increase the levels of circulating catecholamines (Maisel et al., 1990). Prolonged elevation of sympathetic nervous activity by such stressors induces extremely high levels of catecholamines. Additionally, via a β-adrenergic mechanism, it reduces the number of circulating lymphocytes and the activity of natural killer (NK) cells, a subgroup of lymphocytes essential to the cellular immune defense against virus-infected cells, bacteria, and tumor cells (Vivier et al., 2004), thereby decreasing immune defense (Maisel et al., 1990). Furthermore, chronic psychological distress might cause or facilitate diseases such as cancer (Jacobs and Bovasso, 2000). Therefore, it is suggested that negative emotions lead to the deterioration of human immune functions and health.

In contrast, recent findings suggest that positive psychological events are also related to immune functions and health in humans. It has been reported that individuals with a great tendency to experience positive emotions such as happiness and joy are less vulnerable to viral infections (Cohen et al., 2003; Doyle et al., 2006; Marsland et al., 2006). Furthermore, NK cell activity significantly increases after individuals laugh by watching comic films (Takahashi et al., 2001; Berk et al., 2001), and the proportion of circulating NK cells increases after positive emotions are experienced due to sexual arousal (Haake et al., 2004). However, whether the central nervous and immune systems are actually interrelated via neurochemical networks remains obscure because studies that focus on the association between central nervous and immune systems have not yet been conducted. Moreover, it is still obscure whether every positive emotion is related to the immune system.

Seeing one's favorite person such as a love interest or favorite actor/actress may evoke positive emotions and occasionally lead to a feeling of elation (Esch and Stefano, 2005a,b; Stefano and Esch, 2005; Planalp et al., 2006; Aron et al., 2006). Recent neuroimaging studies have demonstrated that such events activate reward-related regions in the brain (Bartels and Zeki, 2004; Aron et al., 2005; Fisher et al., 2005); based on this, attraction toward certain persons may be highly rewarding. In the present study, we attempted to examine positive emotions elicited on seeing a favorite person and the psychological and physiological responses, including central nervous, endocrine, and immune parameters, during these emotions. There have been no investigations on whether seeing a favorite person can indeed evoke positive emotions. In addition, to our knowledge, associations among central nervous, endocrine, and immune systems during experiencing those emotions have not been investigated to date. It is possible that a certain immune parameter is stimulated even when we see our favorite persons. In this study, the participants themselves selected persons whom they found attractive, and positive emotions were manipulated by the viewing of a film featuring these attractive persons. We have recently established a method that can simultaneously record brain activity by using ^{15}O-water positron emission tomography (PET) and peripheral physiological activity including immune activity (Ohira et al., 2006); thus in order to reveal associations among central nervous, endocrine, and immune systems, we simultaneously recorded various parameters such as mood states, brain activity, peripheral circulating NK cell activity, and the serum level of catecholamines when male participants watched films featuring people whom they perceived as attractive.

2. Methods

2.1. Participants

Twelve healthy male volunteers (right handed; age range: 20–29 years) participated in the study. The participants' self-reports in a questionnaire and an interview by a psychiatrist confirmed that they had no past history of psychiatric or neurological illnesses and were not receiving any medication. Two participants were excluded from the catecholamine analyses because of technical difficulties. All the participants provided written informed consent in accordance with the Declaration of Helsinki, and they were paid 15,000 Japanese Yen for participation. The participants received no medication during the experimental period. This study was approved by the Human Studies Committee of Aichi Medical University and the Ethics Committee of Kizawa Memorial Hospital.

2.2. Task procedure

Participants were instructed not to eat 2 h before the scanning session, but they were allowed to consume non-alcoholic and caffeine-free fluids. In the present study, all participants underwent two PET scans. In the last minute of a 3-min rest period (pre-film-watching period), the first blood sample (for assays of endocrine and immune parameters) was obtained, and the participants were asked to evaluate their present mood state on a visual analogue scale (VAS). They then watched either an emotionally neutral film (control film), or a film featuring people they found attractive (positive film). The films were screened for 4 min on a 15-inch. display placed at a distance of approximately 60 cm (film-watching period). In the 2–3 min of the film-watching period, a PET scan (duration: 60 s) was performed. In the last minute of the film-watching period, a second blood sample was obtained. After watching the film, the subjects evaluated their mood state and the watched film on the VAS, and a rest period of 3 min (post-film-watching period) was set. The next pre-film-watching, film-watching, and post-film-watching periods began 5 min after the previous post-film-watching period. The order of the two types of films was counterbalanced across participants.

2.3. Stimuli

We compiled 4-min audiovisual clips. The positive film featured a person whom each participant subjectively considered attractive. By free response, the participants themselves selected this person before the day of the experiment. All the selected persons were famous actresses. By the day of the experiment, we compiled an individual 4-min video film from TV programs and movies for each participant. In order to demonstrate the maximum effect, we did not standardize the actions performed by the actresses in the movies, but the films did not contain erotic and sexually suggestive scenes. For example, one film contained scenes of the favorite person smiling. In addition, we compiled audiovisual clips because we thought that the favorite person's voice was important for participants. The control film was a TV news program with a newscaster whom participants considered not so attractive. Since the newscaster being reported concerned weather in the past, rather than any new information, the participants remained uninterested in the film. In order to delete the activations in the non-emotional brain regions, such as the visual and auditory cortices, the PET images obtained during the control condition

were subtracted from those obtained during the positive condition. The participants watched the edited control and positive films for the first time at the time of the experiment. To evaluate the emotional valence of the films, the participants were asked to evaluate their enjoyment of the film by rating it on the VAS (no pleasantness (0%)–extremely high pleasantness (100%)).

2.4. Whole-blood NK cell activity assay

To determine whether attraction for a favorite person influenced the activity of peripheral circulating NK cells, blood samples were collected in EDTA tubes, and peripheral blood mononuclear cells (PBMCs) were isolated using a Ficoll gradient (Ficoll-Paque PLUS; GE Healthcare Life Sciences, Little Chalfont, England). The NK activity of PBMCs against K562 target cells was determined using a europium-release cytotoxicity assay. Target cells (5×10^6) were washed twice in Hepes buffer (50 mM Hepes, 93 mM NaCl, 5 mM KCl, and 2 mM $MgCl_2$; pH 7.4), incubated for 15 min at 4 °C in 1 ml europium solution [Hepes buffer, 0.1 M sodium dextran sulfate (GE Healthcare), 0.1 mM diethylenetriaminepentaacetic acid (Wako, Osaka, Japan), and 20 mM europium (Aldrich Chemical Co., Inc., WI)], and washed three times in Hepes buffer with 2 mM $CaCl_2$ and 10 mM glucose. The cells were then washed twice in RPMI 1640 medium supplemented with 10% heat-inactivated FBS (RPMI-FBS). Target cells (1×10^4 in 100 μl RPMI-FBS/well) were seeded in duplicate in 96-well tissue culture plates before the addition of 100 μl RPMI-FBS/well (to determine spontaneous lysis), 1% Triton (to determine maximal lysis), or 5×10^5 PBMCs (effector-to-target ratio (E/T) = 50/1). The plates were incubated for 4 h at 37 °C. The cells were centrifuged for 5 min at 500*g*, and 20 μl of the culture supernatant was collected and added to 100 μl/well of the enhancement solution (Perkin-Elmer, MA). After incubation for 5 min at room temperature, fluorescence was determined using a fluorometer (Wallac 1420 multilabel counter; Perkin-Elmer). Specific cytotoxicity was calculated as the % cytotoxicity = (experimental lysis − spontaneous lysis) × 100/(maximal lysis − spontaneous lysis).

2.5. Endocrinological assessment

To determine whether attraction for a favorite person influenced the serum catecholamine levels, blood samples for endocrinological assessment were collected in serum-separator tubes and centrifuged for 10 min at 3000*g*; serum was removed and then stored at −80 °C until analysis. Since blood catecholamine levels, which can influence NK cell activity (McKenna et al., 2002; Bosch et al., 2005), can change in a short time (Bosch et al., 2005), we measured the serum concentrations of dopamine, norepinephrine, and epinephrine using an HPLC-electrochemical detector (ECD) (CoulArray; ESA Biosciences Inc., Chelmsford, MA).

2.6. Image acquisition by PET

During each film-watching period, the distribution of the regional cerebral blood flow (rCBF) was measured using an Advance NXi PET scanner (GE Healthcare) operated in the high-sensitivity three-dimensional mode, as described previously (Ohira et al., 2006). For tracer administration, a venous catheter was inserted in an antecubital fossa vein of the left forearm. After the subject's head was positioned in an inflatable plastic head holder that prevented any head movement, a 10-min transmission scan was conducted using a rotating ^{68}Ge pin source. In each block, after a 370-MBq bolus injection of $H_2{}^{15}O$ was administered over 30 s, scanning was started and continued for 60 s. Initiation of the bolus injection was time-locked to 1 min after the start of the presentation of the first stimulus, and the presentation of stimuli lasted until 1 min after the termination of scanning. The integrated radioactivity accumulated during the 60 s of scanning was used as the index of rCBF. A 15-min interval between successive scans was used to allow the radioactive levels to return to the baseline. A Hanning filter was used to reconstruct images into 35 planes of 4.5-mm thickness and 2 × 2 mm resolution (full width at half maximum).

2.7. Image processing and analysis

The SPM99 software (Friston et al., 1995) implemented in Matlab (version 6.1; Mathworks, Sherborn, MA) was used for spatial preprocessing and statistical analyses as described previously (Ohira et al., 2006). The images were initially realigned using sinc interpolation to remove artifacts before they were transformed into a standard stereotactic space. They were corrected for the whole-brain global blood flow by proportional scaling and smoothed using a Gaussian kernel to a final in-plane resolution of 8 mm at full width at half maximum. To clarify the significant regional changes during the positive condition, the difference between the two conditions (control and positive) was analyzed by subtracting the images obtained during the control condition from those obtained during the positive condition. The effects at each voxel were estimated using a general linear model. Voxel values for each contrast yielded a statistical parametric map of the *t*-statistic (SPM *t*) that was subsequently transformed to a unit normal distribution (SPM *z*). The peak voxel value significance thresholds were set at $p < 0.005$ (uncorrected) and cluster significance thresholds, at 20 voxels.

Further, in order to examine the association between brain activity reflected by the rCBF and the peripheral endocrine and immune activities accompanying positive emotions elicited by attraction for a favorite person, statistical parametric maps were created during the positive condition to identify the brain regions that were activated in synchrony with each of the physiological indices—NK cell activity and peripheral dopamine level. For these maps, the covariates option was selected, generating separate regression analyses that tested the linear relationship between rCBF associated with either NK cell activity or peripheral dopamine level across subjects, yielding a *z* score at each voxel. We entered the values of NK cell activity during watching the positive film and peripheral dopamine level during watching it as covariates. For covariate analyses, the statistical threshold was set at $p < 0.005$ (uncorrected) for height, and clusters larger than 20 contiguous voxels were reported. For ease of discussion, we refer to the findings in terms of significant "correlations", although the analysis formally involved linear regression rather than assessment of correlation.

2.8. Statistical analyses of self-reported and physiological data

Results were expressed as the means ± SEM. The pleasantness of the film was compared using paired *t* test. The mood states, NK cell activities, and serum levels of catecholamines before and after/during film watching were compared using two-way repeated measures ANOVA [condition (control versus positive) × period (baseline versus task)] followed by paired *t* tests. Furthermore, Pearson correlation coefficients were computed between the values of the psychological and peripheral physiological indices to examine the relationships among positive emotions, endocrine, and immune activities.

