killer cells to cultured endothelium. British Journal of Pharmacology 113, 1311–1316.

Benschop, R.J., Jacobs, R., Sommer, B., Schurmeyer, T.H., Raab, J.R., Schmidt, R.E., Schedlowski, M., 1996. Modulation of the immunologic responses to acute stress in humans by beta-blockade or benzodiazepines. The FASEB Journal 10, 517–524.

Bosch, J.A., de Geus, E.J.C., Kelder, A., Veerman, E.C.I., Hoogstraten, J., Nieuw Amerongen, A.V., 2001. Differential effects of active versus passive coping on secretory immunity. Psychophysiology 38, 836–846.

Breznitz, S., Ben-Zur, H., Berzon, Y., Weiss, D.W., Levitan, G., Tarcic, N., Lischinsky, S., Greenberg, A., Levi, N., Zinder, O., 1998. Experimental induction and termination of acute psychological stress in human volunteers: effects on immunological, neuroendocrine, cardiovascular, and psychological parameters.. Brain, Behavior, and Immunity 12, 34–52.

Burleson, M.H., Malarkey, W.B., Casioppo, J.T., Poehlmann, K.M., Kiecolt-Glaser, J.K., Berntson, G.G., Glaser, R., 1998. Postmenopausal hormone replacement: effects on autonomic, neuroendocrine, and immune reactivity to brief psychological stressors. Psychosomatic Medicine 60, 17–25.

Delahanty, D.L., Dougall, A.L., Hawken, L., Trakowski, J.H., Schmitz, J.B., Jenkins, F.J., Baum, A., 1996. Time course of natural killer cell activity and lymphocyte proliferation in response to two acute stressors in healthy men. Health Psychology 15, 48–55.

Dhabhar, F.S., Miller, A.H., McEwen, B.S., Spencer, R.L., 1996. Stress-induced changes in blood leukocyte distribution. Role of adrenal steroid hormones. Journal of Immunology 157, 1638–1644.

Ellard, D.R., Castle, P.C., Mian, R., 2001. The effect of a short-term mental stressor on neutrophil activation. International Journal of Psychophysiology 41, 93–100.

Farag, N.H., Nelesen, R.A., Dimsdale, J.E., Loredo, J.S., Mills, P.J., 2002. The effects of acute psychological stress on lymphocyte adhesion molecule expression and density in cardiac versus vascular reactors. Brain, Behavior, and Immunity 16, 411–420.

Garvy, B.A., Fraker, P.J., 1991. Suppression of the antigenic response of murine bone marrow B cells by physiological concentrations of glucocorticoids. Immunology 74, 519–523.

Gerra, G., Zaimovic, A., Mascetti, G.G., Gardini, S., Zambelli, U., Timpano, M., Raggi, M.A., Brambilla, F., 2001. Neuroendocrine responses to experimentally-induced psychological stress in healthy humans. Psychoneuroendocrinology 26, 91–107.

Gold, S.M., Zakowski, S.G., Valdimarsdottir, H.B., Bovbjerg, D.H., 2004. Higher Beck depression scores predict delayed epinephrine recovery after acute psychological stress independent of baseline levels of stress and mood. Biological Psychology 67, 261–273.

Herbert, T.B., Cohen, S., Marsland, A.L., Bachen, E.A., Rabin, B.S., Muldoon, M.F., Manuck, S.B., 1994. Cardiovascular reactivity and the course of immune response to an acute psychological stressor. Psychosomatic Medicine 56, 337–344.

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

Jennings, K.R., Wood, C.C., 1976. The E-adjustment procedure for repeated measures analysis of variance. Psychophysiology 13, 277–278.

Katsuharu, N., Tadanobu, M., 1982. Development and validation of Japanese version of State-Trait Anxiety Inventory. Shinshin-Igaku 22, 107–112.

Kohm, A.P., Sanders, V.M., 2001. Norepinephrine and beta 2-adrenergic receptor stimulation regulate CD4+ T and B lymphocyte function in vitro and in vivo. Pharmacological Reviews 53, 487–525.

Kuhn, C.M., 1989. Adrenocortical and gonadal steroids in behavioral cardiovascular medicine. In: Schneiderman, N., Weiss, S.M., Kaufmann, P.G. (Eds.), Handbook of Research Methods in Cardiovascular Behavioral Medicine. Plenum, New York, pp. 185–204.

Kunz-Ebrecht, S.R., Mohamed-Ali, V., Feldman, P.J., Kirschbaum, C., Steptoe, A., 2003. Cortisol responses to mild psychological stress are inversely associated with proinflammatory cytokines.. Brain, Behavior, and Immunity 17, 373–383.

Landmann, R.M., Burgisser, E., Wesp, M., Buhler, F.R., 1984. Beta-adrenergic receptors are different in subpopulations of human circulating lymphocytes. Journal of Receptor Research 4, 37–50.

Lutgendorf, S.K., Logan, H., Costanzo, E., Lubaroff, D., 2004. Effects of acute stress, relaxation, and a neurogenic inflammatory stimulus on interleukin-6 in humans. Brain, Behavior, and Immunity 18, 55–64.

Marsland, A.L., Cohen, S., Rabin, B.S., Manuck, S.B., 2001. Association between stress, trait negative affect, acute immune reactivity, and antibody responses to hepatitis B injection in healthy young adults. Health Psychology 20, 4–11.

Mills, P.J., Dimsdale, J.E., Nelesen, R.A., Dillon, E., 1996. Psychologic characteristics associated with acute stressor-induced leukocyte subset redistribution. Journal of Psychosomatic Research 40, 417–423.

Mills, P.J., Farag, N.H., Hong, S., Kennedy, B.P., Berry, C.C., Ziegler, M.G., 2003. Immune cell CD62L and CD11a expression in response to a psychological stressor in human hypertension. Brain, Behavior, and Immunity 17, 260–267.

Northrop, J.P., Crabtree, G.R., Mattila, P.S., 1992. Negative regulation of interleukin 2 transcription by the glucocorticoid receptor. Journal of Experimental Medicine 175, 1235–1245.

Owen, N., Steptoe, A., 2003. Natural killer cell and proinflammatory cytokine responses to mental stress: associations with heart rate and heart rate variability. Biological Psychology 63, 101–115.

Pehlivanoglu, B., Balkanci, Z.D., Ridvanagaoglu, A.Y., Durmazlar, N., Ozturk, G., Erbas, D., Okur, H., 2001. Impact of stress, gender and menstrual cycle on immune system: possible role of nitric oxide. Archives of Physiology and Biochemistry 109, 383–387.

Peters, M.L., Godaert, G.L., Ballieux, R.E., Brosschot, J.F., Sweep, F.C., Swinkels, L.M., van Vliet, M., Heijnen, C.J., 1999. Immune responses to experimental stress: effects of mental effort and uncontrollability. Psychosomatic Medicine 61, 513–524.

Pike, J.L., Smith, T.L., Hauger, R.L., Nicassio, P.M., Patterson, T.L., McClintick, J., Costlow, C., Irwin, M.R., 1997. Chronic life stress alters sympathetic, neuroendocrine, and immune responsivity to an acute psychological stressor in humans. Psychosomatic Medicine 55, 447–457.

Redwine, L., Snow, S., Mills, P., Irwin, M., 2003. Acute psychological stress: effects on chemotaxis and cellular adhesion molecule expression. Psychosomatic Medicine 65, 598–603.

Ring, C., Carroll, D., Willemsen, G., Cooke, J., Ferraro, A., Drayson, M., 1999. Secretory immunoglobulin A and cardiovascular activity during mental arithmetic and paced breathing. Psychophysiology 36, 602–609.

Ring, C., Harrison, L., Winzer, A., Carroll, D., Drayson, M., Kendall, M., 2000. Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic, cold pressor and exercise: effects of alpha-adrenergic blockade. Psychophysiology 37, 634–643.

Rupprecht, R., Wodarz, N., Kornhuber, J., Schmitz, B., Wild, K., Braner, H.U., Muller, O.A., Riederer, P., 1990. In vivo and in vitro effects of glucocorticoids on lymphocyte proliferation in man: relationship to glucocorticoid receptor. Neuropsychobiology 24, 61–66.

Scarpa, A., Luscher, K.A., 2002. Self-esteem, cortisol reactivity, and depressed mood mediated by perceptions of control. Biological Psychology 59, 93–103.

Schedlowski, M., Hosch, W., Oberbeck, R., Benschop, R.J., Jacobs, R., Raab, H.R., Schmidt, R.E., 1996. Catecholamines modulate human NK cell circulation and function via spleen-independent beta 2-adrenergic mechanisms. Journal of Immunology 156, 93–99.

Schmid-Ott, G., Jaeger, B., Adamek, C., Koch, H., Lamprecht, F., Kapp, A., Werfel, T., 2001. Levels of circulating CD8(+) T lymphocytes, natural killer cells and eosinophils increase upon acute psychosocial stress in patients with atopic dermatitis. The Journal of Allergy and Clinical Immunology 107, 171–177.

