**Figure 1.** The region significantly less activated in the high degree of alexithymia group compared with the low degree of alexithymia group (**A**) (thresholded at $p < .001$ uncorrected at the voxel level and at $p < .05$ corrected at the cluster level for significance) and the associated adjusted responses for the between-group comparisons (**B**) (mean percentages of signal changes in the cluster) in the future happy imagery condition compared with the REST condition.
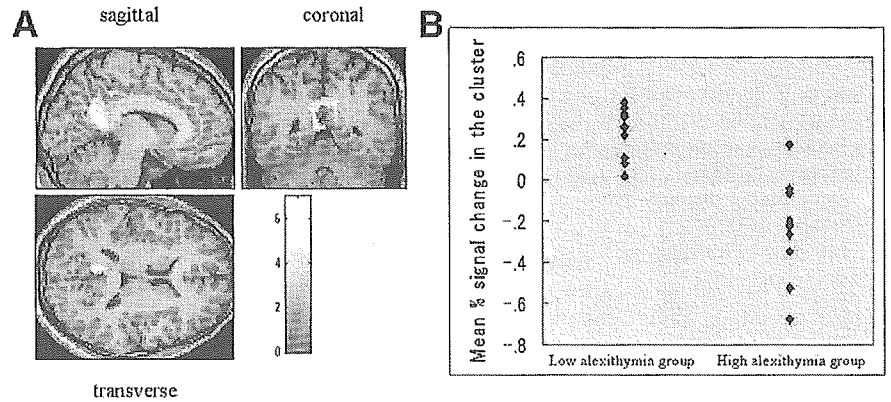
There is considerable evidence that the PCC has functions related to episodic memory (Andreasen et al 1995; Grasby et al 1993; Henson et al 1999; Maddock et al 2001). A review of functional imaging studies showed that the caudal part of the PCC was the cortical region most consistently activated by emotional stimuli compared with nominally matched, emotionally neutral stimuli (Maddock 1999). Moreover, it has been speculated that the PCC plays a role in the modulation of memory by emotionally arousing stimuli (Maddock 1999).

The PCC has strong reciprocal connections with regions engaged in memory processing, such as medial–temporal lobe memory structures and the thalamus (Bentovoglio et al 1993; Suzuki and Amaral 1994). It is also reciprocally connected to regions engaged in emotional processing, such as the ACC and the orbitofrontal cortex (Goldman-Rakic et al 1984; Musil and Olsen 1993; Van Hoesen et al 1993). These neuroanatomic findings also suggest that the PCC is involved in both memory and emotion. It is especially interesting that the ACC and PCC are connected reciprocally, whereas recent neuroimaging studies have suggested that the ACC has neural correlates of alexithymia (Berthoz et al 2002 ; Kano et al 2003). Although in our imagery task the activation of the ACC is not related to the degrees of alexithymia, the disturbance of both the ACC and PCC might comprise the various features of alexithymia having interaction.

One possible explanation for the difference between the groups in brain activation during FH imagery is that people with HDA can construct FH imagery, but in a different way than people with LDA do. It is known that the primary and secondary sensory cortices (such as the visual or auditory cortex) are activated during imagery (Cabeza and Nyberg 2000; Shergill et al 2001). But the activation of these areas did not significantly differ between subjects with HDA and subjects with LDA in any of the imagery conditions. So, we can't consider that people with HDA

are less imaginal, at least on the sensory level. Together with the function of the PCC mentioned in the previous paragraph, our results indicate that subjects with LDA use memories of past emotional events to create FH imagery, but subjects with HDA rarely or never do. On the other hand, the evaluation of an emotionally salient stimulus engages a variety of cognitive processes, many of which have been considered to rely on episodic-memory retrieval (Pratto 1994). Another explanation is that the activation of the PCC is associated with the evaluation of emotional stimuli that depend on episodic memories, and subjects with HDA evaluate their FH imagery as less exciting than do subjects with LDA.

Moreover, the blood oxygen level–dependent response presented during PH and FH imagery compared with REST suggests a deactivation of the PCC in the HDA group. Recently, functional imaging studies have shown that certain brain regions, including the PCC, consistently show greater activity during resting states than during cognitive tasks. Furthermore, it has been hypothesized that these brain regions constitute a default mode network (Greicius et al 2003; Raichle et al 2001). Raichle et al (2001) speculated that in the default state, information broadly arising in the external and internal milieus is gathered and evaluated and that when focused attention is required, activity within these areas might be attenuated. The HDA group demonstrated significant activation in the fusiform gyrus and not in the PCC, whereas the LDA group demonstrated the reverse pattern of activation in the one-sample t test. The fusiform gyrus is related to visual attention (e.g., Mangun et al 1998). Considering the external oriented cognitive style of alexithymia (Nemiah et al 1976; Taylor et al 1997), we speculated that because the HDA subjects might have been more engaged in visual attention to displayed cue letters than in the retrieval of episodic memory, deactivation of the PCC might have been greater in subjects with HDA than in

Figure 2. The region significantly less activated in the high degree of alexithymia group compared with the low degree of alexithymia group (**A**) (thresholded at $p < .001$ uncorrected at the voxel level and at $p < .05$ corrected at the cluster level for significance) and the associated adjusted responses for the between-group comparisons (**B**) (mean percentages of signal changes in the cluster) in the past happy imagery condition compared with the REST condition.
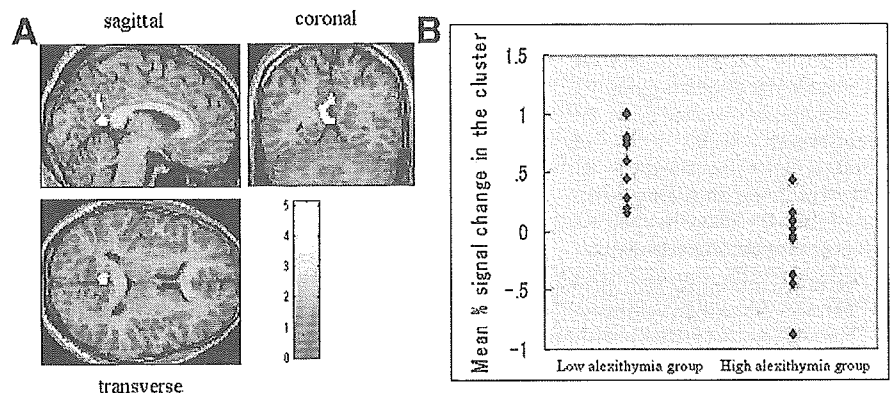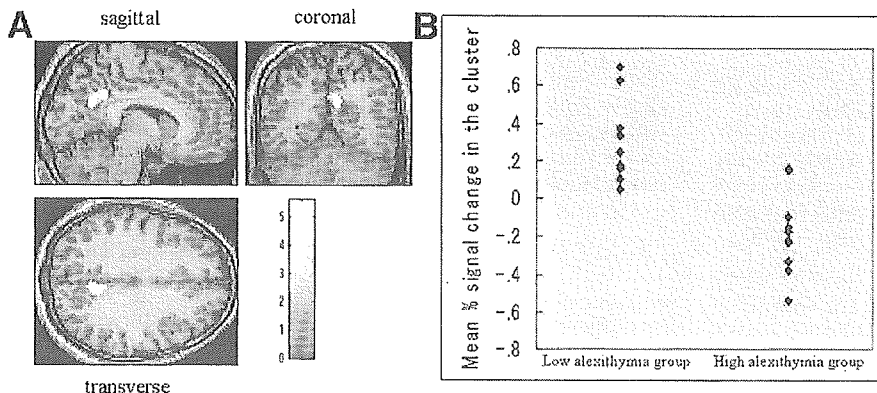
Figure 3. The region significantly less activated in the high degree of alexithymia group compared with the low degree of alexithymia group **(A)** (thresholded at $p < .001$ uncorrected at the voxel level and at $p < .05$ corrected at the cluster level for significance) and the associated adjusted responses for the between-group comparisons **(B)** (mean percentages of signal changes in the cluster) in the future happy imagery condition compared with the future neutral condition.

those with LDA, and the deactivation might likely have contributed to the between-group results.

At a lower level of significance, the PCC was less active in the HDA group than in the LDA group during the PH imagery condition compared with REST. These results, together with the result of the group comparison in FH > REST and FH > FN contrasts, suggest that it is difficult for individuals with HDA to imagine happy events. Our results support the speculation of previous researchers that the restricted imaginal capacities of people with alexithymia limit the extent to which individuals with HDA can modulate negative emotions by imaginative activities that have positive connotations, such as fantasy, dreams, interest, and play (Krystal 1988; Mightes and Cohen 1992). According to Bagby et al (1994b), within the correlation between the TAS-20 and the subscales of extraversion, alexithymia was associated significantly and negatively with the tendency to experience positive emotions. Our data support their idea that alexithymia is associated with a low proneness to experience positive emotions; however, in the PH > PN contrast, there was no significant difference between the groups. Our subjects usually chose daily acts as PN events, and these events were often more recent and more familiar than PH events. Meanwhile, the recency (Piefke et al 2003) and familiarity (Kosaka et al 2003) of autobiographical memory seem to increase PCC activity. The recency and familiarity of PN events might have confounded and diminished the difference between PH and PN conditions in the activation of PCC. To reach a conclusion about the difference in happy imagery between these two groups, further studies controlling these factors are needed.

There is another possible explanation for the groups having differed significantly only in the FH imagery condition. That is, our results might support the speculation of previous researchers that individuals with HDA find it hard to imagine something that they have never experienced. Clinical observations suggest that individuals with HDA seem to be swayed too much by minutiae of superficial "external" things and cannot imagine invisible, intangible things, such as mental content or the future (Marty and de M'Uzan 1963; Taylor et al 1997). They can recall intact their past experience, but they cannot process their experience or imagine events they have never experienced. Our results, however, suggested that the groups did not differ from each other in the FS and FN imagery conditions compared with REST. For FN events, our subjects usually chose daily acts that they had already experienced. We consider that this might explain why the groups did not differ in the FN condition. Moreover, although we did not discuss this in the Results section, the PCC was less active in the HDA group than in the LDA group during FS compared with REST and in FS compared with FN (number of voxels in cluster:

96 and 97, respectively) when the threshold was set at an uncorrected $p < .001$ at the voxel level in an a priori hypothesized region (Elliott et al 2000). On the other hand, there was no difference between the groups in PCC activation during PS compared with REST or during PS compared with PN, even at this lower threshold.