3. Results

3.1. Emotional valence of films and psychological response

In order to assess whether the participants experienced positive emotions, they were asked to evaluate how much they enjoyed the film by rating it on the VAS (0–100%). The rating scores for pleasantness of the film were 25.20 ± 3.02% (control film) and 73.40 ± 4.70% (positive film). The rating score was over 70% for the positive film; thus, the participants enjoyed the positive film. Moreover, positive emotions were seldom evoked while viewing the control film, as indicated by the relatively low rating score. Statistical analyses indicated significant differences in the rating score ($t(11) = -11.42$, $p < 0.01$). This result suggests that the emotional valence of the film featuring the partic-

ipants' favorite persons was positive, while the TV news program was not so positive. Subsequently, in order to assess the changes in mood states accompanying positive emotions, the participants were asked to rate how positive/negative their mood was before and after watching the films on the VAS (not positive/negative (0%)–extremely positive/negative (100%)). ANOVA tests revealed a significant interaction between the condition (control × positive) and period (before × after) in the positive mood score ($F(1,22) = 71.59$, $p < 0.01$; Fig. 1a) and negative mood score ($F(1,22) = 8.06$, $p < 0.01$; Fig. 1b). Further statistical analyses indicated that the mood states of participants became more positive ($t(11) = -8.03$, $p < 0.01$; Fig. 1a) and less negative ($t(11) = 4.43$, $p < 0.01$; Fig. 1b) after watching the positive film. In fact, the positive mood score increased and negative mood score decreased for every participant in the positive condition; thus, the statistic results appear to be appropriate. In addition, the negative mood score did not change ($t(11) = -0.86$, $p = 0.41$), whereas the positive mood score decreased ($t(11) = 2.72$, $p < 0.05$) in the control condition. These results indicated that the participants experienced positive emotions when they watched the positive film, and their mood states became more positive and less negative after watching the film. Those also indicated that the control condition was not positive, but was not negative, either. Probably, it seems that it was emotionally neutral.

3.2. Physiological responses

We subsequently measured various physiological parameters such as the NK cell activity and serum concentrations of dopamine, norepinephrine, and epinephrine before and during film watching. Interestingly, NK cell activity was elevated in the positive condition (two-way ANOVA: $F(1,22) = 4.32$, $p < 0.05$; paired t test: $t(11) = -2.28$, $p < 0.05$; Fig. 2a), but it did not change in the control condition ($t(11) = 1.02$, $p = 0.33$). In fact, 9 of 12 participants exhibited such an increase in NK cell activity in the positive condition, and there is no significant difference in baseline NK cell activity between control and positive conditions ($t(11) = 1.16$, $p = 0.27$); thus, these statistic results appear to be appropriate. Furthermore, as shown in Fig. 2b, the peripheral dopamine level also increased significantly in the positive condition (two-way ANOVA: $F(1,18) = 12.23$, $p < 0.05$; paired t test: $t(9) = -3.54$, $p < 0.01$), whereas it did not change significantly in the control condition ($t(9) = 1.76$, $p = 0.11$). In fact, 9 of 10 participants exhibited such an increase in dopamine level in the positive condition, and there is no significant difference in baseline dopamine level between control and positive conditions ($t(9) = 1.05$, $p = 0.32$); thus, these statistic results appear to be appropriate. The ANOVA tests showed no significant differences in the concentrations of norepinephrine ($F(1,18) = 3.23$, $p = 0.09$; Fig. 2c) and epinephrine ($F(1,18) = 0.56$, $p = 0.46$; Fig. 2d).

3.3. PET data: subtraction analysis

We further investigated brain activity by conducting a PET scan while the participants were watching the films. Subtraction analyses revealed significant increases in the rCBF in the positive condition ($p < 0.005$, uncorrected; Table 1). As compared to the control condition, significant activation in the medial prefrontal cortex (MPFC) was observed during the positive condition (Brodmann's area (BA) 9/10; Fig. 3a); thalamus (Fig. 3a); hypothalamus (Fig. 3b); subcallosal gyrus (BA 25; Fig. 3b); posterior cingulate cortex (PCC) (BA 31; Fig. 3b); superior temporal gyrus (BA 38; Fig. 3c); and cerebellum (Fig. 3c).

3.4. PET data: covariate analysis

We then conducted SPM covariate analyses between the physiological indices and the rCBF in the positive condi-

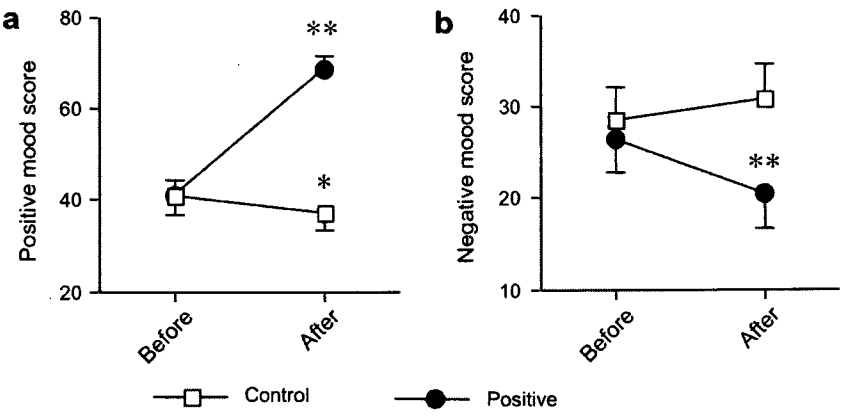

Fig. 1. Psychological response. Each *point* and *vertical line* represents the mean ± SEM VAS score ($n = 12$). (a) Change in positive mood state after watching the films. $^{**}p < 0.01$ and $^{*}p < 0.05$ versus before watching by paired t test. (b) Change in negative mood state after watching the films. $^{**}p < 0.01$ versus before watching by paired t test.

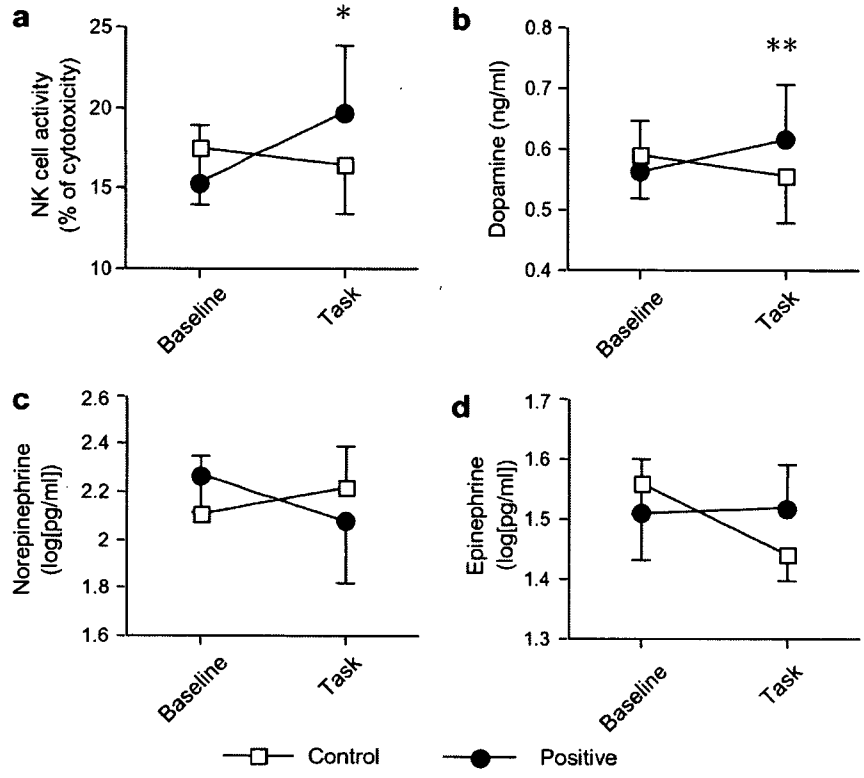

Fig. 2. Physiological responses. Each *point* and *vertical line* represents the mean ± SEM % cytotoxicity (a: $n = 12$) or the mean ± SEM concentration (b, c, and d: $n = 10$). (a) Change in NK cell activity after watching the control and positive films. $^{*}p < 0.05$ versus baseline value in paired t test. (b) Change in the serum dopamine concentration after watching the control and positive films. $^{**}p < 0.01$ versus baseline value by paired t test. (c) Change in the serum norepinephrine concentration after watching the control and positive films. (d) Change in the serum epinephrine concentration after watching the control and positive films.

Table 1
Talairach coordinates of brain regions showing significant increase in the rCBF in the positive condition relative to the control condition

Region	Talairach coordinates (mm)			z score
	x	y	z	
MPFC	−6	48	8	3.80
MPFC	−4	58	12	3.41
MPFC	−26	30	34	3.10
MPFC	4	54	20	3.04
Thalamus	−6	−16	12	3.35
Thalamus	−20	−22	12	2.89
Hypothalamus	−4	−2	−10	3.03
Hypothalamus	4	0	−14	2.93
PCC	6	−36	32	3.33
Subcallosal gyrus	4	20	−12	3.12
Superior temporal gyrus	−32	4	−46	3.28
Superior temporal gyrus	−24	14	−32	3.28
Superior temporal gyrus	28	18	−32	3.13
Superior temporal gyrus	42	18	−30	3.00
Cerebellum	50	−50	−24	3.18

tion. Those analyses delineated components of the neural network associated with NK cell activity ($p < 0.005$, uncorrected; Table 2) and peripheral dopamine level ($p < 0.005$, uncorrected; Table 3). It was revealed that the NK cell activity during watching the positive film was correlated with the rCBF in the MPFC (BA 10; Fig. 4a); PCC (BA 23; Fig. 4a); orbitofrontal cortex (OFC) (BA 11/47; Fig. 4b); cerebellum (Fig. 4b); and superior temporal gyrus (BA 38; Fig. 4c). Furthermore, it was revealed that the peripheral dopamine level during watching the positive film was correlated with the rCBF in the MPFC (BA 9/10; Fig. 5a); PCC (BA 23; Fig. 5b); and superior temporal gyrus (BA 38; Fig. 5c).

3.5. Correlations among positive emotions and peripheral physiological activities

Finally, in order to examine the associations among positive emotions and peripheral physiological activities, the correlations among the positive mood score after watching the positive film, the dopamine level during watching it, and NK cell activity during watching it were computed for the entire sample (Table 4). The analyses indicated that the peripheral dopamine level was positively correlated with NK cell activity ($r(10) = 0.63$, $p < 0.05$; Fig. 6a). In addition, we analyzed the correlation between change from baseline in the dopamine level and positive mood score after watching the positive film. This analysis indicated that change in the dopamine level was positively correlated with positive mood score ($r(10) = 0.73$, $p < 0.05$; Fig. 6b).

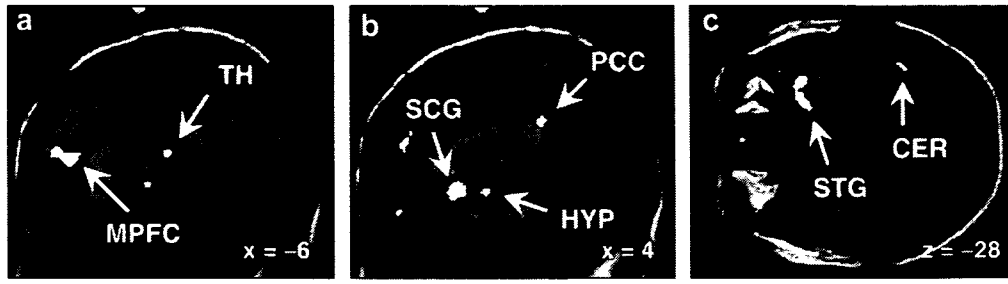

Fig. 3. Statistical parametric maps (SPM99) showing significant increases in the rCBF in the positive condition minus those in the control condition. (a) Activations of the MPFC and thalamus (TH). (b) Activations of the hypothalamus (HYP), subcallosal gyrus (SCG), and PCC. (c) Activations of the superior temporal gyrus (STG) and cerebellum (CER).

Table 2
Talairach coordinates of brain regions showing significant correlation with NK cell activity during watching the positive film

Region	Talairach coordinates (mm)			z score
	x	y	z	
MPFC	−12	68	0	3.81
MPFC	10	64	−8	3.41
OFC	18	20	−28	3.58
OFC	16	12	−26	3.35
PCC	8	−24	26	3.62
Superior temporal gyrus	46	24	−30	3.22
Cerebellum	18	−58	−16	3.73
Cerebellum	34	−58	−36	3.42
Cerebellum	10	−62	−26	3.33
Cerebellum	38	−54	−18	3.28

Table 3
Talairach coordinates of brain regions showing significant correlation with peripheral dopamine level during watching the positive film

Region	Talairach coordinates (mm)			z score
	x	y	z	
MPFC	−18	44	30	3.27
MPFC	14	44	8	3.22
MPFC	8	52	8	2.19
PCC	10	−16	26	3.88
Superior temporal gyrus	32	20	−26	4.35
Superior temporal gyrus	−30	18	−42	3.23
Superior temporal gyrus	−12	40	34	3.03

4. Discussion

The present study aimed to reveal the association between the central nervous, endocrine, and immune systems when positive emotions were elicited as participants watched their favorite persons. When the participants watched a film featuring an actress whom they considered attractive, they subjectively reported having experienced positive emotions. Interestingly, the activity of peripheral circulating NK cells as well as the peripheral circulating dopamine level significantly increased only under the positive condition. The following brain regions were significantly activated in the positive condition relative to the control condition: MPFC (BA 9/10), thalamus, hypothalamus, subcallosal gyrus (BA 25), PCC (BA 31), superior temporal gyrus (BA 38), and cerebellum. Further, SPM covariate analyses indicated that these brain regions were temporally associated with peripheral circulating NK cell activity and dopamine level. It was also indicated that the dopamine level was positively correlated with NK cell activity. These results suggest that while an individual experiences positive emotions, the central nervous, endocrine, and immune systems may be interrelated through neurochemical networks.