Slade, J.D., Hepburn, B., 1983. Prednisone-induced alterations of circulating human lymphocyte subsets. The Journal of Laboratory and Clinical Medicine 101, 479–487.

Speilberger, C.D., Gorusch, R.L., Lushene, R.D., 1970. STAI Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA.

Steptoe, A., Willemsen, G., Kunz-Ebrecht, S.R., Owen, N., 2003. Socioeconomic status and hemodynamic recovery from mental stress. Psychophysiology 40, 184–191.

Steptoe, A., Owen, N., Kunz-Ebrecht, S.R., Brydon, L., 2004. Loneliness and neuroendocrine, cardiovascular, and inflammatory stress responses in middle-aged men and women. Psychoneuroendocrinology 29, 593–611.

Task Force of the European Society of Cardiology, The North American Society of Pacing and Electrophysiology, 1996. Heart rate variability. Standards of measurement, physiological interpretation, and clinical use. Circulation 93, 1043–1065.

Van Tits, L.J., Michel, M.C., Grosse-Wilde, H., Happel, M., Eigler, F.W., Soliman, A., Brodde, O.E., 1990. Catecholamines increase lymphocyte beta 2-adrenergic receptors via a beta 2-adrenergic, spleen-dependent process. The American Journal of Physiology 258, 191–202.

Wahle, M., Stachetzki, U., Krause, A., Pierer, M., Hantzschel, H., Baerwald, C.G., 2001. Regulation of beta2-adrenergic receptors on CD4 and CD8 positive lymphocytes by cytokines in vitro. Cytokine 16, 205–209.

Willemsen, G., Ring, C., Carroll, D., Evans, P., Clow, A., Hucklebridge, F., 1998. Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic and cold pressor. Psychophysiology 35, 252–259.

Willemsen, G., Carroll, D., Ring, C., Drayson, M., 2002. Cellular and mucosal immune reactions to mental and cold stress: associations with gender and cardiovascular reactivity. Psychophysiology 39, 222–228.

Willemsen, G., Ring, C., McKeever, S., Carroll, D., 2000. Secretory immunoglobulin A and cardiovascular activity during mental arithmetic: effects of task difficulty and task order. Biological Psychology 52, 127–141.

Winzer, A., Ring, C., Carroll, D., Willemsen, G., Drayson, M., Kendall, M., 1999. Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic, cold pressor and exercise: effects of beta-adrenergic blockade. Psychophysiology 36, 591–601.

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Immune, endocrine and cardiovascular responses to controllable and uncontrollable acute stress

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Abstract

This study, using a triadic-yoked design, clarified the effects of controllability of acute stress on responses of immune, cardiovascular (heart rate and blood pressure), and cortisol activities. Forty-three women in their follicular phase completed a mental arithmetic task as a stressor in which controllability was manipulated by correct or yoked-bogus feedback. The task decreased proportions of CD3+ T cells, CD4+ T cells, and CD19+ B cells, whereas it increased the numbers of white blood cells, lymphocytes, natural killer (NK) cells, and NK cell activity (NKCA). Our main hypothesis that greater immune and cardiovascular responses to the task would be obtained under the uncontrollable condition than under the controllable condition was not supported. However, the uncontrollable stress condition, but not the controllable situation, led to higher correlations between heart rate or blood pressure, and various immune parameters. On the other hand, parameters of heart rate variability reflecting sympathetic and parasympathetic activities showed significant correlations only with NKCA. These results suggest that immune responses were most directly associated with cardiovascular activities under the uncontrollable condition.

Keywords: Stress; Controllability; Mental arithmetic; Psychoneuroimmunology

1. Introduction

There is much evidence that experimental acute stress tasks such as mental arithmetic influence the function of peripheral immunity. Previous studies have reported that innate immunity and mucosal immunity (e.g., the number of natural killer (NK) cells, NK cell activity (NKCA), and salivary secretory immunoglobulin A (S-IgA)[1]) are enhanced, whereas specific or acquired immunity (e.g., numbers of helper T cells and B cells) either does not change or decreases during mental arithmetic (Delahanty et al., 1996; Pike et al., 1997; Burleson et al., 1998; Willemsen et al., 1998, 2002; Ring et al., 1999, 2000; Bosch et al., 2001, 2002; Isowa et al., 2004; Kimura et al., in press; for review, see Segerstrom and Miller, 2004).

In addition, numerous studies have provided evidence that the nature and magnitude of the immune, endocrine, and cardiovascular responses induced by acute stress may depend on specific situational determinants such as controllability over stressors (Laudenslager et al., 1983; Maier et al., 1986; Weisse et al., 1990; Sieber et al., 1992; Peters et al., 1998, 1999, 2003). The effects of controllability have been widely studied in animal models using a triadic-yoked design (Seligman, 1975). Some previous studies using animal such as rat found that controllability of stress task was effective to physiological responses to it (Laudenslager et al., 1983; Nakata et al., 1996).

Several researchers have tried to extend these findings to human subjects (Weisse et al., 1990; Sieber et al., 1992; Gomez et al., 1994; Peters et al., 1998, 1999, 2003), though the results of these studies have been inconsistent. Weisse et al. (1990) reported decreased lymphocyte proliferation in

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[1] The concentration or secretion rate of S-IgA is an index of specific immunity function in local mucosal sites. However, the roles and characteristics of S-IgA are quite unique compared to those of general specific immunity represented by the production of the other subsets of immunoglobulin. Thus, we specifically refer to mucosal immunity for the value of S-IgA.

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response to concanavalin A (ConA) and PHA in subjects who had control over a stress task. Sieber et al. (1992) found decreased NKCA after uncontrollable stress but not after controllable stress. Gomez et al. (1994) found no effect of uncontrollability on various immune parameters. One possible explanation for the contradictory results may be that these experiments employed slightly different stress tasks of different durations. Furthermore, because most of the previous studies did not measure parameters of all three of the aspects of homeostasis (autonomic nervous, endocrine, and immune systems), it is difficult to examine in detail the interaction of these systems during controllable and uncontrollable acute stress. To our knowledge, only one study by Peters et al. (1999) examined the effects of controllability on the cardiovascular, endocrine, and immune systems of humans. They found that in vitro production of cytokine interleukin-6 (IL-6) was decreased after an uncontrollable stressor, and concluded that this phenomenon was induced by activation of the hypothalamus-pituitary-adrenocortex (HPA) axis. Although their finding is very valuable and important for examining the effect of controllability in acute stress, regrettably, the mediation of activity of the autonomic nervous system in the effects of controllability has not previously been reported.

Many previous experiments have demonstrated that the autonomic nervous system can react more rapidly than the HPA axis (Isowa et al., 2004; Burleson et al., 1998; Pike et al., 1997; Fokkema et al., 1988; Bohous et al., 1987). Moreover, Swenson and Vogel (1983) found that plasma NE and E levels in rats exposed to inescapable shock were higher than those in rats exposed to escapable shock, whereas the corticosterone level was the same in the two groups. Peters et al. (1998) reported that under an uncontrollable stressful condition, the level of plasma NE in humans increased more than it did under a controllable stressful condition, and that blood pressure under the uncontrollable condition was higher than that under the controllable condition. Such evidence suggests that controllability of an acute stressor will have a greater impact on autonomic parameters than on parameters of the HPA axis. In turn, it can be predicted that the autonomic activity induced by the controllability will have a rapid effect on peripheral immune functions. However, no study has directly examined such processes in an experimentally manipulated uncontrollable stressful situation.

Therefore, using a triadic-yoked design, we examined the effects of controllability on the responses of the human autonomic, endocrine, and immune systems to acute stress. For this purpose, we estimated the autonomic (sympathetic versus parasympathetic) activity through heart rate variability (HRV) and examined the effects of cardiovascular activity itself (heart rate (HR) and blood pressure (BP)) on immune parameters. On the basis of the previous studies described above, we predicted that the acute stress task would elicit prompt activation of the autonomic nervous system, and that this system, in turn, would mediate the enhancement of innate immunity (NKCA and proportions of NK cells in blood) and the suppression of specific immunity (proportions of T cells and helper T cells in blood). Specifically, we anticipated that the magnitude of the responses of the autonomic and immune parameters under the uncontrollable condition would be larger than that under the controllable condition.

2. Methods

2.1. Subjects

Forty-three female undergraduates in the Mie Prefectural College of Nursing (age range, 19–34 years; mean = 21.51, S.D. = 2.66) participated in the present study. Each participant was randomly assigned to one of three groups: a controllable stressors (C), an uncontrollable stressors (UC), or a no stressors (control) group. The C and UC groups were assigned 18 subjects, and the control group 7 subjects.

