Effects of Stressor Controllability on Acute Stress Responses: Cardiovascular, Neuroendocrine, and Immune Responses

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（心臓血管系、神経内分泌系、免疫系データに基づく
急性ストレス反応におけるコントロール可能性の効果の検討）

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2008

Declaration

The research in this thesis is the author's own original work. I hereby declare that this thesis has not been submitted, either in the same or different form, to this or any other University for a degree.

Tokiko Isowa

Acknowledgements

Firstly I would like to thank Prof. Dr. Hideki Ohira for his excellent supervision, inspiration, and encouragement. Secondly I would like to thank Prof. Dr. Kiyoshi Ishii and Dr. Shinya Takahashi for their constructive guidance. Thirdly I would like to thank all the people who have helped me with my thesis, Prof. Dr. Nobuko Yamahata, Prof. Dr. Seikou Murashima and Prof. Dr. Masune Sukigara. Finally I would like to thank all my friends who have given me so much support for the PhD.

Abstract

This thesis is concerned with the effects of controllability over acute stressors on psychological and physiological responses intermediated by immune, cardiovascular, neuroendocrine systems. The effects of stressor controllability have been examined in animal studies based on the learned helplessness theory. However, there were few studies in human. Especially, there were remarkably few studies that examined the effects of stressor controllability on immunological system. In addition, results of these studies were inconsistent among researchers for methodological reasons. The present research was conducted to assess differences of physiological responses between two coping styles, namely passive coping and active coping, and to provide their psychophysiological evidence by using immune, cardiovascular, neuroendocrine indices.

Based on previous findings out, hypothesis was that the active coping situation should elicit typical acute stress responses such as an increase of innate immunity and a decrease of acquired immunity. Furthermore, it was predicted that the active coping situation should have a greater impact on peripheral immune cells than a passive coping situation. This hypothesis was tested in experiment 1 that used a mental arithmetic task and a cold pressor task as active and passive coping situations respectively during which immune and cardiovascular responses were measured. As we predicted, under the active coping situation, innate immunity (natural killer (NK) cells) increased and acquired immunity (CD3 + T cells) decreased. Under the passive coping situation, immune responses were remarkably lower. The results suggest that immune responses under the active coping situation were evoked by increase of heart rate (HR) and blood pressure (BP) caused by adrenaline and noradrenaline. In contrast, those responses under the passive situation were elicited mainly by the increase of BP only caused by noradrenaline.

For the experiment 2, the triadic-yoked design including controllable, uncontrollable, and control conditions were used according to typical animal study in literature. In addition, for manipulation of controllability, correct-error feedback to participants' answers was used during mental arithmetic task.

Contrary to the prediction, no effect of controllability was observed in any immune parameters, though the experimental manipulation of controllability was valid. This was explained by the fact that at the beginning of stressor exposure, stress responses of the active coping type were evoked regardless of stressor controllability. In addition, under 15 minutes of the uncontrollable stress situation, both active and passive coping responses were mixed degree of which is affected by individual difference. Thus, effects of uncontrollability might have been present as strong correlations between immune and cardiovascular reactivity, but not as a difference of mean values.

The experiment 3 was designed to examine the time course of the uncontrollable acute stressor effect on immune responses up to 24 hours. For this purpose, participants performed four sessions of the acute stress task for two days. They performed mental arithmetic task under controllable or uncontrollable situations, and then performed again the same acute stress task several times, but under the controllable condition. Results supported hypothesis that the effects of an uncontrollable acute stressor should be present as the down-regulation of immune reactivity caused by inhibited cardiovascular and autonomic nervous responses. Among those participants who were exposed to the uncontrollable stressor in the first session, the increase of NK cells was lower than those in participants exposed to the controllable stressor. Especially, although the inhibition of immune activation in the uncontrollable condition did not appear in the first session, it appeared after the second session and became remarkable in the fourth session.

In the final part of this thesis, the findings from the three experimental studies are combined to establish a new model concerning stressor controllability and acute stress response. With this new model, I discuss the possibility that the mechanism that down-regulate immune response to uncontrollable situation may one of the effective functions to adapt to the varying environment for survival.

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Effects of stressor controllability on acute stress responses

1.1 Preface

The environment surrounding an organism changes from moment to moment, and occasionally becomes very stressful. In order to survive, organisms have to adapt to stressors within changing environments by evoking appropriate behavioral and physiological responses. To have an appropriate behavioral response to a stressor, in other words, to exhibit “coping behavior”, evaluation of the stressor is vital. A uniform strategy of coping in response to various stressors is less effective than responding dynamically according to the stressor properties. Especially, recognizing whether the stressor can be overcome or not by the organism’s own means, that is, evaluation of stressor controllability, is a key factor in deciding coping behaviors for survival.

Control means "having influence over, regulating or having power over" (*Webster's Collegiate Dictionary*). While “control” entails an objective reality, it also has a subjective dimension. Thus, the notion of controllability includes both one's ability to influence and regulate the environment in such a way as to fulfill needs and desires, and to protect oneself from danger and one’s confidence about this ability. From this perspective, a stable sense of control would depend on three broad sets of expectations: (a) that overall goals can be achieved, (b) that one can protect oneself effectively from most threats, and (c) that some failures in achieving goals and in protecting oneself from harm are likely to occur but that these do not signal an overall failure of controllability. In contrast, lack of control, “uncontrollability”, would include a sense of one’s lack of ability to influence the world to achieve the overall desired aims and effectively protect against danger. A sense of lacking control might occur if there were unrealistic expectations of success or failure in accomplishing these aims (Shear, 1991).

In addition to behavioral responses or coping behaviors, there are biological responses to a stressor that can also change dynamically according to stressor properties and the organism’s evaluation of them. Both dynamic biological responses and coping behaviors are effective for adaptation. Thus, stressor controllability is an extremely important factor for adaptation that associates strongly with coping behaviors and biological responses to an environmental

stressor.

In all biological systems that respond to environmental stressors, the immune system plays a key role; it exists for biological defense and is necessary for survival. Therefore, elucidation of the mechanisms of biological responses to evaluated stressors, especially mechanisms of the immune system, will enable us to understand biological principles of organisms' adaptation to environments.

The present study focuses on the effects of controllability over stressors on peripheral immune responses to an acute stressor. For this purpose, psychological and physiological responses to an acute stress task in controllable and uncontrollable conditions are compared. The present thesis begins with an overview of previous findings on the relationship between immune responses to acute stressors and controllability, including important theories with regard to these studies. Based on this review, the hypotheses of this work are presented. Subsequently, acute-stress tasks to evoke typical stress responses are described, and the revealed mechanism of peripheral immune responses during the acute stress task is presented. Then, the results of two additional experiments which examine effects of stressor controllability on the immune responses are reported. Finally, conclusions are drawn from the results of these three experiments, and the contribution of the present findings to an understanding of the effects of controllability is discussed.

1.2 Review of previous findings about acute stress and controllability

There are many reports that acute stressors influence the autonomic nervous, cardiovascular, endocrine, and immune systems. The physiological responses to acute stressors are divided into two patterns from the viewpoint of cardiovascular responses. These responses seem to be associated with coping styles determined by controllability over the stressors. The present study is concerned with the effects of controllability on acute-stress responses; therefore, it is important to understand the mechanism of typical physiological responses to acute stressors. The factor of controllability influences changes of the typical activation level of the autonomic nervous, cardiovascular, endocrine, and immune system responses to acute stressors. In this section, mechanisms of typical physiological stress response are described before studies about effects of controllability on acute-stress responses are reviewed.