Lesion and functional imaging studies have predominantly related emotional processing to the medial prefrontal cortical regions, such as the MPFC and the medial OFC (Damasio, 1999; Davidson and Irwin, 1999; Phan et al., 2002; Burgdorf and Panksepp, 2006). Considering this information, the medial prefrontal cortical regions are

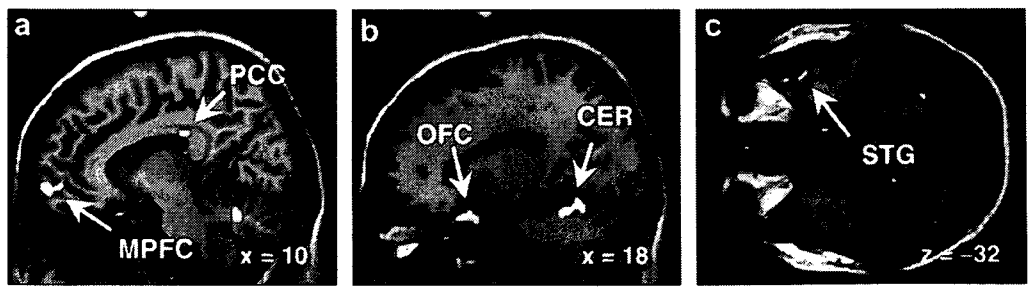

Fig. 4. SPM99 covariate analysis of rCBF during the positive condition with NK cell activity as the covariate of interest. (a) Correlations with the MPFC and PCC. (b) Correlations with the OFC and cerebellum (CER). (c) Correlation with the superior temporal gyrus (STG).

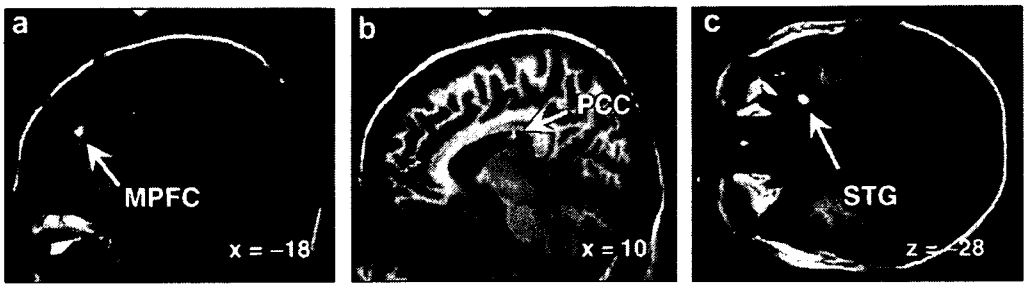

Fig. 5. SPM99 covariate analysis of rCBF during the positive condition with peripheral dopamine level as the covariate of interest. (a) Correlation with the MPFC. (b) Correlation with the PCC. (c) Correlation with the superior temporal gyrus (STG).

Table 4
Correlations among NK cell activity during watching the positive film (NKCA), peripheral dopamine level during watching it (DA), and positive mood score after watching it (PMS)

	NKCA	DA	PMS
NKCA	—	0.63 ($p < 0.05$)	0.14 ($p = 0.44$)
DA		—	0.31 ($p = 0.86$)

Pearson correlation coefficients were computed among the values of psychological and physiological indices.

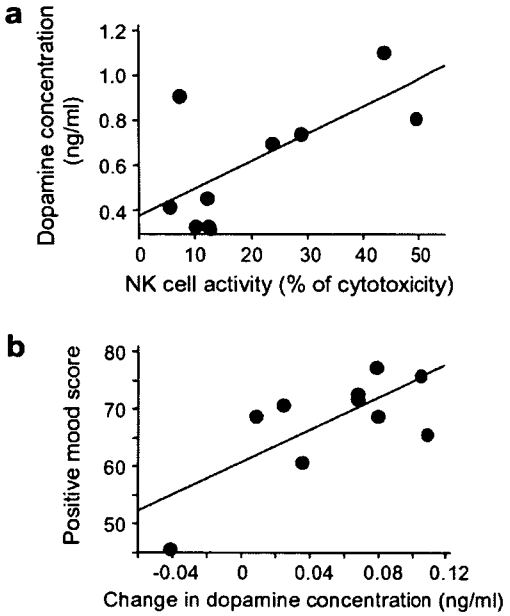

Fig. 6. (a) Scatterplots of NK cell activity and dopamine concentration during watching the positive film. (b) Scatterplots of change from baseline in the dopamine concentration and positive mood score after watching the positive film.

likely to process the positive subjective emotion—attraction for a favorite person. Further, in the positive condition, the thalamus, hypothalamus, subcallosal gyrus, PCC, superior temporal gyrus, and cerebellum were significantly activated relative to the control condition. The PCC receives direct afferents from the hippocampus and thalamus (Vogt et al., 1979); thus, it has been regarded, on connectional grounds, as a part of the limbic system and is therefore considered to serving emotional and motivational functions. A report suggests that PCC activation by emotional stimuli may reflect an interaction between emotions and memory, such as the enhancement of memory for emotional information (Maddock et al., 2003). The superior temporal gyrus has also been implicated in autobiographical memory in normal subjects (Fink et al., 1996). Furthermore, human brain imaging investigations of anxiety, fear, dysphoria, depression, and pain, and studies involving exposure to pleasant or unpleasant images, music, touch, or taste have implicated the subcallosal anterior cingulate region (George et al., 1995; Royet et al., 2000). Thus, the rCBF increases found in the subcallosal gyrus in response to emotional stimuli provides a convergent image. The association between the cerebellum and emotional experience has been also indicated recently (Turner et al., 2007). The anatomical connections of the cerebellum with limbic and brainstem regions which regulate the mood regulation and can influence autonomic nervous activity has been also revealing (Anand et al., 1959; Dum and Strick, 2003; Turner et al., 2007). Study using the patients of cerebellum stroke revealed that cerebellar lesions were associated with reduced pleasant experience in response to happiness-evoking stimuli (Turner et al., 2007). Based on these previous observations, it is suggested that the neural network of the thalamus, hypothalamus, subcallosal gyrus, PCC, superior temporal gyrus, and cerebellum, together with the prefrontal areas, is engaged in the ecphory of affect-laden autobiographical information, and it may enhance the fortunate memory for a favorite person.

Dopamine is known to play an important role in the expression of positive emotions (Aron et al., 2005; Bartels and Zeki, 2004; Verhoeff et al., 2003). The brain dopaminergic network, which projects to the prefrontal cortex from the midbrain region via hypothalamus, is known as the "brain reward system" (Aron et al., 2005; Bartels and Zeki, 2004). It has been demonstrated in the animal studies that stimulation of the brain reward system increases peripheral circulating NK cell activity (Wenner et al., 2000; Wrona and Trojniar, 2003; Wrona et al., 2004). Furthermore, a previous study indicated a positive correlation between the increases in the peripheral dopamine level and NK cell activity accompanying subjective positive emotions in humans (Mizuno et al., 2003). The peripheral

actions of dopamine may be functionally related to the observed global activation of the brain dopamine system (Gilbert, 1995). In addition, peripheral dopamine can modulate NK cell activity because NK cells have many dopamine receptors on their surface (McKenna et al., 2002), and dopamine receptor antagonists inhibit NK cell-mediated cytotoxicity in normal mice (Fiserova et al., 2002). The present study demonstrated that while the subjects watched the positive film, the dopamine related brain reward regions such as the MPFC and hypothalamus were activated, the serum dopamine concentration was increased, and it was positively correlated with NK cell activity. Therefore, it is suggested that the central nervous network activated by perceiving a favorite person may increase central and peripheral circulating dopamine level, and consequently elevate NK cell activity. Associations among central nervous, endocrine, and immune activities in positive psychological situations may be by means of the dopaminergic network.

Unfortunately, the correlation between absolute values of circulating dopamine level and positive mood score was not observed. As described above, it is thought that the dopamine activity is necessary for subjective experience of positive emotion; thus, we further analyzed the correlation between change from baseline in the dopamine level and positive mood score after watching the positive film, and positive correlation was observed. In such a short time, in order to experience positive emotions, the perception of change in the dopamine level may be important. In addition, the positive mood score was not correlated with both circulating NK cell activity and change from baseline in NK cell activity (data not shown). NK cell activity is increased not only in the positive condition but also in the stress situation (Isowa et al., 2004); thus increased NK cell activity may not be necessary for positive emotion perception.

It has been observed that experimental exposure to acute psychological stressors, such as public speaking and short-term mental arithmetic, increases the heart rate, blood pressure, and blood levels of norepinephrine and epinephrine by facilitating the activation of the peripheral autonomic nervous system and HPA axis (Isowa et al., 2004, 2006; Kimura et al., 2005; Bosch et al., 2005; Abo and Kawamura, 2002). In such stress situation, the number of circulating NK cells was also increased in a short time (in about 2 min) (Isowa et al., 2004, 2006; Kimura et al., 2005). This is not enough time for new cells to be created, and thus they must be mobilized from lymph nodes, bone marrow, or somewhere into blood stream (Abo and Kawamura, 2002). The present study also indicated the rapid change of NK cell activity; thus it is possible that the increased NK cell activity may be due to increased number of circulating NK cells. Furthermore, the brain limbic system, usually in concert with the prefrontal area, influences autonomic brainstem nuclei controlling the activity of sympathetic and parasympathetic systems, via hypothalamic centers (Beauchaine, 2001). The peripheral autonomic nervous activity tends to peak within minutes after stimulus onset, and regulates NK cell activity (Isowa et al., 2004, 2006; Kimura et al., 2005; Bosch et al., 2005; Abo and Kawamura, 2002). NK cells are known to attack malignant tumor cells by using perforin, and the secretion of perforin is suppressed under conditions of sympathetic nerve activation (Abo and Kawamura, 2002). In general, it is considered that parasympathetic nervous activity increases and sympathetic nervous activity decreases under the positive situation (Sokhadze, 2007); therefore, it is possible that the parasympathetic nervous system influenced NK cell activity in the present study although it was not exactly measured.

Certain limitations of this study must be recognized. First, although previous studies have reported the brain-body association in a small sample size (Ottowitz et al., 2004; Ohira et al., 2006) and short-time changes in endocrine and immune parameters (Ohira et al., 2006; Isowa et al., 2004, 2006; Kimura et al., 2005), the relatively small sample size ($n = 12$ samples) and short experiment time (film-viewing period = 4 min) were insufficient to determine the effects of such visual stimulation on daily health. Thus, the generalizability of the present findings must be further tested using a larger sample size and longer experiment time. Second, it is possible that the effects observed in the present study were simply a byproduct of arousal. Third, interaction effect may exist with the order in which the two types of films were presented; however, we can not examine the effect because of small sample size. In a future study, we will attempt to investigate the interaction effect with order. Nevertheless, the present study demonstrates that seeing a favorite person can influence one's mental and physical state and can activate the innate immune system.

By simultaneously assessing the brain, endocrine, and immune activities, we revealed the association between these systems when positive emotions are elicited as individuals look at their favorite person. These results may expand the scope of clinical literature that addresses the links between positive emotions and immunity.