The mean BMI of the subjects was 21.03 kg/m^2 (S.D. = 2.22). None of the subjects were suffering from any chronic or oral illness, and none were taking medication known to influence immunity. In addition, no subjects were using oral contraceptives. Considering the effects of the menstrual cycle on the immune system, all subjects were required to measure their basal body temperature (BBT) daily for more than 3 months before the experimental sessions. They participated in the experiments during the late luteal and early follicular phases. In these periods, secretion of female sex hormones (progesterone and estrogen) is at low levels; thus, the influence of these hormones on the autonomic nervous and immune systems was minimized. For confirmation of the periods of the menstrual cycle, participants reported about both their current menstrual cycles and their BBT, and serum levels of estradiol (E_2) and progesterone were measured in all subjects on the days of the experiment. The mean value of estradiol was 50.87 pg/ml (S.D. = 55.31), and the mean value of progesterone was 0.96 ng/ml (S.D. = 1.78). In most cases, the hormone levels matched the levels expected based on the participant's self report. All subjects provided written informed consent. The Ethics Committee of the Mie Prefectural College of Nursing approved the present study.

2.2. Immunological measures

Blood samples for immunological determinations were collected in heparinized tubes. The numbers of total white blood cells (WBC), lymphocytes, monocytes, and granulocytes per sample were determined by standard means. Percentages of lymphocyte subsets were determined by flow cytometry (FACS Calibur; Becton-Dickinson, San Jose, CA). A whole-blood lysis method was used to stain the cell with the following pairs of Fluorescein isothiocyanate (FITC)/Phycoerythrin (PE) conjugated, isotype-matched

monoclonal antibodies (DAKO Inc., Carpinteria, CA): mouse IgG1, CD3+ T cells, CD3+/CD4+ helper T cells, CD3+/CD8+ cytotoxic T cells, CD3+/CD19+ B cells, and CD3+/CD16+/CD56+ NK cells.

A chromium release assay was used to determine natural killer cell activity (NKCA). Effector and ^{51}Cr-labeled K562 target cells were incubated for 3.5 h in 96-well round-bottomed plates. Wells contained effector and target cells at ratios of 20:1. Wells with K562 in medium alone or with 1N HCL were used to assess spontaneous and maximum release. Radioactivity was counted in a γ-counter and the percentage-specific lysis was determined according to the formula: (mean experimental cpm − mean spont. release cmp)/(mean maximal cmp − mean spont. release cmp) × 100.

To determine the volume of secreted saliva and the concentration of S-IgA, samples of unstimulated saliva were collected using cotton swabs (Salivettes; Sarstedt, Ltd., Leicester, UK). A cotton swab was placed underneath the tongue of each participant for 5 min. Subsequently, the cotton swab was removed and saliva was extracted from the cotton by centrifugation at 3.5×10^3 rpm for 10 min. Saliva was stored frozen in capped test tubes at −20 °C until assay. The S-IgA concentration (in salivain micrograms per milliliter) was determined by enzyme-linked immunoabsorbent assay using an IgA test (MBL Inc., Nagoya, Japan). The thawed saliva aliquots (10 μl) were diluted 40 times. Saliva samples were reacted with polystyrene beads that labeled the antihuman secretory component. After incubation at 37 °C for 1 h, the beads were washed twice and reacted with peroxidase standard antihuman IgA (rabbit IgG/Fab′) (second reaction). After incubation at room temperature for 1 h, the beads were washed three times, and then enzyme metrical fluid (orthophenylenediamine + 4 mM H_2O_2) (third reaction) was added. After incubation at room temperature for 30 min, the reaction was stopped by the addition of H_2SO_4. The reaction product was quantified spectrophotometrically at 492 nm with a microplate reader (model 550; Bio-Rad Inc., Hercules, CA). The S-IgA secretion rate (in micrograms per min) was calculated as the product of S-IgA concentration and saliva flow rate.

In this study, we measured the proportions of lymphocyte subsets and S-IgA at five points (baseline, just after the task, and 15 min, 30 min, and 1 h after the task) and NKCA at three points (baseline, just after the task, and 1 h after the task). Sampling points for NKCA were fewer mainly due to the limits of the Mie Prefectural College of Nursing on the total allowable quantity of blood drawn: 5 ml blood is necessary for the assay of NKCA, but only 1 ml blood is necessary to measure other immune indices. In addition, the sampling timing for the NKCA assay was determined based on a previous study in which NKCA was increased immediately after a 15-min stress task and decreased 15 min after termination of the task (Peters et al., 1999).

2.3. Cortisol measures

Samples of saliva for measurement of the concentration of salivary cortisol were obtained by the same method as that used for S-IgA and stored at −20 °C until assay. The concentration of cortisol in the saliva (in micrograms per milliliter) was determined by an enzyme-linked immunoabsorbent assay. Ninety-six-well Costar™ microplates precoated with anti-Cortisol rabbit antibody were prepared. A saliva sample of 50 μl was added to each well in triplicate. Then an enzyme conjugate of 50 μl was added to each well and incubated at room temperature for 1 h. After incubation, each well was washed three times. A substrate of 50 μl was added to each well and incubated at room temperature for 30 min. Subsequently, 1N HCL 50 μl was added to each well to stop the enzyme reaction. To measure the concentration of cortisol, the plate was read at 450 nm with a microplate reader (model 550; Bio-Rad Inc.).

2.4. Cardiovascular measures

Cardiodynamic activity was recorded by electrocardiography (ECG) and non-invasive finger blood pressure (FINAP) measurements. To determine HR, ECG was recorded using an MP 100 system (BIOPAC Systems Inc., Santa Barbara, CA). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded through the finger cuff of a Portapres Model 2 (TNO Biomedical Instrumentation Inc., Amsterdam, The Netherlands) attached to the third finger of the non-dominant arm of each subject. Each indicator was recorded continuously during the task and the rest periods. The ECG data were subsequently analyzed to yield HRV. The data were first subject to visual inspection, and only completely artifact-free data were used for estimation of the R–R intervals. The R–R interval data were resampled at 4 Hz to obtain equidistant time series values. A power spectrum density was then obtained through a fast Fourier transformation of the tachogram. In connection with the fast Fourier transformation, the data were detrended linearly and filtered through a rectangular window. We studied the integral of the power spectrum in two major frequency bands, a high frequency band (HF, 0.15–0.5 Hz) and a low frequency band (LF, 0.05–0.15 Hz). The former is correlated with respiratory sinus arrhythmia and is exclusively attributable to parasympathetic influence, and the latter mirrors the baroreceptor feedback loop that controls blood pressure and appears to reflect both sympathetic and parasympathetic activity (Sayers, 1973). In the present study, we examined the HF component expressed as a percentage of the total power in the spectrum (Perini et al., 2000) and the relative contributions of LF and HF power (LF/HF), which reflects sympathovagal balance. Two subjects in the C group, two subjects in the UC group, all subject in the control group were excluded from analyses of HRV due to technical problems. Thus the HRV analyses were performed for 16

subjects in the C group and 16 subjects in the UC group. Analyses of ECGs and FINAP waveforms were performed using the software package AcqKnowledge for the MP 100.

2.5. Psychological measures

The subjects were asked to evaluate subjectively the intensity of their stress, physical fatigue, and mental fatigue on visual–analog scales (0–100%). Additionally, they were asked to rate their sense of control over the task on a scale from 0% (not controllable at all) to 100% (perfectly controllable). In addition, they completed a Japanese version (Kazuhito et al., 1990) of the Profile of Mood States (POMS) (Usala and Hertzog, 1989) and a Japanese version (Katsuharu and Tadanobu, 1982) of the State-Trait Anxiety Inventory (STAI) (Speilberger et al., 1970). POMS is composed of six sub-scales (Tension-Anxiety (T-A), Depression-Dejection (D), Anger-Hostility (A-H), Vigor (V), Fatigue (F), and Confusion (C); 65 items). STAI is composed of two sub-scales to measure state and trait anxiety and consists of 40 items.

2.6. Stress task and manipulation of controllability

The subjects in the C and the UC groups performed mental arithmetic for 15 min. The subjects were told to add the currently displayed number (from 2 to 9) to the next one shown on the PC monitor, and to indicate the last digit of the resulting number by pressing a key (from 0 to 9). Each number was displayed for 500 ms and followed by a 1500-ms interval. The task included 34 sets (one set consists of 10 answers). During the task, bursts of aversive noise (approximately 100 dB) were delivered continuously when the error rate exceeded 20% in a set, and the noise was stopped when the rate of correct answers exceeded 80% in the next set. Subjects in the C and UC groups were told that they had to calculate a correct answer 90% of the time, and that if their accuracy rate was lower than this, none of their data would be used for the analysis.

As a manipulation of controllability, the noise was administered to the subjects in the C group. On the other hand, in the UC group the noise yoked to the C group was administered irrespective of the subjects' performance. Thus, subjects in the UC group could not stop the noise by achieving a high rate of correct answers. The subjects in the control group did not perform the mental arithmetic task and thus received no aversive noise.

2.7. Procedure

The subjects were instructed to eat a light breakfast on the morning of the experiment, and caffeine-containing beverages were not allowed. Also, the subjects were told to paste a monoanesthetic seal (PENLES; Wyeth Lederle Inc., Tokyo, Japan) at the location of the cannula insertion in their arms about 1 h before the experimental sessions to reduce pain. Subjects suffering from an infectious illness within 2 weeks of the experiment were rescheduled.