1.2.1 Impact of acute stressors

1.2.1.1 Impact of acute stressors on autonomic nervous, endocrine, and immune systems

As for the responses of immune system to acute stressors, activation of both the rapid sympathetic-adrenal-medullary (SAM) axis and the relatively slow hypothalamic-pituitary-adrenocortical (HPA) axis play central roles, and they are caused by processes in the cerebral cortex and cerebral limbic system of the central nervous system (CNS) (Figure 1.1). The CNS receives feedback from the activated peripheral immune system. Under an acute-stress situation, changes in peripheral lymphocytes are caused by the SAM axis. Responses of the autonomic nervous system result in the release of adrenaline and noradrenaline from terminal arborization of the sympathetic nerves and the adrenal medulla, respectively, by activation of the sympathetic nervous system and affect receptor-bearing target organs. The cardiovascular system shows rapid responses such as increases of heart rate (HR) and blood pressure (BP). Meanwhile, activation of the vagal nerve, which is a part of the parasympathetic nervous system and shows antagonistic actions against the sympathetic nervous system, is suppressed during an acute-stress situation and contributes to the increase of HR. In this case, the immune system, especially redistribution of lymphocytes in blood, is influenced by adrenaline and noradrenaline (Landmann et al., 1984) and by increased blood flow and blood pressure (Benschop et al., 1993, 1996; Mills et al., 1995).

On the other hand, the HPA axis response during an acute-stress situation shows secretion of cortisol from the adrenal cortex with secretion of adrenocorticotropic hormone (ACTH) by the pituitary gland via the release of corticotrophin-releasing hormone (CRH) from the hypothalamus. Cortisol has the functions of increasing blood glucose levels and suppressing the immune function. These hormones are regulated by a negative feedback system. Under the acute-stress situation, the autonomic nervous, cardiovascular, endocrine, and immune systems interact with each other to maintain homeostasis for the purpose of survival.

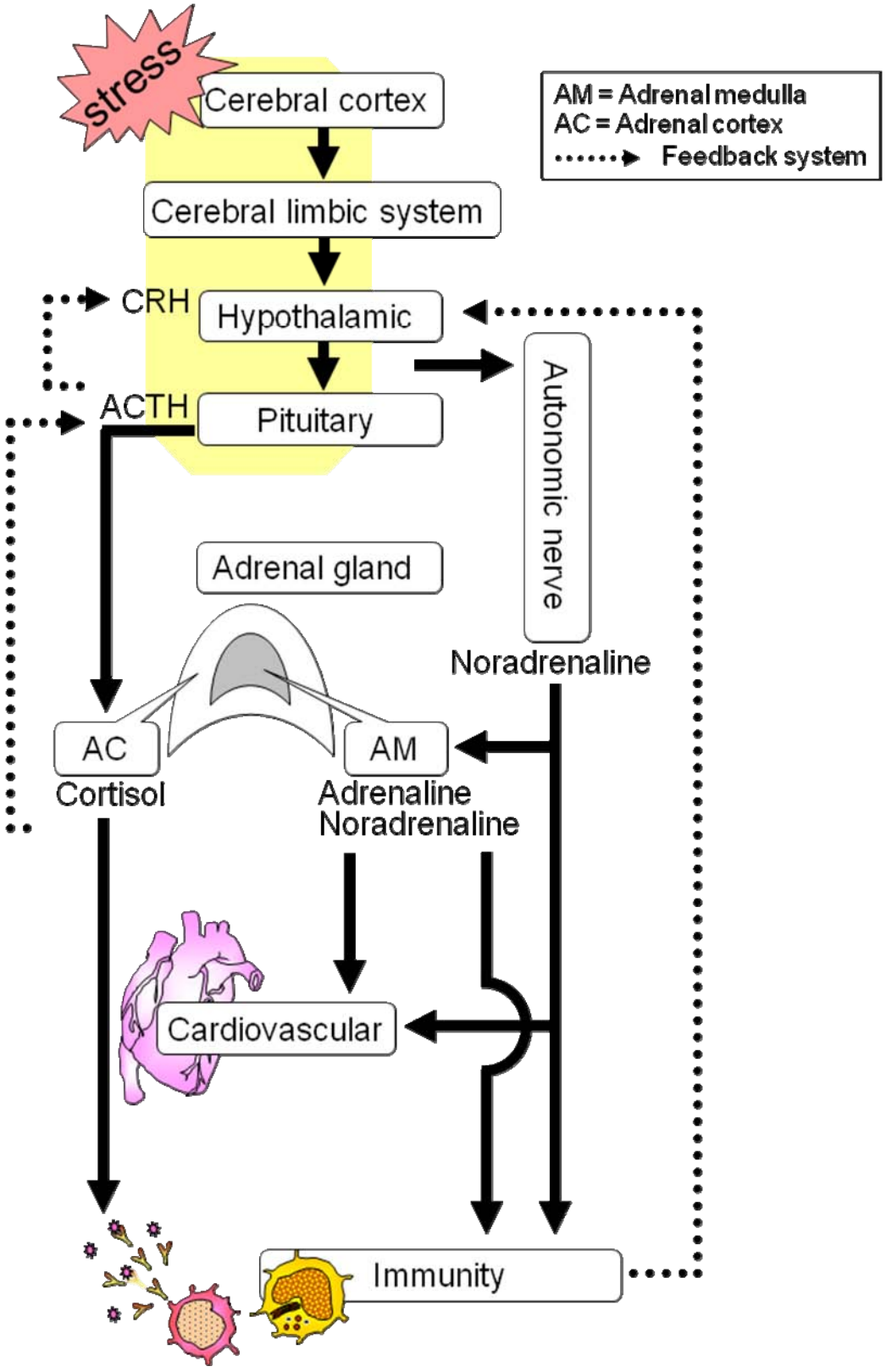

Figure 1.1 Mechanism of acute stress responses

1.2.1.1.1 Components of the immune system

To understand the relationship of psychosocial stressors and immune functioning, it is necessary to recognize the distinction between innate and acquired immunity. Innate immunity is an immune subtype that is characteristic of not only mammals but also lower-order organisms such as sponges. Cells involved in innate immunity do not provide defense against any particular pathogen; rather, they are all-purpose cells that can attack a number of different pathogens and do so in a relatively short time frame (minutes to hours) when challenged. The largest group of cells involved in innate immunity is granulocytes, and cells eat their targets. Another group of cells involved in innate immunity are the natural killer (NK) cells. NK cells recognize the lack of a self-tissue molecule on the surface of cells (characteristic of many kinds of viral infections and some cancerous cells) and lyse those cells by releasing toxic substances on them. NK cells are thought to be important in limiting the early phases of viral infections, before acquired immunity becomes effective, and in attacking self-cells that have become malignant (Segerstrom &Miller, 2004).