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Imaging brain and immune association accompanying cognitive appraisal of an acute stressor

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Acute stress elicits multiple responses in autonomic, endocrine, and immune systems. Cognitive appraisal is believed to be one important modulator of such stress responses. To investigate brain substrates of crosstalks between the homeostasis-maintaining systems accompanying appraisal of stressor controllability, we simultaneously recorded regional cerebral blood flow (rCBF) using ^{15}O-water positron emission tomography, cardiovascular indices (heart rate (HR) and blood pressure (BP)), neuroendocrine indices (concentrations of epinephrine, norepinephrine, and adrenocorticotropic hormone (ACTH) in blood), and immune indices (proportions of subsets of lymphocytes (NK cells, helper T cells, cytotoxic T cells, and B cells) in blood), in 11 male subjects who performed a mental arithmetic task with either high controllability (HC) and low controllability (LC). The LC task resulted in less sense of control in subjects than the HC task. Significant increases of rCBF in the medial and lateral orbitofrontal cortices (OFC), and in the medial and lateral prefrontal cortices (MPFC, LPFC) were observed by subtracting the HC task from the LC task. More importantly, significant positive correlations between rCBF and HR, BP, and NK cells were commonly found in the OFC and MPFC during the LC tasks, but not during the HC tasks. The present results showed for the first time that the prefrontal neural network including the OFC and MPFC might be one pivotal region for bi-directional functional association between the brain and peripheral autonomic and immune activities accompanying appraisal of an acute stressor.

Keywords: Stress; Appraisal; Controllability; Immunity; PET

Introduction

Studies in psychoneuroimmunology revealed that the central nervous, and autonomic nervous, endocrine, and immune systems were interrelated, and influenced each other's functions through complex biochemical pathways (Ader et al., 2001). One characteristic of the rapid changes in peripheral immune functions caused by psychosocial factors such as acute stress is redistribution of lymphocytes in blood. Specifically, circulating numbers of lymphocytes representing innate immunity such as natural killer (NK) cells increase, and numbers of lymphocytes representing acquired immunity such as T cells and B cells do not change or even decrease during acute phases of psychological stress (Dhabhar et al., 1995; Bosch et al., 2003; Isowa et al., 2004, 2006; Kimura et al., 2005; Landmann et al., 1984; Meehan et al., 1993; Minton and Blecha, 1990; Schedlowski et al., 1993, 1996; Stefanski, 2000). It is recognized that such trafficking of lymphocytes between various body areas is critical for efficient immune responses, and for survival (Engler et al., 2004). Indeed, increasing numbers of peripheral innate immune cells which can non-specifically react with any antigens might be interpreted as a preparation step for potential invasion by bacteria from injuries accompanying fight/flight behaviors, whereas decreasing numbers of acquired immune cells might represent trafficking of such cells into lymph nodes where helper T cells are sensitized to antigens, and cascades of antigen-specific immune responses can start. Numerous studies showed that redistribution of blood lymphocytes during acute stress situations was mediated by activation of both the rapidly working sympathetic nervous system (SNS) and the relatively slowly working hypothalamic-pituitary-adrenocortical (HPA) axis (Mills et al., 1995; Pike et al., 1997; Stevenson et al., 2001; Bauer et al., 2001, 2002; Bosch et al., 2005).

Rapid changes in numbers of circulating lymphocytes should be adaptive and beneficial for survival. However, a stable pattern of immune responses to acute stress would be less effective.

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Rather, continuous assessment of environmental demands and dynamic modulation of responses to deal with those demands are critical for adaptation. Psychological models of stress adaptation (Lazarus and Folkman, 1984; Blascovich et al., 1999) have focused on cognitive appraisal of such processes. In particular, in response to a stressful event, whether the event is impactful is firstly assessed (primary appraisal). Then, controllability of the event and the individual's coping resource to the event, or whether the event is a threat or challenge to the individual is evaluated (secondary appraisal). As a result of such a series of appraisal processes to the stressor, subjective feelings and behaviors can be affected. Furthermore, autonomic, endocrine, and immune systems can react differently to a particular stressor (Peters et al., 1999, 2003; Gaab et al., 2003; Isowa et al., 2006; Maier and Watkins, 2005).

All those phenomena suggest that activities of several nuclei in the hypothalamus or the midbrain determining peripheral physiological systems to maintain homeostasis are dynamically modulated by higher brain cortices to cope with demands from environments. However, to date, details of the neural basis of such modulation of immune functions accompanying appraisal of psychological acute stressors in humans remain to be explored. Though previous neuroimaging studies (Wik et al., 1998; Lekander et al., 2000; Tashiro et al., 2001) reported association of brain activity with some immune parameters, all these studies did not examine dynamic associations between brain and immune functions, and effects of cognitive processes such as appraisal.

Therefore, the present study examined the neural basis of corticolimbic modulations according to peripheral redistribution of lymphocytes accompanying appraisal of controllability of an acute stressor. For this purpose, we simultaneously measured regional cerebral blood flow (rCBF) using ^{15}O-water positron emission tomography (PET) and physiological parameters of cardiovascular, neuroendocrine, and immune activities for a typical laboratory acute stressor, a continuous mental arithmetic task with time pressure. Degree of controllability of the task was manipulated by feedback about subjects' performance in each trial: feedback indicating a correct answer or feedback indicating an error exactly corresponding to the subject's performance represented a high controllability (HC) condition whereas bogus feedback was irrelevantly given to the subject's performance with some probability in a low controllability (LC) condition. In the LC condition, subjects would experience a gap between subjective perception about their performance and feedback about performance, resulting in experiences of lower controllability for the task. We hypothesized that regions in the prefrontal cortex (PFC), especially the orbitofrontal cortex (OFC), medial prefrontal cortex (MPFC), and anterior cingulate cortex (ACC) were involved in cognitive appraisal of controllability of the acute stressor, and in modulation of peripheral physiological responses. The following previous results have provided rationales for our hypothesis. (1) Human neuroimaging studies (O'Doherty et al., 2001, 2003; O'Doherty, 2004) and animal studies (Roberts, 2006) clarified that the OFC played critical roles in evaluation of contingencies between actions and outcomes, and the MPFC and ACC were involved in monitoring one's own actions and action regulation (Bush et al., 2000; Ridderinkhof et al., 2004). (2) The MPFC and ACC have direct neural projections to limbic and midbrain areas which regulate autonomic and endocrine activities (Kringelbach and Rolls, 2004; Kringelbach, 2005). Human neuroimaging studies indicated that activation in subdivisions of the MPFC and ACC correlated with autonomic activities as seen in cardiovascular activity and skin conductance during cognitive and stress tasks (Critchley et al., 2000a, 2000b, 2003, 2005; Gianaros et al., 2004, 2005; Matthews et al., 2004). (3) Animal studies revealed that secretion of dopamine and serotonin in the OFC and MPFC areas was a key factor for behavioral changes in uncontrollable stress situations (Bland et al., 2003; Amat et al., 2005).

Materials and methods

Subjects

Twelve male volunteers (right-handed Japanese undergraduate and graduate students; age range, 20–24 years; mean age, 21.15 years, SD=1.28) participated in the present study. One subject was excluded from analyses because PET imaging revealed that he had arteriovenous malformation in the brain. The remaining subjects were healthy, had no past history of psychiatric or neurological illness, and were not taking any medications. Only men were studied to avoid contamination in endocrine and immune variations caused by the menstrual cycle in women. All subjects gave written informed consent in accordance with the Declaration of Helsinki. This study was approved by the Ethics Committee of Kizawa Memorial Hospital.

Design of experiment

All subjects performed a mental arithmetic task for 2 min in HC conditions followed by LC conditions. The "learned helplessness theory" argues that animals and human who are exposed to unavoidable or uncontrollable aversive situations exhibit less activity and show a poorer performance in subsequent tasks (Peterson et al., 1993). Thus, order of conditions was not counterbalanced to avoid any carry-over influences from experiences of uncontrollability to the subsequent arithmetic task in HC conditions. Instead, to control effects from any orders of HC and LC conditions, subjects were randomized into 2 groups: (1) the "early low controllability" group (N=6) performed a HC task in 3 early blocks, and performed a LC task in 5 late blocks; and (2) the "late low controllability" group (N=6) performed a HC task in 5 early blocks, and performed a LC task in 3 later blocks. By comparing data from self-reports, behavioral performance, endocrine and immune indices, and PET images during the fourth and fifth blocks, we could examine whether changes in such indices were associated with experimental conditions or resulted from effects of the order of tasks. A subject in the "late low controllability" group was excluded from analyses of PET images for the reason described above.

Task and manipulation of controllability

For the mental arithmetic task, subjects were told to add the currently displayed number (from 2 to 9) to the next one shown on a PC monitor, and to orally report the sum of the numbers as a single digit (from 0 to 9). Incases that the sum equaled a number with 2 digits, subjects were asked to only report the last digit of the number. Each number was displayed for 500 ms, and followed by a 1500-ms interval before another one was displayed. In each trial, feedback from correctness/error of each subject's answer by displaying symbols such as a circle or a cross was evaluated. To maintain motivation, subjects were instructed that an error rate more than 10% per block would result in failure of the experiment,

and collected data would be invalid, thus subjects would have to mark correct answers more than 90% in each block. To manipulate controllability, feedback was correctly given to subjects in the HC blocks. On the other hand, during the LC blocks, bogus feedback was given irrespective of subjects' real performance. Standard of bogus feedback was independently set for each subject in the following manner: mean rate of correct responses in blocks of the HC condition was determined for each subject. In the following LC blocks, feedback indicating a correct answer was randomly given at a rate below 15% of the mean rate of correct responses by the subject in the HC blocks. For example, if a subject performed the task with a rate of 90% accuracy in the HC blocks, feedback indicating a correct answer was delivered at a rate of 75%, randomly in the LC blocks.

Procedures

Subjects were instructed to eat a light breakfast on the morning of the experiment, and caffeine-containing beverages were not allowed. Subjects suffering from an infectious illness within 2 weeks of the experiment were rescheduled. All subjects had 8 PET scans of 60 s each, and at 13-min intervals.

Subjects performed 8 blocks of the mental arithmetic task using HC and LC conditions as described above. Just before and immediately at the end of each block, subjects were asked to rate the strength of experienced stress on a 10-point scale (1: not at all; 10: extremely stressful). In addition, to evaluate subjective sense of control over the task, subjects were asked to rate how much (as a percentage) of their correct answers could be attributed to their own ability and effort but not to incidental factors (0–100%) at the end of each block. Just before the start of each block, and just after the end of each block, blood samples were taken from each subject using a heparinized cannula inserted in the right forearm vein to measure endocrine and immune indices. Cardiovascular indices were continuously recorded throughout experimental sessions. At the end of each experimental session, subjects were fully debriefed about purpose and manipulation of the experiment, and were thanked. All subjects were paid 15,000 yens for participation.

Measurement of physiological data

Cardiovascular indices

Cardiodynamic activity was recorded using electrocardiography (ECG) and non-invasive finger blood pressure (FINAP) measurements. To determine heart rate (HR), ECG was recorded using a MP 100 system (BIOPAC Systems, Inc.). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded using the finger cuff of a Portapres Model 2 (TNO Biomedical Instrumentation Inc.) attached to the third finger of the non-dominant arm of each subject. Analyses of ECGs and FINAP waveforms were performed using the software AcqKnowledge for the MP 100 system. Mean values of HR, SBP, and DBP were determined for 2 min just before the task as baseline, and during 2 min of the task in each block.

Neuroendocrine indices

Blood was collected in ethylenediaminetetraacetic acid (EDTA)-containing tubes to measure epinephrine, norepinephrine, and ACTH. Tubes were kept on ice, centrifuged for 10 min, and plasma was removed and frozen at −80 °C until analysis. Concentrations of plasma epinephrine and norepinephrine were determined by high performance liquid chromatography. The intra-assay coefficient of variation was less than 5%, and inter-assay variations were less than 6% for measurements of epinephrine and norepinephrine. ACTH was assayed in triplicates using an immunoradiometric assay (Mitsubishi Chemical, Inc.). The intra-assay coefficient of variation was less than 6%, and inter-assay variations were less than 7% for measurements of ACTH.

Immune indices

Blood samples for immunological determinations were collected in heparinized tubes. The number of total lymphocytes per sample was determined using a standard means. Percentages of lymphocyte subsets were determined by flow cytometry (FACS Calibur, Becton-Dickinson). A whole-blood lysis method was used to stain cells with the following pairs of Fluorescein isothiocyanate/Phycoerythrin-conjugated monoclonal antibodies (DAKO, Inc.). Isotype-matched antibodies were mouse IgG1, CD3+/CD4+helper T cells, CD3+/CD8+cytotoxic T cells, CD3−/CD19+B cells, and CD3−/CD16+/CD56+NK cells.