The experimental sessions were composed of a mental arithmetic task and four rest periods. The subjects were tested individually between 9:00 a.m. and 12:00 p.m. in a temperature- and humidity-controlled room. After a subject entered the experiment room, a cannula was inserted into the forearm vein of her non-dominant arm. Next, electrodes for electrocardiographic measurements and a finger cuff for blood pressure recording were attached. For the next 15 min, the subject filled out psychological questionnaires. After a rest period of 15 min, the first blood sample (for assays of female sex hormones and immunological parameters) and the first saliva sample (for assays of S-IgA and cortisol) were taken. Next, instructions were given for the mental arithmetic task, and the subjects were allowed to practice the mental arithmetic task for 2 min. The subjects then performed the mental arithmetic task for 15 min. Immediately after the task, the second blood and saliva samples were taken, and each subject filled out the questionnaire again. After each rest period (15, 30, and 60 min), the third, fourth, and fifth blood and saliva samples were taken, respectively, and the questionnaires were filled out. Autonomic indices (ECG and BP) were measured continuously throughout the experimental session. After the end of the procedure, the electrodes, blood-pressure cuff, and cannula were removed, and the subjects were fully debriefed and thanked. Each subject was paid 2400 Japanese yen for participating in the study.

2.8. Statistical analysis

Prior to statistical analysis, the mean values of HR, SBP, and DBP were calculated for the last 5 min of the pre-experimental baseline period, for the periods during the stress task (0–5 min, 5–10 min, and 10–15 min), and for the last 5 min of each rest period after the task. Means of the cardiovascular parameters were determined every 5 min to examine their temporal variations during the stress task (e.g., habituation to the task). The cardiovascular data were analyzed using repeated-measures analyses of variance (ANOVAs): Group (C, UC, and control group) × Period (baseline, $\text{stress}_{5\ \text{min}}$, $\text{stress}_{10\ \text{min}}$, $\text{stress}_{15\ \text{min}}$, $\text{rest}_{15\ \text{min}}$, $\text{rest}_{30\ \text{min}}$, $\text{rest}_{60\ \text{min}}$). The immune, endocrine, and psychological measures and the HF components of HRV and the LF/HF ratio were analyzed using repeated-measures ANOVAs: Group × Period (baseline, stress, $\text{rest}_{15\ \text{min}}$, $\text{rest}_{30\ \text{min}}$, $\text{rest}_{60\ \text{min}}$). The components of HRV were calculated during 15-min epochs of each baseline, stress and rest period. The Greenhouse–Geisser epsilon correction factor, ε (Jennings and Wood, 1976), was used where appropriate. Corrected degrees of freedom are reported; the P-values reflect the epsilon correction. In cases where significant interactions were found in the ANOVAs, post hoc analyses using LSD tests ($P < 0.05$) were conducted to examine which combinations of data points differed significantly. For perception of subjective controllability,

a Student's t-test was used on only the C and UC groups, both of which had performed the mental arithmetic task. For each group, Pearson correlation coefficients were computed among change scores (scores at the stress period − scores at the baseline) of these indices to examine the relationship between immune, cardiovascular, and endocrine reactivity. Additionally, analyses comparing the strength of correlation coefficients between the C and UC groups were carried out using z-scores of the normal distribution for all correlation coefficients that showed significant correlations in the C group, the UC group, or both.

3. Results

3.1. Immunological measures

The immune data at the baseline, stress, and rest periods are summarized in Table 1. Fig. 1 illustrates changes in the percentages of CD3+ T cells, NK cells, B cells, and NKCA. The main effects of Period were significant for WBCs, lymphocytes, NK cells, NKCA, CD3+ T cells, CD19+ B cells, CD4+ T cells, and CD8+ T cells (Fs(1–2,34–113) = 9.47–50.11, Ps < 0.001).

A previous study has shown that salivary flow is often correlated with a change in the concentration of S-IgA (Stone et al., 1987). Because the present data also yielded a significant negative correlation between S-IgA concentration and salivation ($r(42) = -0.523$, $P < 0.01$), a finding in accord with previous research (Herbert and Choen, 1993), the secretion rate of S-IgA was calculated by multiplying the saliva S-IgA concentration (μg/ml) and saliva volume (ml/min) for statistical analysis. In parameters of mucosal immunity, the main effects of Period were significant for S-IgA concentration and S-IgA secretion rate ($F(34,109) = 2.78$, $P < 0.05$, $F(3,111) = 3.59$, $P < 0.05$). In addition, the main effects of Group were significant for S-IgA concentration ($F(2,36) = 5.65$, $P < 0.01$).

Table 1
Means (S.D.s) of immunological measures and results of ANOVAs

	Group	Baseline	Stress	Rest 15 min	Rest 30 min	Rest 60 min	N	Effect[a]
WBC ($\times 10^3/\mu l$)	C	5.01 (1.46)	5.29 (1.45)	5.19 (1.42)	5.37 (1.44)	5.38 (1.47)	17	
	UC	4.45 (1.10)	4.73 (1.06)	4.62 (1.03)	4.71 (1.03)	4.78 (1.04)	18	Period**
	Control	3.85 (0.45)	3.95 (0.48)	4.08 (0.46)	4.13 (0.50)	4.17 (0.46)	6	
Lymphocyte ($\times 10^3/\mu l$)	C	1.59 (0.45)	1.76 (0.51)	1.61 (0.46)	1.72 (0.50)	1.77 (0.55)	17	
	UC	1.44 (0.23)	1.66 (0.33)	1.57 (0.25)	1.59 (0.28)	1.69 (0.33)	18	Period**
	Control	1.15 (0.36)	1.23 (0.12)	1.33 (0.15)	1.38 (0.12)	1.69 (0.42)	6	
Monocyte ($\times 10^3/\mu l$)	C	0.46 (0.21)	0.51 (0.20)	0.47 (0.19)	0.45 (0.18)	0.47 (0.16)	17	
	UC	0.44 (0.18)	0.43 (0.19)	0.40 (0.15)	0.46 (0.19)	0.41 (0.16)	18	n.s.
	Control	0.43 (0.12)	0.43 (0.15)	0.42 (0.13)	0.40 (0.06)	0.30 (0.10)	6	
Granulocyte ($\times 10^3/\mu l$)	C	2.95 (1.08)	3.02 (1.01)	3.11 (1.11)	3.2 (1.16)	3.14 (1.11)	17	n.s.
	UC	2.56 (1.02)	2.64 (1.03)	2.65 (0.98)	2.66 (0.91)	2.67 (0.86)	18	
	Control	2.27 (0.42)	2.28 (0.41)	2.33 (0.48)	2.3 (0.49)	2.3 (0.43)	6	
CD3+ CD4+ (%)	C	49.68 (7.53)	42.47 (10.48)	47.09 (7.55)	45.90 (7.21)	44.85 (6.32)	17	
	UC	48.29 (6.68)	39.57 (7.78)	44.45 (7.03)	44.58 (8.07)	43.81 (6.98)	18	Period** Group ×
	Control	46.73 (3.45)	45.45 (4.28)	44.62 (3.33)	43.86 (3.68)	42.64 (4.34)	6	Period*
CD3+ CD4+ (%)	C	28.66 (6.85)	27.25 (6.16)	28.52 (6.21)	28.11 (5.84)	27.87 (5.72)	17	
	UC	30.41 (5.74)	29.09 (5.53)	30.20 (5.58)	29.59 (5.26)	29.40 (5.33)	18	Period*
	Control	28.69 (2.94)	27.38 (3.42)	27.92 (3.50)	27.58 (3.69)	27.40 (3.44)	6	
Saliva flow rate (ml/min)	C	0.24 (0.16)	0.29 (0.10)	0.27 (0.14)	0.22 (0.13)	0.21 (0.11)	17	
	UC	0.28 (0.14)	0.30 (0.10)	0.28 (0.12)	0.23 (0.12)	0.24 (0.14)	18	n.s.
	Control	0.20 (0.14)	0.17 (0.16)	0.13 (0.14)	0.15 (0.15)	0.22 (0.12)	7	
sIgA secretion rate (μg/min)	C	12.98 (7.41)	15.23 (4.45)	13.86 (6.55)	10.97 (5.97)	11.03 (5.57)	17	
	UC	15.92 (5.73)	16.03 (2.84)	14.96 (5.04)	13.11 (5.09)	14.62 (8.50)	16	Period*
	Control	11.96 (9.16)	11.17 (12.11)	7.81 (8.05)	8.25 (7.90)	13.24 (7.60)	7	
sIgA concentration (μg/ml)	C	56.54 (8.15)	54.59 (7.40)	52.18 (5.37)	52.46 (5.03)	54.39 (6.09)	17	
	UC	54.20 (8.19)	51.97 (4.83)	51.08 (3.87)	53.28 (4.93)	56.03 (8.02)	16	Group**, Period*
	Control	56.58 (9.14)	67.04 (16.64)	61.83 (11.25)	60.88 (11.12)	62.23 (9.93)	7	
Cortisol (ng/ml)	C	2.83 (0.67)	2.79 (0.59)	2.60 (0.76)	2.53 (0.76)	2.95 (0.58)	17	
	UC	2.79 (0.56)	2.61 (0.62)	2.52 (0.72)	2.68 (0.59)	2.68 (0.57)	17	Period*
	Control	2.89 (0.39)	2.73 (0.66)	2.52 (0.79)	2.48 (0.92)	2.77 (0.96)	5	

C: controllable group; UC: uncontrollable group; Control: control group.
[a] Main effects and interactions as results of ANOVAs.
* $P < 0.05$.
** $P < 0.01$.