Meanwhile, discrepancies were observed between brain activation and subjective rating results. In general, the different responses to sad and happy imagery conditions were as follows. There were no significant differences in brain activation between the HDA and LDA groups in sad imagery conditions, but there were such differences in subjective ratings between the groups. For happy imagery conditions, on the other hand, the opposite was found: there were no significant differences between the groups in subjective ratings, but such differences were found in brain activation. These results seem to be paradoxical. We speculate that there were no significant differences between the groups in brain activation during sad imagery in the scan, but there might have been differences between the groups in brain activation when the subjects reported the subjective ratings after the scan. Several investigators suggested that alexithymia might involve a "decoupling" of the subjective and physiologic components of the emotional response to stressful stimuli—that is, a higher degree of alexithymia was associated with fewer subjective responses and greater physiologic reactivity (Martin and Pihl 1986; Papciak et al 1985). The results of these previous studies might suggest that subjects with alexithymia have deficiencies in conscious awareness of emotion. In previous neuroimaging studies of alexithymia, the ACC and MPFC have been reported as the neural correlates of conscious awareness of emotion. Studies of decoupling theory have focused exclusively on negative emotion. Berthoz et al (2002) reported that the activation of the ACC/MPFC was lower during negative stimuli and higher during positive stimuli in people with alexithymia than in people without it. Kano et al (2003) found that although the activation of ACC was lower in subjects with alexithymia than in those without it in response to an angry face, there was no difference between groups in the ACC activation in response to a happy face. These results also suggest that "decoupling" occurs in relation to negative emotion but not to positive emotion. If so, when brain activations differ between HDA and LDA subjects during happy imagery, subjective ratings should also differ between HDA and LDA subjects. In this study, however, no significant differences were found in subjective ratings of intensity of emotion during the happy imagery conditions. This might have been influenced by the difference in effect size between brain activation and subjective rating. Neuroimaging is a much more powerful tool than traditional behavior methods for detecting subtle relation-

ships between two variables (Canli and Amin 2002). In fact, in spite of the small sample size, subjective ratings of the intensity of emotion tended to be higher in the LDA group than in the HDA group for PH and FH, although not significantly.

On the other hand, to our surprise, there was no significant difference in the activation of the ACC/MPFC region between the groups for which we had an a priori hypothesis. The small sample size might explain the absence of such a difference. In fact, a qualitative comparison of brain activation by the one-sample t test suggested that the LDA group had significantly greater activity than the HDA group in the ACC/MPFC region during PS imagery. And if the subjects with HDA had poorer imaginal capacity than those with LDA, the activation of this area during the control condition, that is, REST condition (during which free recall could occur) and the neutral imagery condition, could be greater in the LDA group. In fact, ACC activation in the LDA group was significantly greater in PN than in REST in this study, whereas no ACC activation was found in the HDA group during PN. Furthermore, the brain activity detected by the one-sample t test was poorer than it was in George et al (1995), which showed bilateral limbic and paralimbic activation, including that of the ACC/MPFC. We considered that factors such as the shorter time interval among tasks, which might have resulted in mutual influence, or the shorter duration of imagery generation in this study than in the PET study of George et al (1995) might have influenced these differences in results between the two studies. Next, no difference between the groups was observed in the limbic structure (i.e., the amygdala, the hippocampal formation, and the hypothalamus), which plays a central role in emotional responses to simple perceptual aspects of stimuli. This finding is consistent with previous studies that found that the limbic area is not associated with alexithymia (Berthoz et al 2002; Kano et al 2003). Furthermore, no difference between the groups was observed in the insular cortex or in the orbitofrontal cortex; these cortices have been discussed in numerous neuroimaging studies about emotional recall/imagery (Phan et al 2002) and general emotional processing (Bechara et al 2000). This absence of activity might be attributable to the imaging method used. Whereas activation of these regions has been reported mainly in PET studies, it is known to be difficult to detect the activation of these areas by fMRI for susceptibility artifact (Ojemann et al 1997). Thus, our study cannot conclude that there is no relationship between emotional imagery disturbance related to alexithymia and these important brain regions, except for the PCC. Further studies considering these points are needed.

There are some limitations to this study. First, because of the small sample size, we might have failed to identify activation differences between HDA and LDA in other imagery conditions. Second, the sensory modalities of imagery (e.g., auditory, olfactory) involved in each event, in addition to visual sensation, differed not only between subjects but within each subject. This might have been a confounding factor, although it is difficult to control these factors because autobiographical memory is usually multimodal and because imagery, in which sensory modality is restricted, is different from daily experiences, especially emotional ones. Third, the subjects' retrospective ratings of their imagery and intensity of emotion might have been inaccurate, especially if the subjects with HDA had trouble with episodic memory. Finally, some subjects might have been unable to refrain from imagery and emotion or other cognitive activity during the rest periods. The level of each subject's cognitive activity during these rest periods might also be a confounding factor. Further studies considering these points are needed.

In conclusion, the present study revealed that the reduced activation of PCC in subjects with HDA was associated with the disturbance of FH imagery. The disturbance of FH imagery can reduce motivation and hope, and it might be an important factor in the construction of deficits in the emotional regulation of alexithymia. We suggest that PCC might play a crucial role in alexithymia-related imagery disturbance. Although this study has several limitations, the present results are meaningful as the first report to demonstrate neural correlates of imagery disturbance in alexithymia.

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ORIGINAL PAPER

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Gender differences in brain activity toward unpleasant linguistic stimuli concerning interpersonal relationships: an fMRI study

■ **Abstract** Women are more vulnerable to psychosocial stressors such as interpersonal conflicts than men, and are more susceptible to some psychiatric disorders. We hypothesized that there are differences in the brain activity of men and women while perceiving unpleasant linguistic stimuli concerning interpersonal relationships, and that they underlie the different sensitivity toward these stressful stimuli.

We carried out a functional magnetic resonance imaging (fMRI) study on 13 young female adults and 13 young male adults who performed an emotional decision task including sets of unpleasant words concerning interpersonal relationships and sets of neutral words.

In the women, the unpleasant words more significantly activated the bilateral caudate nuclei and left putamen than the neutral words. However, among the men, there was no difference in the level of activation of any brain area induced by the unpleasant or neutral word stimuli. Upon performing the task, there was a significant gender difference in brain activation. Moreover, among the female subjects, the activation in the bilateral caudate nuclei and left thalamus was negatively correlated with the average rating of pleasantness of the words concerning interpersonal conflicts by the subject.

These results demonstrate gender differences in brain activity in processing unpleasant linguistic stimuli related to interpersonal conflicts. Our data suggest that the bilateral caudate nuclei and left putamen play an important role in the perception of words concerning interpersonal conflicts in women. The bilateral caudate nuclei and left thalamus may regulate a woman's sensitivity to unpleasant information about interpersonal difficulties.

■ **Key words** human · brain imaging techniques · stress · language

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Introduction

There are diverse stressors around us, and they are roughly classified into two categories: physical and psychological stressors. Physical stressors include trauma, injury, physical exertion, noise, overcrowding, excessive heat or cold, and so on. Psychological stressors include stressful experiences such as time-pressured tasks, interpersonal conflict, isolation, and other types of traumatic life events [14]. As to psychiatric disorders, the available data are consistent with the view that social stress can trigger the onset of many psychiatric illnesses including major depression, anxiety disorders and eating disorders [19, 23, 31]. Interpersonal difficulties predict the propensity of depressive episodes to follow a chronic course [6].

As to susceptibility to psychiatric disorders, women are approximately three times more likely than men to experience a lifetime episode of depression [32]. The predominance of depression among women is a cross-cultural phenomenon and one of the most robust findings in psychiatric epidemiology [4]. Eating disorders are another category of psychiatric illnesses having a larger incidence among women than among men; up to 10 women for every 1 man develop an eating disorder [32]. These data indicating high susceptibility of women to psychiatric illnesses in which stress may play an im-

portant role suggest that women are more vulnerable to psychosocial stressors such as interpersonal conflicts than men.

With regard to the neural substrates underlying the cognition of unpleasant stimuli concerning interpersonal relationships, many lesion studies and functional brain imaging studies using facial expressions, which symbolize human emotions or what people think and which are necessary as one of the ways in which people communicate with each other, of fear, anger or disgust [1, 3, 15, 16, 20, 21, 26] and vocal expressions of fear, disgust or sadness [17, 20], suggested the involvement of the amygdala and basal ganglia including the caudate and putamen. To date, however, only a few studies have examined the gender differences in brain activation while perceiving facial expressions [9, 13]. These studies analyzed the brain activation of subjects of each gender separately, but the data were not directly compared. Moreover, the neural substrates underlying the cognition of linguistic stimuli concerning interpersonal conflict remain unknown.

To investigate which areas of the brain play an important role in the perception of stressful word stimuli concerning interpersonal relationships and whether the activation of brain regions shows gender differences, we performed a functional magnetic resonance imaging (fMRI) study with a modified emotional decision task based on the task used by Tabert et al. [28].

Methods

Subjects

Thirteen men (mean age, 25.3 y; S. D., 2.8 y; range, 21–30 y) and 13 women (mean age, 24.9 y; S. D., 3.3 y; range, 21–30 y) participated in this study. All of the subjects were right-handed and native Japanese speakers. Handedness was determined using the Edinburgh Handedness Inventory [18]. The subjects had no history of psychiatric, neurological, nor other major medical illness, and had never been treated with a psychotropic medication. To eliminate age-related effects, the subjects of the two genders were age-matched. This study was conducted using a protocol that was approved by the Ethics Committee of Hiroshima University School of Medicine. All subjects provided written informed consent for participation in the study.