Neuroimaging by PET

Image acquisition

During each block, distribution of regional cerebral blood flow (rCBF) was measured with a General Electric ADVANCE NXi PET scanner operated in a high-sensitivity three-dimensional mode. A venous catheter for administering the tracer was inserted in an antecubital fossa vein in the left forearm. After the subject's head was positioned in the inflatable plastic head-holder that prevented possible head movements, a 10-min transmission scan using a rotating ^{68}germanium pin source was completed. In each block, following a 370-MBq bolus injection of $H_2^{15}O$ over 30 s, scanning was started and continued for 60 s. Initiation of bolus injection was time-locked to the start of presentation of the stimulus, and presentation of the stimulus lasted until 30 s after termination of scanning. The integrated radioactivity accumulated during 60 s of scanning was used as the index of rCBF. Eight scans were acquired per subject, and interval between successive scans was 15 min in order to allow for radioactive levels to return to baseline level. A Hanning filter was used to reconstruct images into 35 planes with 4.5-mm thickness and a resolution of 2 mm × 2 mm (full width half maximum).

Image processing and analysis

SPM 99 (Friston et al., 1995) implemented in Matlab (version 5.3, Mathworks, Sherborn, Massachusetts) was used for spatial pre-processing and statistical analyses. Images were initially realigned using sinc-interpolation to remove artifacts before being transformed into a standard stereotactic space. Images were corrected for whole brain global blood flow by proportional scaling, and smoothed using a Gaussian kernel to a final in-plane resolution of 10 mm at full width at half maximum.

Differences within conditions (HC vs. LC) were the primary focus of this study, and subtraction analyses of images (HC (1–3 blocks) minus LC (6–8 blocks), and LC minus HC) were conducted to reveal significant increases of rCBF. Effects at each voxel were estimated using a general linear model. Voxel values for each contrast yielded a statistical parametric map of the *t* statistic (SPM *t*) and were subsequently transformed to a unit normal distribution (SPM *Z*). Peak voxel-value significance

thresholds were set at $p<0.001$ (uncorrected), and cluster significance thresholds were set at 20 voxels.

Furthermore, to examine functional associations between brain activity reflected by increase of rCBF and peripheral cardiovascular, endocrine, and immune activities, a correlation map was composed for each condition to identify brain regions that were activated in synchrony with each physiological index. Changes in values of HR, SBP, and DBP were computed by subtracting means of values at baseline from means of values during the mental arithmetic task. Changes in values of epinephrine, norepinephrine, ACTH, and percentages of lymphocyte subsets were calculated by subtracting values before each block from values after each block. We conducted correlation analyses using such change values of physiological indices. Additionally, to examine psychological effects on brain activity accompanying appraisal processes, we conducted similar correlation analyses between rCBF and self-report data of sense of control and change values of subjective stress from before each block to after each block. These correlation analyses were conducted between the rCBF increase and each physiological and psychological index in the whole brain in the HC (1–3 blocks) and LC (6–8 blocks) conditions across subjects, separately. For the correlation analyses, statistical threshold was set at $p<0.001$ (uncorrected) for height, and clusters larger than 20 contiguous voxels were reported. Those relatively conserved thresholds were determined considering inflation of type I error rates in many comparisons, correlation analyses in the present study, and expansion of cluster sizes of activated voxels by image smoothing. The above thresholds would keep the probability of a false positive to a minimum according to analytical conditions used in the present study (Forman et al., 1995).

Results

Self-report and behavioral data

For each subject, means of sense of control and subjective stress were calculated using rating scores in HC (1–3) blocks, middle (4 and 5) blocks, and LC (6–8) blocks, in the "early low controllability" and the "late low controllability" groups (Table 1). Both groups commonly conducted the HC task in HC blocks, and conducted the LC task in LC blocks. The "early low controllability" group conducted the LC task whereas the "late low controllability" group conducted the HC task in the middle blocks. A two-way mixed (Group (early low controllability vs. late low controllability)×Block (HC, middle, LC)) analysis of variance (ANOVA) revealed a significant interaction between Group and Block for sense of control ($F(2, 18)=6.28$, $p<0.01$). Additional analyses using LSD tests ($p<0.05$) indicated that subjects in both groups had a lower sense of control in the LC blocks than in the HC blocks, and subjects in the "early low controllability group" reported lower sense of control than subjects in the "late low controllability" group in the middle blocks. No significant effects ($F<1.46$) were observed for change values of subjective stress. These results suggested that we could successfully manipulate controllability of an acute stressor, independently from subjective severity of stress.

Table 1
Means and standard deviations for self-report and behavioral parameters

	Block	Early low controllability	Late low controllability
Sense of control	HC	84.39 (7.82)	79.00 (9.01)
	Middle	76.67 (13.20)	86.00 (11.26)
	LC	80.83 (7.05)	75.33 (10.23)
Subjective stress	HC	2.78 (9.41)	3.87 (3.54)
	Middle	7.33 (7.29)	0.50 (6.22)
	LC	7.61 (6.40)	5.67 (8.29)
Accuracy of task (%)	HC	93.29 (2.72)	91.45 (3.32)
	Middle	88.68 (5.81)	93.59 (4.19)
	LC	82.22 (12.04)	86.79 (9.27)

HC, high controllability; LC, low controllability.

Mean rates of correct responses in the mental arithmetic task during the HC, middle, and LC blocks were calculated (Table 1). Although bogus feedback was provided in the LC condition, net performance of the task for each subject was evaluated. A main effect of Block was significant ($F(2, 18)=3.91$, $p<0.05$), indicating that subjects' performance was poorer in LC blocks than in HC blocks. Additionally, LSD tests ($p<0.05$) revealed that task accuracy was reduced in the "early low controllability" group whereas accuracy was maintained in the "late low controllability" group in the middle blocks. These results suggested that experimental manipulation of uncontrollability of the task interfered with performance of subjects, and these effects could not be attributed solely to effects of the order of tasks.

Physiological data

For each subject, means of physiological (cardiovascular, neuroendocrine, and immune) data at each observation point were calculated for HC, middle, and LC blocks. A three-way mixed ANOVA (Group (early low controllability vs. late low controllability)×Block (HC, middle, LC)×Period (baseline vs. task)) was conducted for each parameter. Means, standard deviations, and all significant effects in ANOVAs are summarized in Table 2.

For cardiovascular indices, significant interactions between Block and Period were observed in HR, SBP, and DBP ($F(2, 18)=16.97$, $p<0.001$; $F(2, 18)=7.08$, $p<0.01$; $F(2, 18)=6.51$, $p<0.01$, respectively). Additional analyses using LSD tests ($p<0.05$) revealed that such cardiovascular activities were significantly enhanced during the task compared to baseline activity in each condition of blocks (HC, middle, LC). However, degree of enhancement was more prominent in HC blocks than in LC blocks. A significant interaction between Group, Block, and Period was found for HR ($F(2, 18)=3.93$, $p<0.05$), suggesting that degree of increase in HR was larger in the "late low controllability" group than in the "early low controllability" group in the middle blocks. Thus, at least for HR, observed effects of controllability of tasks (HC vs. LC) on cardiovascular activity did not result from order of tasks and from habituation to the stressor. For neuroendocrine indices, concentrations of epinephrine, norepinephrine, and ACTH significantly or statistically marginally increased after the task compared to baseline ($F(1, 9)=10.08$, $p<0.05$; $F(1, 9)=3.58$, $p<0.10$; $F(1, 9)=4.77$, $p<0.10$, respectively). Furthermore, a significant interaction between Block and Period was observed for epinephrine ($F(2, 18)=5.69$, $p<0.05$), suggesting that increment in epinephrine level was more salient following the HC blocks than following the LC blocks. Although an interaction between Group, Block, and Period for epinephrine level was statistically marginal ($F(2, 18)=2.64$, $p<0.10$), LSD tests ($p<0.05$) showed that an increase in epinephrine concentration after the task from baseline in the middle blocks was significant in the "late low controll-

Table 2
Means and standard deviations of cardiovascular, neuroendocrine, and immune parameters

		Block	Early low controllability		Late low controllability	
			Baseline	Task	Baseline	Task
Cardiovascular	HR (bpm)	HC	68.70 (8.02)	89.96 (18.49)	74.67 (11.18)	102.13 (14.85)
		Middle	65.46 (5.64)	83.13 (11.47)	76.56 (10.12)	93.50 (14.57)
		LC	68.88 (8.57)	84.41 (12.66)	76.23 (8.07)	91.02 (11.22)
	SBP (mmHg)	HC	120.48 (19.70)	136.10 (21.61)	138.53 (28.20)	153.21 (27.53)
		Middle	117.84 (18.82)	130.10 (24.41)	136.56 (25.70)	149.71 (24.47)
		LC	124.44 (22.57)	134.99 (22.82)	141.16 (19.92)	148.91 (22.64)
	DBP (mmHg)	HC	63.15 (12.32)	73.99 (18.49)	69.07 (12.32)	77.70 (9.98)
		Middle	60.05 (17.61)	69.96 (17.41)	67.31 (11.75)	76.08 (9.46)
		LC	64.03 (20.42)	71.71 (19.39)	70.72 (7.93)	75.30 (7.45)
Neuroendocrine	ACTH (pg/ml)	HC	26.94 (8.43)	30.78 (14.42)	25.13 (8.28)	26.80 (8.87)
		Middle	22.42 (6.03)	23.67 (8.41)	20.70 (4.83)	20.10 (4.57)
		LC	23.94 (7.52)	26.44 (7.11)	19.27 (4.23)	21.27 (5.07)
	E (pg/ml)	HC	38.33 (17.35)	47.78 (32.23)	34.67 (4.47)	66.67 (31.00)
		Middle	36.67 (10.80)	41.67 (21.60)	36.00 (5.48)	52.00 (20.49)
		LC	35.56 (12.05)	40.00 (16.87)	41.33 (11.93)	58.67 (25.88)
	NE (pg/ml)	HC	187.22 (26.11)	206.11 (44.84)	260.67 (75.48)	256.67 (71.38)
		Middle	174.17 (41.40)	196.67 (32.20)	235.00 (73.06)	248.00 (76.04)
		LC	169.44 (33.96)	197.78 (38.80)	250.00 (80.52)	250.67 (59.32)
Immune	NK cells (%)	HC	18.63 (10.33)	21.23 (9.09)	16.04 (3.55)	20.40 (5.77)
		Middle	14.30 (6.18)	17.45 (8.57)	13.18 (1.98)	14.33 (2.97)
		LC	16.68 (4.28)	18.23 (7.82)	12.76 (3.59)	15.34 (4.00)
	Helper T cells (%)	HC	34.27 (6.47)	33.31 (5.48)	44.71 (5.37)	42.09 (4.46)
		Middle	37.77 (5.37)	36.46 (5.20)	47.46 (5.69)	47.31 (4.87)
		LC	37.70 (4.45)	36.38 (5.48)	48.31 (7.08)	46.94 (8.04)
	Cytotoxic T cells (%)	HC	31.56 (8.67)	30.70 (8.84)	23.63 (9.56)	23.02 (9.90)
		Middle	32.90 (8.37)	31.58 (8.10)	25.71 (8.04)	24.14 (9.43)
		LC	31.63 (8.30)	30.82 (8.42)	25.18 (9.08)	24.92 (9.13)
	B cells (%)	HC	14.47 (5.96)	15.00 (5.12)	14.47 (5.96)	15.00 (5.12)
		Middle	16.60 (4.13)	16.18 (4.13)	16.60 (4.13)	16.18 (4.13)
		LC	14.39 (2.58)	14.00 (2.82)	16.33 (3.65)	1572 (3.49)

HC, high controllability; LC, low controllability; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACTH, adrenocorticotropic hormone; E, epinephrine; NE, norepinephrine; NK, natural killer cells.

ability" group but not in the "early low controllability" group. Thus, effects of controllability of the stressor on changes in epinephrine level seemed beyond the simple order effects of tasks or habituation. For immune indices, main effects of Period were significant in NK cells, helper T cells, and cytotoxic T cells ($F(1, 9)=27.94$, $p<0.001$; $F(1, 9)=8.46$, $p<0.05$; $F(1, 9)=6.52$, $p<0.05$, respectively), indicating that the acute stressor increased the proportion of NK cells, but decreased proportions of helper T cells and cytotoxic T cells. Furthermore, main effects of Block were also significant in NK cells and helper T cells ($F(2, 18)=8.93$, $p<0.01$; $F(2, 18)=19.94$, $p<0.001$, respectively), indicating that increase in numbers of NK cells and decrease in numbers of helper T cells were more salient in the HC blocks than in the LC blocks.