Acquired immunity is characterized by greater specificity and lead time than the innate immune response. Lymphocytes have receptor sites on their cell surfaces. The receptor on each cell fits with one and only one small molecular shape, or antigen, on a given invader and therefore responds to one and only one kind of invader. When activated, these antigen-specific cells divide to create a population of cells with the same antigen specificity in a process called *clonal proliferation*, or the *proliferative response*. Although this process is efficient in terms of the number of cells that have to be supported on a day-to-day basis, it creates a delay of up to several days before a full defense is mounted, and the body must rely on innate immunity to contain the infection during this time. There are three types of lymphocytes that mediate acquired immunity: helper T cells, cytotoxic T cells, and B cells. The main function of helper T cells is to produce cytokines that direct and amplify the rest of the immune responses. Cytotoxic T cells recognize antigens expressed by cells that are infected with viruses or otherwise compromised (e.g., cancer cells) and lyse those cells. B cells produce soluble proteins called *antibodies* that can perform a number of functions, including neutralizing bacterial toxins, binding to a free virus to prevent its entry into cells, and opsonization, in which a coating of antibody increases the effectiveness of innate immunity.

1.2.1.1.2 Relationship between acute stress and the immune system

In an acute-stress situation, peripheral immune cells are affected by activation of both the rapid SAM axis reaction and the relatively slow HPA axis reaction (Bauer et al., 2001, 2002; Bosch et al., 2005; Mills et al., 1995; Pike et al., 1997; Stevenson et al., 2001). The sympathetic fibers descend from the brain into both primary (bone marrow and thymus) and secondary (spleen and lymph nodes) lymphoid tissues (Felten & Felten, 1994). These fibers can release a wide variety of substances that influence immune responses by binding to receptors on white blood cells (Ader et al., 1995; Felten & Felten, 1994; Kemeny et al., 1992; Rabin, 1999). Though all lymphocytes have adrenergic receptors, the differential density and sensitivity of adrenergic receptors on lymphocytes may determine responsiveness to stress among cell subsets. For example, NK cells have both high-density and high-affinity for beta-2-adrenergic receptors, B cells have high density but lower affinity, and T cells have the lowest density (Anstead et al., 1998; Landmann, 1992; Maisel et al., 1989). Furthermore, the HPA axis, the SAM axis, and the hypothalamic-pituitary-ovarian axis secrete adrenal hormones such as adrenaline, noradrenaline, and cortisol. These substances bind to specific receptors on white blood cells and have diverse regulatory effects on their distribution and function (Ader et al., 2001).

Under an acute-stress situation, the number of circulating lymphocytes representing innate immunity such as NK cells increases, while the 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 (Figure 1.2) (Dhabhar et al., 1995; Bosch et al., 2003; Landmann et al., 1984; Meehan et al., 1993; Minton & Blecha, 1990; Schedlowski et al., 1993, 1996; Stefanski, 2000). This trafficking of the lymphocytes has been shown to be mainly mediated by adrenaline and noradrenaline through surface receptors (mainly, beta-2- and alpha-adrenoreceptors: Landmann et al., 1984) and to be caused by increased blood flow and blood pressure (Benschop et al., 1993, 1996; Mills et al., 1995). An increase of peripheral NK cells is beneficial for survival under an acute-stress situation because of nonspecific and rapid reaction to any pathogen that effectively prevents bacteria from entering a wound by fight/flight behaviors (Engler et al., 2004; Stefanski and Engler, 1998). On the other hand, a decrease of T cells might represent homing to local immune organs and lymph nodes where T cells can be

sensitized to signals of antigens. This response might also be understood as beneficial. These responses are not rigid or stereotyped but flexible. It allows the organism to adapt dynamically to various environments and stimulations.

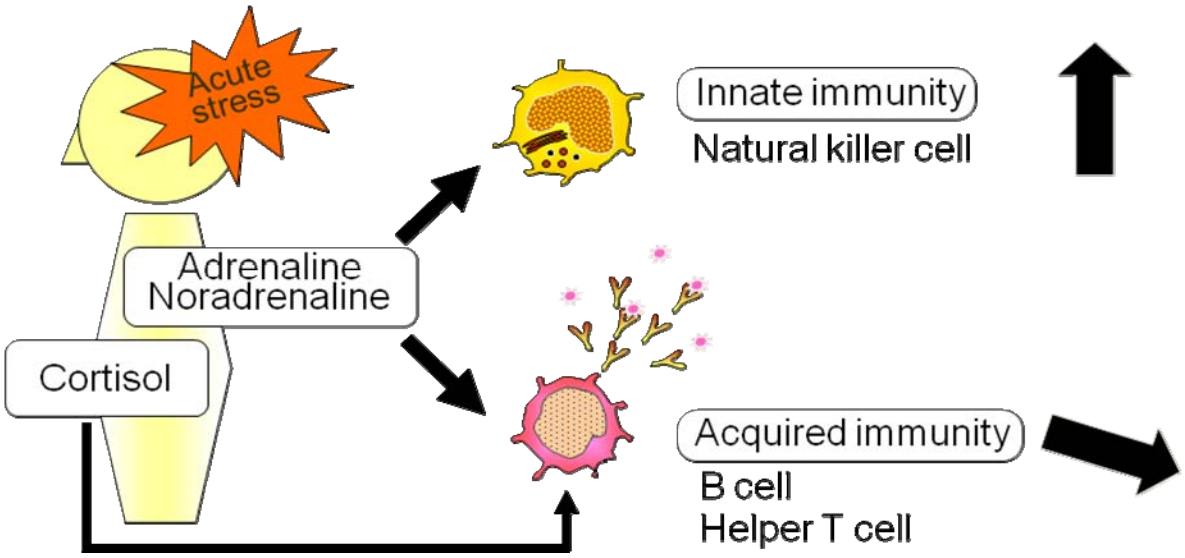

Figure 1.2 Trafficking of the lymphocytes to acute stress responses

1.2.1.2 Two patterns of acute-stress responses and coping types

Obrist et al. (1978) argued that coping strategies to stressors were divided into two types depending on the controllability of the stressor. These two coping strategies are called active and passive coping strategies, respectively, and evoke two different patterns of cardiovascular stress responses (Table 1.1) (Schneiderman, et al., 1989; Williams, 1986). In previous studies examining the difference in physiological responses to the two coping strategies, challenging tasks and gaze or patience tasks were used, respectively (Obrist et al., 1987; Schneiderman et al., 1989; Sherwood et al., 1990; Williams, 1986). As tasks requiring active coping, for example, a mental arithmetic task and a reaction time task for avoidance of aversive stimulus were used. In the case of these tasks, subjects could avoid a stressor by their own effort; thus, the situation was controllable. By contrast, for passive coping, the task of enduring an aversive stimulus (e.g. cold pressor, noise, and film) was used. In the case of these tasks, subjects could not avoid the stressor by their own effort; thus, the situation was uncontrollable. Put it all together, when organisms have controllability, active coping seem to be evoked, which enables them to defiantly and competitively challenge the stressor. In contrast, when organisms do not have controllability over the stressor, passive coping seem to be elicited to endure stressors patiently while monitoring it.

