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Psychiatry Research 153 (2007) 61–67
PSYCHIATRY RESEARCH
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The new GRID Hamilton Rating Scale for Depression demonstrates excellent inter-rater reliability for inexperienced and experienced raters before and after training

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Received 16 January 2006; received in revised form 15 May 2006; accepted 23 July 2006

Abstract

The Hamilton Rating Scale for Depression (HAMD) is the *de facto* international gold standard for the assessment of depression. There are some criticisms, however, especially with regard to its inter-rater reliability, due to the lack of standardized questions or explicit scoring procedures. The GRID-HAMD was developed to provide standardized explicit scoring conventions and a structured interview guide for administration and scoring of the HAMD. We developed the Japanese version of the GRID-HAMD and examined its inter-rater reliability among experienced and inexperienced clinicians ($n=70$), how rater characteristics may affect it, and how training can improve it in the course of a model training program using videotaped interviews. The results showed that the inter-rater reliability of the GRID-HAMD total score was excellent to almost perfect and those of most individual items were also satisfactory to excellent, both with experienced and inexperienced raters, and both before and after the training. With its standardized definitions, questions and detailed scoring conventions, the GRID-HAMD appears to be the best achievable set of interview guides for the HAMD and can provide a solid tool for highly reliable assessment of depression severity.
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Keywords: Depressive disorder; Psychiatric status rating scales; Mental status schedule; Reproducibility of results

1. Introduction

The Japanese Society of Clinical Psychopharmacology has long realized the need to standardize the administration of the Hamilton Rating Scale for Depression

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0165-1781/$ - see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.psychres.2006.07.004

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(HAMD) (Hamilton, 1960), the *de facto* international standard for the assessment of depression (Furukawa et al., 2005), within Japan and appointed a team headed by Dr. Higuchi to develop a model training program in 2000. In the course of these efforts, we learned that a group of researchers had met in the USA in 1999 and proposed to establish a common set of standards for scoring and administering the HAMD that would be acceptable to the Food and Drug Administration and be used by pharmaceutical, academic and clinical researchers. This proposal led to the formation of the Depression Rating Scale Standardization Team (DRSST), a group of individuals representing clinicians, academia, government and the pharmaceutical industry. The goal of this group was to standardize the administration and scoring of the HAMD without significantly altering the original intent of Hamilton's items or the scoring profile rather than to develop a new instrument (Kalali et al., 2002; Bech et al., 2005).

The product of their efforts is the GRID-HAMD, which has three components: the GRID scoring system, (scoring intensity and frequency separately to obtain the severity score), the manual of scoring conventions with detailed anchor descriptions and more behavioral exemplars, and a semi-structured interview guide. The DRSST clarified and operationalized ambiguous anchor descriptions and incorporated the new definitions into the individual items. The GRID-HAMD can be downloaded free of charge at the International Society for CNS Drug Development homepage (http://www.iscdd.org). Given the many versions of the scale in use, the DRSST concluded that standardization would improve the current scale and lay the groundwork for development of a new scale.

The Japanese team felt that the GRID-HAMD would set a new standard in depression rating and decided to develop the Japanese training program around it. We developed the Japanese version of the GRID-HAMD (see Section 2) and then conducted a model training course for the GRID-HAMD in March 2004. The primary purpose of the study is to examine the inter-rater reliability of the Japanese version of the GRID-HAMD among experienced and inexperienced Japanese psychiatrists and psychologists, how rater characteristics may affect it, and how training can enhance it.

2. Methods

2.1. Participants

Psychiatrists ($n=52$), clinical psychologists ($n=12$) and medical students ($n=6$) from three university medical schools in Japan (Nagoya City University, Nagoya University and Fujita Health University) took part in a full day training course for the newly developed Japanese version of the GRID-HAMD. Of the 70 participants, 20 had no previous experience with any version of the HAMD, whereas 17 had administered it between one and five times and 33 had administered it six or more times. However, only 16 of the last group had ever received formal training in the administration of the instrument. The mean (S.D.) of clinical experience was 6.3 (6.1) years for the psychiatrists and 3.5 (3.0) years for the clinical psychologists.

2.2. Instrument

The Japanese version of the GRID-HAMD was developed in collaboration with the DRSST. The original English version of the GRID-HAMD was translated into Japanese by TAF. A team of seven psychiatrists, all of them experts in depression treatment and research, checked the translation and amended it where necessary, based upon the consensus of the team. Two research assistants, both proficient in English and one with a Bachelor's degree in psychology, and both blind to the original English version, then back-translated the Japanese translation of the probe questions into English. AK checked the backtranslation and pointed out possible discrepancies, based upon which TAF retranslated the questioned sentences into Japanese. This process was repeated three times, until AK was able to ascertain semantic equivalence between the original and back-translated versions.

2.3. Procedure

We used three pairs of videotapes of pre- and post-treatment administration of the HAMD. Two pairs used simulated Japanese patients (one man and one woman) and the other pair used a simulated English patient. The Japanese man, woman and their interviewers were played by professional actors and actresses, based on rough scenarios but including a substantial amount of ad lib interactions. The participants' general impression was that the patients were very well played and appeared natural, but that the interviewers appeared rather stiff. The English patient's interviews had Japanese subtitles. Each interview lasted between 15 and 40 min. The experts' consensus total scores for the six videotapes were 26 for the Japanese man pre-treatment, 10 post-treatment, 37 for the Japanese woman pre-treatment, 19 post-treatment, 21 for the English woman pre-treatment and 0 post-treatment. These videotapes were prepared independently of and before our training workshop for the GRID-

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HAMD. The interviewers in these videotapes by and large followed the conventions of the Structured Interview Guide for the Hamilton Depression Rating Scale (SIGH-D) (Williams, 1988), which sometimes did not probe specifically enough into the frequency of the symptoms during the last week.

The participants in the workshop used GRID-HAMD to rate each interview. When the videotape failed to ask for the frequency, the participants were instructed to assume that the frequency was 50% of the time. This was the case for items 2, 3, 7, 10, 11, 12, and 13 of the pre-treatment videotape of the Japanese man, for items 2, 11, and 13 of the post-treatment videotape of the Japanese man, for items 2, 3, 6, 7, 10, 12, 13, and 15 of the pre-treatment video of the Japanese woman, and for items 2, 5, 7, 10, 12, and 13 of the post-treatment video of the Japanese woman. In other words, 24 out of the 68 items (35%) required participants to rely on this rating convention.