Emotional decision task

We used the emotional decision task [28], with some modifications. The words used in the task were selected from the database of Toglia and Battig [30], which includes 2854 words that have been rated on several items such as familiarity and pleasantness, from one (very unfamiliar; very unpleasant) to seven (very familiar; very pleasant) with four as the midpoint. For the current study, 30 highly unpleasant words concerning interpersonal relationships and 30 neutral words were selected from the database and translated into Japanese. The highly unpleasant words and neutral words did not significantly differ with regard to word length (mean word length: Interpersonal relationships vs. Neutral = 2.8 vs. 3.1 in Japanese letters; P = 0.293 by two-tailed two-sample Student's t-test) nor familiarity (mean, 4.2 vs. 4.3; P = 0.808 by two-tailed Wilcoxon single-rank test) [25]. The words in each of the two groups consisted of nouns, verbs, adjectives and adverbs.

The selected words were used to generate word sets of unpleasant

words concerning interpersonal relationships and word sets of neutral words. Each word set was comprised of a unique combination of three words. The word sets were presented in six alternating blocks of word sets composed of unpleasant words concerning interpersonal relationships and neutral word sets (three cycles; Fig. 1A). Each block began with a 3-s cue indicating whether the block consisted of word sets of unpleasant words concerning interpersonal relationships or neutral word sets. Five word sets were presented in each block. Each word set was shown for 4 s with a 1.4-s inter-stimulus interval (ISI) (Fig. 1B and C). The blood oxygen level-dependent (BOLD) response was recorded during three blocks of unpleasant words concerning interpersonal relationships and to three blocks of neutral words. During each ISI, a fixation-cross placed centrally on the screen replaced the word set. Baseline functional magnetic resonance (fMR) images were obtained during a 9-s interval prior to the first block of trials, during which the subject viewed a centrally placed fixation-cross. During each trial, the word set was projected to the center of the subject's field of view via an SVGA computer-controlled projection system. The timing of presentation of word sets was controlled by Presentation Software Version 0.51 (Neurobehavioral Systems, Inc., San Francisco, CA) and the word sets were presented in a randomized order.

Immediately before fMRI scanning was begun, the subject was given 10 practice trials (5 unpleasant word sets and 5 neutral word sets). The words presented in the practice trials did not overlap with the experimental words.

The subject was given the instructions before fMRI scanning was started. The subject was instructed to select the most unpleasant word from each word set of unpleasant words based on his/her personal knowledge and experience. For each word set of neutral words, the subject was instructed to select the word that he/she thought was the most neutral. The subject was asked to respond by pressing one of three buttons on a response pad in the MRI scanner.
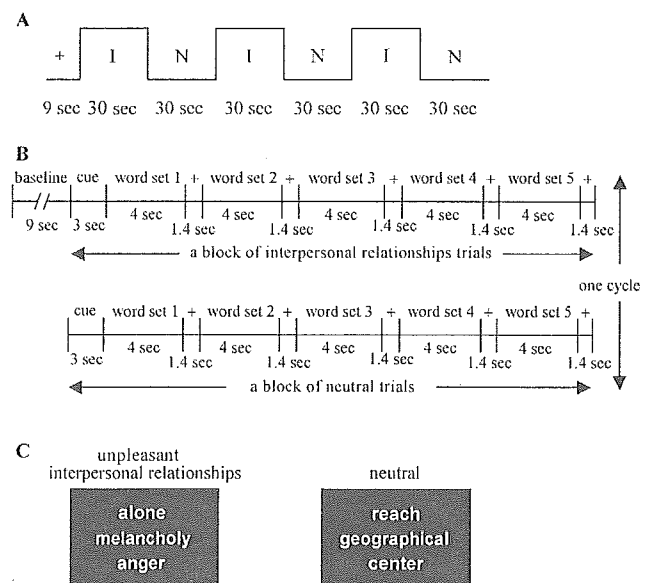
Fig. 1 The design of the task used in this study. **A** Overview of block-designed stimulus presentation paradigm for the tasks. Six alternating blocks of word sets of unpleasant words concerning interpersonal relationships (I) and neutral words (N) were successively presented. The total scan time of each task was 189 s (3 min and 9 s), while yielding 63 images of 28 axial slices (1764 images). **B** Blocks of word sets of unpleasant words concerning interpersonal relationships and neutral word sets preceded by baseline fMRI measurement. Each block began with a cue indicating "unpleasant words concerning interpersonal relationships" or "neutral words". The subject was instructed to select the word that he/she judged to be the most unpleasant or neutral, respectively, in each word set, by pressing one of the three buttons. **C** Typical examples of word sets presented in this study that are translated into English. The actual word sets consisted of Japanese words

Image acquisition and processing

Functional MRI was performed with a MAGNEX ECLIPSE 1.5T Power Drive 250 (Shimadzu Medical Systems, Kyoto, Japan). A time-course series of 63 volumes was acquired with T2*-weighted, gradient echo, echo planar imaging (EPI) sequences. Each volume consisted of 28 slices, and the thickness of each slice was 4.0 mm with no gap, encompassing the entire brain. The interval between two successive acquisitions of the same image (TR) was 3000 ms, the echo time (TE) was 55 ms, and the flip angle was 90°. The field of view was 256 mm and the matrix size was 64*64, giving voxel dimensions of 4.0*4.0* 4.0 mm. After functional MRI scanning, structural scans were acquired using a Tl-weighted gradient echo pulse sequence (TR = 12 ms; TE = 4.5 msec; Flip angle = 20°; FOV = 256 mm; voxel dimensions of 1.0*1.0*1.0 mm), and they facilitated localization and coregistration of the functional data.

Image processing and statistical analysis were performed using Statistical Parametric Mapping 99 (SPM99) software (Wellcome Department of Cognitive Neurology, London, UK) implemented in Matlab (Mathworks, Inc., Natick, MA). The first two volumes of the fMRI run (pre-task period) were discarded because the magnetization was unsteady, and the remaining 61 volumes were used for the statistical analysis. Images were corrected for motion and realigned with the first scan of the session, which served as the reference. The T1 anatomical images were coregistered to the first functional images in each subject and aligned to a standard stereotaxic space, using the Montreal Neurological Institute (MNI) T1 template in SPM99. The calculated nonlinear transformation was applied to all functional images for spatial normalization. Finally, the functional MR images were smoothed with a 12-mm full-width, half-maximum (FWHM) Gaussian filter.

Using group analysis according to a random effect model that allowed inference to the general population [8], we first identified brain regions that showed a significant response to word sets of unpleasant words concerning interpersonal relationships in comparison with the response to neutral word sets among the male subjects and among the female subjects, as brain areas related to the cognition of stimuli of unpleasant words concerning interpersonal relationships in males and females, respectively. Next, we directly compared the activation of the entire brain of the subjects of each gender, using the two-sample Student's t-test. The resulting set of voxel values for each contrast constituted an SPM map. The SPM maps were then interpreted by referring to the probabilistic behavior of Gaussian random fields. The data were initially thresholded at $P < 0.001$ uncorrected at the voxel level and at $P < 0.05$ corrected at the cluster level.

The x-, y- and z-coordinates provided by SPM, which were in Montreal Neurological Institute brain space, were converted to the x-, y-, and z-coordinates in Talairach and Tournoux's (TT) brain space [29] using the following formula: {TT_x = MNI_x · 0.88 – 0.8; TT_y = MNI_y · 0.97 – 3.32; TT_z = MNI_y · 0.05 + MNI_z · 0.88 – 0.44}. Labels for brain activation foci were obtained in Talairach coordinates using the Talairach Daemon software (Research Imaging Center, University of Texas, TX), which provides accuracy similar to that of neuroanatomical experts [10]. The labeling of areas given by this software was then confirmed by comparison with activation maps overlaid on MNI-normalized structural MR images.

Evaluation of pleasantness and familiarity with the word stimuli

Each subject was asked to rate the pleasantness and his/her familiarity with all of the words presented in the tasks on a 7-point scale from one (very unfamiliar; very unpleasant) to seven (very familiar; very pleasant), immediately after scanning. All of the words used in the tasks were printed in a table in randomized order.

Results

Rating of words

The rating of familiarity with the two categories of words did not significantly differ among all of the subjects (mean, Interpersonal relationships vs. Neutral = 4.2 vs. 4.3; P = 0.798 by two-tailed Wilcoxon single-rank test), among women (4.1 vs. 4.3; P = 0.695), and among men (4.3 vs. 4.4; P = 0.700). However, the subjects rated the unpleasant words concerning interpersonal relationships as significantly more unpleasant than the neutral words (all subjects, Interpersonal relationships vs. Neutral = 2.3 vs. 4.1, P = 0.000083; women, 2.3 vs. 4.1, P = 0.0015; men, 2.4 vs. 4.1, P = 0.0015).

Neither the rating of pleasantness nor the rating of familiarity in each word category significantly differed between the male and female subjects.

Functional MRI scan: brain activation in the subjects of each gender

Among the female subjects, there was significantly greater activation of the bilateral caudate body, left putamen and left parahippocampal gyrus when performing the emotional decision task on unpleasant words concerning interpersonal relationships than when performing the task on neutral words. However, among the male subjects, there was no significant difference in the level of activation of any brain region when performing the task on unpleasant words concerning interpersonal relationships or when performing the task on neutral words (Table 1, Fig. 2).

In the female subjects, the two-sample Student's t-test revealed that there was a significantly higher BOLD response than the male subjects in the bilateral caudate body and left putamen when performing the task on unpleasant words concerning interpersonal relationships than when performing the task on neutral words (Table 1, Fig. 3). No brain area was more significantly activated in the male subjects than in the female subjects during any of the tasks.

We could not attribute these activations to a particular structure with the resolution of our data since we performed the smoothing procedure to facilitate intersubject averaging.