Each coping type evokes a different pattern of cardiovascular responses with a different pattern of autonomic nervous reactivity. In case of active coping, BP increases with increase of cardiac output (CO) and HR (pattern 1). Increase of CO is caused by enhancement of the sympathetic nervous reactivity (beta-adrenergic effect) and suppression of the vagal activity (cholinergic effect) (Thayer and Brosschot, 2005). The increase of HR is caused by an increase of adrenaline secreted from the adrenal medulla controlled by the sympathetic nerve. On the other hand, in the case of passive coping, BP increases with an increase of total peripheral resistance (TPR) (pattern 2). The increase in TPR is caused by enhancement of the sympathetic nervous reactivity (alpha-adrenergic effect) under which HR and CO tend to decrease by activation of the vagal activity (cholinergic effect). Thus, when the stressor is controllable, active coping is evoked and BP increases with an increase of HR and CO. When the stressor is uncontrollable, in contrast, passive coping is selected, and BP increases with an increase of TPR (Bandler et al., 2000; Keay and Bandler, 2001).

Peripheral immune responses should alter under patterns of cardiovascular response depending on coping style, because these responses to stressors are mediated by the autonomic nervous and cardiovascular system. Namely, when the stressor can be evaluated as controllable, active coping is selected, and an increase of NK cells for innate immunity and a decrease of T cells and helper T cells for acquired immunity seem to be induced via activation of adrenaline and noradrenaline through surface receptors and increased blood flow and blood pressure. On the other hand, when the stressor seems to be evaluated as uncontrollable, passive coping is selected, and a blunted pattern of lymphocyte redistribution takes place via only increased blood pressure.

Table 1.1 Immune responses with patterns of cardiovascular reactivity under coping styles to stressor

	Coping type	
	Active coping	Passive coping
Patterns of cardiovascular reactivity	Pattern 1	Pattern 2
Cardiovascular parameters		
Blood pressure	++	++
Cardiac output	++	-
Heart rate	++	-
Total peripheral resistance	-	++
Endocrine parameters		
Adrenaline	++	±
Noradrenaline	+	+
Immune parameters		
NK cells	++	+
Citotoxic T cells	+	±
T cells	- ~ ±	±
Helper T cells	- ~ ±	±

+ ~ ++; increase, ±; no change, -; decrease

1.2.2 Effects of stressor controllability on acute-stress response

1.2.2.1 Previous studies of stressor controllability on animals

The effects of stressor controllability on immunological responses to the acute stressors have been widely studied based on the learned helplessness theory using animals. These studies suggested that the degree of control over a stressor was an important modulator of the immune responses (Fleshner et al., 1992, 1995, 1996, 1998; Gazda et al., 2003; Laudenslager et al., 1983; Nakata et al., 1996; Shavit et al., 1983; Spehner et al., 1996). Additionally, immune responses depend on many other factors, including their types and the time-point of immune function assessment (Fleshner et al., 1995, 1996; Laudenslager et al., 1994; Maier et al., 1998; for a reviews, Moynihan and Ader, 1996). In this section, I review some of the recent results and typical experimental designs of these studies.

Laudenslager et al. (1983) were the first to examine whether and how stressor controllability modulates immune system reactions. They found a decrease of proliferation of peripheral blood lymphocytes to mitogens such as phytohemagglutinin (PHA) or concanavalin A (conA) among a group subjected to uncontrollable electric-shock group but not one subjected to controllable shock. Shavit et al. (1983) also reported their findings with rats of a decrease of splenic NK cell activity among an uncontrollable-shock condition group. Fleshner et al. (1992) reported that uncontrollable shocks increased the proportion of CD4+ helper T cells and the CD4+/CD8+ ratio in the mesenteric lymph nodes. Nakata et al. (1996) reported that CD4+ helper T cells, white blood cells, and the CD4+/CD8 ratio in the peripheral blood, spleen, and thymus in rats under an uncontrollable-stressor condition were significantly smaller than those under controllable and a no-shock conditions. Spehner et al. (1996) reported that macrophage phagocytic function in mice decreased remarkably under an uncontrollable-stressor condition. Gazda et al. (2003) reported that T cell proliferation after keyhole limpet hemocyanin (KLH) restimulation was markedly suppressed in rats under an uncontrollable condition, and that anti-KLH IgG, IgG1 and IgG2a were also significantly suppressed.

In general, a triadic design has been widely used in animal studies (Seligman, 1975). The experiment is composed of a two-phase procedure in which, for example, sets of three rats are exposed to escapable shock or yoked inescapable

shock, or face simple restraint in wheel-turn boxes. The first rat in this triad receives a series of escapable electric shocks through electrodes affixed to its tail. Trials occur with a variable inter-trial interval. Once shock begins on a trial, the master rat is required to turn the wheel to terminate shock. The wheel is easily turned and the escape contingency is acquired rapidly. Shock duration for the second rat is yoked to the escape behavior of the master rat. Shock begins simultaneously for both rats on a trial. Once the master rat completes the escape contingency, shock terminates simultaneously for both animals. Wheel-turn responses by the rat receiving yoked, inescapable shock have no effect on shock onset or termination. Thus, both rats receive the same intensity, pattern and duration of shocks during the pretreatment session. The two rats differ, however, in the extent to which they can exert behavioral control over the termination of the stressor. The third rat in each triad is restrained in the chamber and receives no shock. This restrained rat serves as a control for any differential effects of stressor controllability during later testing, which typically occurs 24 hr after stress pretreatment (Maier and Jackson, 1977; Minor and Hunter, 2002).

Many of the behavioral effects of inescapable shock are typically measured 24 hours after the shock exposure rather than soon after that. It is suggested from these reports that immunological effects by the uncontrollable-stressor condition were associated with an opioid form of analgesia which can be restored by reinstating the shocks (Drugan et al., 1981; Jackson et al., 1979; Laudenslager et al., 1983; Maier et al., 1982; Shavit et al., 1982, 1983). Jackson et al. (1979) reported that inescapable shock subjects became analgesic by re-exposure to a small amount of shock (itself insufficient to produce analgesia) after 24 hours of the inescapable shock. Subjects for whom the initial shocks were escapable didn’t become analgesic when shocked after 24 hours. However, both escapable- and inescapable shock subjects became analgesic immediately after shock (Maier et al., 1982). Shavit et al. (1982) found footshock that produces an opioid form of analgesia to be immunosuppressive, whereas footshock that produces a nonopoid form of analgesia was not. The analgesia that emerges upon brief re-exposure to shock 24 hours after inescapable but not escapable shock is completely reversed by opiate antagonists and completely cross-tolerant with morphine (Drugan et al., 1981). Furthermore, many other changes produced by uncontrollable stress, such as reduced activity (Jackson et al., 1978), opioid analgesia (Grau et al., 1981), norepinephrine depletion (Weiss et al., 1981), and potentiation of

morphine-conditioned place preference (Will et al., 1988) were also reported after re-exposure to shock 24 hours after the initial shock treatment.

In summary, effects of uncontrollable stressors have appeared as immunosuppressive (e.g. decrease in the proliferation of lymphocytes, NKCA, T cells, and B cells), whereas effects of controllable stressors have appeared as immune-activating. For a typical design of animal experiments, in uncontrollable or inescapable conditions, passive coping was evoked and immune responses were suppressed. In controllable or escapable conditions, active coping was evoked and immune responses were activated. Thus, coping styles change depending on stressor controllability, and as a consequence, immune responses to the stressor also change.