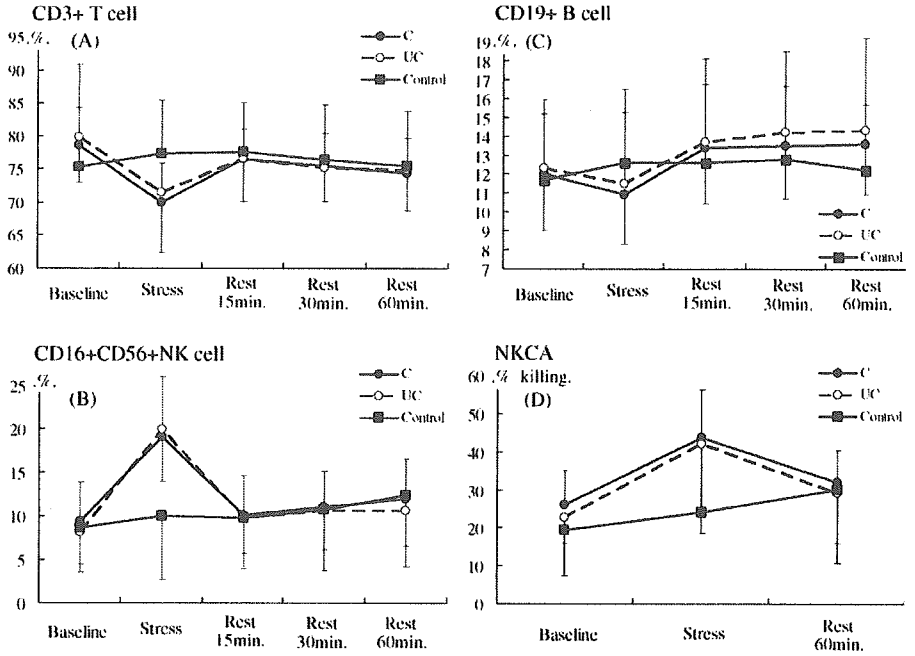

Fig. 1. Percentage of CD3+ (A); NK cells (B); CD19+ (C); and NKCA (D) at the five measurement points. Vertical bars indicate standard deviations. C: controllable group; UC: uncontrollable group; Control: control group.

In the C and the UC groups, indices of innate immunity (percentages of NK cells and NKCA) were significantly higher, and indices of specific immunity (percentages of CD3+, 19+, CD4+ and CD8 cells) were significantly lower, after the stress task than at the respective baseline (see Fig. 1). The C and UC groups differed significantly from the control group in all immune measures during the task periods (Fs(4–8,34–156) = 2.28–9.56, $Ps < 0.001$–0.05) but not during the rest periods ($Ps > 0.01$). Notably, there were no significant differences between the C and the UC groups in any indices.

3.2. Cardiovascular measures

Changes in cardiovascular indices are illustrated in Fig. 2. ANOVAs yielded significant main effects of Group for HR and DBP ($F(2,39) = 4.09$, $P < 0.05$, $F(2,39) = 4.90$, $P < 0.05$). Additionally, there were significant main effects of Period for HR, SBP, and DBP (Fs(1–2,64–83) = 25.62–38.85, $P < 0.001$). In the C and UC groups, all cardiovascular parameters were significantly higher during the stress task than at the respective baseline. These parameters remained at high levels and did not change during the task, suggesting that habituation did not take place during the 15-min task. The C and UC groups differed significantly from the control group in all cardiovascular measures during the task periods ($Fs(12,234) = 4.33$–20.93, $P < 0.001$) but not during the rest periods ($Ps > 0.01$). On the other hand, the main effects of Period were shown in HRV parameters: the HF component of HRV was significantly reduced during the task period compared with the baseline ($F(1,30) = 13.26$, $P < 0.01$), and the LF/HF ratio showed a significant increase in the task period ($F(1,30) = 6.07$, $P < 0.01$). The decrease in the HF component and the increase in the LF/HF ratio suggest that the mental arithmetic task induced a dominant state of sympathetic activity. For the cardiovascular measures, there were no significant differences between the C and the UC groups in any indices.

3.3. Cortisol measures

The cortisol levels at the baseline, after the stress task, and during the rest periods are presented in Table 1. In all groups, the cortisol level continued to significantly decrease to the $rest_{30\ min}$ compared with the baseline, and significantly increased after the $rest_{60\ min}$ compared with the baseline. There were no significant main effects of Group and no significant interactions between Group and Period. Notably, there were no significant differences between the C and the UC groups.

3.4. Psychological measures

The psychological data at baseline, after the stress task, and during the rest periods are presented in Table 2. Concerning the perceived controllability of the groups, the mean value of this parameter was 49.22 (S.D. = 17.20) in the C group, and 38.56 (S.D. = 16.64) in the UC group. That of the C group was marginally higher than that of the UC group ($t(34) = 1.77$, $P = 0.08$). ANOVAs yielded significant main effects of Group for perception of stress, state anxiety, and T-A, D, A-H, C of POMS (Fs(2,34–39) = 3.53–6.72,

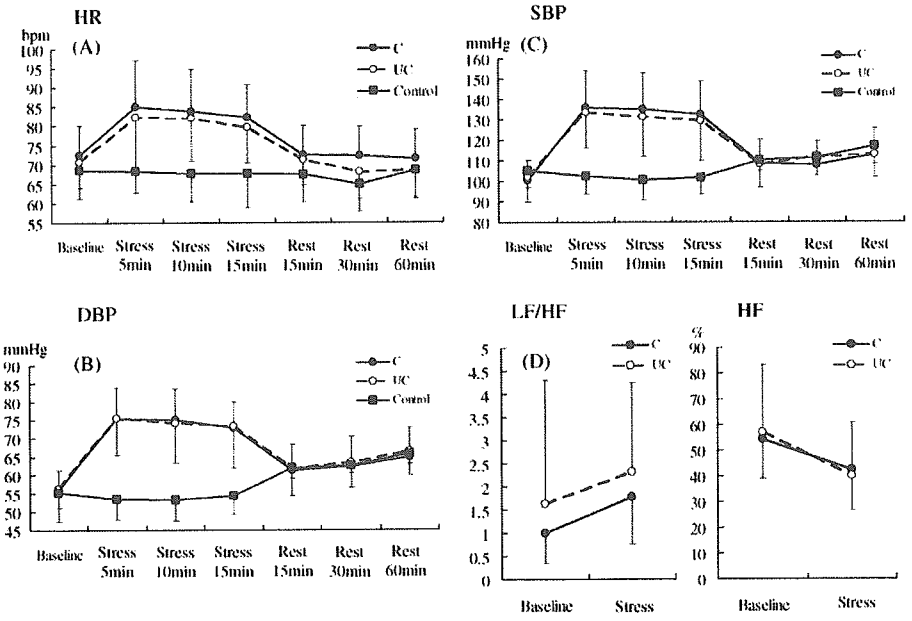

Fig. 2. HR (A); DBP (B); and SBP (C) at the seven measurement points; and HF and LF/HF (D) during baseline and task periods. Vertical bars indicate standard deviations. Vertical bars indicate standard deviations. C: controllable group; UC: uncontrollable group; Control: control group.

$Ps < 0.01$–0.05). In addition, there were significant main effects of Period for physical fatigue, mental fatigue, perception of stress, state anxiety, and T-A, D, A-H, V, C of POMS ($Fs(1–2,54–109) = 3.83–18.44$, $Ps < 0.001$–0.05). Significant Group × Period interactions were observed for mental fatigue, perception of stress, state anxiety, and T-A, V, C of POMS ($Fs(4–8,68–156) = 2.72–4.92$, $Ps < 0.001$–0.05). Post hoc analyses ($P < 0.05$) indicated that mental