### Correlation between psychological data and brain activation

Among the female subjects, simple regression analysis considering the entire brain revealed that the average rating of pleasantness of the unpleasant words concerning interpersonal relationships by a subject was negatively correlated with the BOLD response in some brain areas including the bilateral caudate nuclei and left thal-

Table 1 Relative increases in brain activity associated with unpleasant words concerning interpersonal relationships (task) and neutral words (control)

	Cluster	BA[a]	t-score	x	y	z
Female subjects (n = 13)						
Left putamen	1503		7.82	−22	−1	17
Left caudate body			7.47	−6	1	19
Right caudate body			6.17	6	−1	19
Left parahippocampal gyrus	437	36	5.89	−22	−42	−8
		35	5.82	−24	−23	−16
Right fusiform gyrus	161	37	5.41	29	−46	−11
		37	4.77	26	−52	−19
			4.12	20	−56	−26
Female subjects (n = 13) > Male subjects (n = 13)						
Left caudate body	1146		5.78[b]	−6	1	17
Left putamen			5.58[b]	−20	−1	17
Right caudate			4.53	6	3	10
Female subjects (n = 13) − inversely correlated with pleasantness of word stimuli						
Left caudate body	412		6.07	−11	6	11
Right caudate body			6.01	6	4	18
Left thalamus			5.71	−1	−5	8

Stereotaxic coordinates were derived from the human atlas of Talairach and Tournoux [23] and refer to the medial-lateral position (x) relative to the midline (positive = right), anterior-posterior position (y) relative to the anterior commissure (positive = anterior), and superior-inferior position (z) relative to the commissural line (positive = superior). [a] BA Brodmann area; [b] corrected P < 0.05 at the voxel level and all other areas; [c] corrected P < 0.05 at the cluster level

Fig. 2 Significant brain activation associated with unpleasant words concerning interpersonal relationships than to the neutral words among the female subjects. Three-dimensional "look-through" projections of statistical parametric maps of the brain regions are shown ([a] One-sample Student's t-test; corrected P < 0.05 at the cluster level; n = 13; df = 12)
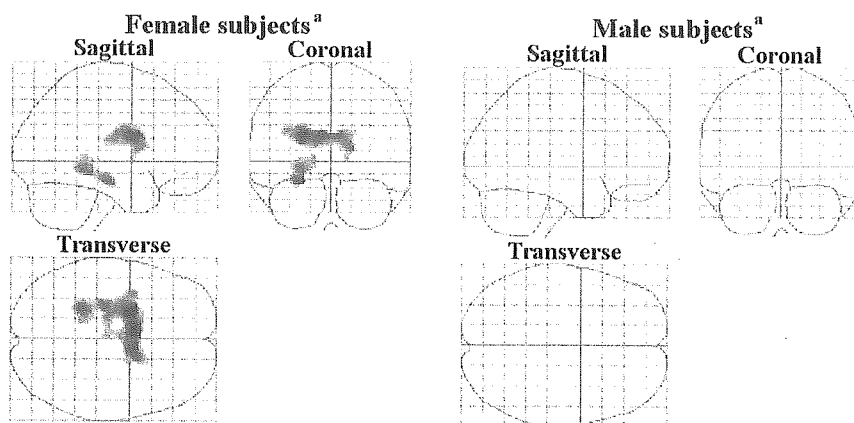

amus (P < 0.05 corrected in extent) (Table 1, Fig. 4). Among the male subjects, the average rating of pleasantness of the unpleasant words concerning interpersonal relationships by a subject was not significantly correlated with the BOLD response in any brain area.

Discussion

In the present study, we used the emotional decision task with unpleasant words concerning interpersonal relationships and neutral words to examine the brain areas engaged in the perception of unpleasant words concerning interpersonal relationships and to compare the pattern of brain activation between the female and male subjects. Our results showed that the bilateral caudate nuclei and left putamen are important in processing unpleasant words concerning interpersonal relationships, specifically in women. In our study, the male subjects did not have significantly greater activation in any area of the brain toward stimuli of unpleasant words concerning interpersonal relationships than toward the stimuli of neutral words. One possible reason for the difference is gender differences in specific cognitive functions. Men show superior spatial memory and women demonstrate superior verbal memory, and women rely on emotional content to a greater degree in the processing of information [5]. Men and women may take different strategies and may show different patterns of the brain activation during the same task. From this viewpoint, the women were considered to have processed word stimuli in this task with more emotional context than men. To further clarify these points, additional studies that directly compare the brain acti-

A

B BOLD response of each subject in the cluster
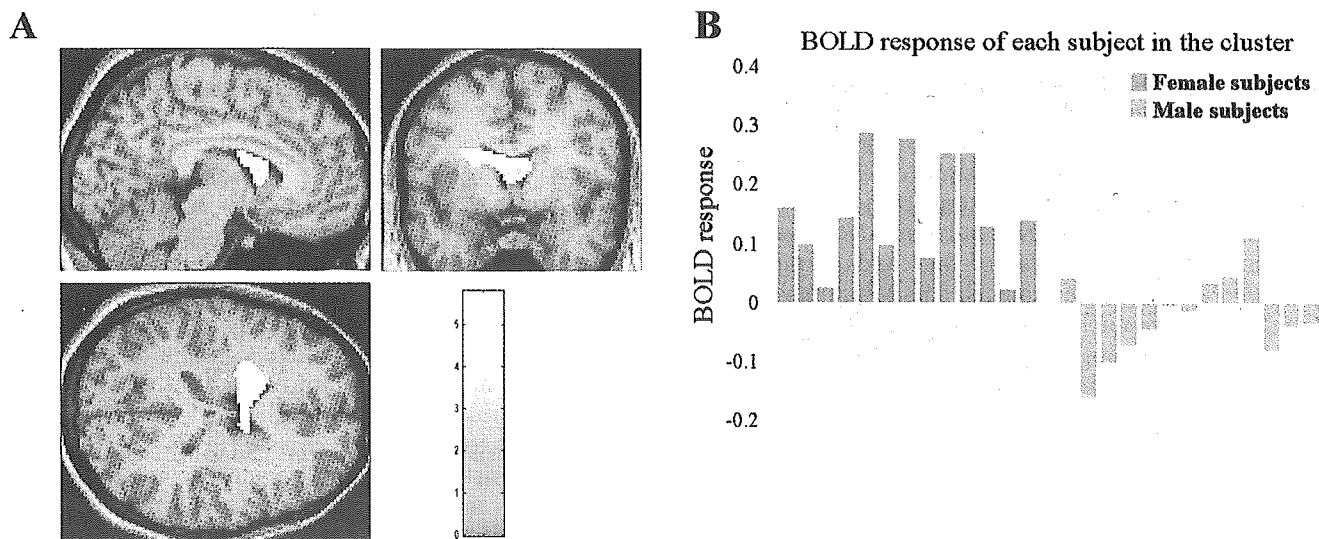
Fig. 3 Brain regions showing significantly greater activation in the female subjects than in the male subjects while performing the emotional decision task on unpleasant words concerning interpersonal relationships compared to neutral words. **A** Clusters of activation are overlaid onto a T1-weighted anatomical MR image. The T-levels of activation are color-coded from red to yellow. Two-sample Student's t-test; corrected $P < 0.05$ in extent; n = 26 (13 male and 13 female subjects); df = 24. **B** Each bar in the graph indicates the raw data of each subject in the cluster. Pink bars represent activation in the female subjects, and pale blue bars represent activation in the male subjects

A

B Correlation between BOLD response and word[a]

Pleasantness of words concerning interpersonal relationships
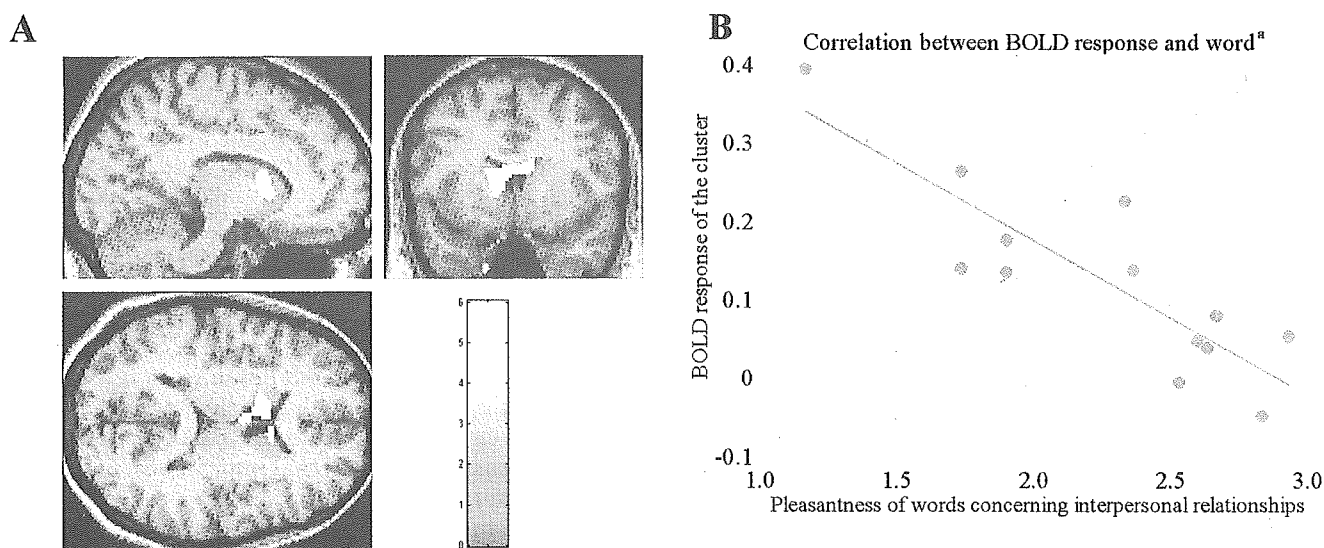
Fig. 4 Relationship between the degree of activation of brain areas and the average rating of pleasantness of words concerning interpersonal relationships among the 13 female subjects. **A** Clusters of activation are overlaid onto a T1-weighted anatomical MR image. The T-levels of activation are color-coded from red to yellow. Correlation analysis was performed on the entire brain and several brain areas showed a significant correlation. Simple regression analysis; corrected $P < 0.05$ in extent; n = 13; df = 11. **B** The scatter diagram demonstrates the correlation between the BOLD response of this cluster and the average rating of pleasantness of the words concerning interpersonal relationships among the 13 female subjects ([a] Spearman's rank-order correlation analysis; correlation coefficient = −0.851; P = 0.00068; n = 13)

vation of female and male subjects while processing visual stimuli such as unpleasant facial expressions as nonverbal stimuli concerning negative interpersonal relationships are needed. Another possible reason for the gender differences in brain activation is the involvement of some behavioral differences between men and women. In this study, we only have two behavioral data, rating of familiarity and pleasantness of the words used in this study, and these parameters did not explain the gender differences of the brain activity. Further studies are needed to make clear what kinds of behavioral differences exist between men and women which affect the brain activations.