Because the rating difficulty might differ between the videotapes of the Japanese man and woman, the participants were randomly divided into two groups, and each group saw either the man's videos or the woman's videos first. There was no discussion immediately following the two videos. The videos therefore served as pre-training and post-training assessments of the raters' reliability. After this pre-training assessment in the reliability of the GRID-HAMD, their training began with a lecture on the history of the Hamilton Rating Scale for Depression and a general discussion of assessment in psychiatry. The training of the GRID-HAMD formed the core of the workshop and used the English woman's videotapes. After scoring each English woman's videotapes, possible discrepancies and questions were discussed among the participants and the trainers. The three pairs of videotapes were therefore presented during this 1-day course as shown in Fig. 1.

2.4. Analyses

The inter-rater reliability for each item of the GRID-HAMD and for its total score was estimated by way of the ANOVA intraclass correlation coefficient (ICC) (one-way random effects model, single rater) of the SPSS (SPSS Inc., 2002). Because of its intrinsic paradoxical characteristic whereby we obtain low ICC despite high agreement (Feinstein and Cicchetti, 1990), we did not calculate ICC

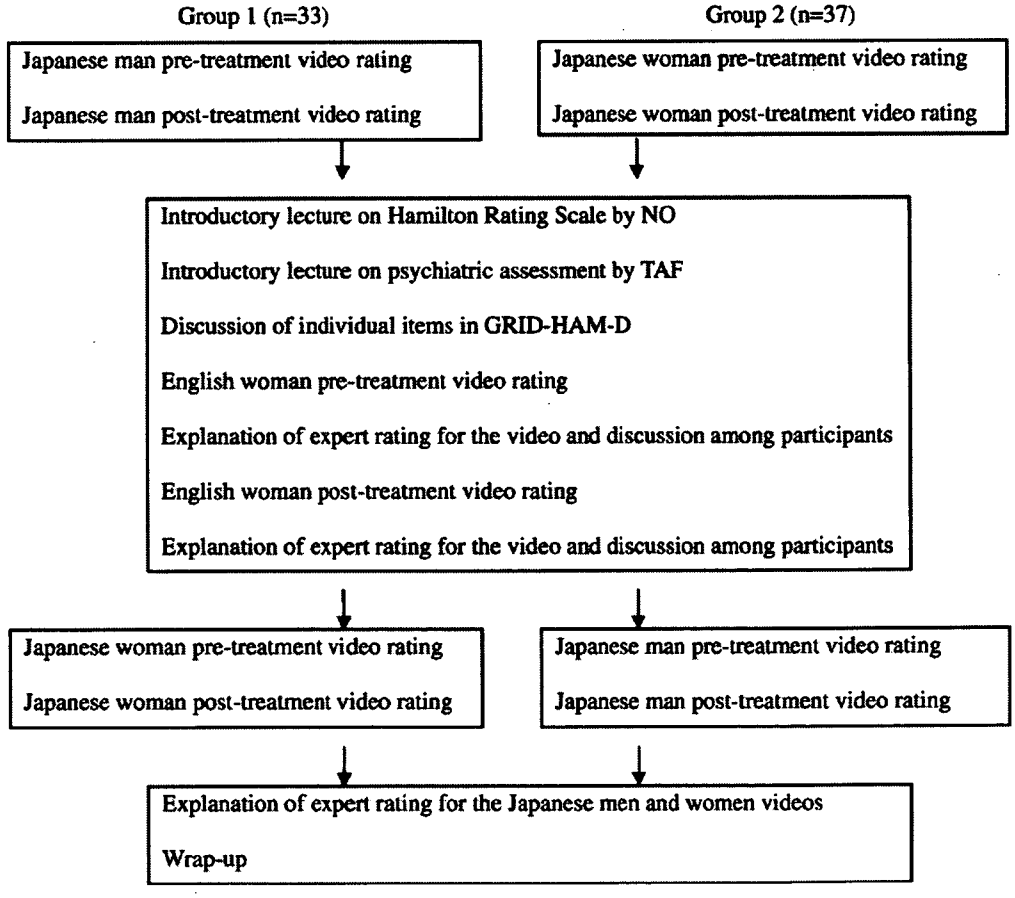

Fig. 1. Procedure of the model training program.

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when one rating predominated (≥0.90 of all the ratings) for a particular item. It has been suggested that ICCs between 0.61 and 0.80 are "substantial" and those greater than 0.80 are "almost perfect" (Landis and Koch, 1977).

Because we were interested in the effects of experience and training, we subgrouped our participants based on their previous experience with the HAMD as follows.

Group A (n=20) No previous experience with the HAMD
Group B (n=17) Have administered the HAMD between one and five times
Group C (n=17) Have administered the HAMD six or more times, but have never had formal training in its administration
Group D (n=16) Have administered the HAMD six or more times, and have received formal training in its administration.

To examine the influence of the rating convention of assigning 50% frequency to such items as where the interviewer failed to ask for frequency in the videotape, we ran a supplementary sensitivity analysis by comparing

Table 1
ANOVA ICC for each item and the total score of the GRID-HAMD for the four subgroups of participants before and after training