Cardiovascular–neuroendocrine–immune pathways

Theoretically, variations of immune functions in acute stress situations are mediated by autonomic and neuroendocrine activities. To elucidate such pathways, we conducted regression analyses using a change scoring system (task—baseline) of each immune parameter as a dependent variable, and change score systems of cardiovascular (HR, SBP, and DBP) and neuroendocrine (epinephrine, norepinephrine, and ACTH) parameters as dependent variables in HC and LC blocks, separately. Results showed that epinephrine significantly predicted increase in NK cells, and decreases in helper T cells and cytotoxic T cells in the LC blocks; whereas no parameters accounted for variations of immune functions in the HC blocks (Table 3). Thus, autonomic activity (secretion of epinephrine) might directly regulate redistribution of lymphocytes when the subjects are

Table 3
Regression analyses for immune parameters

	HC				LC			
	NK	hT	cT	B	NK	hT	cT	B
HR	0.17	−0.19	0.65	0.55	0.11	−0.01	0.45	0.23
SBP	−0.46	0.21	−0.53	0.52	−0.20	−0.05	0.09	−0.70
DBP	0.33	−0.17	0.62	−0.73	0.05	0.19	−0.51	0.14
E	0.40	**−0.87**	−0.56	−0.31	**0.90**	**−0.77**	**−0.84**	−0.45
NE	−0.02	0.10	−0.04	0.24	0.25	−0.37	0.11	0.16

HC, high controllability; LC, low controllability; HR, heart rate; SBP, systolic blood pressure; DBP, diastolic blood pressure; ACTH, adrenocorticotropic hormone; E, epinephrine; NE, norepinephrine; NK, natural killer cells; hT, helper T cells; cT, cytotoxic T cells; B, B cells.
Values represent beta coefficients, and bold values represent statistically significant results ($p<0.05$).

faced with reappraisal of stressor controllability, but autonomic, neuroendocrine, and immune systems might be activated relatively independently when the stress situation is evaluated as controllable.

PET data: subtraction analyses

Results of subtraction analyses are summarized in Table 4. Subtraction of HC minus LC blocks revealed a significant increase in rCBF in the middle temporal (BA 22, $x=56$, $y=-42$, $z=-14$) and fusiform (BA 20, $x=-54$, $y=-36$, $z=-22$) gyri in the temporal lobe, the middle occipital gyrus (BA 19, $x=-50$, $y=-58$, $z=-10$) and the cuneus (BA 18, $x=-2$, $y=-82$, $z=16$) in the occipital lobe, and the bilateral cerebellum ($x=-26$, $y=-64$, $z=-46$; $x=32$, $y=-54$, $z=-44$) (Fig. 1a). No increase in rCBF was observed in the PFC. The reversed pattern of subtraction (LC–HC) revealed a significant increase in rCBF in a wider range of loci including the medial and right lateral OFC (BA 10, coordinates of peak, $x=32$, $y=54$, $z=-2$). Furthermore, increased rCBF was observed in the dorsal ACC (BA 32, $x=-6$, $y=24$, $z=42$) expanding to the adjacent MPFC, the right lateral PFC (LPFC; $x=20$, $y=44$, $z=26$; BA 8, $x=46$, $y=24$, $z=42$), and the thalamus ($x=14$, $y=2$, $z=12$) (Fig. 1b). Additional activation was found in several loci in the PFC and temporal areas (Table 4).

In addition to self-report, behavioral, and physiological data, means of rCBF at each peak activation location observed for comparisons of HC minus LC and LC minus HC were determined for HC, middle, and LC blocks. A two-way mixed ANOVA (Group (early low controllability vs. late low controllability) × Block (HC, middle, LC)) was conducted for rCBF at each location. No rCBF in activated sites observed from subtraction of HC minus LC showed a significant interaction between Group and Block, suggesting that activations in temporal and occipital cortices, and the cerebellum observed in the HC blocks were independent of the manipulation of uncontrollability, and changes in rCBF in those sites were probably attributed to habituation (Fig. 2a). On the other hand, rCBF in the OFC, loci in the LPFC, and ACC observed by comparing LC minus HC values showed a significant or marginal interaction between Group and Block ($F(2, 18)=4.26, 5.54, 2.55, 4.27$; $p<0.05, 0.05, 0.10, 0.05$, respectively; Fig. 2b). These results clearly indicated that activation in PFC areas observed in LC minus HC blocks were caused by manipulation of uncontrollability.

PET data: correlation analyses

High controllability

Since we were interested in functional association between brain regions including the PFC, limbic, and midbrain areas, and peripheral physiological responses, we limited ourselves to report significant correlations in such regions. We revealed that changes in HR positively correlated with rCBF in the midbrain ($x=-2$, $y=-34$, $z=-18$, $Z=4.68$) and changes in SBP correlated with rCBF in the pons ($x=6$, $y=-14$, $z=-24$, $Z=3.75$) (Fig. 4a) in the HC blocks. For immune parameters, increase in numbers of NK cells positively correlated with rCBF in the right hippocampus ($x=30$, $y=-10$, $z=-14$, $Z=3.49$) (Fig. 5a) and the pons ($x=8$, $y=-40$, $z=-34$, $Z=3.13$). Analyses using other physiological and psychological (sense of control and subjective stress) parameters showed no significant correlations with rCBF.

Low controllability

Overall, larger ranges showed significant correlations with cardiovascular, neuroendocrine, and immune parameters in the LC blocks than in the HC blocks. Changes in HR showed a positive correlation with increases in rCBF in several loci in the MPFC (BA 8, $x=-6$, $y=24$, $z=42$, $Z=5.53$; BA 10, $x=-2$, $y=64$, $z=12$, $Z=4.68$; BA 9, $x=-16$, $y=46$, $z=30$, $Z=4.36$), an anterior lateral part of the right OFC (BA 11; $x=26$, $y=58$, $z=-22$, $Z=4.37$) (Fig. 3b), and the thalamus ($x=4$, $y=-6$, $z=4$, $Z=3.95$) in the LC blocks. Changes in SBP significantly correlated with increases in rCBF in an anterior part of the ACC (BA 32, $x=-6$, $y=40$, $z=31$, $Z=4.10$), the

Table 4
Significant increases in rCBF between High controllability and Low controllability blocks

Salience	Region	Side	BA	x	y	z	Z score
a. HC–LC							
	Middle temporal gyrus	R	22	56	−42	−14	4.32
	Middle occipital gyrus	L	19	−50	−58	−10	4.09
	Cerebellum	L		−26	−64	−46	3.88
	Cuneus	L	18	−2	−82	16	3.88
	Fusiform gyrus	L	20	−54	−36	−22	3.58
	Cerebellum	R		32	−54	−44	3.51
b. LC–HC							
	Orbitofrontal cortex	R	10	32	54	−2	5.32
	Anterior cingulate cortex	L	32	−6	38	42	4.78
	Medial prefrontal cortex	R	8	16	32	42	4.61
	Lateral prefrontal cortex	R	8	20	44	26	4.47
	Lateral prefrontal cortex	R	8	46	24	42	4.39
	Superior temporal gyrus	L	40	−56	−60	28	4.37
	Lateral prefrontal cortex	L	10	−32	54	6	4.31
	Anterior cingulate cortex	R	32	14	30	28	4.00
	Thalamus	R		14	2	12	3.97

Coordinates are in MNI space (SPM99). HC, high controllability; LC, low controllability; R, right; L, left; BA, Brodmann's area; x, y, z, three-dimensional coordinates used to determine a voxel referring to medial–lateral (x: positive=right), anterior–posterior (y: positive=anterior), and superior–inferior (z: positive=superior) positions; rCBF, regional cerebral blood flow.

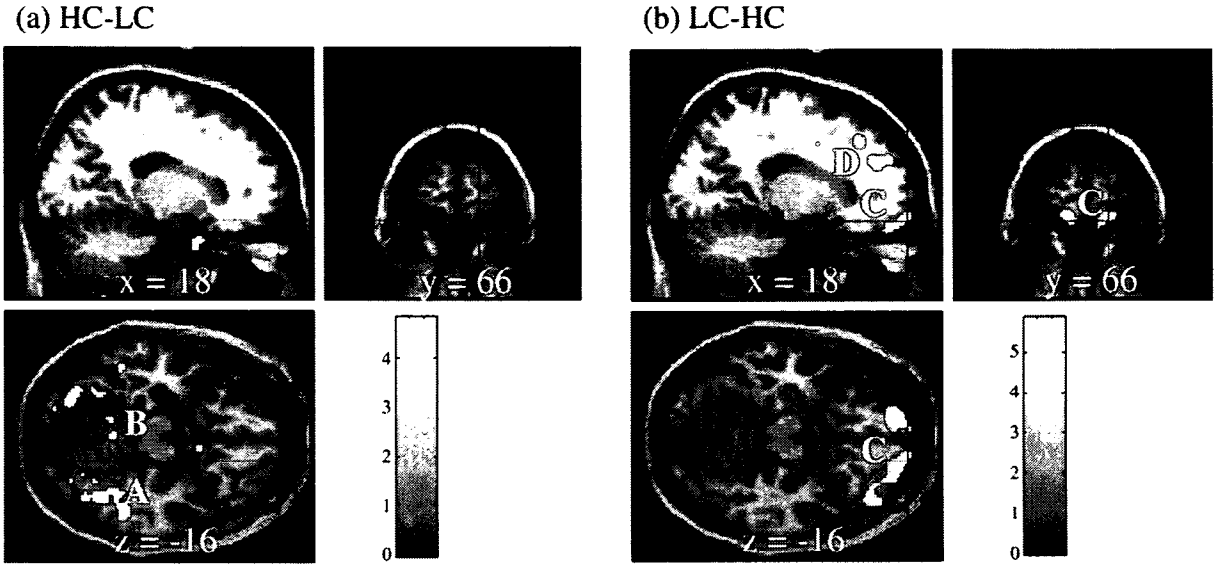

Fig. 1. (a) Statistical parametric map (SPM99) showing significant increases of regional cerebral blood flow (rCBF) in the High controllability blocks minus the Low controllability blocks. (b) Statistical parametric map (SPM99) showing significant increases of rCBF in the Low controllability blocks minus the High controllability blocks. An uncorrected p value of 0.001 was used as threshold for each subtraction analysis. HC, high controllability; LC, low controllability; A, visual areas; B, cerebellum; C, orbitofrontal cortex; D, medial prefrontal cortex.

right lateral OFC (BA 47, $x=40$, $y=40$, $z=-2$, $Z=3.87$) (Fig. 4b), and the pulvinar ($x=22$, $y=-30$, $z=10$, $Z=4.14$). Scatter plotting showed strength of these correlations between brain activity and cardiovascular parameters (Figs. 3 and 4).

For immune parameters, increase in proportion of NK cells correlated with increase in rCBF in the medial OFC (BA 11; $x=-12$, $y=30$, $z=-28$, $Z=4.33$), the bilateral OFC (BA 47, $x=34$, $y=36$, $z=-24$, $Z=3.90$; BA 47; $x=-34$, $y=22$, $z=-18$, $Z=3.09$) (Fig. 5b). Additionally, correlations were observed in several loci in the anterior MPFC (BA 10, $x=24$, $y=62$, $z=10$, $Z=3.96$; BA 10, $x=-8$, $y=50$, $z=10$, $Z=3.84$; BA 10, $x=-26$, $y=56$, $z=14$, $Z=3.63$), the left insula ($x=-38$, $y=-14$, $z=14$, $Z=3.99$), and the left hippocampus ($x=-30$, $y=-32$, $z=-8$, $Z=3.86$). Decrease in proportion of helper T cells correlated with decrease in rCBF in the medial OFC (BA 11, $x=18$, $y=16$, $z=-18$, $Z=3.61$), and the right insula ($x=48$, $y=16$, $z=-10$, $Z=3.49$) (Fig. 6b). Correlations were found also in the MPFC (BA8, $x=16$, $y=52$, $z=40$, $Z=4.39$). Scatter plotting indicated that correlations between brain activity and immune parameters were not necessarily clear and linear (Figs. 5 and 6), though statistically significant. These results suggested that associations between brain and immune activities should not be direct but indirect (see Discussion).