Table 2
Means (S.D.s) of psychological measures and results of ANOVAs

	Group	Baseline	Stress	Rest 15min	Rest 30min	Rest 60min	N	Effect[a]
Perception of stress	C	26.29 (25.80)	46.59 (21.36)	35.59 (21.60)	36.29 (25.40)	38.89 (26.27)	17	Group*, Period**,
	UC	26.06 (21.53)	53.83 (27.89)	26.22 (15.44)	20.94 (14.74)	20.72 (17.83)	18	Group × Period**
	Control	10.43 (9.68)	16.86 (12.39)	18.29 (14.45)	19.43 (14.26)	18.43 (15.75)	7	
Mental fatigue	C	27.29 (23.59)	41.71 (21.67)	36.94 (20.64)	37.29 (24.89)	39.59 (24.62)	17	Period**,
	UC	33.67 (24.15)	53.33 (23.93)	33.22 (18.27)	25.83 (16.88)	25.39 (19.01)	18	Group × Period**
	Control	16.29 (14.60)	21.86 (17.49)	23.14 (18.22)	24.29 (17.54)	25.43 (17.89)	7	
STAI								
State-anxiety	C	41.82 (8.20)	48.53 (8.55)	40.76 (7.44)	40.41 (7.95)	39.35 (8.02)	17	Group**, Period**,
	UC	38.83 (5.26)	50.83 (10.06)	37.78 (4.81)	37.17 (4.63)	34.72 (5.49)	18	Group × Period**
	Control	36.57 (3.15)	33.86 (4.34)	32.71 (4.31)	33.14 (5.27)	31.14 (5.37)	7	
POMS								
Tension-anxiety	C	10.00 (6.77)	10.21 (7.01)	–	–	6.71 (5.89)	14	Group**, Period**,
	UC	13.19 (4.83)	18.13 (8.34)	–	–	7.75 (3.40)	16	Group × Period**
	Control	8.71 (5.47)	4.57 (2.57)	–	–	3.14 (2.79)	7	
Vigor	C	12.25 (6.07)	8.38 (6.04)	–	–	9.25 (6.14)	16	Period**,
	UC	13.76 (4.28)	8.35 (5.20)	–	–	12.41 (5.36)	17	Group × Period*
	Control	10.14 (4.41)	10.14 (4.41)	–	–	10.14 (3.53)	7	
Fatigue	C	8.06 (6.94)	7.56 (7.04)	–	–	8.13 (5.86)	16	Group × Period*
	UC	12.53 (7.09)	10.24 (7.00)	–	–	7.47 (4.62)	17	
	Control	4.86 (3.53)	5.29 (3.55)	–	–	5.14 (3.93)	7	

Only the parameters that interaction was found in is shown in Table 2; An effect of periods was found in the following parameters: Physical fatigue** and Depression-Dejection**, Confusion** and Anger-Hostility** of POMS; An effect of group was found in the following parameters: Depression-Dejection* and Anger-Hostility* of POMS; There was no significantly all effect for Trait-anxiety. C: controllable group; UC: uncontrollable group; Control: control group.

[a] Main effects and interactions as results of ANOVAs.

* $P < 0.05$.

** $P < 0.01$.

Table 3
Correlations between changes in immune, cardiovascular, and endocrine measures (each group)

Controllable	$HR_{5\ min}$	$HR_{10\ min}$	$HR_{15\ min}$	$SBP_{5\ min}$	$SBP_{10\ min}$	$SBP_{15\ min}$	$DBP_{5\ min}$	$DBP_{10\ min}$	$DBP_{15\ min}$	HF	Cortisol
Granulocyte											0.07
CD3+	0.01	−0.15		−0.04	−0.16	−0.14	0.00	−0.04	−0.04		
CD4+				−0.24	−0.28	−0.28	−0.14	−0.19	−0.16		
CD8+											
CD19+	−0.46	−0.43	−0.41	−0.35	−0.39	−0.42	−0.19	−0.12			
NKcell	0.19	0.33	0.32	0.19	0.33	0.32	0.08	0.10	0.15		0.20
NKCA	0.78*									0.23	
Cortisol	0.09	−0.04									
Granulocyte											−0.55*
CD3+	−0.54*	−0.51*		−0.63**	−0.66**	−0.64**	−0.55**	−0.69**	−0.68**		
CD4+				−0.58**	−0.74**	−0.70**	−0.51**	−0.70**	−0.73**		
CD8+											
CD19+	−0.46**	−0.44*	−0.42**	−0.43**	−0.45*	−0.42**	−0.32*	−0.31*			
NKcell	0.64**	0.58**	0.52**	0.65**	0.69**	0.68**	0.49*	0.62**	0.60**		0.59*
NKCA	0.33									−0.79**	
Cortisol	0.54*	0.53*									

The underline indicates significant differences between correlation coefficients in the controllable vs. uncontrollable groups. In control group, there were significant correlations between $SBP_{10\ min}$ and Monocyte ($r = 0.80^*$), CD19+ ($r = -0.77^*$), or NKCA ($r = 0.91^*$).

* $P < 0.05$.

** $P < 0.01$.

fatigue of the UC group and perception of stress and state anxiety of the C and the UC groups were higher than those of the control group after the task. In POMS, TA of the UC groups was higher than that of the C and control groups after the task.

3.5. Associations among immune, cardiovascular, and endocrine reactivity

Controllability was not shown to have any effect on cardiovascular or immune parameters by ANOVA. Thus, to further examine the effects of controllability on functional associations among the autonomic nervous, endocrine, and immune systems during the acute stress task, we performed correlation analyses among changes in cardiovascular, endocrine, and immune parameters in each experimental group separately. Furthermore, to examine the temporal characteristics of the influences of autonomic activity on the immune functions, we determined the mean changes of cardiovascular parameters in three time windows during the task: 0–5 min, 5–10 min, and 10–15 min; the correlations between these cardiovascular parameters and the immune parameters were then calculated for each time window. The results in the C and UC groups are presented in Table 3. There were no significant correlations except those between HR and NKCA in the C group. On the other hand, in the UC group, there were many strong correlations among the endocrine, cardiovascular, and immune measures (see Table 3). To prove that these correlations in the UC group were not merely artifacts, we performed a scatterplotting of the SBP and immune parameters in the UC and the C group, respectively, as shown in Fig. 3. All cardiovascular measures in the UC group correlated positively with the change in the percentage of NK cells, and negatively with the change in the percentage of CD3+ T cells and CD19+ B cells, and blood pressure correlated positively with the change in the percentage of NK cells, and negatively with the change in the percentages of CD3+ T cells, CD4+ T cells, and CD19+ B cells. Further, remarkably high correlations of the autonomic and the immune parameters were found continuously from the initiation to the end of the acute stress task. The HF component of HRV showed a remarkably significant negative correlation with NKCA ($r = -0.79$, $P < 0.01$) only in the UC group, suggesting that reduction of vagal activity led directly to upward regulation of the cytotoxicity of NK cells in the uncontrollable stress condition.

Analyses comparing the strength of the correlation coefficients between the C and UC groups showed that the correlation coefficients relating SBP or DBP at all time points and CD3+ T cells, CD4+ T cells, or NK cells in the UC group were significantly larger than those in the C group ($zs = 1.99$–2.39, $P < 0.05$).

4. Discussion

4.1. Cardiovascular and immune reactions to acute stress

During the mental arithmetic task, enhanced cardiovascular responses (HR, SBP, and DBP) were observed. Additionally, results of HRV parameters (LF/HF ratio and HF) suggested activation of the sympathetic nervous system and deactivation of the parasympathetic nervous system. Furthermore the proportion of NK cells in peripheral blood increased and the proportions of T cells, helper T cells, and B cells in blood decreased. Such lymphocyte trafficking by

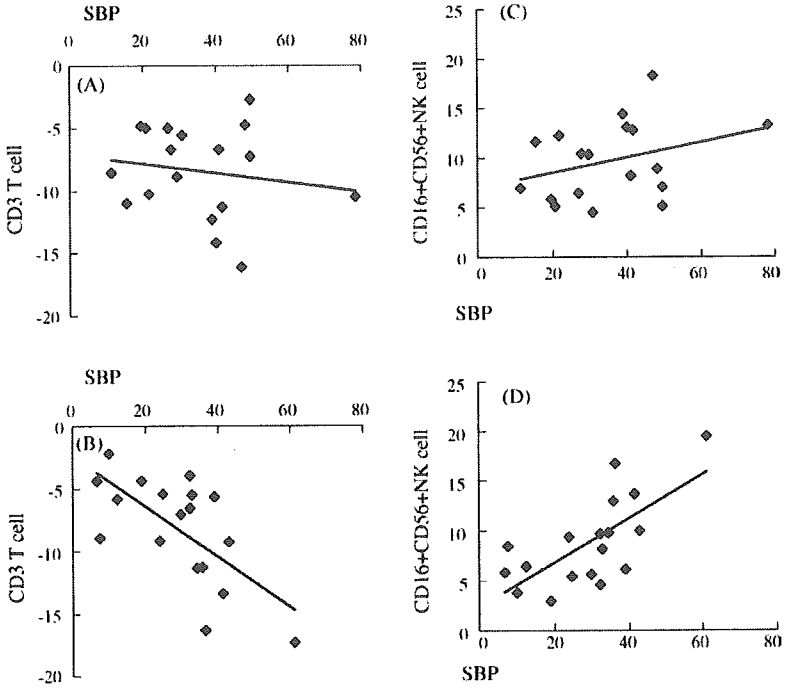

Fig. 3. Scatterplotting of SBP and immune parameters in controllable and uncontrollable groups. A and C: controllable group; B and D: uncontrollable group.

acute stress has been suggested to be adaptive for survival (Engler et al., 2004; Segerstrom and Miller, 2004). An increase of antigen-nonspecific peripheral innate immune cells, here represented by NK cells, might be interpreted as preparation for the potential invasion of bacteria or other antigens by injuring accompanying fight/flight behaviors. A decrease of T cells and B cells might represent trafficking of such cells into the lymph nodes, where helper T cells are sensitized to antigens and cascades of antigen-specific immune responses are initiated. Additionally, the increased NKCA and S-IgA secretion rate by the acute stress task might represent enhancement of functional aspects of innate and mucosal immunity, which might also be adaptive for survival under acute stress. These responses in cardiovascular and immune parameters are consistent with previous studies (Delahanty et al., 2000, 1996; Willemsen et al., 2002; Isowa et al., 2004; Kimura et al., 2005).

