.

Using fMRI, others have reported regional BOLD signal decreases during pain, which have generally been interpreted as evidence for stimulus-induced decreases in brain activity. Porro and associates (48) noted decreases in medial parietal cortex, perigenual anterior cingulate, and medial PFC in response to prolonged burning pain induced by injection of a dilute ascorbic acid solution into the dorsum of the foot. The spatial extent of these BOLD signal decreases increased with both pain intensity and duration. Bonaz and colleagues (2) reported that rectal distension elicited BOLD signal increases in expected pain areas in healthy subjects, but BOLD signal decreases within the right insula, amygdala, and striatum in IBS patients (10). The surprising predominance of BOLD signal decreases compared with increases in this study is consistent with the sex differences reported herein, in that 92% of the IBS patients were women, whereas 63% of the healthy subjects in the earlier study (2) were men. More recent studies have also reported BOLD signal decreases in response to pharmacological interventions during emotional (44) and pain-producing stimuli (25).

Sex Differences in Brain Responses to Aversive Stimuli

In the random-effects analysis, no statistically significant sex differences in BOLD signal changes (activations) were observed. However, when the number of activated and deactivated voxels were quantified, a greater proportion of affected voxels were deactivations in women compared with men in all a priori regions during all three levels of pressure. Furthermore, in the mixed-effects analysis, women had a significantly higher proportion of deactivated voxels during the 25-mmHg distension in the amygdala, ventral striatum, thalamus, and dorsal brainstem. In contrast, men showed more activations in the insula.

Sex differences in the human central nervous system response to pain is a young field and has yet to reach consensus on a number of issues. The initial study by Paulson and associates (43) found striking similarity between the responses of men and women to noxious heat in 12 of 16 brain areas studied with PET measures of blood flow. Women showed greater activation of contralateral insula and thalamus, and prefrontal activation was more ipsilateral in men and contralateral in women. Also using ^{15}O-PET, Derbyshire and associates (18) noted that the prior study did not equate subjective heat intensity across subjects. When they did so, their study found greater insula activation in men compared with women. Using PET blood flow images in multiple studies, our group previously noted in different IBS patient samples (4, 41) more insula activation during rectal inflation in male patients, compared with female patients. However, Kern and associates (27) reported that healthy women, but not men, showed increased insula activation during rectal distension. No deactivations were reported in this study.

Moulton and associates [(39), also see article by Moulton et al. in this volume (39a)] recently reported that noxious heat applied to the dorsum of the left foot produced sex differences very similar to those we report herein in response to visceral distension. It is important to note that their analysis, like the current one, combined data across subjects at the ROI level. Although they did not assess the subcortical structures where the current effects are strongest, men showed suggestively or significantly more activation than women, or women more deactivation than men in four of the eight cortical ROIs they interrogated. There were no sex differences in the opposite direction. In addition, the percentage of affected voxels that constituted deactivations (the ratio measure used in the current paper) was suggestively or significantly larger in women than men in 3 ROIs, including the most prominent pain activation regions in the anterior insula and midcingulate.

Possible Mechanisms Underlying BOLD Signal Decreases

There are many possible reasons for BOLD decreases (22). While the BOLD signal was shown to reflect input to an area, as inferred from local field potentials, better than spike output (34), recent data have confirmed that like BOLD increases, BOLD decreases are correlated with local activity measured via both action potentials and multiunit activity (50, 55). Because brain physiology uses layered inhibition and disinhibition of distributed functional networks, it is reasonable that increased activity in one region can produce hyperpolarization leading to decreased activity in other regions. Although BOLD decreases could also be artifacts of activations, because of an initial signal dip that can be maximal in the spatial surround of a focal activation, spatial dissociation of activations from deactivations, as we have recently demonstrated in multiple structures (5, 6, 8), argues against the widespread acceptance of this explanation. It has been proposed that the rCBF decreases during intense pain, which would also be expected to produce BOLD decreases, could be due to increased sympathetic activity or hyperventilation (12). In a recent review of fMRI studies of human somatosensory function, Porro and associates (49) suggest that the BOLD decreases noted during pain in perigenual anterior cingulate and prefrontal cortex can be related to the interruption of ongoing brain activity because of the intrusive nature of pain on consciousness. In the same manner, it has been posited that regional BOLD decreases during a range of goal-directed behaviors are the result of temporary suspension of an organized, baseline "default mode" of brain activities (51). Vogt et al. (60) suggest that reduced rCBF during noxious heat in rostrofrontal areas may enhance pain perception in the perigenual cingulate, whereas that in the posterior cingulate may disengage visually guided processes. Petrovic and Ingvar (46) propose that in response to pain, limbic deactivations are likely to be functionally antinociceptive. If so, they would be detectable in fMRI studies as BOLD signal decreases.

Neurophysiological mechanisms underlying pain stimulus-induced deactivations include the effect of ascending monoaminergic systems on brain activity, activation of endogenous opioid systems, or the interaction of these mechanisms. For example, norepinephrine release in ascending pathways from the locus coeruleus during pain stimuli, such as rectal distention (28, 30), have been proposed to increase the signal-to-

noise ratio in target regions by inhibiting neuronal activity in areas around the excitation (54). The administration of opioid agonists has recently been shown to increase the BOLD-fMRI signal in all of the pain-associated regions we assessed in this report (32). Zubieta and associates (62), using the μ-opioid ligand [^{11}C] carfentanil, demonstrated larger magnitudes of μ-opioid system activation in men compared with women in anterior thalamus, ventral basal ganglia, and amygdala. Interestingly, these are subcortical areas substantially congruent with those where a 25-mmHg distension elicited a greater relative degree of activation in men, and deactivation in women, in the current study.

Limitations of Study

Because of the small sample size relative to the number of regions and conditions investigated, the mixed-effects analysis has to be considered as a hypothesis-generating effort that requires replication. However, the recent presentation of similar sex-related differences in response to a somatic pain stimulus by Moulton and associates (39) and the previous report of greater BOLD signal decreases in women in response to rectal distension (2, 10) increase our confidence in these findings. In addition, we have not discussed sex differences in the peak signal amplitude within each region as we have signal spatial extent, nor have we explored possible sex differences in signal time-course and within-region localization of activations relative to deactivations. Although previous studies (27, 39) and our own investigations to date have not found strong sex differences in these measures (5–8), they are important in characterizing sex differences in brain response and further analyses, as well as additional studies, are in progress.

In summary, brain imaging studies provide evidence that during pain or the anticipation of pain, local deactivation within limbic structures and possibly other pain-associated structures may represent antinociceptive coping responses. Several recent fMRI studies have found that in response to discomfort and low-level pain, a relatively larger proportion of total brain response constitutes activations in men and deactivations in women. It is conceivable that the greater familiarity of women with physiologically occurring pelvic visceral discomfort during the menstrual cycle and during pregnancy may allow them to down-regulate the alerting function of central arousal systems during low levels of visceral discomfort. In view of our earlier findings in IBS patients of greater limbic and paralimbic activation in female patients, one may speculate that a breakdown in these counter regulatory mechanisms in women results in a maladaptive activation of arousal systems during physiological visceral stimuli. In combination with the results of Moulton and associates (39), the present data suggest that the tendency of healthy women to elicit more deactivation, or inhibition, may be a general sex-related characteristic and applies to both cortical and subcortical structures. Further research is needed with larger samples to ascertain the relative strength of these effects in different structures.

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