Item		Group A		Group B		Group C		Group D	
		Before training	After training	Before training	After training	Before training	After training	Before training	After training
1	Depressed mood	0.83 (0.64–0.95)	0.89 (0.76–0.97)	0.78 (0.50–0.96)	0.84 (0.61–0.97)	0.91 (0.74–0.98)	0.84 (0.62–0.97)	0.84 (0.57–0.97)	0.92 (0.55–0.99)
2	Guilt	0.69 (0.41–0.89)	0.60 (0.30–0.86)	0.41 (0.07–0.84)	0.71 (0.40–0.94)	0.67 (0.34–0.93)	0.59 (0.27–0.91)	0.70 (0.34–0.94)	0.58 (0.19–0.91)
3	Suicide	0.89 (0.75–0.97)	0.87 (0.70–0.96)	0.92 (0.79–0.99)	0.90 (0.73–0.98)	0.92 (0.78–0.99)	0.89 (0.72–0.98)	0.94 (0.83–0.99)	0.97 (0.91–1.00)
4	Insomnia, early	0.90 (0.76–0.97)	0.90 (0.77–0.97)	1.00 (1.00–1.00)	1.00 (1.00–1.00)	0.72 (0.41–0.95)	0.85 (0.63–0.97)	0.86 (0.62–0.98)	1.00 (1.00–1.00)
5	Insomnia, middle	0.78 (0.55–0.93)	0.75 (0.50–0.92)	0.66 (0.32–0.93)	0.79 (0.51–0.96)	0.78 (0.50–0.96)	0.70 (0.39–0.94)	0.87 (0.64–0.98)	0.78 (0.47–0.96)
6	Insomnia, late	0.86 (0.69–0.96)	0.93 (0.83–0.98)	0.91 (0.74–0.98)	0.97 (0.91–1.00)	0.87 (0.66–0.98)	0.95 (0.86–0.99)	0.92 (0.77–0.99)	0.96 (0.8–0.99)
7	Work and activities	0.63 (0.34–0.87)	0.69 (0.42–0.90)	0.70 (0.37–0.94)	0.72 (0.40–0.94)	0.73 (0.42–0.95)	0.75 (0.47–0.95)	0.73 (0.39–0.95)	0.71 (0.35–0.94)
8	Psychomotor retardation	0.67 (0.39–0.89)	0.80 (0.68–0.94)	0.67 (0.34–0.93)	0.78 (0.50–0.96)	0.85 (0.63–0.97)	0.83 (0.61–0.97)	0.59 (0.20–0.91)	0.61 (0.22–0.92)
9	Psychomotor agitation	na	na	−0.05 (−0.18–0.43)	−0.05 (−0.18–0.43)	0.13 (−0.10–0.67)	na	na	0.00 (−0.21–0.57)
10	Anxiety, psychic	0.75 (0.50–0.92)	0.38 (0.08–0.74)	0.64 (0.30–0.92)	0.75 (0.45–0.95)	0.73 (0.41–0.95)	0.74 (0.45–0.95)	0.82 (0.54–0.97)	0.96 (0.88–0.99)
11	Anxiety, somatic	0.88 (0.73–0.96)	0.82 (0.61–0.94)	0.80 (0.53–0.96)	0.87 (0.66–0.98)	0.89 (0.70–0.98)	0.86 (0.65–0.97)	0.93 (0.80–0.99)	0.89 (0.69–0.98)
12	Loss of appetite	0.87 (0.72–0.96)	0.91 (0.78–0.97)	0.89 (0.71–0.98)	0.95 (0.84–0.99)	0.88 (0.69–0.98)	0.82 (0.58–0.97)	0.91 (0.74–0.99)	0.87 (0.64–0.98)
13	Somatic symptoms, general	0.53 (0.21–0.82)	0.50 (0.20–0.81)	0.64 (0.30–0.93)	0.48 (0.13–0.87)	0.57 (0.21–0.90)	0.36 (0.07–0.81)	0.63 (0.25–0.93)	0.44 (0.05–0.86)
14	Sexual interest	na	na	na	na	na	na	na	na
15	Hypochondriasis	0.60 (0.30–0.86)	0.62 (0.33–0.87)	0.53 (0.18–0.89)	0.73 (0.42–0.95)	0.69 (0.37–0.94)	0.83 (0.60–0.97)	0.85 (0.59–0.97)	0.73 (0.39–0.95)
16	Loss of weight	0.63 (0.34–0.87)	0.65 (0.36–0.88)	0.64 (0.30–0.92)	0.79 (0.51–0.96)	0.71 (0.39–0.94)	0.84 (0.73–0.98)	0.77 (0.46–0.96)	0.71 (0.35–0.94)
17	Loss of insight	na	na	na	na	0.03 (−0.15–0.55)	na	na	na
Total		0.95 (0.87–0.98)	0.95 (0.87–0.91)	0.93 (0.82–0.99)	0.95 (0.86–0.99)	0.97 (0.91–1.00)	0.95 (0.85–0.99)	0.97 (0.90–1.00)	0.99 (0.96–1.00)

Figures in parentheses indicate the 95% confidence intervals.
na = not applicable due to too little variation because the particular score predominated and more than 90% of the obtained ratings were the same.
Group A (n=20): No previous experience with the HAMD.
Group B (n=17): Have administered the HAMD between one to five times.
Group C (n=17): Have administered the HAMD six or more times, but have never had formal training in its administration.
Group D (n=16): Have administered the HAMD six or more times, and have received formal training in its administration.

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the average ANOVA ICCs between items for which the interviewers did not ask about frequency in more than half of the videotapes (items 2, 7, 10, 12, and 13) and those for which the interviewers asked (items 1, 4, 5, 6, and 15).

3. Results

Table 1 shows the ANOVA ICCs and their 95% confidence intervals for each item and the total score of the GRID-HAMD as applied to the Japanese man and woman's videotapes, for Groups A through D, both before and after training with lectures and practice with the English woman's videotapes. Excluding items 9, 14, and 17 (Psychomotor agitation, Sexual interest, and Loss of insight), which showed too little variation among raters to calculate meaningful chance-corrected agreement coefficients, and item 13 (Somatic symptoms, general), which often had ANOVA ICCs below 0.60, the inter-rater reliability of individual items was already largely in the substantial to excellent range before the training and did not show much increase after the training. Thus the respective averages of the ANOVA ICCs for individual items were 0.75 and 0.74 for Group A before and after training, 0.73 and 0.81 for Group B, 0.78 and 0.79 for Group C, and 0.81 and 0.79 for Group D. The ANOVA ICCs for the total score were almost perfect for all groups both before and after the training (range: 0.93 to 0.99). The average ICC for the items where the interviewers asked for frequency was 0.83 (range: 0.70 to 0.92) and that for the items where they failed to ask and where therefore the subjects were instructed to assume 50% frequency was 0.69 (range: 0.52 to 0.89).

4. Discussion

Our results suggest that when we relied on the GRID-HAMD scoring conventions, the inter-rater reliability of the total score was excellent to almost perfect and that satisfactory inter-rater reliability for individual items was also achievable, even with inexperienced raters and even without training. These findings are at variance with some previous studies on inter-rater reliability for HAMD items, which often reported poor reliability at the individual item levels. Cicchetti and Prusoff (1983) assessed reliability before treatment initiation and 16 weeks later at trial end. Before treatment, only one item was sufficiently reliable and 13 items had coefficients below 0.50. After treatment, again only one item was sufficiently reliable and 11 items had coefficients below 0.50. Craig et al. (1985) also found that only one item had adequate inter-rater reliability. On the other hand, Moberg et al. (2001) reported that nine items showed adequate reliability when the standard HAMD depression scale was administered, but all items showed adequate reliability when the scale was administered with the SIGH-D interview guidelines of Williams (1988). Our findings appear to extend theirs. Narita et al. (2002) pointed out specific weaknesses/ambiguities in the rating instructions in the SIGH-D, especially with regard to items for middle insomnia, somatic anxiety, loss of weight, depersonalization/derealization, and loss of insight; all of these are well anchored in the GRID-HAMD.

On the other hand, our results suggested that inter-rater reliability for general somatic symptoms may be low. However, we suspect that this was due to the difference in emphasis between SIGH-D item 13 and GRID-HAMD item 13, the former following the traditional HAM-D interpretation and focusing on heaviness and aches and the latter emphasizing fatigue and anergia in accordance with DSM-IV criterion symptoms.