No endocrine parameters showed significant correlations with rCBF at the rigorous threshold ($p<0.001$). However, considering the limited imaging spatial resolution using PET, and the above reported results of regression analyses showing influences of epinephrine on NK cells and helper T cells, we conducted correlation analyses for endocrine parameters using a more liberal threshold ($p<0.005$) to explore possible functional associations between the brain and endocrine activities. As a result, changes in epinephrine concentration positively correlated with rCBF in foci in the OFC (BA 47, coordinates of peak, $x=42$, $y=44$, $z=-6$, $Z=2.91$), foci in the MPFC (BA9, $x=-16$, $y=52$, $z=30$, $Z=4.06$; BA 10, $x=14$, $y=56$, $z=10$, $Z=3.47$), the midbrain ($x=6$, $y=-32$, $z=-18$, $Z=3.70$), and the parahippocampal gyrus ($x=34$, $y=-18$, $z=-18$, $Z=2.97$) in the LC blocks (Fig. 7), but not in the HC blocks. Scatter plotting is not shown because this correlation analysis is just exploratory and preliminary. Psychological (sense of control and subjective stress) parameters showed no significant correlations with rCBF.

Some activation showing correlations between brain and physiological activity expanded to out of brain areas (Figs. 3–7). Relatively long time of experimental session (approximately 2 h) might cause such motion artifacts. However, it was confirmed that each peak of activation was in brain areas, thus the above results about correlations should be interpretable.

Discussion

Functional association between brain and cardiovascular, neuroendocrine, and immune responses accompanying appraisal of stressor controllability

The major finding in this study was that the neural network within the OFC, MPFC, ACC, and LPFC was activated when subjects had to update appraisal of stressor controllability. Furthermore, the present study indicated that PFC regions including the OFC and MPFC were commonly associated with peripheral immune responses, i.e., redistribution of lymphocytes, and with cardiovascular and neuroendocrine activities which probably mediated changes in immune functions, accompanying appraisal of stressor controllability. The PFC does not directly regulate peripheral immune cells but does so indirectly via autonomic and neuroendocrine pathways. Specifically, The OFC and MPFC might firstly affect activities of nuclei in stress-related brain structures such as the thalamus, hypothalamus, and midbrain through direct neural projections, and secondly such centers of autonomic and endocrine systems might affect immune functions through modulation of cardiovascular parameters, and secretion of catecholamines and acetylcholine (Maier and Watkins, 1998; Tracey, 2002). Observed activations of the thalamus, pulvinar, and midbrain correlating with HR, SBP, and epinephrine, respectively, might indicate involvement

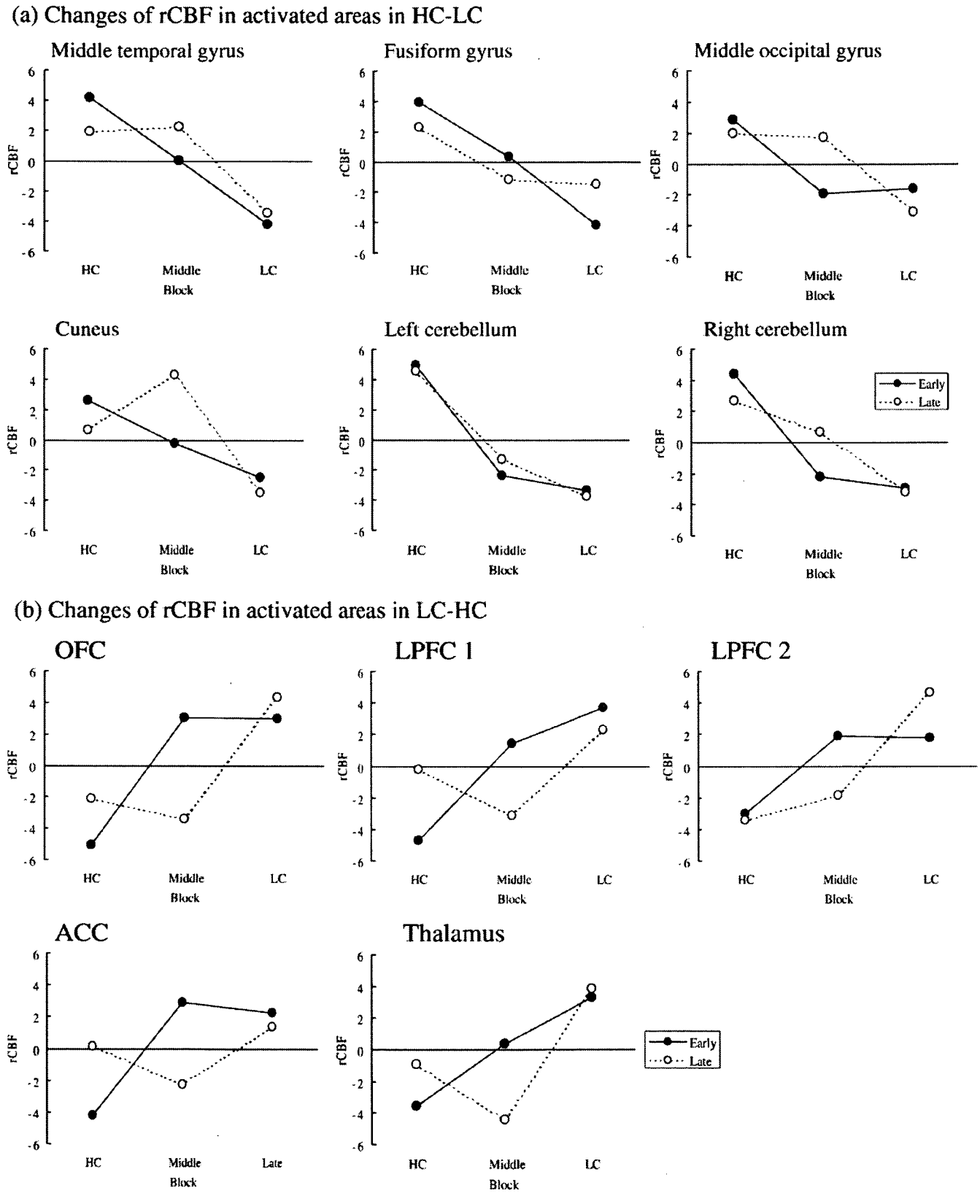

Fig. 2. (a) Changes of regional cerebral blood flow (rCBF) in activated brain areas across HC, middle, and LC blocks in "early low controllability" group and "late low controllability" group in subtraction of HC minus LC. (b) Changes of rCBF in activated brain areas across HC, middle, and LC blocks in "early low controllability" group and "late low controllability" group in subtraction of LC minus HC. HC, high controllability; LC, low controllability.

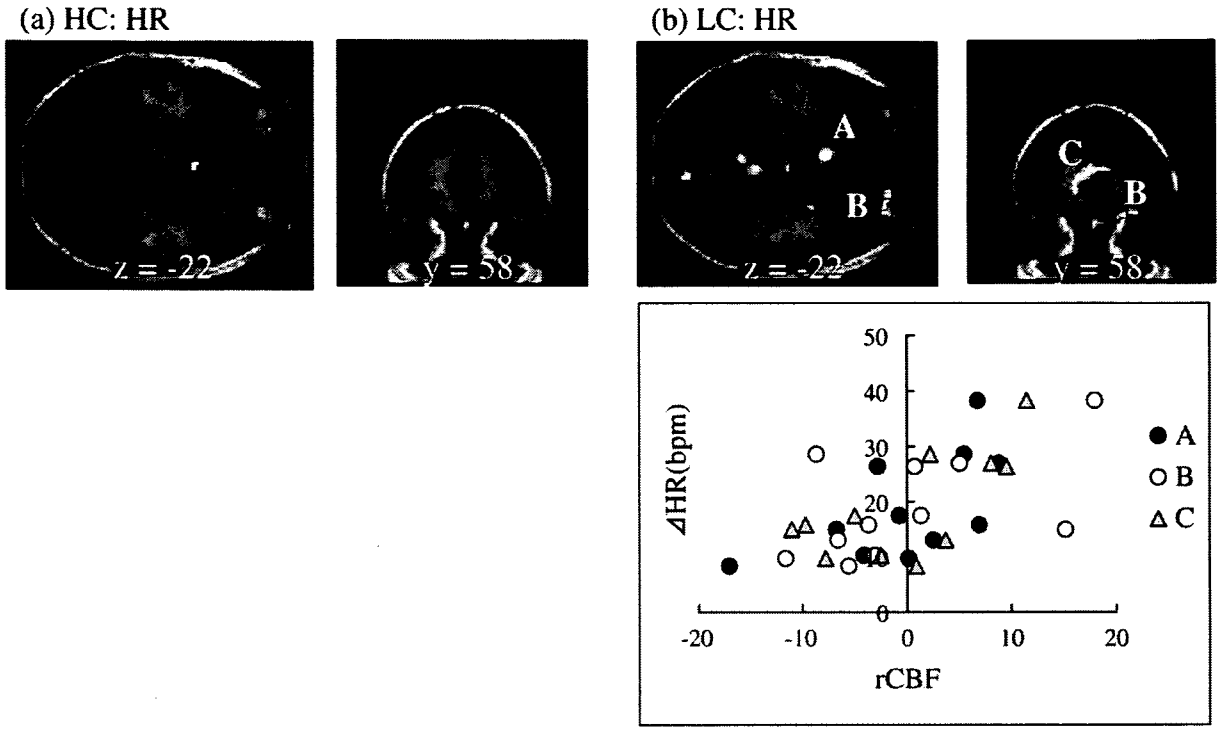

Fig. 3. Results of correlation analyses in High controllability (a) and Low controllability (b) blocks showing significant positive correlations between heart rate and regional cerebral blood flow (rCBF). An uncorrected p value of 0.001 was used as threshold. HC, high controllability; LC, low controllability; A, medial orbitofrontal cortex; B, lateral orbitofrontal cortex, C, medial prefrontal cortex.

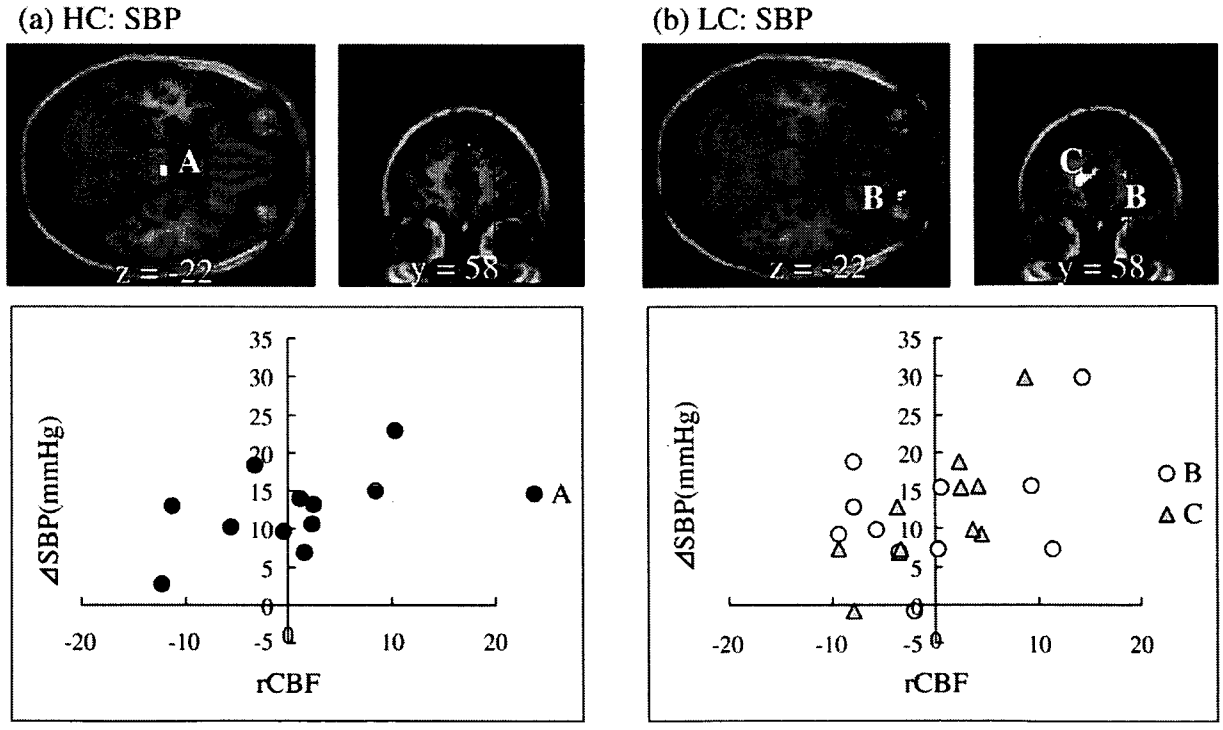

Fig. 4. Results of correlation analyses in High controllability (a) and Low controllability (b) blocks showing significant positive correlations between systolic blood pressure and regional cerebral blood flow (rCBF). An uncorrected p value of 0.001 was used as threshold. HC, high controllability; LC, low controllability; A, Pons; B, medial orbitofrontal cortex; C, medial prefrontal cortex.