Several studies have indicated the involvement of the caudate nucleus and putamen in processing negative facial expressions of disgust. For instance, a neuropsychological study reported that patients with Huntington's disease, which is characterized by specific lesions in the caudate nuclei, showed impaired responses to facial expressions of disgust [27], and thereafter it was reported in an fMRI study that facial expressions of disgust activated the putamen in healthy subjects [26]. Also, in an-

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other functional MRI study using four different stimuli, i.e., facial and vocal expressions of each of fear and disgust, only the facial expressions of disgust activated the caudate-putamen [20]. These studies indicate that the caudate nuclei and putamen play an important role in the perception of facial expressions of disgust. We used unpleasant linguistic stimuli concerning interpersonal relationships in the present study and detected significant activation in the area of the caudate nuclei and putamen in the female subjects. This result may suggest that the caudate nuclei and putamen play an important role in the processing of disgust with regard to interpersonal conflict regardless of the sort of stimulus, whether it is verbal or nonverbal.

Regarding the relationship between the degree of brain activation and the ratings of pleasantness and familiarity with the word stimuli by the subjects, simple regression analysis among the 13 female subjects showed that the average rating of pleasantness of the unpleasant words concerning interpersonal relationships by a subject was inversely correlated with the BOLD response in the bilateral caudate nuclei and left thalamus. As described above, the caudate nuclei are involved in the processing of stimuli related to disgust. Furthermore, the intensity of the BOLD response of this area was correlated with the subjective sensitivity of the individual towards the unpleasant words concerning interpersonal relationships, and this suggests that the caudate nucleus may play a key role in the regulation of sensitivity to external stimuli of disgust. As to the thalamus, an fMRI study revealed that the BOLD signal in the left thalamus was significantly increased during processing of high-valence facial expressions including happy and angry expressions [7], and several functional neuroimaging studies reported that the thalamus was activated when viewing films, viewing sets of pictures, or recalling personal experiences that evoked happiness, sadness or disgust [11, 12, 22]. These results suggest that the thalamus is activated rather unspecifically during the processing of both pleasant and unpleasant stimuli that span a variety of different emotions including anger, sadness and disgust. Although the thalamus may be activated regardless of the emotional valence, it is interesting that the more unpleasant the female subject felt the word stimuli were, the greater the magnitude of activity in the bilateral caudate nuclei and left thalamus.

Previous studies on patients with localized brain lesion have provided evidence that the human amygdala plays a role in evaluation of information containing negative emotion [2, 3, 24, 33]. However, these results suggest that activation of the amygdala may be particularly associated with fear, anger and threat rather than with all negative emotions.

There are some limitations in the present study. First, the resolution of our data was relatively low because we performed the smoothing procedure in order to facilitate intersubject averaging. This made it difficult to attribute the activation to a particular brain structure. Second, we did not perform a structured interview in se-

lecting the subjects for participation in this study. Nevertheless, they had no psychiatric nor neurological illness at the time of their participation, although we can not predict their occurrence in the future. Third, this study was targeted to only young adults to improve the statistical power; therefore, it is unclear whether these results apply to all age groups. Finally, although our data suggest that there is differential activation of the brain of men and women when perceiving unpleasant stimuli related to interpersonal conflicts and that women are more sensitive to stimuli concerning interpersonal conflicts, these data are not sufficient to conclude that bilateral caudate nuclei, left putamen and left thalamus are the neural substrates that underlie the high susceptibility of women to psychiatric illnesses in which stress may play an important role. Further studies are needed to confirm that these brain areas are involved in the susceptibility of women to psychiatric illnesses.

Further studies are also needed to reveal the differences in brain activation in response to stressful word stimuli concerning interpersonal relationships between psychiatric patients or psychiatric patients in remission and healthy subjects in each gender.

In conclusion, fMRI revealed that the bilateral caudate nuclei and left putamen were activated in 13 young female adults while performing the emotional decision task with unpleasant words concerning interpersonal relationships, and that these brain areas were more strongly activated in women than in men. In addition, the magnitude of activation of the bilateral caudate nuclei and left thalamus was negatively correlated with the subject's average rating of pleasantness of the unpleasant words concerning interpersonal relationships, only among the female subjects. These results suggest the involvement of qualitative factors in the activation of the bilateral caudate nuclei and left putamen in response to stimuli evoking disgust and the possibility of the role of the bilateral caudate nuclei and left thalamus in the regulation of sensitivity to external information of disgust related to interpersonal relationships.

Further studies that compare psychiatric subjects and healthy subjects are needed to elucidate the vulnerability to psychiatric illnesses in which stress may play a major role.

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Distorted Images of One's Own Body Activates the Prefrontal Cortex and Limbic/Paralimbic System in Young Women: A Functional Magnetic Resonance Imaging Study

Mitsuhaya Kurosaki, Naoko Shirao, Hidehisa Yamashita, Yasumasa Okamoto, and Shigeto Yamawaki

Background: Our aim was to study the gender differences in brain activation upon viewing visual stimuli of distorted images of one's own body.

Methods: We performed functional magnetic resonance imaging on 11 healthy young men and 11 healthy young women using the "body image tasks" which consisted of fat, real, and thin shapes of the subject's own body.

Results: Comparison of the brain activation upon performing the fat-image task versus real-image task showed significant activation of the bilateral prefrontal cortex and left parahippocampal area including the amygdala in the women, and significant activation of the right occipital lobe including the primary and secondary visual cortices in the men. Comparison of brain activation upon performing the thin-image task versus real-image task showed significant activation of the left prefrontal cortex, left limbic area including the cingulate gyrus and paralimbic area including the insula in women, and significant activation of the occipital lobe including the left primary and secondary visual cortices in men.

Conclusions: These results suggest that women tend to perceive distorted images of their own bodies by complex cognitive processing of emotion, whereas men tend to perceive distorted images of their own bodies by object visual processing and spatial visual processing.

Key Words: Body image, functional MRI, prefrontal cortex, amygdala, gender differences, eating disorders

Young women not only are more concerned about body shape and size, but also more harshly judge their own body shape and size than men. Eating disorders (EDs) are one category of psychiatric illness having a larger incidence among women than among men; women are approximately 10 times more likely to experience a lifetime episode of ED than men (Weissman et al 1995). One possible cause of this gender difference in susceptibility to ED may be that the cognitive processing of visual information about body image differs between men and women, and women are more sensitive to information about body image than men.

Several functional magnetic resonance imaging (fMRI) studies on EDs have investigated brain activation upon presentation of images of foods or body shape. In fMRI studies, women with an ED showed an activation of the limbic/paralimbic system and medial prefrontal cortex (PFC) upon viewing images of high caloric foods (Ellison et al 1998; Uher et al 2003, 2004). Functional MRI studies in which female subjects were shown their own distorted images indicated that women with ED showed activation of the limbic system, PFC and the parietal lobe (Seeger et al 2002; Wagner et al 2003). A few neuroimaging studies

specifically investigated gender differences in brain activation. Shirao et al (2005) reported that in an emotional decision task involving unpleasant word stimuli about body image, men showed increased PFC activation whereas women showed only amygdala activation.

However, there has been no study on gender differences in the pattern of brain activation upon viewing distorted images of one's own body. We hypothesized that the differences in brain activation while processing body image stimuli between the two genders involve a discrepancy in activation of the limbic area including the amygdala, paralimbic area and prefrontal cortex. We used fMRI to investigate the brain activity of young men and young women while they were engaged in a "body image task" using distorted images and real images of the subject's own body.

Methods and Materials

Subjects

Eleven women and 11 men participated in this study. All of the subjects were right-handed as determined by the Edinburgh Handedness Inventory (Oldfield 1971). Subjects were asked to fill out a written questionnaire regarding his/her own medical history. According to the self-reported responses, the subjects had no history of psychiatric, neurological, or other medical illness, and had never been treated with a psychotropic medication. We limited the female subjects in this study to women between the ages of 20 and 30 years, since young women even in the nonclinical population are often sensitive to body image and since ED commonly occurs in young women. To eliminate age-related effects, the subjects of the two genders were age-matched. To minimize the effect of the body shape proportion of the subjects in the two groups, we matched the body mass index (BMI) of the male and female subjects. Also, to minimize the effects of eating behavior, body image distortion and psychological features of the subjects in the two groups, we matched the score of the total Eating Disorder Inventory-2 (EDI-2) of the male and female subjects. The Clinical characteristics of the subjects

Table 1. Characteristics of the Subjects

	Women ($n = 11$)	Men ($n = 11$)	t-value	p-value
Age (y)	24.5 ± 3.4	24.8 ± 3.1	−.194	.8478
Range	21–30	20–30		
BMI (kg/m^2)	19.9 ± 1.3	20.8 ± 1.4	−1.533	.1409
Range	18.1–22.8	18.5–23.8		
EDI-2	45.5 ± 21.4	35.8 ± 14.5	1.247	.2268
Range	21–89	8–60		

p-values were obtained with the unpaired t-test. EDI-2, Eating Disorder Inventory-2; BMI, body mass index.

are summarized in Table 1. The scores on the subscales of EDI-2 are summarized in Table 2. The protocol of this study was approved by the Ethics Committee of Hiroshima University School of Medicine. After a complete description of the study was provided to the subjects, written informed consent was obtained.