With regard to the total score of the HAMD, most of the previous studies reported substantial to satisfactory inter-rater reliability, with ICCs ranging from 0.46 to 0.99 (Bagby et al., 2004). Some investigators provided evidence that the skill level or expertise of the interviewer and the provision of structured queries and scoring guidelines affect reliability (O'Hara and Rehm, 1983; Hooijer et al., 1991). Our findings suggest that with the use of explicit scoring conventions as outlined in the GRID-HAMD, even inexperienced raters can achieve satisfactory inter-rater reliability. We failed to show a significant effect of expertise or training, possibly because of the ceiling effect of these already high baseline reliability coefficients, although the raw scores do hint at even higher reliability coefficients after training and for more experienced users.

Weaknesses of the present study may be as follows: Firstly, the present study is based on videotaped interviews with simulated patients. Although the actor and actress played their roles naturally, with much ad lib interaction, the generalizability of the present findings to bona fide patients cannot be taken for granted and warrant another study. However, it should be pointed out that experienced physicians have been reported to be unable to differentiate standardized patients from real patients when they were sent unannounced into a physician's office, even when the physician was told in advance that this would be occurring (Kobak et al., 2003). The videotaped reliability study with simulated patients may also have inflated reliability estimates in comparison with test–retest design with real patients, which would more accurately reproduce clinical realities. Secondly, we used videotapes that had been made prior to and independently of our workshop for the GRID-HAMD.

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The interviewers in the videotapes therefore did not abide by the GRID-HAMD conventions but roughly followed the SIGH-D questions. They therefore did not probe specifically enough about the frequency of some symptoms. The rating convention of assigning a 50% frequency to such items may have inflated the reliability estimates, but our sensitivity analysis did not support this possibility. Had the interviewers in the videotapes followed the GRID-HAMD interview guides, it is safe to assume that reliability could have been even higher. Thirdly, the videotaped interviews in the present study were such that there was little variation for three out of 17 items of the GRID-HAMD. We could therefore not ascertain satisfactory reliability for these items. In future studies we need to prepare videotapes that allow more variation in ratings for these items. Fourthly, although the ICCs did not change materially before and after the training, it must be pointed out that the present findings do not obviate the need for clinical expertise in depression assessment, as almost all the participants had substantial clinical experience already. In order to assure satisfactory rater performance, the raters' ability to conduct assessments on real patients is important in itself, in addition to the reliability of the instrument (Lipsitz et al., 2004). Lastly, the present study was conducted in Japanese with the Japanese version of the GRID-HAMD. The Japanese version was developed in strict adherence to the established back-translation procedure to ensure its linguistic equivalence with the English original, and we believe the present findings can be replicated with the original version as well, as it is thanks to the well-structured, adequately explained nature of the GRID-HAMD and not to any particularities of its Japanese version that we could achieve satisfactory reliability. Strictly speaking, however, the cross-cultural generalizability of the present findings must await independent replication studies in English and other languages and cultures.

Recently, a comprehensive review of the HAMD by Bagby et al. (2004, 2005) concurred that the GRID-HAMD is a major improvement over the previous versions in developing clear structured interview prompts and scoring guidelines, and in standardizing the scoring system. However, the retention of "loss of insight" that makes neither a conceptual nor an empirical contribution to the severity of depression or the lack of such DSM-IV criterion symptoms as "loss of concentration" remain major difficulties with the GRID-HAMD. Also the report from a 2002 National Institute of Health sponsored conference in the US on the assessment of depression and anxiety in clinical trials recommended the GRID-HAMD as the optimal way to administer the HAMD. A recent National Institutes of Health sponsored conference on assessment of suicidality also recommended the GRID-HAMD as the preferred version of the HAMD for assessing suicidality.

We feel that the GRID-HAMD is the best achievable set of semi-structured guides for the HAMD, the *de facto* standard in depression rating for over four decades, and this fact was corroborated in the present study by its robust reliability findings. In conclusion, the GRID-HAMD appears to provide a solid tool for highly reliable assessment of depression severity for both experienced and inexperienced mental health professionals.

References

Bagby, R.M., Ryder, A.G., Schuller, D.R., Marshall, M.B., 2004. The Hamilton Depression Rating Scale: has the gold standard become a lead weight? American Journal of Psychiatry 161, 2163–2177.

Bagby, R.M., Schuller, D.R., Ryder, A.G., Marshall, M.B., 2005. Dr. Bagby and colleagues reply. American Journal of Psychiatry 162, 2397–2398.

Bech, P., Engelhardt, N., Evans, K.R., Gibertini, M., Kalali, A.H., Kobak, K.A., Lipsitz, J.D., Williams, J.B., Pearson, J.D., Rothman, M., 2005. Why the Hamilton Depression Rating Scale endures. American Journal of Psychiatry 162, 2396.

Cicchetti, D.V., Prusoff, B.A., 1983. Reliability of depression and associated clinical symptoms. Archives of General Psychiatry 40, 987–990.

Craig, T.J., Richardson, M.A., Pass, R., Bregman, Z., 1985. Measurement of mood and affect in schizophrenic inpatients. American Journal of Psychiatry 142, 1272–1277.

Feinstein, A.R., Cicchetti, D.V., 1990. High agreement but low kappa: I. The problems of two paradoxes. Journal of Clinical Epidemiology 43, 543–549.

Furukawa, T.A., Streiner, D.L., Azuma, H., Higuchi, T., Kamijima, K., Kanba, S., Ozaki, N., Aoba, A., Murasaki, M., Miura, S., 2005. Cross-cultural equivalence in depression assessment: Japan–Europe–North American study. Acta Psychiatrica Scandinavica 112, 279–285.

Hamilton, M., 1960. A rating scale for depression. Journal of Neurology, Neurosurgery and Psychiatry 23, 56–62.

Hooijer, C., Zitman, F.G., Griez, E., van Tilburg, W., Willemse, A., Dinkgreve, M.A., 1991. The Hamilton Depression Rating Scale (HDRS); changes in scores as a function of training and version used. Journal of Affective Disorders 22, 21–29.

Kalali, A., Williams, J., Kobak, K.A., Lipsitz, J., Engelhardt, N., Evans, K., Olin, J., Pearson, J., Rothman, M.L., Bech, P., 2002. The new GRID HAM-D: pilot testing and international field trials. International Journal of Neuropsychopharmacology 5, S147–S148.

Kobak, K.A., Lipsitz, J.D., Feiger, A., 2003. Development of a standardized training program for the Hamilton Depression Scale using internet-based technologies: results from a pilot study. Journal of Psychiatric Research 37, 509–515.