1.2.2.2 Previous studies of stressor controllability on human

Experimental studies in humans focusing on the effects of controllability over acute stressors are heavily outnumbered by animal studies. In particular, there are very few studies investigating the effects of stressor controllability on the immune system. In this section, previous experimental studies with humans investigating the effects of stressor controllability under stress situations are reviewed to examine the experimental methodology in this thesis.

Table 1.2 shows previous human studies on the effect of stressor controllability. Weisse et al. (1990) reported decreased lymphocyte proliferation in response to concanavalin A (ConA) and phytohemagglutinin (PHA) among subjects who had control over a stress task, but the decrease was absent for those who did not have control over it. Sieber et al. (1992) found decreased NK cell activity (NKCA) after an uncontrollable stress task, but not after the controllable stress task. Gomez et al. (1994) found no effect of uncontrollability on various immune parameters. Brosschot et al. (1998) found decreased helper T cells in an uncontrollable group and increased B cells in a controllable group. Peters et al. (1999, 2003) found that the *in vitro* production of cytokine interleukin-6 (IL-6) decreased after exposure to an uncontrollable stressor. As described above, the results from these studies are somewhat inconsistent.

There are some explanations for the inconsistency. First, the experimental time course and duration of the task varied among previous studies. Weisse et al. (1990) used auditory noise with mild electric shock for 30 minutes as an acute stressor.

The controllable group was able to terminate the stressor by pressing a button, while the uncontrollable group was administered an identical pattern of noise and electric shock from the previous subject from the controllable condition group: Thus, the pair of groups was yoked. Sieber et al. (1992) divided participants into four groups: Escapable Noise (EN), Inescapable Noise/Response (IN/R), Inescapable Noise/ No Response (IN/NR), and No Noise (NN). They used auditory noise lasting for 20 minutes as an acute stressor, and it was to be controlled by pressing a button. The EN group was able to terminate the noise by pressing the button. For the IN/R group, the noise stopped randomly, regardless of what responses they made. For the IN/ NR group, the noise was not stopped. The length of the tone sequences between the IN/R and the IN/ NR groups were yoked to those of the EN group. Gomez et al. (1994) also used a noise task with button pressing. The controllable group received bogus success feedback, while the uncontrollable group received bogus failure feedback. In their paper, however, the stress task and manipulation of controllability weren't detailed. Brosschot et al (1998) used a three-dimensional puzzle as a task and manipulation of controllability. Participants were asked to solve the puzzle within 8 minutes. The puzzle was manipulated in such a way that every subject fails. During the next 12 minutes, the subjects had to explain how to solve the puzzle to another subject. After the task, participants were divided into two groups depending on how much controllability they perceived, by subjective evaluation of controllability. In the study by Peters et al. (1999, 2003), participants received one of two different stress tasks: either a mental arithmetic task or key-press task with auditory noise for 15 minutes. As the manipulation of controllability, the intensity of the noise varied depending on their performance. In the controllable condition, participants succeeded in most of the trials; thus, they could choose the noise intensity and also could predict the noise. To match the amount of aversive stimulation between conditions, the performance was yoked to the uncontrollable condition.

Second, measured parameters were different among previous studies (see Figure 1.2). Especially, 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 assess in detail the interaction of these systems under controllable and uncontrollable acute stress situations. To my knowledge, only one study by Peters et al. (1999) examined the effects of controllability on the cardiovascular, endocrine, and immune systems in humans. They found that *in vitro*

production of cytokine interleukin-6 (IL-6) was lower after the experience of an uncontrollable stressor. On the other hand, Peters et al. (1998) also reported that under an uncontrollable stress condition, the level of plasma NE in humans increased more than that under a controllable stress condition, and that blood pressure under the uncontrollable condition was higher than that under the controllable condition. These results suggest that controllability over an acute stressor has a greater impact on autonomic parameters than on parameters of the HPA axis.

Finally, the timing of measurement was different among previous studies. In typical experimental procedures in animal studies, subjects were first exposed to an inescapable stressor, and then re-exposed to the same but escapable stressor over 24 hours after exposure to the first stressor (Laudenslager et al., 1983; for a review, Maier and Watkins, 2005). The effects of controllability were measured 24 hours after the stressor exposure rather than during or immediately after exposure. Additionally, some of the animal studies reported that rats presented the same response just after the shock regardless of whether it was escapable or not, but they exhibited different reactions when re-exposed to the same, but escapable stressor 24 hours after the first stressor (Drugan et al., 1984; Jackson et al., 1979; Maier et al., 1990; Weiss et al., 1981). However, in human studies, the effects of controllability have been measured during the first experience of an acute stressor or immediately after. Sieber et al. (1992) evaluated the effects of controllability 24 and 72 hours after the experience of an uncontrollable stressor, and found decreased NK cell activity (NKCA) in the uncontrollable stressor group but not in the controllable stressor group. However, they did not measure responses to the re-exposed stressor after the experience of uncontrollability. Thus, as far as I know, there is no human study that has examined the response to re-exposure to an uncontrollable stressor after 24 hours.

The results from human studies are inconsistent. From the viewpoint of coping style, three studies reported immunosuppressive responses under passive coping with uncontrollable conditions (Brosschot et al., 1998; Sieber st al., 1992; Peters et al., 1999, 2003), and two studies reported immune activation or no change under passive coping with uncontrollable conditions (Gomez et al., 1994; Weisse et al., 1990). Thus, in terms of the relationship between immune responses and coping style depending on stressor controllability, at this time the results of human studies differ from those of animal studies.

Table 1.2 Previous studies of effects of controllability on immune responses to acute experimental stressor

Authors	Task	Aversive stimulation	Task duration (minutes)	Physiological Parameters	Results of uncontrollable condition
Weisse et al (1990)	Key-press	Electric shock, Noise	30	Lympocyte proliferation to ConA and PHA, helper T cells, supressor T cells, B cells	No effect
Sieber et al (1992)	Key-press	Noise	20	Lymphocyte, NK cells, NKCA, CD3, CD4, CD8, CD19	Decrease of NKCA
Gomez et al (1994)	Key-press	Noise		Lymphocyte, NK cells, CD3, CD4, CD45RO, CD19, CD11, NKCA, IgA, IgG, IgM	No effect
Brosschot et al (1998)	Puzzule	Nothing	8	Lympocyte proliferation to PHA and PWM, NK cells,	Decrease of helper T cells
Peters et al (1998, 1999, 2003)	Key-press, mental atirhmetic	Noise	15	Lympocyte proliferation to PHA, NKcells, CD3, CD4, CD8, CD14, NKCA, , IL-6, IL-4, cortisol, inter beat interval, blood pressure, epinephrine,	Decrease of IL-6, increase of cortisol, blood pressure, and norepinephrine

1.3 Previous models of stress and coping

Previously, several models or theories about biological responses (Cannon, 1935; Selye, 1936) and the stress of coping (Lazarus & Folkman, 1984; Seligman, 1975) have been proposed. In particular, learned helplessness theory (Seligman, 1975) and psychological models of stress adaptation (Lazarus & Folkman, 1984) have been very important and influential for this study.

The models and theories regarding stressor controllability and coping style are reviewed in the following sections.