Paradigm

The aim of this study was to investigate the neuronal processing of visual stimulation of fat or thin body image. We developed and created the paradigm, which consisted of a pair of distorted body image and real image, so that the subjects would recognize their own body image on comparison of two images. We thought that it was possible to elucidate the cognitive and emotional processing of visual stimulation of fat or thin body image by analyzing fMRI data on performing our paradigm.

Before the fMRI examination, we took digital photographs of each subject's whole body against a monotonous, light-colored background. The subject wore a white T-shirt and blue jeans, and was in the standing position. We changed the width of the image of the individual from −25 to +25% of the width in the original image by using Paint Shop Pro Version 8.02 (Jasc Software, Inc., Eden Prairie, Minnesota), to obtain ten distorted images with different degrees of thinness or fatness. For each subject, we used the following images of the subject: undistorted original image, fat-body images (width at +5, +10, +15, +20, or +25% of the width of the individual in the original image), undistorted original image with a red cross on the body, and thin-body images (width at −5, −10, −15, −20, or −25% of the width of the individual in the original image).

Using these distorted images and original images, we created image sets which consisted of three types of pairs of images: 1) pairs of a fat-body-image (gaining weight image) and real-image (undistorted original image); 2) pairs of the real-image and the real-image with a red cross; and 3) pairs of a thin-body-image (losing weight image) and real-image (Figure 1A). We used the block-designed fMRI paradigm which consisted of three different blocks of tasks: fat-image task, real-image task, thin-image task (Figure 1B). Each block began with a 3-sec cue indicating

whether the block consisted of the fat-image task, real-image task, or thin-image task. Five image sets were presented in each block. Each image set was shown for 5 sec with a 1-sec inter-stimulus-interval (ISI). Three different blocks were presented in one cycle. Our paradigm consisted of three cycles (Figure 1C). The blood oxygen level-dependent (BOLD) response was recorded during the three blocks of fat-image task, real-image task, or thin-image task. During each ISI, a fixation-cross placed centrally on the screen replaced the image set. Baseline functional magnetic resonance images were obtained during a 9-sec interval prior to the first block of trials, during which the subject viewed a centrally placed fixation-cross. During each trial, the image set was projected to the center of the subject's field of view via a super video graphics array (SVGA) computer-controlled projection system. The timing of presentation of image sets was controlled by Presentation Software Version .76 (Neurobehavioral Systems, Inc., San Francisco, California) and the image sets were presented in random order.

Immediately before fMRI scanning was begun, the subject was given 2 practice trials (1 fat-image task and 1 real-image task). In the practice trials, sample image sets of a girl were presented instead of pictures of the subject (Figure 1A). The subject was given instructions before the practice trials. In the fat-image tasks and thin-image tasks, the subject was instructed to select the more unpleasant image from each image set. In the real-image tasks, the subject was instructed to select the image with the red cross on the body. The subject was asked to respond by pressing one of two buttons on a response pad in the MRI scanner.

MRI Acquisition and Processing

Functional MRI of the brain was performed with a MAGNEX ECLIPSE 1.5T Power Drive 250 (Shimadzu Medical Systems, Kyoto, Japan). A time-course series of 102 volumes was acquired with T2*-weighted, gradient echo, echo planar imaging (EPI) sequences. Each volume consisted of 28 slices, and the thickness of each slice was 4.0 mm with no gap, encompassing the entire brain. The interval between two successive acquisitions of the same image (TR) was 3000 msec, the echo time (TE) was 55 msec, and the flip angle was 90°. The field of view was 256 mm
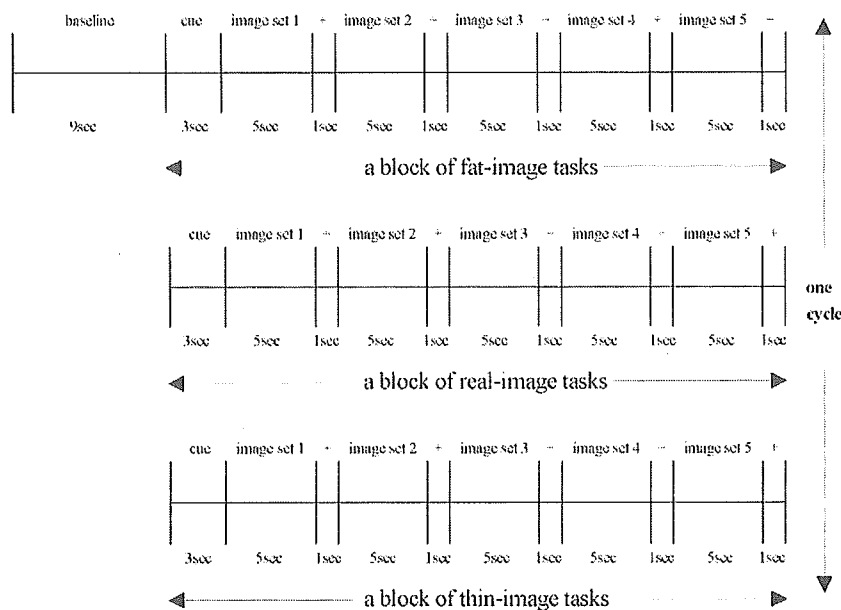
Table 2. Scores of the Subscales of EDI-2

	Total	Drive for Thinness	Body Dissatisfaction	Bulimia	Ineffectiveness	Perfectionism
Women	45.5	4.09	9.18	1.82	5.09	.45
Men	35.8	1.27	4.91	.73	4.73	3.73

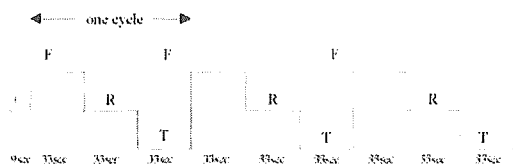|	Interpersonal Distrust	Interoceptive Awareness	Maturity Fears	Asceticism	Impulse Regulation	Social Insecurity
Women	4.18	3.91	2.45	3.18	2.73	3.55
Men	4.82	1.45	4.00	4.64	.82	5.82
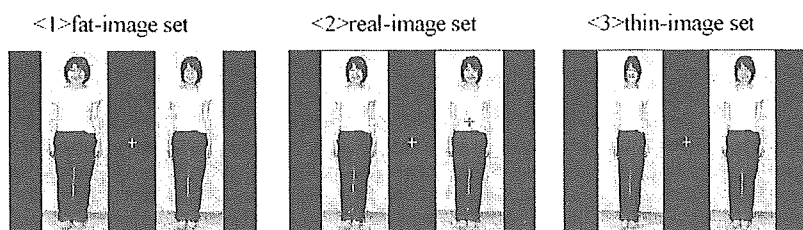
Figure 1. The design of the paradigm used in this study. (A) Sample image sets of the subject's own body that were presented to the subject. (B) Blocks of fat-image, real-image and thin-image tasks were preceded by baseline. Each block began with a cue indicating "fat," "real" or "thin." The subject was instructed to select the more unpleasant image from each image set for the fat-image task or thin-image task, and to select the image with a red cross on the body for the real-image task, by pressing one of two buttons. (C) Overview of block-designed stimulus presentation paradigm for the tasks. Nine alternating blocks of fat-body image (F), real-body image (R), and thin-body image (T) tasks were presented successively. The total scan time was 306 sec (5 min and 6 sec), while yielding 102 images of 28 axial slices (2856 images).

and the matrix size was 64 × 64, giving voxel dimensions of 4.0 × 4.0 × 4.0 mm. After functional MRI scanning, structural scans were acquired using a T1-weighted gradient echo pulse sequence (TR = 12 msec; TE = 4.5 msec; Flip angle = 20°; field of view (FOV) = 256 mm; voxel dimensions of 1.0 × 1.0 × 1.0 mm), and they facilitated localization and coregistration of the functional data.

Data Analysis

Image processing and statistical analysis were performed using Statistical Parametric Mapping 99 (SPM99) software (Wellcome Department of Cognitive Neurology, London, United Kingdom) implemented in Matlab (Mathworks, Inc., Natick, Massachusetts). The first two volumes of the fMRI run (pre-task period) were discarded because the magnetization was unsteady, and the remaining 100 volumes were used for the statistical analysis. Images were corrected for motion and realigned with the first scan of the session, which served as the reference. The T1

anatomical images were coregistered to the first functional image in each subject and aligned to a standard stereotaxic space, using the Montreal Neurological Institute (MNI) T1 template in SPM99. The calculated nonlinear transformation was applied to all functional images for spatial normalization. Finally, the functional MR images were smoothed with a 12-mm full-width, half-maximum (FWHM) Gaussian filter.

Using group analysis according to a random effect model that allowed inference to the general population (Friston et al 1999), we first identified brain regions that showed a significant response to image sets containing a distorted body image in comparison with the response to image sets containing two real body images among the female subjects and among the male subjects, as brain areas related to the cognition of body image stimuli in women and men, respectively. Next, we directly compared the activation of the entire brain of the subjects of each gender, using the two-sample Student's t-test. The resulting set of voxel values for each contrast constituted an SPM {T} map. The

SPM {T} maps were then interpreted by referring to the probabilistic behavior of Gaussian random fields. The data were initially thresholded at $p < .001$ uncorrected at the voxel level, and regions about which we had an a priori hypothesis were reported at this threshold (Elliott et al 2000). For regions about which there was no clear a priori hypothesis, a more stringent threshold of $p < .05$ corrected at the cluster level for multiple comparisons was used. Only regions that survived at this threshold were reported.

The x-, y- and z-coordinates provided by SPM, which were in MNI brain space, were converted to the x-, y-, and z-coordinates in Talairach and Tournoux's (TT) brain space (Talairach and Tournoux 1988) using the following formula: {TT-x = MNI−x * .88 − .8; TT−y = MNI−y * .97 − 3.32; TT−z = MNI−y * .05 + MNI−z * .88 − .44}. Labels for brain activation foci were obtained in Talairach coordinates using the Talairach Daemon software (Research Imaging Center, University of Texas, San Antonio, Texas), which provides accuracy similar to that of neuroanatomical experts (Lancaster et al 2000). The labeling of areas given by this software was then confirmed by comparison with activation maps overlaid on MNI-normalized structural MR images.