Landis, J.R., Koch, G.G., 1977. The measurement of observer agreement for categorical data. Biometrics 33, 159–174.

Lipsitz, J., Kobak, K., Feiger, A., Sikich, D., Moroz, G., Engelhard, A., 2004. The Rater Applied Performance Scale: development and reliability. Psychiatry Research 127, 147–155.

Moberg, P.J., Lazarus, L.W., Mesholam, R.I., Bilker, W., Chuy, I.L., Neyman, I., Markvart, V., 2001. Comparison of the standard and structured interview guide for the Hamilton Depression Rating Scale

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in depressed geriatric inpatients. American Journal of Geriatric Psychiatry 9, 35–40.
Narita, T., Kim, N., Nakane, Y., Ozaki, N., 2002. Reliability and validity of the Structured Interview Guide of the Hamilton Depression Rating Scale (SIGH-D). Japanese Journal of Clinical Psychopharmacology 6, 77–82 [in Japanese].
O'Hara, M.W., Rehm, L.P., 1983. Hamilton Rating Scale for Depression: reliability and validity of judgments of novice raters. Journal of Consulting and Clinical Psychology 51, 318–319.
SPSS Inc., 2002. SPSS for Windows Version 11.5. SPSS Inc., Chicago.
Williams, J.B., 1988. A structured interview guide for the Hamilton Depression Rating Scale. Archives of General Psychiatry 45, 742–747.

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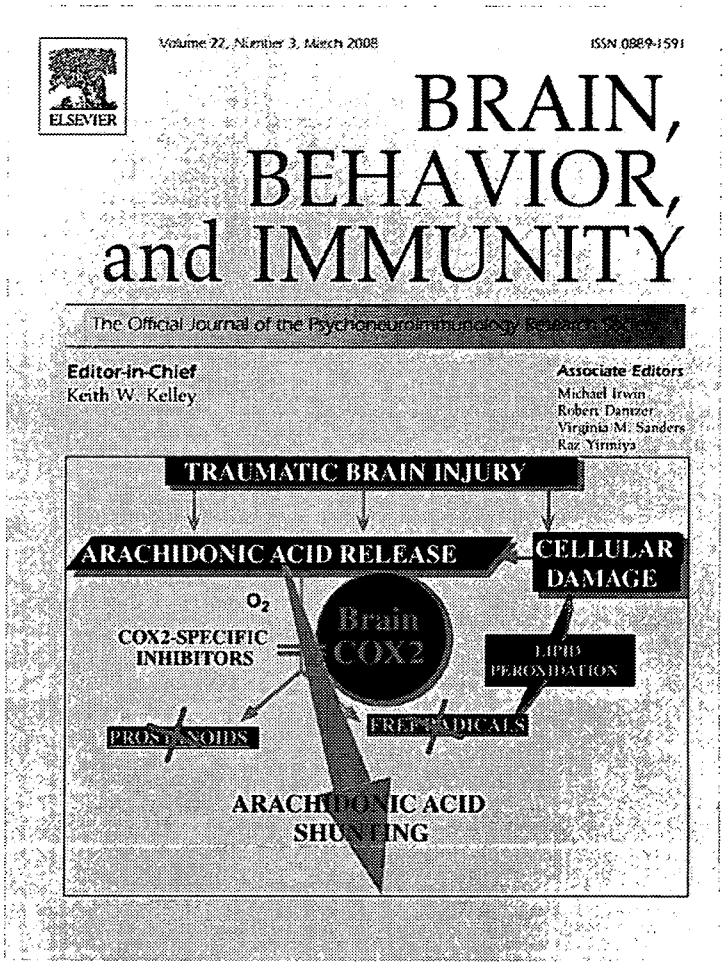

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BRAIN, BEHAVIOR, and IMMUNITY
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Associations among central nervous, endocrine, and immune activities when positive emotions are elicited by looking at a favorite person

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Received 3 August 2007; received in revised form 14 September 2007; accepted 21 September 2007
Available online 30 October 2007

Abstract

Recent studies on psychoneuroimmunology have indicated that positive psychological events are related to immune functions; however, limited information is available regarding associations among the central nervous, endocrine, and immune systems when positive emotions are elicited. In the present study, we demonstrated associations among these systems by simultaneously recording brain, endocrine, and immune activities when positive emotions were evoked in participants as they watched films featuring their favorite persons. Interestingly, the activity of peripheral circulating natural killer cells and the peripheral dopamine level were elevated while participants experienced positive emotions, and these values were positively correlated. The following brain regions were significantly activated in the positive condition relative to the control condition: medial prefrontal cortex, thalamus, hypothalamus, subcallosal gyrus, posterior cingulate cortex, superior temporal gyrus, and cerebellum. Further, covariate analyses indicated that these brain regions were temporally associated with endocrine and immune activities. These results suggest that while an individual experiences positive emotions, the central nervous, endocrine, and immune systems may be interrelated and attraction for favorite persons may be associated with the activation of the innate immune function via the dopaminergic system.
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Keywords: Positive emotion; Positron emission tomography; Natural killer cell activity; Dopamine

1. Introduction

Studies on psychoneuroimmunology have revealed that the central nervous, peripheral autonomic nervous, endocrine, and immune systems are interrelated via complex biochemical pathways (Ader, 2000). Some have also revealed that acute psychosocial stressors such as public speaking, examinations, and even short-term mental arithmetic are sufficient to effect changes in immunological parameters (Goebel and Mills, 2000; Downing and Miyan, 2000; Isowa et al., 2004, 2006; Kimura et al., 2005). These stressors can activate the sympathetic nervous system and the hypothalamus–pituitary–adrenal (HPA) axis and

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doi:10.1016/j.bbi.2007.09.008

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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

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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-

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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-

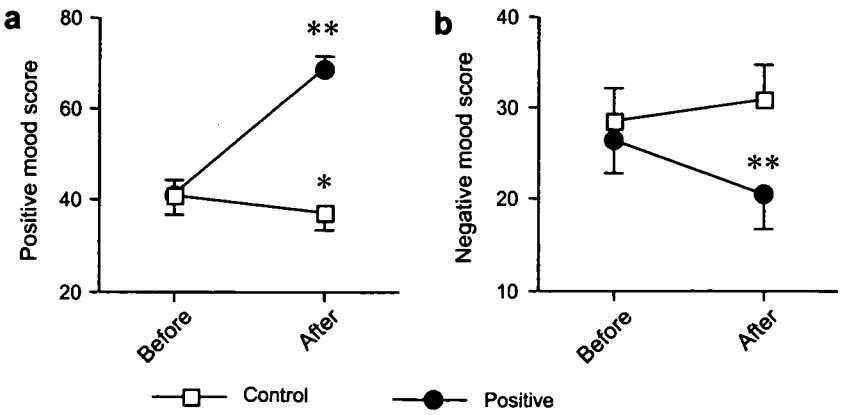

Fig. 1. Psychological response. Each *point* and *vertical line* represents the mean ± SEM VAS score ($n = 12$). (a) Change in positive mood state after watching the films. $^{**}p < 0.01$ and $^{*}p < 0.05$ versus before watching by paired t test. (b) Change in negative mood state after watching the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