1.3.1 Selye's general adaptation syndrome (Selye, 1936)

The concept of stress was proposed by Hans Selye in the field of medicine (Selye, 1936). He defined a harmful stimulus from the environment as a stressor and physical responses to the stressor as stress. Additionally, Selye postulated a general adaptation syndrome (GAS), a stereotyped physiological response (swelling of the adrenal cortex, atrophy of the thymus, gastric and duodenal ulcers) that takes the form of a series of three universal stages of coping in response to a stressor. According to Selye, organisms facing a stressor will immediately enter into the first stage where a series of complex physiological changes take place, exhibiting such symptoms as increased HR and breathing. The first stage is an "alarm reaction", in which the adrenal medulla releases epinephrine and the adrenal cortex produces glucocorticoids, both of which help to restore homeostasis. Homeostasis, as coined in 1932 by Walter Bradford Cannon, is a systematic regulation of an organism's internal environment so as to maintain a stable, constant condition. Restoration of homeostasis leads to the second stage, resistance, in which defense and adaptation are sustained and optimal. If the stressor persists, the stage of exhaustion follows, and adaptive response ceases; the consequence may be illness and death.

Selye's model postulates that the same coping strategy and the same biological response are evoked in response to all types of stressors. Additionally, this model does not include a viewpoint of stressor controllability. However, clearly, a rigidly stereotyped set of coping strategy and biological responses to the stressor would be ineffective for adaptation. Thus, the model is not suitable for the present study.

1.3.2 Learned helplessness theory (Seligman, 1975)

Overmier & Seligman (1967) proposed the learned helplessness theory regarding the effects of inescapable stressors. Learned helplessness can explain a psychological condition in which an organism has come to believe that it has no control over its situation and that whatever it does is futile. As a result, the organism will stay passive in the face of an unpleasant, harmful or damaging situation, even when it does actually have the power to change its circumstances (Overmier & Seligman, 1967; Seligman & Maier, 1967; Seligman, 1975). Concretely, the shocks delivered in a Pavlovian conditioning experiment are inescapable, and so escapable and yoked inescapable shocks were compared in their effectiveness in producing later failure to learn to escape. The result was that only the animals that had received the yoked inescapable shocks later showed a learning deficit (Seligman & Maier, 1967). Furthermore, the phenomenon remains for some days from 24 hours after the first exposure of an uncontrollable stressor, and elicits the same response regardless of stress situations from the first situation of uncontrollable stress (generalization effect) (for a review, see Maier and Watkins, 2005).

The theory behind the learned helplessness phenomenon consists of three factors, non-contingency between individual behavior and outcome, expectation of non-contingency in the future, and passive behavior. Thus, a focused point of this theory is eventual passive behavior that was induced by an uncontrollable stressor. However, the focus of the present study is the effects of stressor controllability on acute-stress responses, especially, changes in biological responses that are induced by evaluation of controllability over an acute stressor.

1.3.3 Psychological model of adaptation (Lazarus & Folkman, 1984)

Central concepts of this model are appraisal and coping. Lazarus and Folkman (1984) reported that cognitive and behavioral processes (cognitive appraisal and coping) in response to environmental demands (stressors) affect the aspect and degree of stress response. Cognitive appraisals are divided into two forms, evaluation of a harmful and intimidating effect from the stressor (primary appraisal) and evaluation in terms of the amount of the organism’s individual resources (secondary appraisal). Psychological as well as behavioral reactions are

determined by the result of cognitive appraisals of comparisons between individual resources and environmental demands, and also as a function of time. Thus, their insistence is that cognitive appraisals contribute to determining coping strategy and physical reaction to the stressor. Furthermore, continuation of an uncontrollable situation in which the degree of environmental demand is higher than that of the individual's resources induces psychological and physiological illness.

Unlike in the case of learned helplessness as eventual passive behavior by an inescapable stressor, the model focuses on change of coping style depending on evaluation of stressor controllability. Thus, for the purpose of this study, the model proposed by Lazarus seems appropriate, at least in part. However, this model does not predict biological responses depending on coping styles and stressor controllability.

1.4 The goal of the present study

The aim of the present study was to examine the effects of controllability over an acute stressor on physiological responses, especially on the peripheral immune system. Specifically, this study attempts to elucidate how stressor controllability affects the peripheral immune system and cardiovascular and autonomic nervous system as mediators of the immune system, and how these responses change with time. Ultimately, the goal is to construct an adaptive model of biological responses to acute stress situations including factors of environmental evaluation and time course based on the current research and previous reports. As far as I know, there has been no such human study to date.

From previous reports, it can be predicted that passive coping is usually evoked under uncontrollable stress situations (Bandler et al., 2000; Keay and Bandler, 2001). However, it seems that stress responses of the active coping would be evoked at the beginning of exposure to a new stressor, even if the situation was uncontrollable, because an organism would actively challenge the stressor while seeking an appropriate coping strategy. After that, the active coping would shift to passive coping as the uncontrollable situation persists. Rationales for such a prediction come, for example, from a work by Obrist et al (1976). In their study using reaction time task of three kinds of task difficulty (easy, hard, and impossible conditions), acute stress responses in the three conditions were evoked equally at the beginning of exposure to the task, however responses in the easy and

impossible conditions decreased as time advanced. Furthermore, in typical experimental procedures in animal studies on stressor controllability, the animals were first exposed to an inescapable stressor, and then re-exposed to the same, but escapable stressor, over 24 hours after exposure to the first stressor (Laudenslager et al., 1983; for a review, Maier and Watkins, 2005). The effects of controllability were measured 24 hours after the stressor exposure rather than during, or soon after, exposure, and were observed as passive coping stress responses.

Here, I examine the effect of controllability on psychological and physiological responses to acute stressors in humans including the factor of time.

1.5 Structure of this thesis

After an overview and discussion of previous studies in the first chapter, I explain three experiments from the second to the fourth chapter. In Chapter 2, I investigate physiological responses by using a typical acute-stress task to elicit the two patterns of acute stress discussed in section 1.2.1.2 (experiment 1). In Chapter 3, effects of controllability over the acute stressor on peripheral immune responses are addressed (experiment 2). In Chapter 4, I report the time course of immune responses to an uncontrollable acute stressor up to 24 hours (experiment 3). Finally, in Chapter 5, I conduct a comprehensive discussion about these results from the viewpoint of adaptive significance.

Chapter 2

Responses and mechanism of cardiovascular and immune system to acute stressor (Experiment 1)

The purpose of this thesis was to examine effects and the mechanism of stressor controllability on peripheral immune responses to acute stressor. My hypothesis was that typical acute stress responses, fight-or-flight reaction, should be inhibited under a situation of uncontrollable stressor, because passive coping situation should suppress acute stress responses under active coping situation (Delahanty et al., 1996; Willemsen et al., 2002). To demonstrate the hypotheses, firstly I will prove that the typical acute stress responses differ from both coping situations, and will clarify mechanism of immune changes depending on both coping situations.

Reactions of immune system should be differentiated by either an active coping or a passive coping situation, whereas most previous studies have focused on the influences of active coping situation. Furthermore, there were remarkably few studies that examined relationship between peripheral immune responses and autonomic nervous system as mediators of immune responses on both coping situations. To our knowledge, only three studies have compared immune reactions to active and passive coping situations directly (Patterson et al., 1994 ; Delahanty et al., 1996; Willemsen et al., 2002). These studies predicted that active coping situation would have a greater impact on immunity than passive scoping situation (Delahanty et al., 1996; Willemsen et al., 2002). However, Patterson et al. (1994) examined only the reactivity of platelets, and Delahanty et al. (1996) measured only NKCA and lymphocyte proliferation to these stressors.