Results

Comparison of brain activation upon performing the fat-image task versus the real-image task showed significant activation of the bilateral prefrontal cortex (PFC) (Brodmann area (BA) 13, 47, 9), the left limbic area including the amygdala, and the right cerebellum in the women, and significant activation of the right occipital lobe including the primary and secondary visual cortices (BA 17, 18), the right temporal lobe and parietal lobe, and the left cerebellum in the men while performing the fat-image task.

Comparison of brain activation upon performing the thin-image task versus the real-image task showed significant activation of the left PFC (BA 32, 13), the left limbic area including the cingulate gyrus, the paralimbic area including the insula, and the left cerebellum in the women, and significant activation of the occipital lobe including the left primary and secondary visual cortices (BA 17, 18, 19), the temporal lobe including the bilateral fusiform gyrus, and the right parietal lobe in the men while performing the thin-image task.

Regarding the behavior of the subjects, on each fat-image task, the subjects in both genders tended to select the fat image as more unpleasant image and the women tended to select the fat image more frequently than did men (see Figure 2; Tables 3 and 4).

Discussion

In the present study, upon performing the fat-image task, the PFC and limbic/paralimbic lobe were activated in the women but not in the men, and the dorsal and ventral pathways of the visual system in the occipital, temporal and parietal lobes were activated in the men but not in the women. Our data are the first to demonstrate clear gender differences in brain activation pattern when men and women are confronted with their own distorted body image. These results suggest that the gender differences in brain activation pattern may explain the differences in cognition of distorted body image.
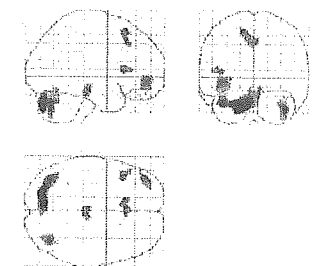
It has been suggested that the amygdala is involved in fear conditioning (Buchel et al 2000; LaBar et al 1998; LeDoux et al 2000; Morris et al 1998), in the recognition of fearful facial expressions (Adolphs et al 1995, 1999), and in evocation of fearful emotional responses from direct stimulation (Halgren et al
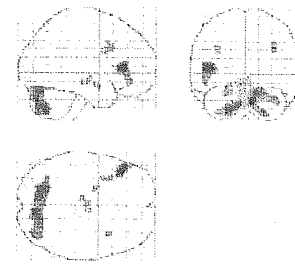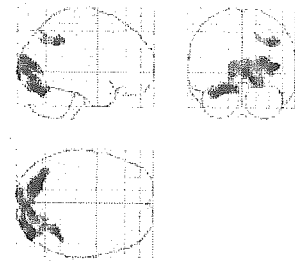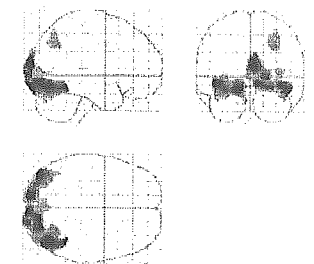

Figure 2. Differences in brain activation pattern between the two genders upon comparison of brain activation while performing the fat-body task and thin-body task. Three-dimensional "look-through" projections of statistical parametric maps of the brain regions are shown. One-sample Student's t-test; corrected $p < .05$ at the cluster level; $n = 11$; $df = 10$.

1978). Using fMRI, Ellison et al (1998) found that adult female anorectic patients, when viewing pictures of high-calorie drinks, had increased signal changes in the left amygdala-hippocampal region. They discussed that visual images of high-calorie food are fearful stimuli for patients with anorexia nervosa (AN). An fMRI study on brain reaction to distorted body image reported that female AN patients showed activation of the right amygdala (Seeger et al 2002). Our finding in the present study that the amygdala was activated in the female subjects upon viewing one's own fat body image suggested that a fat shape of her own body was interpreted as fearful information not only in patients with ED, but also in healthy women.

Previous studies suggested that the anterior cingulate gyrus is involved in attention tasks (Casey et al 1997), and in self-monitoring, conflict-resolution, and reward-based decision making (Bush et al 2002; Carter et al 1998; Devinsky et al 1995). Functional MRI studies in female AN patients revealed that the anterior cingulate gyrus was activated when the subjects viewed food stimuli or distorted body images (Ellison et al 1998; Uher et al 2003; Wagner et al 2003). Our finding that the anterior cingulate (BA 32) was activated upon viewing the thin body image only in the women indicates that the attention network was activated in the women and suggests that women tend to perceive their own body image with attentional and self-monitoring processes.

An fMRI study reported that the dorsolateral prefrontal cortex (DLPFC) and orbitofrontal cortex (OFC) are essential for volitional regulation of emotional impulses mediated by limbic/paralimbic structures (Lévesque et al 2003). The medial PFC (MPFC) has connections to limbic/paralimbic structures and

Table 3. Relative Increases in Brain Activity Associated with the Distorted-Body-Image Task Compared with the Real-Image Task

	Cluster	BA	t-Score	x	y	z
Female subjects (n = 11)						
Fat > Real						
Left inferior frontal gyrus[a]	330	13	13.12	−40	26	6
Left middle frontal gyrus[a]		47	4.68	−45	34	−6
Right cerebellum	1456		8.93	6	−79	−34
Right cerebellum			8.00	12	−77	−24
Right inferior frontal gyrus	44	9	5.84	31	10	28
Left amygdala	32		5.42	−20	−3	−15
Left middle frontal gyrus	64	9	4.99	−31	12	29
Left precentral gyrus		6	4.59	−31	3	25
Thin > Real						
Left cerebellum	1028		8.72	−17	−79	−38
Left cerebellum			8.69	−22	−79	−33
Left cerebellum			7.67	−8	−77	−31
Left superior frontal gyrus	169	6	5.81	−13	20	50
Left cingulate gyrus		32	5.31	−1	20	39
Left cingulate gyrus		32	5.05	−8	18	43
Left insula	90	13	5.78	−38	16	8
Left inferior frontal gyrus		13	4.69	−45	26	8
Left inferior frontal gyrus	279	10	5.60	−36	43	−2
Male Subjects (n = 11)						
Fat > Real						
Right middle occipital gyrus[a]	1568	18	8.25	36	−85	4
Right lingual gyrus[a]		17	6.40	3	−89	−5
Right lingual gyrus[a]		18	6.28	12	−81	−11
Left cerebellum	711		8.17	−20	−75	−22
Left cerebellum			6.41	−29	−69	−25
Right superior temporal gyrus	307	39	6.07	31	−52	31
Right precuneus		7	4.61	26	−67	31
Right precuneus		7	4.52	17	−67	37
Thin > Real						
Left inferior occipital gyrus[a]	1255	18	10.93	−38	−81	−11
Left lingual gyrus[a]		17	10.93	−13	−93	−17
Left fusiform gyrus[a]		18	7.20	−29	−83	−17
Right cuneus[a]	2482	18	10.82	3	−93	7
Right fusiform gyrus[a]		19	9.06	34	−69	−13
Right fusiform gyrus[a]		37	8.73	36	−50	−15
Right precuneus[a]	389	7	5.83	26	−63	30

Stereotaxic coordinates were derived from the human atlas of Talairach and *Tournoux* (6) and refer to the medial-lateral position (x) relative to the midline (positive = right), anterior-posterior position (y) relative to the anterior commissure (positive = anterior), and superior-inferior position (z) relative to the commissural line (positive = superior). Only the activated brain areas within the gray matter are displayed. t-scores were obtained with one-sample t-test. BA, Brodmann area.

[a]Exceeded the extent threshold of P < .05 corrected at the cluster level. All other areas that are shown exceeded the height threshold of P < .001 uncorrected at the cluster level and belonged to a cluster of activation with an extent of at least 30 voxels.

constitutes an interaction zone between emotional processing and cognitive processing (Drevets et al 1998). When individuals turn their attention inwards to themselves, the activity in the MPFC increases (Johnson et al 2002; Gusnard et al 2001; Kelley et al 2002; Zysset et al 2002). Our finding that the PFC (DLPFC: BA 9,10; OFC: BA 13, 17; MPFC: BA 32) and limbic/paralimbic lobes were activated in the female subjects upon performing both the fat-image task and thin-image task suggests that a woman tends to perceive her body shape with cognitive and emotional

processing, regulate emotional stimulation by herself, and turn attention inwards to assess emotional awareness about herself.

On the other hand, there was no significantly greater activation in the PFC and the limbic/paralimbic system upon performing the fat-image task versus the real-image task in the male subjects; on the contrary, the occipital lobe including the primary and secondary visual cortices, the temporal lobe and parietal lobe were activated in the male subjects. Within the visual cortex, the occipitotemporal pathway, or "ventral stream," is crucial for

Table 4. Percentage of Subjects who Selected the Real Image as the More Unpleasant Image in Each Gender (%)

Task	+25	+20	+15	+10	+5	−5	−10	−15	−20	−25
Women	.0	.0	.0	.0	13.3	80.0	60.0	46.7	53.3	13.3
Men	.0	6.1	6.1	18.2	30.3	51.5	33.3	33.3	39.4	30.3

the identification of objects including identification of features such as color, shape and faces, whereas the occipitoparietal pathway, or "dorsal stream," is crucial for spatial perception and observation of the movements of objects in space such as motion and information about location (Ungerleider et al 1998). The activation of the dorsal and ventral pathways was detected only among the men in this study as a subtraction between the reactions to both stimulations in this kind of analysis. These results suggest that men tended to pay attention to differences in size and shape between the distorted images of their own bodies and real images of their bodies, while women tended to pay the same attention to the distorted images of their own bodies and real images of their bodies.