It has been suggested that increase of NK cells during acute stress should be mediated by increased blood flow and blood pressure, and effects by E and NE through surface receptors (mainly, β2- and α-adrenoreceptors) (Benschop et al., 1993; Mills et al., 2000; Farag et al., 2002). Although we did not measure catecholamines directly, HRV parameters were evaluated as indirect indices of autonomic activity. As our results indicated, changes in the proportion of NK cells robustly correlated with cardiovascular indices whereas they showed only a limited correlation with HRV. These results suggest that the redistribution of NK cells observed in this study is considered to be mainly caused by an increase of blood pressure rather than an enhancement of autonomic activity. Because blood pressure levels are regulated not only by autonomic activity but also many other endocrine factors (e.g., vasopressin, atrial natriuretic peptide, opioids, oxytocin, angiotensin and so on), the observed increase of peripheral NK cells likely took place as the result of the integrated effects of such cardiovascular and neuroendocrine activities. On the other hand, because the physical effects of increased blood pressure cannot explain the decrease of T cells and B cells in peripheral circulation and the increase of NKCA, such immune changes under acute stress might be mediated mainly by neuroendocrine factors. This speculation is supported by reports that T cells and B cells have receptors for various neuroendocrine substances (Landmann et al., 1984; Van Tits et al., 1990; Kohm and Sanders, 2001).

4.2. *Effects of controllability*

Contrary to our prediction, no effects of controllability of the acute stress task were observed in any immune parameters. These results are consistent with the previous study by Peters et al. (1998), who reported that there were no effects of stress controllability on immune parameters except IL-6 concentration. On the other hand, although Peters et al. (1998, 1999) suggested that an effect of controllability appeared in cortisol level as an index of the HPA axis, we did not observe any effects of stress controllability in cortisol. Considering the null effect in the present study and the inconsistent and mixed results in previous studies (Weisse et al., 1990; Sieber et al., 1992; Gomez et al., 1994; Peters et al., 1998, 1999, 2003), immune and endocrine reactivity to acute stress might not be as sensitive to the controllability of stressors as previously thought.

However before reaching any definitive conclusions, some caveats regarding the present study must be recognized. First, in this study, the difference of perceived controllability between the C and UC conditions was only marginally significant, and thus we must suspend the conclusion that the experimental manipulation of controllability was substantially valid. Second, effects of controllability on the immune system and the HPA axis might have been concealed by the relatively wide inter-individual differences and the small sample size. Third, as regards to the HPA axis, circadian variation might have affected the results of cortisol in this study. The observed trend that the concentration of cortisol decreased according to the progress of the experimental session in all groups suggests such effects of circadian variation. During the time period in the present experiment (i.e., 9:00 a.m.–2:00 p.m.), cortisol levels usually drop; this circadian variation might be contaminated in the reported results. Further studies to control such factors more rigorously are awaited.

4.3. Associations among immune, cardiovascular, and endocrine reactivity in uncontrollable acute stress

As unpredicted results, correlations between the cardiovascular parameters, specifically SBP and DBP, and the immune parameters, especially T cells, helper T cells, and NK cells, were prominent in the UC condition, whereas few and slight correlations among those parameters were found under the C condition (see Table 3). In addition, beginning at 5 min after the start of the task and continuing until the end of the task, uncontrollability served to consistently strengthen the association between cardiovascular and immune parameters. Further, the scatterplotting of indices of blood pressure and immune parameters (see Fig. 3) appeared to suggest that those effects were not just artifacts. Taken together, the findings in the present study for the first time suggest that uncontrollability of acute stress, at least in some situations, might have the effect of strengthening the correspondence between the autonomic nervous and immune systems rather than increasing the reactivity in each system.

Recent neuroanatomical and functional neuroimaging findings can offer suggestions in considering the mechanisms underlying such effects. Much evidence has indicated that the contingency between a stimulus and reward or punishment in a situation should be represented in the orbitofrontal cortex (OFC) in the frontal lobe (O'Doherty et al., 2001a,b). The OFC receives inputs from sensory associative cortices of all modalities and from limbic structures such as the amygdala and the hippocampus (Carmichael et al., 1994; Carmichael and Price, 1995), and has rich connections to the hypothalamus and the periaqueductal gray matter (PAG), that have been implicated in the modulation of autonomic and endocrine functions (Price, 1999; Kringelbach and Rolls, 2004). Based on this neuroanatomical architecture, the OFC might evaluate how one can control the current situation and regulate autonomic and endocrine systems to optimize levels of their activity to meet the current demand. In an uncontrollable situation, when the situation is evaluated as still somewhat controllable and worthy of allocating more resources, sympathetic nervous and endocrine systems might be more activated, and thus enhancement of innate immunity and suppression of specific immunity might be emphasized through increased secretion of catecholamines and glucocorticoid (Peters et al., 1998; Swenson and Vogel, 1983). On the other hand, when the situation is evaluated as totally uncontrollable, the OFC might tune the activity of autonomic and endocrine systems to minimum levels to avoid allocation of resources in vain, in order to increase the chances of survival. In such a case, influences on innate and specific immunity should also decrease. Such processes will lead to relatively wide individual differences in levels of activity in the autonomic, endocrine, and immune systems. Consequently, the correspondence between autonomic activity and immune activity might be strengthened in an uncontrollable stress situation. While, this is speculation because measurement of brain activity was not conducted in this study, our previous research has documented significant activation in lateral and medial OFC under uncontrollable compared with controllable stress using position emission tomography (PET) (Ohira et al., 2004).

4.4. Limitations of the study

First, the relatively small sample size ($N = 43$) and large standard deviation in each parameter suggest that the observed effects in this study might not be robust, although they were statistically significant. Second, the levels of S-IgA secretion at baseline and after stress treatment in the current study were lower than those observed in previous studies (Willemsen et al., 1998; Ring et al., 1999). This inconsistency may be attributable to differences in the methodologies. The S-IgA assay employing ELISA in the studies of Willemsen et al. (1998) and Ring et al. (1999) measured the volume both of IgA monomer of fragment of IgA and S-IgA. However, we measured only the volume of S-IgA secreted in saliva. In addition, values of S-IgA reported in our previous experiments (Isowa et al., 2004; Kimura et al., 2005) are similar to values of S-IgA in the present study. Third, it is still unclear whether the reported results are limited for the mental arithmetic task or can be generalized for other acute stress tasks. The present findings must be replicated using other acute stress tasks and manipulations of controllability over the tasks.

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References

Benschop, R.J., Oostveen, F.G., Heijnen, C.J., Ballieux, R.E., 1993. β2-Adrenergic stimulation causes detachment of natural killer cells from cultured endothelium. European Journal of Immunology 23, 3242–3247.

Bohous, B., Benus, R.F., Fokkema, D.S., Koolhaas, J.M., Nyakas, C., van Oortmerssen, G.A., Prins, A.J.A., de Ruiter, A.J.H., Scheurink, A.J.W., Steffens, A.B., 1987. Neuroendocrine states and behavior and physiological stress responses. Progress in Brain Research 72, 57–70.

Bosch, J.A., de Geus, E.J.C., Kelder, A., Veerman, E.C.I., Hoogstraten, J., Nieuw Amerongen, A.V., 2001. Differential effects of active versus passive coping on secretory immunity. Psychophysiology 38, 836–846.

Bosch, J.A., Ring, C., de Geus, E.J.C., Veerman, E.C.I., Nieuw Amerongen, A.V., 2002. Stress and secretory immunity. International Review of Neurobiology 52, 153–213.

Burleson, M.H., Malarkey, W.B., Casioppo, J.T., Poehlmann, K.M., Kiecolt-Glaser, J.K., Berntson, G.G., Glaser, R., 1998. Postmenopausal hormone replacement: effects on autonomic, neuroendocrine, and immune reactivity to brief psychological stressors. Psychosomatic Medicine 60, 17–25.

Carmichael, S.T., Clugnet, M.C., Price, J.L., 1994. Central olfactory connections in the macaque monkey. The Journal of Comparative Neurology 346, 403–434.

Carmichael, S.T., Price, J.L., 1995. Limbic connections of the orbital and medial prefrontal cortex in macaque monkeys. The Journal of Comparative Neurology 363, 615–641.

Delahanty, D.L., Dougall, A.L., Hawken, L., Trakowski, J.H., Schmitz, J.B., Jenkins, F.J., Baum, A., 1996. Time course of natural killer cell activity and lymphocyte proliferation in response to two acute stressors in healthy men. Health Psychology 15, 48–55.