The study by Willemsen et al. (2002) was the first to systematically examine peripheral immune responses to both types of coping situations using mental arithmetic and cold pressor. They showed that the numbers of NK cells and CD8+ cytotoxic T cells increased after a mental arithmetic task. In contrast, the number of CD4+ T cells was reduced by both active and passive stress. Furthermore, only in the mental arithmetic task, significant correlations were shown between immune parameters (the number of NK cells) and autonomic nervous system parameters.

Data in experiment 1 was published in Isowa, Ohira, and Murashima (2004).

However, although study of Willemsen et al. (2002) is very important in understanding the relationship between immune and autonomic nervous responses, some problems in their study need to be examined. They used a relatively brief interval of 10 minutes between tasks, yet other studies have found that levels of norepinephrine (NE) and epinephrine (E), when raised by acute stress tasks, did not recover to baseline even 10 minutes after the task (Galvez et al., 1996; Richer et al., 1996). Thus, it is possible that the second task in the study of Willemsen et al. (2002) might have begun while effects of the first task remained. Especially, the effects of the cold pressor task on immunity by the second task might be contaminated by residual effects of the preceding mental arithmetic task because levels of NE and E that might be elevated by the first task could have remained elevated during the second task. Therefore, further evidence in an experiment with longer intervals is needed to distinguish between the effects of active and passive coping situations on immunity.

Thus, the purpose of the first experiment is to establish peripheral immune responses and the mechanism of active and passive coping before examining effects of stressor controllability on acute stress responses. For the purpose, I used mental arithmetic task as active coping situation and cold pressor task as passive coping situation, and measured lymphocyte subsets in peripheral blood and cardiovascular parameters as mediator of peripheral immunity.

2.1 Introduction

The purpose of this section is to establish peripheral immune responses and mechanism of the immune response to active and passive coping situations.

As identified at 1.2.1.1, facing acute stress, circulating numbers of lymphocytes representing innate immunity such as natural killer (NK) cells increase, but the numbers of lymphocytes representing acquired immunity such as T cells and B cells do not change or even decrease (Bosch et al., 2003; Herbert et al., 1993; Landmann et al., 1984; Marsland et al., 2002; Mills et al., 1996; Willemsen et al., 2002). This trafficking of the lymphocytes has been shown to be mainly mediated by adrenaline and noradrenaline through surface receptors (mainly, β2- and α-adrenoreceptors: Landmann et al., 1984) and to be caused by increased blood flow and blood pressure (Benschop et al., 1993, 1996; Mills et al., 2000). Thus, to examine mechanism under peripheral immune reaction to acute stressor,

lymphocyte subsets in peripheral blood and cardiovascular parameters (HR and BP) as mediator of immune response are used in this experiment.

According to review on 1.2.1.2, controllable stress situation evokes active coping response whereas uncontrollable stress situation evokes passive coping response. Therefore, in this experiment, mental arithmetic task with time pressure was used as active coping situation and cold pressor task as passive coping situation. Additionally, to demonstrate whether both stress tasks evoke specific patterns of stress responses depending on personal differences, within-subject design was used.

Based on previous findings, I hypothesized that active coping situation should elicit typical acute stress responses such as an increase of NK cells and a decrease of helper T cells and B cells. Furthermore, the active coping situation would have a greater impact on peripheral immune cells than a passive coping situation. In particular, an increase in the percentage of NK cells and in NKCA as innate immunity under an active coping situation may be remarkable, as either increase might be caused by involvement of NK cells' beta2-adrenoreceptors, which should be related to active stressors.

2.2 Methods

2.2.1 Participants

Twenty-six female undergraduates (range of ages 20 to 33 years; mean age = 22.38 years) participated in the present study. The mean BMI of the subjects was 21.22 kg/m^2 (SD = 1.81). None of them was suffering from any chronic illness and was taking medication known to influence immunity. Considering the effects of the menstrual cycle on the immune system, they participated in the experiments during the late luteal and follicular first phases only. In these periods, the secretion of female sex hormones is low, and thus the influences of these hormones on the autonomic nervous and immune systems were minimized. Levels of estradiol and progesterone in serum were measured on the day of the experiment in order to confirm that each woman was at one of these acceptable phases of the menstrual cycle. The mean value of estradiol was 44.74pg/ml (SD = 41.51) and the mean value of progesterone was 1.42ng/ml (SD = 3.67). All subjects provided written informed consent. The Ethics Committee of the Mie Prefectural College of Nursing

approved the present study.

2.2.2 Acute stress task

All subjects conducted both a mental arithmetic task as active coping condition and a cold pressor task as passive coping condition. In the mental arithmetic task, subjects added numbers (1-9) displayed on a PC monitor continuously for 15 minutes. Each digit was displayed for 500 ms and followed by a 1500-ms interval. The subject answered each question by pressing a key (0-9) with her dominant hand. Before beginning the exercise, each subject was instructed to try to achieve at least 95% accuracy in calculation. After this instruction, each subject took a practice task for two minutes. In the cold pressor task, the subjects soaked their dominant hands for 10 minutes in water kept at 10 ^{o}C in a constant-temperature bath. Before the test, each subject was instructed to remove her hand from the water if she felt sick. However, all subjects completed the full 10 minutes of the task. The duration of both tasks (15 minutes for mental arithmetic and 10 minutes for cold pressor) was determined following the previous study (Willemsen et al., 2002), in which the mental arithmetic tasks lasted longer (8 minutes) than the cold pressor task (4 minutes). Our pilot study showed that subjects rated the difficulty of two tasks that lasted for above-mentioned durations (15 and 10 minutes) as being almost identical.

2.2.3 Experimental procedure

The subjects had been instructed to eat light breakfast and avoid drinking caffeine-containing beverages. To reduce pain, subjects were also instructed to paste a monoanesthetic seal (PENLES; Wyeth Lederle, Inc.) at the location of the cannula insertion of the nondominant arm about 1 hour before the experiment. Subjects who suffered from any infectious illnesses within two weeks prior to the experiment were rescheduled.

The experimental sessions were composed of two stress tasks and two rest periods. The interval between tasks was about one hour. The order of the mental arithmetic and the cold pressor tasks was counterbalanced. Subjects were tested individually between 9:00 AM and 2:00 PM in a temperature- and humidity-controlled room. First, a cannula was inserted into the forearm vein of

her non-dominant arm. Next, electrodes for electrocardiographic measurements and a finger cuff for recording blood pressure were attached. For the next 15 minutes, subjects filled in psychological questionnaires. After 15 minutes rest period, the first blood sample (for assays of female sex hormones and immunological parameters) was taken. Then, instructions were given for one of the two tasks. Subjects were allowed to practice the mental arithmetic task for two minutes, but there was no such practice for the cold pressor task. After that, the subjects performed either the mental arithmetic task for 15 minutes or the cold pressor task for 10 minutes. Immediately after each task, the second blood sample was taken, and each subject filled in the questionnaire again. After 15 minutes rest period, the third blood sample was taken, and the questionnaires were filled out for the third time. After an intermission of 45 minutes, the second task was conducted, followed by a rest period of 15 minutes. Before and after the second task and after the rest period, blood sample and psychological data were taken. Autonomic indexes (ECG and BP) were measured continuously through the experimental session. After the end of the procedure, the electrodes, blood-pressure cuff, and cannula were removed, and the subjects were fully debriefed. Three thousand Japanese yen was paid to subjects for reward.