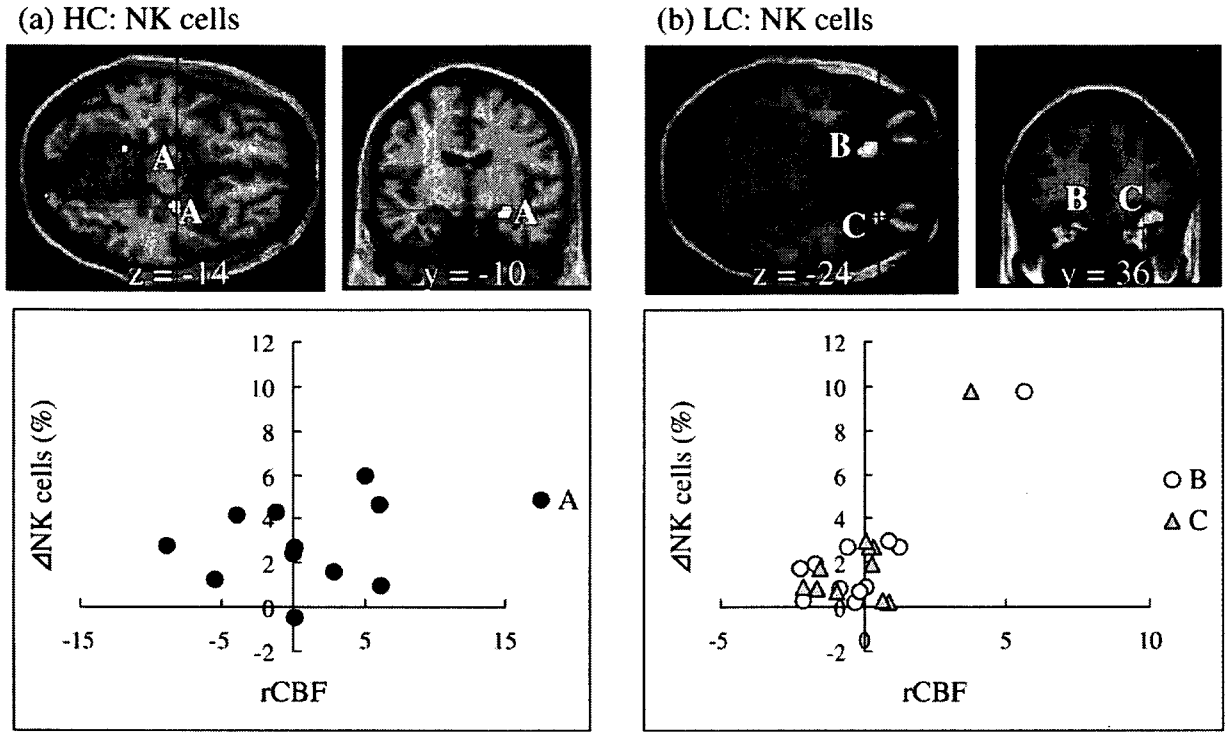

Fig. 5. Results of correlation analyses in High controllability (a) and Low controllability (b) blocks showing significant positive correlations between proportions of natural killer cells and regional cerebral blood flow (rCBF). An uncorrected p value of 0.001 was used as threshold. HC, high controllability; LC, low controllability; A, hippocampus; B, C, lateral orbitofrontal cortex.

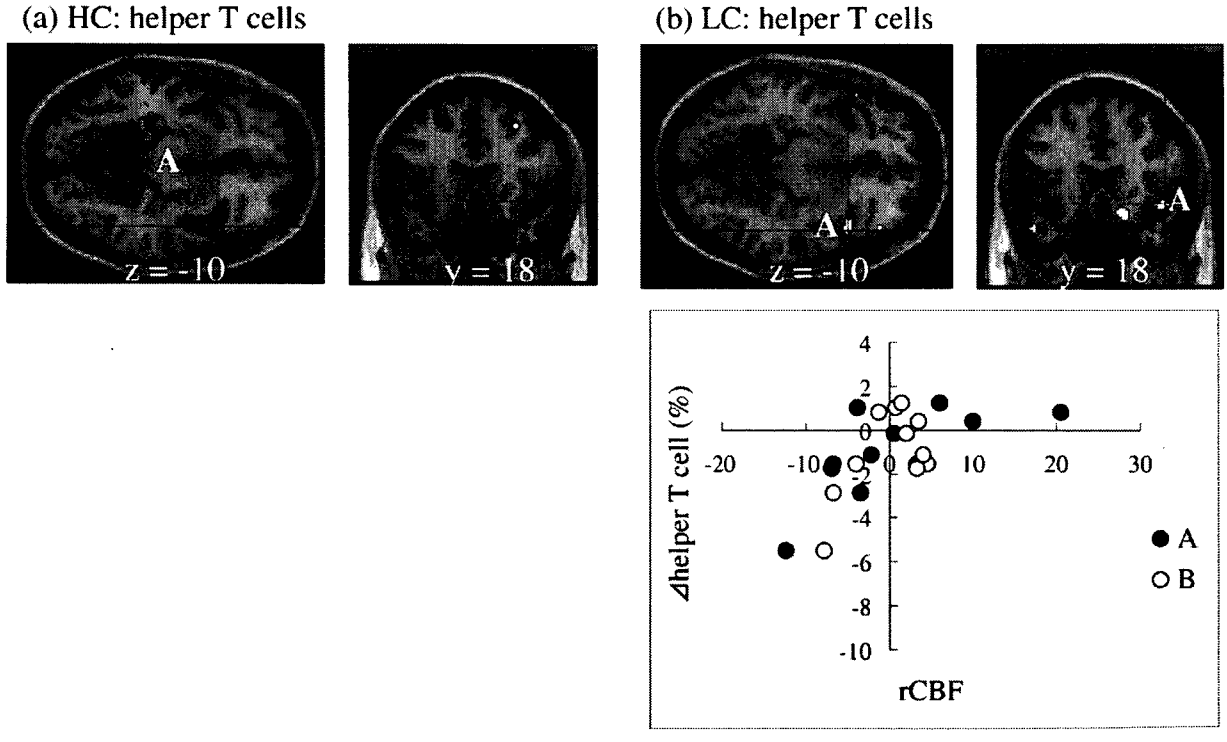

Fig. 6. Results of correlation analyses in High controllability (a) and Low controllability (b) blocks showing significant positive correlations between proportions of helper T cells, and regional cerebral blood flow (rCBF). An uncorrected p value of 0.001 was used as threshold. HC, high controllability; LC, low controllability; A, insula; B, medial orbitofrontal cortex.

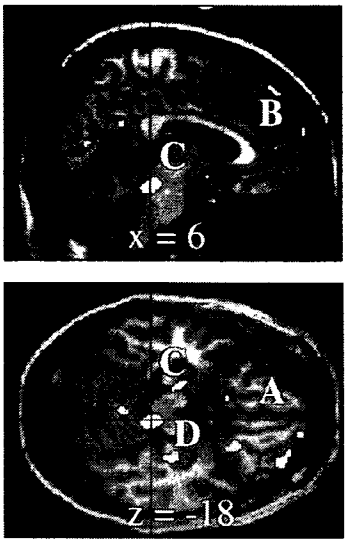

Fig. 7. Results of correlation analyses in Low controllability blocks showing a significant positive correlation between concentration of blood epinephrine and regional cerebral blood flow (rCBF). An uncorrected p value of 0.005 was used as threshold. A, orbitofrontal cortex; B, medial prefrontal cortex; C, midbrain; D, parahippocampal gyrus.

of parts of such neural routes. Significant but non-linear correlations between rCBF and immune parameters shown in scatter plotting (Figs. 5 and 6) suggested such indirect association of brain activity and peripheral immune functions.

Another possibility for the association of PFC activation and immune parameters is the afferent feedback from peripheral immune activity to the brain. Increase in number of NK cells during acute stress tasks leads to enhancement of activation of the inflammatory transcription factor NF-kappaB (Richlin et al., 2004) and should result in enhanced production of inflammatory cytokines such as IL-1β and tumor necrosis factor (Lisowska and Witkowski, 2003; Liang et al., 2004). Those cytokines can result in changes in visceral or interoceptive senses in local sites, and information about these changes can be conveyed to the brain via vagal pathways, and is finally projected to the insula and OFC (Craig, 2003; Critchley, 2005). In addition, information on cytokines themselves can be carried via the sensory vagus to the hypothalamus and the dorsal vagal complex in the midbrain (Hosoi et al., 2000; Tracey, 2002). The observed correlations between activation in the insula and OFC and changes of NK and helper T cells might, at least partly, reflect such an immune function as the "sixth sense" (Blalock, 1984, 1994). Thus, the present results suggested that bi-directional functional association between the brain and peripheral immune functions might become dominant when a stressor is less controllable, and thus, when more efficient coping is needed for the challenging environment.

Although the order of conditions of high and low controllability was not counterbalanced, the design of this study allowed us to confirm that neither effects of the order of conditions nor effects of habituation and adaptation to the stress situation completely explained changes in brain activation and physiological responses. Changes in subjects' ratings on sense of control, behavioral accuracy in the mental arithmetic task, HR, epinephrine, and rCBF in loci in the PFC corresponded to timing of initiation of manipulation of low controllability. Furthermore, because subjects' ratings of intensity of stress were not significantly different between the HC and LC blocks, the results described above can not be attributed to accumulated stress or increased fatigue which the subjects might have felt as the stress task progressed. Thus, these results showed that experimental manipulation of stressor controllability was valid, and we suppose that subjects adapted to the mental arithmetic task in the HC blocks at once, and later had to make reappraisal of controllability of the task after introduction of manipulation of low controllability in the following LC blocks.

Functional associations between discrete regions in the PFC and peripheral physiological responses in stress situations have been reported in earlier human neuroimaging studies. Wang et al. (2005) indicated that rCBF in the right OFC and dorsal ACC measured by perfusion functional magnetic resonance imaging (fMRI) showed positive correlations with perceived stress during a mental arithmetic task. Locations of peaks in those activated areas are very close to those observed in subtraction of LC minus HC in the present study. Since Wang et al. (2005) did not manipulate stressor controllability, subjects' ratings of perceived stress in their study might have reflected primary appraisal of impact of the stressor (Lazarus and Folkman, 1984), but not secondary appraisal of controllability or possibility of coping with the stressor. However, consistent activations in the OFC and ACC in their study and ours suggested that those regions of the PFC were involved in appraisal processes of stressors. Furthermore, in Wang et al.'s study (2005), activations in the right OFC and dorsal ACC positively commonly correlated with changes in HR and cortisol level as a result of the task. Critchley et al. (2000a) also revealed that rCBF measured by PET in the right anterior lateral OFC and dorsal ACC correlated with cardiovascular activities reflected by HR and blood pressure changes during stress tasks including mental arithmetics. Another study by the same group (Critchley et al., 2000b) showed that activations in the right OFC and anterior MPFC measured by fMRI were associated with sympathetic arousal indexed by skin conductance responses (SCR) during a gambling task. Although influences of appraisal of stressor controllability have not been examined in previous studies, locations of PFC sites where activation was observed substantially overlap with brain regions showing positive correlations with autonomic and even immune activities (e.g., HR, SBP, epinephrine, and NK cells) observed in the LC blocks in the present study. Taken together, our present results confirmed previous findings on functional associations between brain, cardiovascular, and neuroendocrine activities in acute stress situations, and further expanded these findings by showing that common brain regions could regulate functions including peripheral immune functions. It should be noted that the OFC and anterior insula are important centers both for efferent modulation over peripheral visceral and somatic responses and for afferent feedback projection from the body (Craig, 2003; Critchley, 2005). Thus, brain activity observed in the present study might reflect both of such processes. Unfortunately, limited temporal resolution of PET imaging prevented from dissociation of brain activity for efferent and afferent processes. As Critchley et al. (2000b) dissociated neural activity related to generation and afferent representation of SCR, event-related fMRI might be helpful to test this issue further.

Anatomical evidence indicates that the OFC, MPFC, and ACC may be the appropriate brain regions for a highly complex processing for adaptation (Kringelbach and Rolls, 2004; Kringelbach, 2005). The OFC, especially its posterior parts receives inputs from all sensory modalities including visceral afferents, and thus is