Delahanty, D.L., Wang, T., Maravich, C., Forlenza, M., Baum, A., 2000. Time-of-day effects on response of natural killer cells to acute stress in men and women. Health Psychology 19, 39–45.

Engler, H., Bailey, M.T., Engler, A., Sheridan, J.F., 2004. Effects of repeated social stress on leukocyte distribution in bone marrow, peripheral blood and spleen. Journal of Neuroimmunology 148, 106–115.

Farag, N.H., Nelesen, R.A., Dimsdale, J.E., Loredo, J.S., Mills, P.J., 2002. The effects of acute psychology stress on lymphocyte adhesion molecule expression and density in cardiac versus vascular reactors. Brain, Behavior, and Immunity 16, 411–420.

Fokkema, D.S., Smit, K., van der Gugten, J., Koolhaas, J.M., 1988. A coherent pattern among social behavior, blood pressure, corticosterone and catecholamine measures in individual male rats. Physiology and Behavior 42, 485–489.

Gomez, V., Zimmermann, G., Froehlich, W.D., Hnop, J., 1994. Stress, control experience, acute hormonal and immune reactions. Psychological Beitrage 36, 74–81.

Herbert, T.B., Choen, S., 1993. Stress and immunity in humans: a meta-analytic review. Psychosomatic Medicine 55, 364–379.

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

Jennings, K.R., Wood, C.C., 1976. The E-adjustment procedure for repeated measures analysis of variance. Psychophysiology 13, 277–278.

Katsuharu, N., Tadanobu, M., 1982. Development and validation of Japanese version of State-Trait Anxiety Inventory. Shinshin-Igaku 22, 107–112.

Kazuhito, Y., Shunichi, A., Norito, K., Tatsuya, T., 1990. Production of the Japanese edition of profile of mood states (POMS): assessment of reliability and validity. Japanese Journal of Public Health 37, 913–918.

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

Kohm, A.P., Sanders, V.M., 2001. Norepinephrine and beta 2-adrenergic receptor stimulation regulate CD4+ T and B lymphocyte function in vitro and in vivo. Pharmacological Reviews 53 (4), 487–525.

Kringelbach, M.L., Rolls, E.T., 2004. The functional neuroanatomy of the human orbitofrontal cortex: evidence from neuroimaging and neuropsychology. Progress in Neurobiology 72, 341–372.

Landmann, R.M., Burgisser, E., Wesp, M., Buhler, F.R., 1984. Beta-adrenergic receptors are different in subpopulations of human circulating lymphocytes. Journal of Receptor Research 4, 37–50.

Laudenslager, M.L., Ryan, S.M., Hyson, R.L., Maire, S.F., 1983. Coping and immunosuppression: inescapable but not escapable shock suppresses lymphocyte proliferation. Science 221, 568–570.

Maier, S.F., Ryan, S.M., Barksdale, C.M., Kalin, N.H., 1986. Stressor controllability and the pituitary-adrenal system. Behavioral Neuroscience 100, 669–674.

Mills, P.J., Goebel, M., Rehman, J., Irwin, M.R., Maisel, A.S., 2000. Leukocyte adhesion molecule expression and T cell naive/memory status following isoproterenol infusion. Journal of Neuroimmunology 102, 137–144.

Nakata, A., Araki, S., Tanigawa, T., Sakurai, S., Yokoyama, M., 1996. Effects of uncontrollable and controllable electric shocks on T lymphocyte subpopulations in the peripheral blood, spleen, and thymus of rats. Neuroimmunomodulation 3, 336–341.

O'Doherty, J., Kringelbach, M.L., Rols, E.T., Hornak, J., Andrew, C., 2001a. Abstract reward and punishment representations in the human orbitofrontal cortex. Nature Neuroscience 4, 95–102.

O'Doherty, J., Rolls, E.T., Francis, S., Bowtell, R., McGlone, F., 2001b. Representation of pleasant and aversive taste in the human brain. Journal of Neurophysiology 85, 1315–1321.

Ohira, H., Isowa, T., Nomura, M., Ichikawa, N., Kimura, K., Miyakoshi, M., Iidaka, T., Fukuyama, S., Nakajima, T., Yamada, J., 2004. Neural Basis of Modulation of Autonomic and Immune Responses under Uncontrollable Stress: Simultaneous Measurement of Brain Activity with PET and Peripheral Autonomic and Immune Activity. Poster Presented at 44th Annual Meeting of Society for Psychophysiological Research, Santa Fe, NM.

Perini, R., Milesi, S., Fisher, N.M., Pendergast, D.R., Veicsteinas, A., 2000. Heart rate variability during dynamic exercise in elderly males and females. European Journal of Applied Physiology 82, 8–15.

Peters, M.L., Godaert, G.L.R., Ballieux, R.E., Vliet, M.V., Willemsen, J.J., Sweep, F.C.G.J., Heijnen, C.J., 1998. Cardiovascular and endocrine responses to experimental stress: effects of mental effort and controllability. Psychoneuroendocrinology 23, 1–17.

Peters, M.L., Godaert, G.L.R., Ballieux, R.E., Brosschot, J.F., Sweep, F.C.G.J., Swinkels, L.M.J.W., Vliet, M.V., Heijnen, C.J., 1999. Immune responses to experimental stress: effects of mental effort and uncontrollability. Psychosomatic Medicine 61, 513–524.

Peters, M.L., Godaert, G.L.R., Ballieux, R.E., Heijnen, C.J., 2003. Moderation of physiological stress responses by personality traits and daily hassles: less flexibility of immune system responses. Biological Psychology 65, 21–48.

Pike, J.L., Smith, T.L., Hauger, R.L., Nicassio, P.M., Patterson, T.L., McClintick, J., Costlow, C., Irwin, M.R., 1997. Chronic life stress alters sympathetic, neuroendocrine, and immune responsivity to an acute psychological stressor in human. Psychosomatic Medicine 55, 447–457.

Price, J.L., 1999. Prefrontal cortical networks related to visceral function and mood. Annals of the New York Academic of Science 877, 383–396.

Ring, C., Carroll, D., Willemsen, G., Cooke, J., Ferraro, A., Drayson, M., 1999. Secretory immunoglobulin A and cardiovascular activity during mental arithmetic and paced breathing. Psychophysiology 36, 602–609.

Ring, C., Harrison, L., Winzer, A., Carroll, D., Drayson, M., Kendall, M., 2000. Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic, cold pressor and exercise: effects of alpha-adrenergic blockade. Psychophysiology 37, 634–643.

Sayers, B.McA., 1973. Analysis of heart rate variability. Ergonomics 16, 17–32.

Segerstrom, S.C., Miller, G.E., 2004. Psychological stress and the human immune system: a meta-analytic study of 30 years of inquiry. Psychological Bulletin 130, 601–630.

Sieber, W.J., Rodin, J., Larson, L., Ortega, S., Cummings, N., 1992. Modulation of human natural killer cell activity by exposure to uncontrollable stress. Brain, Behavior, and Immunity 6, 141–156.

Seligman, M.E.P., 1975. Helplessness: On Depression, Development and Death. Freeman & Co., San Francisco.

Speilberger, C.D., Gorusch, R.L., Lushene, R.D., 1970. STAI Manual for the State-Trait Anxiety Inventory. Consulting Psychologists Press, Palo Alto, CA.

Stone, A.A., Cox, D.S., Valdimarsdottir, H., Neale, J.M., 1987. Secretory IgA as a measure of immunocompetence. Journal of Human Stress Fall 136–140.

Swenson, R.M., Vogel, W.H., 1983. Plasma catecholamine and corticosterone as well as brain catecholamine changes during coping in rats exposed to stressful footshock. Journal of Pharmacology and Experimental Therapeutics 190, 193–209.

Usala, P.D., Hertzog, G., 1989. Evaluation of an adjective rating scale instrument. Research on Aging 11, 403–436.

Van Tits, L.J., Michel, M.C., Grosse-Wilde, H., Happel, M., Eigler, F.W., Soliman, A., Brodde, O.E., 1990. Catecholamines increase lymphocyte beta 2-adrenergic receptors via a beta 2-adrenergic, spleen-dependent process. The American Journal of Physiology 258, 191–202.

Weisse, C.S., Pato, C.N., McAllister, C.G., Littman, R., Breier, A., Paul, S.M., Baum, A., 1990. Differential Effects of controllable and uncontrollable acute stress on lymphocyte proliferation and leukocyte percentages in human. Brain, Behavior, and Immunity 4, 339–351.

Willemsen, G., Ring, C., Carroll, D., Evans, P., Clow, A., Hucklebridge, F., 1998. Secretory immunoglobulin A and cardiovascular reactions to mental arithmetic and cold pressor. Psychophysiology 35, 252–259.

Willemsen, G., Carroll, D., Ring, C., Drayson, M., 2002. Cellular and mucosal immune reactions to mental and cold stress: associations with gender and cardiovascular reactivity. Psychophysiology 39, 222–228.