In a previous study using positron emission tomography (PET), Bailer et al (2004) reported ED patients showed parietal lobe activity on the resting-state experiments. Wagner et al (2003) also reported parietal lobe activity in anorectic patients who viewed distorted body images, but they used a mosaic image as the control. It is difficult to compare our results with the results of previous studies because of differences in methodology.

Another finding of the present study was that the cerebellum was activated in both women and men upon performing the body image task. Neuroimaging studies have indicated that cerebellar regions are not only important in motor control, but also involved in several different types of higher cognitive operations (Kim et al 1994; Allen et al 1997). However, the distinct role of the cerebellar regions in these diverse and complex cognitive functions remains unclear.

Based on comparison of the fMRI images of the brain activation upon performing the fat- versus real-image task or thin- versus real-image task, our study showed that women tend to perceive their own distorted body images by complex cognitive processing of emotion, whereas men tend to perceive their own distorted body images by object and spatial visual processing. On the behavioral level, the female subjects were more sensitive to their fat body image with respect to unpleasant emotion than the male subjects (Table 4). These results and our finding that the amygdala or PFC was activated upon performing the distorted-image task in the women suggest that certain distorted body images in our paradigm may be fearful stimuli to the subjects. There were no significant differences in age, BMI or the EDI-2 total score between the male and female subjects (Table 1). Therefore, the areas of the brain that were activated in each gender may explain their style of cognition of distorted body shape. Taking into consideration the results of previous studies on EDs (Seeger et al 2002; Wagner et al 2003), our study suggests that the cognitive style of visual stimuli of body image in healthy women is more similar to that in ED patients with weight phobia, than to that in healthy men. These results may be related to the fact that women are approximately 10 times more likely to experience a lifetime episode of ED than men and the fact that women tend to be more sensitive to information about body image in daily life than men.

Our study has some limitations. First, we did not perform a structured interview during the selection of subjects for participation in the study. Nevertheless, they had no psychiatric nor neurological illness at the time of their participation in this study, although we can not predict their occurrence in the future. Second, our paradigm was appropriate for detecting neuronal processing of body image in the brain. However, there were some uncertainties in our paradigm: (1) Were the results about the neuronal processing of the distorted self body image, the distorted body image, or just the distorted image? (2) Was the cue itself the

negative reward? (3) Was there a difference in the difficulty of the active and control tasks? These are points that are worth studying in the future. Third, although our data suggest that there is differential activation pattern of the brain of men and women when processing distorted body image, these data are not sufficient to conclude that the PFC and the limbic/paralimbic area, which were activated in the women but not the men upon viewing distorted body images of one's own body, are the focal areas responsible for susceptibility to ED.

In conclusion, our neuroimaging results provide evidence that women process visual stimuli of their own body images differently from men, and that women are more emotionally preoccupied with the appearance of their own body shapes than men. Further studies that compare the brain activation of ED patients and healthy controls while performing our tasks are needed to confirm that these brain areas are involved in the mechanism of the onset of ED.

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Research Report

Age-related disturbance of memory and CREB phosphorylation in CA1 area of hippocampus of rats

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Abstract

In the early process of long-term memory formation, cyclic AMP response element-binding protein (CREB), a transcription factor on which multiple signal transduction pathways converge, has been implicated. We examined whether the age difference in the performance of contextual fear conditioning (CFC) is associated with a change in activation of CREB in the hippocampus which is an important neural structure for long-term memory. The activation of CREB in the hippocampus in young (15 weeks old) and old (120 weeks old) male rats was determined immunohistochemically with an antibody that specifically recognizes the phosphorylated form of CREB (pCREB). Young rats exhibited better performance than old rats with respect to the freezing time in CFC. Phosphorylation of CREB as revealed by the ratio of the pCREB-immunoreactive cell number to the CREB-immunoreactive cell number was increased in the CA1 region, but not in other hippocampal regions following training for CFC. The close relationship between behavioral performance and CREB phosphorylation in the CA1 region suggests that hippocampal CREB is involved in age-related decline of learning and memory.
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Theme: Neural basis of behavior
Topic: Aging

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1. Introduction

Impairment of learning and memory in the aging process has been widely studied in various animal models as well as in humans. The hippocampus is well known to play a significant role in the process of learning and/or memory [4,18,20]. In aged rats, there is evidence of marked impairment of hippocampus-dependent tasks, such as spatial memory tasks [7,36], Morris water-maze [9,21,27], contextual fear conditioning (CFC), radial maze [22], and passive avoidance [27]. There is, on the other hand, no

impairment in solving a simple cued discrimination task [22] or immediate memory even though short-term memory is impaired [33]. To understand the molecular mechanisms of the impairment of learning and memory in the aging process, rat hippocampus-dependent tasks are reasonably valid and useful animal models.

Previous studies have suggested some mechanisms underlying the aging-related cognitive changes in the rat brain. One of the mechanisms is a reduction in the duration of experimentally induced long-term potentiation (LTP) of hippocampal synapses [16,17], which is correlated with faster forgetting of spatial information [4]. The impairment of LTP was thought to be caused by an increase of intracellular calcium by aging [6,17]. Another is loss of synaptic connectivity and integration among hippocampal

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cells, which can lead to a reduced probability or magnitude of LTP induction [6,7,15,21]. Earlier studies proposed neuronal loss in the hippocampus as one mechanism of learning impairment in aging [17]. However, recent studies using more precise stereotactic methods demonstrated no significant age-related changes in the number of pyramidal cells in the ammon horn, granule cells in the dentate gyrus, or astrocytes in the hilus [7,20,21].

In addition to the major role of the hippocampus, other brain areas may also be implicated in learning and memory circuitry such as the cerebral cortex and amygdala. Soon after short-term memory is converted to long-term memory, it is stored in a different site in the cerebral cortex [13]. Aside from hippocampus, neocortex has been shown to play a role to store contextual memory [8].

The cerebral cortex is another site undergoing such modification by aging, as loss of neurons in cerebral cortex [32] and reduction in the number of NADPH-diaphorese-positive cells, which secrete nitric oxide [38]. In long-term implicit memory that is related to a cue or sound fear, the amygdala plays an important role in generating and storing long-term memory, with special emphasis on the basolateral nuclei and central nucleus [12].

One of the hippocampus-dependent tasks that encompasses other forebrain structures is contextual fear conditioning (CFC). Previous studies by Fos immunohistochemical staining and lesioning revealed the involvement of the dorsal hippocampus [2,11], amygdala, and parietal cortex [26] in CFC. Corticosterone is a key hormone in the expression of conditioned freezing behavior, which is mediated specifically by mineralocorticoid receptor [11]. Plasma corticosterone level is markedly elevated in aged rats [17,19], which may also explain the age-related decline in performance of CFC.

Cyclic AMP response element binding protein (CREB), a transcription factor, when activated (phosphorylated in serine 133), has been implicated in a wide variety of biological processes, and there has been considerable interest in its role in the conversion of short-term memory to long-term memory [1]. In *Drosophila*, CREB family members are likely to be involved in the long-term memory formation, which is cycloheximidine (CXM)-sensitive [39]. In rodents, bilateral infusion of Sp-cAMP, an analogue of cAMP, into the CA1 region produced a 30% increase in PKA activity and was able to increase hippocampal phosphorylated CREB (pCREB), thus attenuating novelty-induced amnesia in avoidance training [34]. A phosphorylated or active form of this transcription factor is selectively recruited in the CA1 and dentate gyrus (DG), but not in the CA3, and showed dose dependency. Initial recruitment of pCREB is thought to depend on calcium influx via the NMDA receptor [29], and subsequent activation of PKC, PKA, and MAPK [28]. Mutant animals lacking the alpha and delta isoforms of this transcription factor, which showed a 75% reduction in CREB level, showed impairment of LTP and long-term memory [5].

Therefore, to investigate the influence of aging on learning ability and hippocampal functions in rats, we trained young (15 weeks) and aged (120 weeks) rats using CFC, and quantified freezing behavior as well as the number of pCREB-positive cells in the hippocampus.

2. Materials and methods

2.1. Animals

Fifteen-week-old (240–250 g) and 120-week-old (600–900 g) male SD rats were purchased from Japan SLC (Shizuoka, Japan). The rats were maintained individually in a light/dark cycle-controlled (lights from 0600 h to 1800 h) and temperature-controlled animal room with free access to laboratory chow and tap water. The rats were allowed to rest undisturbed in their transportable home cages for at least 5 days prior to experiments. All experimental procedures were conducted in accordance with the guidelines of the Ethical Committee for Animal Experiments of Yamanashi Medical University. Every possible effort was made to minimize the number of animals used and their suffering.

2.2. Contextual fear conditioning

Contextual fear conditioning (CFC) was conducted according to the method of Silva et al. [31]. The details were described in our previous paper [14]. The CFC task was performed in a conditioning chamber housed in a sound-attenuating box during the light phase of the cycle. The conditioning chamber (28W × 21H × 22D cm) was constructed of clear Plexiglas. The floor of the chamber was lined with 18 stainless steel bars (4 mm in diameter; 1.5 cm spacing), which formed a footshock grid to deliver scrambled shock produced by a stimulator. The footshock was 2-s direct current of 0.75 mA and served as the aversive unconditioned stimulus (US). The sound-attenuating box (48W × 48H × 48D cm) was provided with 20 W house light, and a ventilation fan was located on the top of the box, supplying background white noise (74 dB). A discrete tone conditioned stimulus (tone CS) was given in addition to a general contextual stimulus. The tone cue (800 Hz, 20-s duration, 80 dB) was delivered by two speakers located in the lower corner of the sound-attenuating box.

Prior to the conditioning, all the rats received 3-day habituation, in which they were placed in the conditioning chamber for 1 min and returned to their home cages once a day. On the day of conditioning, the rats were placed in the conditioning chamber and allowed to explore for 3 min. A footshock was delivered 18 s after the tone CS. The rats were then allowed to recover for 30 s in the conditioning chamber and returned to their home cages.

Firstly, we measured short-term memory. Immediately after the rats were returned to their home cages, they were once again placed in the conditioning chamber and their