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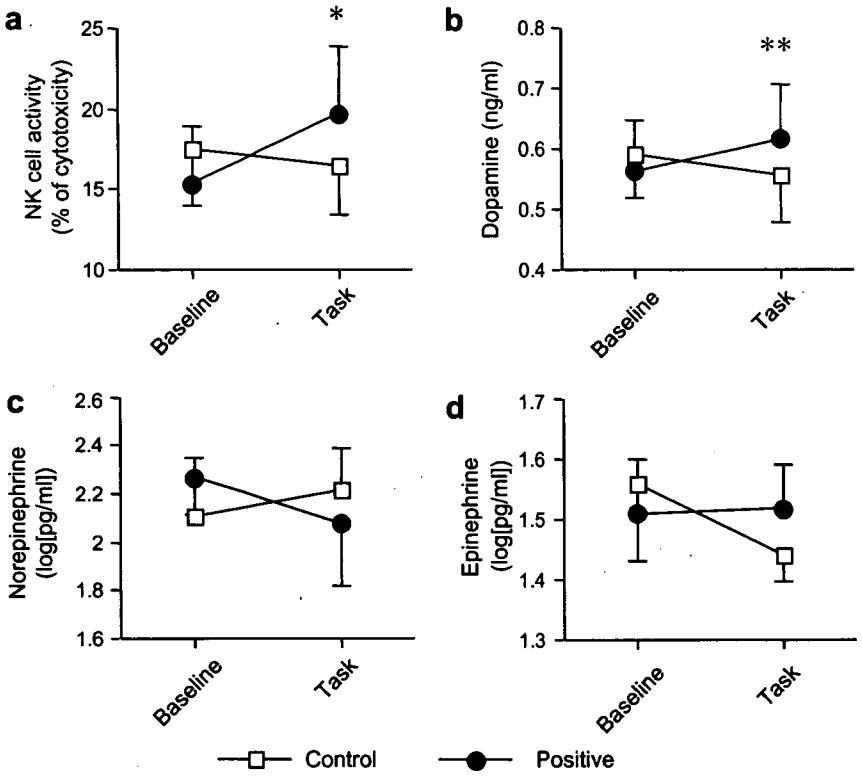

Fig. 2. Physiological responses. Each *point* and *vertical line* represents the mean ± SEM % cytotoxicity (a: $n = 12$) or the mean ± SEM concentration (b, c, and d: $n = 10$). (a) Change in NK cell activity after watching the control and positive films. $^{*}p < 0.05$ versus baseline value in paired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

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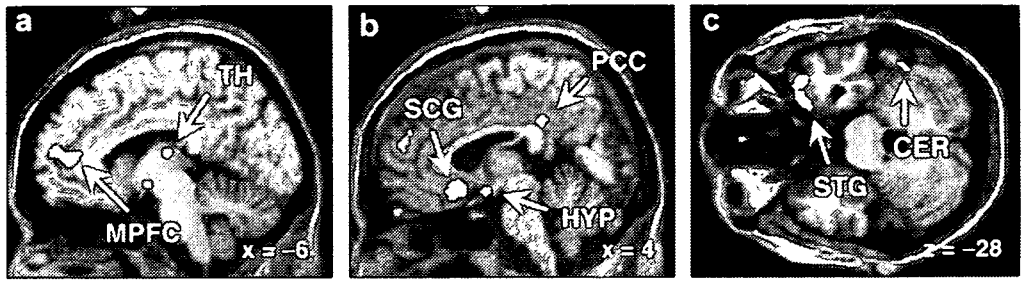

Fig. 3. Statistical parametric maps (SPM99) showing significant increases in the rCBF in the positive condition minus those in the control condition. (a) Activations of the MPFC and thalamus (TH). (b) Activations of the hypothalamus (HYP), subcallosal gyrus (SCG), and PCC. (c) Activations of the superior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

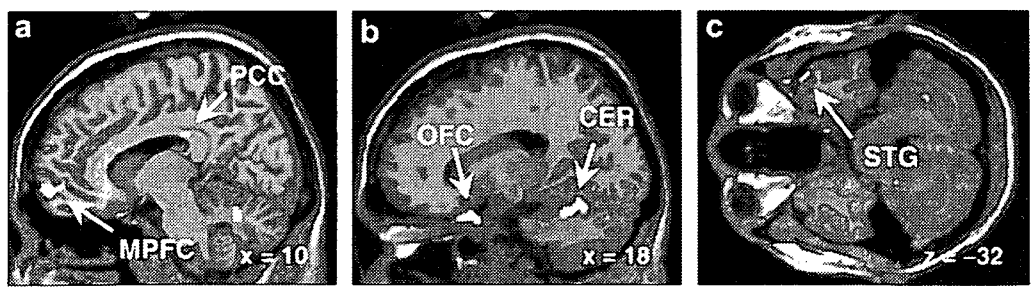

Fig. 4. SPM99 covariate analysis of rCBF during the positive condition with NK cell activity as the covariate of interest. (a) Correlations with the MPFC and PCC. (b) Correlations with the OFC and cerebellum (CER). (c) Correlation with the superior temporal gyrus (STG).

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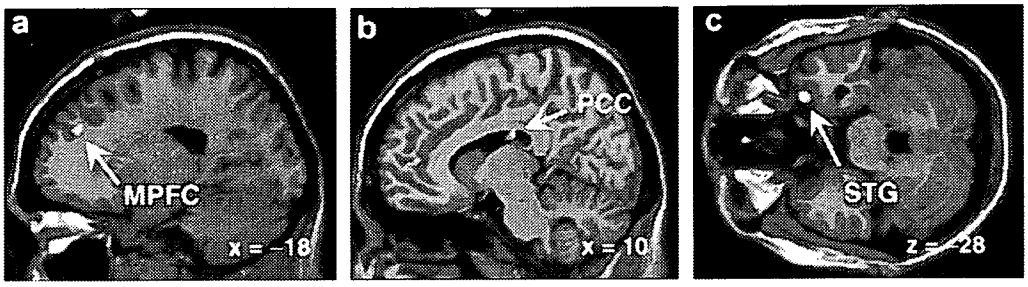

Fig. 5. SPM99 covariate analysis of rCBF during the positive condition with peripheral dopamine level as the covariate of interest. (a) Correlation with the MPFC. (b) Correlation with the PCC. (c) Correlation with the superior temporal gyrus (STG).

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.

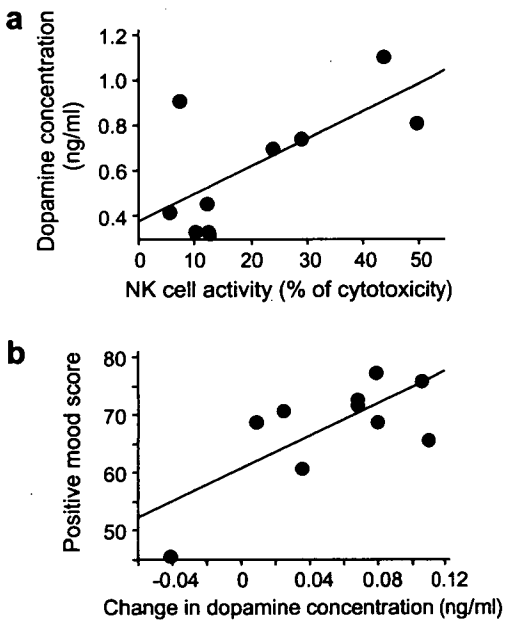

Fig. 6. (a) Scatterplots of NK cell activity and dopamine concentration during watching the positive film. (b) Scatterplots of change from baseline in the dopamine concentration and positive mood score after watching the positive film.

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

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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.

Acknowledgments

We thank Prof Shinici Kakumu (Division of Gastroenterology, Department of Internal Medicine, Aichi Medical University School of Medicine, Japan) for his suggestions and encouragement throughout this study, and Dr Michio Nomura (Department of Psychology, Tokai Gakuin University, Japan) for his technical assistance. This work was supported in part by Grant-in-Aid for Scientific Research of the Japan Society for the Promotion of Science (No. 16330136), and Health and Labour Sciences Research Grant on Research on Occupational Safety and Health from Japan Ministry of Health, Labour, and Welfare (No. H17-RODO-5). Masahiro Matsunaga has received a Research Fellowship of the Japan Society for the Promotion of Science for Young Scientists.

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References

Abo, T., Kawamura, T., 2002. Immunomodulation by the autonomic nervous system: therapeutic approach for cancer, collagen diseases, and inflammatory bowel diseases. Ther. Apher. Dial. 6, 348–357.

Ader, R., 2000. On the development of psychoneuroimmunology. Eur. J. Pharmacol. 405, 167–176.

Anand, B.K., Malhotra, C.L., Singh, B., Dua, S., 1959. Cerebellar projections to limbic system. J. Neurophysiol. 22, 451–457.

Aron, A., Fisher, H., Mashek, D.J., Strong, G., Li, H., Brown, L.L., 2005. Reward, motivation, and emotion systems associated with early-stage intense romantic love. J. Neurophysiol. 94, 327–337.

Aron, A., Fisher, H., Strong, G., 2006. Romantic love. In: Vangelisti, A., Perlman, D. (Eds.), The Cambridge Handbook of Personal Relationships. Cambridge University Press, Cambridge, pp. 595–614.

Bartels, A., Zeki, S., 2004. The neural correlates of maternal and romantic love. NeuroImage 21, 1155–1166.

Beauchaine, T., 2001. Vagal tone, development, and Gray's motivational theory: toward an integrated model of autonomic nervous system functioning in psychopathology. Dev. Psychopathol. 13, 183–214, Review.

Berk, L.S., Felten, D.L., Tan, S.A., Bittman, B.B., Westengard, J., 2001. Modulation of neuroimmune parameters during the eustress of humor-associated mirthful laughter. Altern Ther. Health Med. 7, 62–76.

Bosch, J.A., Berntson, G.G., Cacioppo, J.T., Marucha, P.T., 2005. Differential mobilization of functionally distinct natural killer subsets during acute psychologic stress. Psychosom. Med. 67, 366–375.

Burgdorf, J., Panksepp, J., 2006. The neurobiology of positive emotions. Neurosci. Biobehav. Rev. 30, 173–187.

Cohen, S., Doyle, W.J., Turner, R.B., Alper, C.M., Skoner, D.P., 2003. Emotional style and susceptibility to the common cold. Psychosom. Med. 65, 652–657.

Damasio, A.R., 1999. The Feeling of What Happens: Body and Emotion in the Making of Consciousness. Harcourt Brace, New York.

Davidson, R.J., Irwin, W., 1999. The functional neuroanatomy of emotion and affective style. Trends Cogn. Sci. 3, 11–21.

Doyle, W.J., Gentile, D.A., Cohen, S., 2006. Emotional style, nasal cytokines, and illness expression after experimental rhinovirus exposure. Brain Behav. Immun. 20, 175–181.

Downing, J.E., Miyan, J.A., 2000. Neural immunoregulation: emerging roles for nerves in immune homeostasis and disease. Immunol. Today 21, 281–289.

Dum, R.P., Strick, P.L., 2003. An unfolded map of the cerebellar dentate nucleus and its projections to the cerebral cortex. J. Neurophysiol. 89, 634–639.

Esch, T., Stefano, G.B., 2005a. Love promotes health. Neuroendocrinol. Lett. 26, 52–55.

Esch, T., Stefano, G.B., 2005b. The neurobiology of love. Neuroendocrinol. Lett. 26, 175–192.

Fink, G.R., Markowitsch, H.J., Reinkemeier, M., Bruckbauer, T., Kessler, J., Heiss, W.D., 1996. Cerebral representation of one's own past: neural networks involved in autobiographical memory. J. Neurosci. 16, 4275–4282.

Fiserova, A., Starec, M., Kuldova, M., Kovaru, H., Pav, M., Vannucci, L., Pospisil, M., 2002. Effects of D_2-dopamine and alpha-adrenoceptor antagonists in stress induced changes on immune responsiveness of mice. J. Neuroimmunol. 130, 55–65.

Fisher, H., Aron, A., Brown, L.L., 2005. Romantic love: an fMRI study of a neural mechanism for mate choice. J. Comp. Neurol. 493, 58–62.

Friston, K.J., Holmes, A.P., Worsley, K.J., Poline, J.-P., Frith, C.D., Frackowiak, R.S.J., 1995. Statistical parametric maps in functional imaging: a general linear approach. Hum. Brain Mapp. 2, 189–210.

George, M.S., Ketter, T.A., Parekh, P.I., Horwitz, B., Herscovitch, P., Post, R.M., 1995. Brain activity during transient sadness and happiness in healthy women. Am. J. Psychiatry 152, 341–351.

Gilbert, C., 1995. Optimal physical performance in athletes: key roles of dopamine in a specific neurotransmitter/hormonal mechanism. Mech. Ageing Dev. 84, 83–102.

Goebel, M.U., Mills, P.J., 2000. Acute psychological stress and exercise and changes in peripheral leukocyte adhesion molecule expression and density. Psychosom. Med. 62, 664–670.

Haake, P., Krueger, T.H., Goebel, M.U., Heberling, K.M., Hartmann, U., Schedlowski, M., 2004. Effects of sexual arousal on lymphocyte subset circulation and cytokine production in man. NeuroImmuno-Modulation 11, 293–298.

Isowa, T., Ohira, H., Murashima, S., 2004. Reactivity of immune, endocrine and cardiovascular parameters to active and passive acute stress. Biol. Psychol. 65, 101–120.

Isowa, T., Ohira, H., Murashima, S., 2006. Immune, endocrine and cardiovascular responses to controllable and uncontrollable acute stress. Biol. Psychol. 71, 202–213.

Jacobs, J.R., Bovasso, G.B., 2000. Early and chronic stress and their relation to breast cancer. Psychol. Med. 30, 669–678.

Kimura, K., Isowa, T., Ohira, H., Murashima, S., 2005. Temporal variation of acute stress responses in sympathetic nervous and immune systems. Biol. Psychol. 70, 131–139.

Maddock, R.J., Garrett, A.S., Buonocore, M.H., 2003. Posterior cingulate cortex activation by emotional words: fMRI evidence from a valence decision task. Hum. Brain Mapp. 18, 30–41.

Maisel, A.S., Knowlton, K.U., Fowler, P., Rearden, A., Ziegler, M.G., Motulsky, H.J., Insel, P.A., Michel, M.C., 1990. Adrenergic control of circulating lymphocyte subpopulations. Effects of congestive heart failure, dynamic exercise, and tertbutaline treatment. J. Clin. Invest. 85, 462–467.

Marsland, A.L., Cohen, S., Rabin, B.S., Manuck, S.B., 2006. Trait positive affect and antibody response to hepatitis B vaccination. Brain Behav. Immun. 20, 261–269.

McKenna, F., McLaughlin, P.J., Lewis, B.J., Sibbring, G.C., Cummerson, J.A., Bowen-Jones, D., Moots, R.J., 2002. Dopamine receptor expression on human T- and B-lymphocytes, monocytes, neutrophils, eosinophils and NK cells: a flow cytometric study. J. Neuroimmunol. 132, 34–40.

Mizuno, T., Oda, S., Takeda, H., Mizuno, M., Moriya, K., 2003. Immunological, hormonal, and psychological effects of comfortable self-paced running as compared to bed-resting relaxation in untrained healthy men. Med. Sci. Sports Exerc. 35 (suppl. 1), S379.

Ohira, H., Nomura, M., Ichikawa, N., Isowa, T., Iidaka, T., Sato, A., Fukuyama, S., Nakajima, T., Yamada, J., 2006. Association of neural and physiological responses during voluntary emotion suppression. NeuroImage 29, 721–733.

Ottowitz, W.E., Dougherty, D.D., Sirota, A., Niaura, R., Rauch, S.L., Brown, W.A., 2004. Neural and endocrine correlates of sadness in women: implications for neural network regulation of HPA activity. J. Neuropsychiatry Clin. Neurosci. 16, 446–455.

Phan, K.L., Wager, T., Taylor, S.F., Liberzon, I., 2002. Functional neuroanatomy of emotion: a meta-analysis of emotion activation studies in PET and fMRI. NeuroImage 16, 331–348.

Planalp, S., Fitness, J., Fehr, B., 2006. Emotion in theories of close relationships. In: Vangelisti, A., Perlman, D. (Eds.), The Cambridge Handbook of Personal Relationships. Cambridge University Press, Cambridge, pp. 369–384.

Royet, J.P., Zald, D., Versace, R., Costes, N., Lavenne, F., Koenig, O., Gervais, R., 2000. Emotional responses to pleasant and unpleasant olfactory, visual, and auditory stimuli: a positron emission tomography study. J. Neurosci. 20, 7752–7759.

Sokhadze, E.M., 2007. Effects of music on the recovery of autonomic and electrocortical activity after stress induced by aversive visual stimuli. Appl. Psychophysiol. Biofeedback 32, 31–50.

Stefano, G.B., Esch, T., 2005. Love and stress. Neuroendocrinol. Lett. 26, 173–174.

Takahashi, K., Iwase, M., Yamashita, K., Tatsumoto, Y., Ue, H., Kuratsune, H., Shimizu, A., Takeda, M., 2001. The elevation of

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natural killer cell activity induced by laughter in a crossover designed study. Int. J. Mol. Med. 8, 645–650.

Turner, B.M., Paradiso, S., Marvel, C.L., Pierson, R., Boles Ponto, L.L., Hichwa, R.D., Robinson, R.G., 2007. The cerebellum and emotional experience. Neuropsychologia 45, 1331–1341.

Verhoeff, N.P., Christensen, B.K., Hussey, D., Lee, M., Papatheodorou, G., Kopala, L., Rui, Q., Zipursky, R.B., Kapur, S., 2003. Effects of catecholamine depletion on D2 receptor binding, mood, and attentiveness in humans: a replication study. Pharmacol. Biochem. Behav. 74, 425–432.

Vivier, E., Nunes, J.A., Vely, F., 2004. Natural killer cell signaling pathways. Science 306, 1517–1519.

Vogt, B.A., Rosene, D.L., Pandya, D.N., 1979. Thalamic and cortical afferents differentiate anterior from posterior cingulate cortex in the monkey. Science 204, 205–207.

Wenner, M., Kawamura, N., Ishikawa, T., Matsuda, Y., 2000. Reward linked to increased natural killer cell activity in rats. NeuroImmunoModulation 7, 1–5.

Wrona, D., Trojniar, W., 2003. Chronic electrical stimulation of the lateral hypothalamus increases natural killer cell cytotoxicity in rats. J. Neuroimmunol. 141, 20–29.

Wrona, D., Klejbor, I., Trojniar, W., 2004. Chronic electric stimulation of the midbrain ventral tegmental area increases spleen but not blood natural killer cell cytotoxicity in rats. J. Neuroimmunol. 155, 85–93.

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NeuroImage 39 (2008) 500–514

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

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Received 16 April 2006; revised 24 July 2007; accepted 17 August 2007
Available online 25 August 2007

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.
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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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Available online on ScienceDirect (www.sciencedirect.com).

1053-8119/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.neuroimage.2007.08.017

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,