2.2.4 Measures

2.2.4.1 Immunological measures

Blood samples for immunological determinations were collected in heparinized tubes. The numbers of total white blood cells (WBCs), lymphocytes, monocytes, and granulocytes per sample were determined by standard means. The percentages of lymphocyte subsets were determined by flow cytometry (FACSCalibur, Becton-Dickinson). A whole-blood lysis method was used to stain the cells with the following pairs of Fluorescein isothiocyanate (FITC)/Phycoerythrin (PE) conjugated, isotype-matched monoclonal antibodies (DAKO, Inc., Carpinteria, CA): mouse IgG1, CD3+ indicating T cells (T cell), CD3+ / CD4+ indicating helper T cells (helper T cell), CD3+ / CD8+ indicating cytotoxic T cells (cytotoxic T cell), CD3- / CD19＋ indicating B cells (B cell), and CD3- / CD16＋ / CD56＋ indicating NK cells (NK cell).

The chromium release assay was used to determine NKCA. Effector and

^{51}Cr-labeled K562 target cells were incubated for 3.5 hours in 96-well round-bottomed plates. The wells contained effector and target cells at ratios of 20:1. Wells with K562 in medium alone or including 1N-HCL were used to assess spontaneous and maximum releases. Radioactivity was counted in a γ-counter, and the percentage of specific lysis was determined according to the formula: (mean experimental cpm - mean spontaneous release cmp) / (mean maximal cmp - mean spontaneous release cmp) × 100.

2.2.4.2 Cardiovascular measures

Cardiodynamic activity was evaluated by measurements of electrocardiography (ECG) and non-invasive finger blood pressure (FINAP). ECG was recorded using an MP 100 system (Biopac Systems, Inc.). Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were recorded through the finger cuffs of a Portapres Model 2 (TNO Biomedical Instrumentation, Inc.) attached to the third finger of the non-dominant arm. Each indicator was recorded continuously during the two tasks and rest periods. Analyses of ECG and FINAP waveforms were performed using software (AcqKnowledge) for the MP 100.

2.2.4.3 Psychological measures

The subjects were asked to evaluate subjectively intensity of the stress, physical fatigue, and mental fatigue on analog-visual scales (0% to 100%). In addition, they completed a Japanese version (Yokoyama et al., 1990) of the Profile of Mood States (POMS) (Usala and Hertzog, 1989) and a Japanese version (Nakazato and Mizuguchi, 1982) of the State-Trait Anxiety Inventory (STAI) (Speilberger et al., 1970). POMS is composed of six sub-scales (Tension-Anxiety, Depression-Dejection, Anger-Hostility, Vigor, Fatigue, and Confusion), with a total of 65 items. STAI is composed of two sub-scales to measure state and trait anxiety and consists of 40 items).

2.2.5 Statistical analysis

Prior to statistical analysis, mean values of HR, SBP and DBP data were calculated for the last 5 minutes of the pre-experimental baseline period, two

periods during the stress tasks (0 to 5 minutes and 5 to 10 minutes), and the last 5 minutes of the rest period after each task. The cardiovascular data were analyzed using repeated-measures analyses of variance (ANOVAs) with a within-subjects factor of task (mental arithmetic vs. cold pressor) and a within-subjects factor of period (baseline, $stress_{5min}$, $stress_{10min}$, rest). Immune and psychological data were analyzed using repeated-measures ANOVAs with a within-subjects factor of task and a within-subjects factor of period (baseline, stress, rest). The Greenhouse-Geisser epsilon correction factor, ε (Jennings & Wood, 1976), was used where appropriate. In cases where significant interactions were found in the ANOVAs, post hoc analyses using LSD tests ($p < .05$) were conducted to examine which combinations of data points differed significantly. Based on the study by Willemsen et al. (2002), Pearson correlation coefficients were computed among change scores (scores at the stress period – scores at the baseline) of these indexes to examine the relationship between immune and cardiovascular reactivity.

2.3 Results

2.3.1 Immunological measures

The immune data at the baseline, stress, and rest periods are summarized for each task in Table 2.1. Figure 2.1 illustrates changes in the proportion of T cells, B cells, NK cells, and NKCA.

ANOVAs yielded significant main effects of Task for WBCs, $F(1,23) = 6.61$, $p < .05$, meaning that there were more WBCs during the cold pressor task than during the mental arithmetic task. In addition, the main effects of Period, reflecting temporal variation of indexes during the experimental session, were significant for WBCs, lymphocytes, granulocytes, T cells, B cells, helper T cells, NK cells, and NKCA ($F(2,46) = 20.72$, $p < .01$; $F(2,46) = 26.65$, $p < .01$; $F(2,46) = 4.16$, $p < .05$; $F(2,48) = 29.90$, $p < .01$; $F(2,48) = 21.96$, $p < .01$; $F(2,48) = 29.53$, $p < .01$; $F(2,48) = 39.82$, $p < .01$; $F(1,11) = 37.60$, $p < .01$). WBCs, lymphocytes, granulocytes, NK cells, and NKCA were higher after both stress tasks than at the baseline. In contrast, T cells, B cells, and helper T cells were lower after both stress tasks than at the baseline. Furthermore, interaction between Task and Period was significant for NK cells and T cells ($F(2,48) = 3.80$, $p < .05$; $F(2,48) = 29.90$ $p < .05$). Post hoc analyses using LSD tests ($p < .05$) indicated increased

proportion of NK cells after the mental arithmetic task compared to after the cold pressor task, and the decreased proportion of T cells was more prominent after the mental arithmetic task than after the cold pressor task.

These results indicate that acute stressor increased innate immunity as represented by NK cells and NKCA, and decreased acquired immunity as represented by T cell and helper T cells. Especially, the degree of these immune responses to mental arithmetic as active coping condition were higher than cold pressor as passive coping condition.

Table 2.1 Means (SDs) of immunological measures and results of ANOVAs

	Task	Baseline	Stress	Rest	N	[a]Effect
WBC (×10^3/μl)	[b]MA	4.26(.83)	4.49(.82)	4.41(.79)	24	Task*, Period**
	[c]CP	4.47(.88)	4.65(.91)	4.69(.90)	24	
Lymphocyte (×10^{3}μ/l)	MA	1.47(.32)	1.63(.28)	1.58(.27)	24	Period**
	CP	1.61(.37)	1.73(.38)	1.75(.36)	24	
Monocyte (×10^{3}μ/l)	MA	.37(.13)	.37(.12)	.35(.15)	24	n.s
	CP	.35(.13)	.37(.13)	.35(.14)	24	
Granulocyte (×10^{3}μ/l)	MA	2.42(.69)	2.49(.68)	2.48(.67)	24	Period*
	CP	2.51(.66)	2.55(.70)	2.59(.78)	24	
Helper T cells (%)	MA	45.27(8.32)	41.07(8.76)	44.01(7.45)	25	Period**
	CP	44.19(7.40)	41.52(7.67)	43.60(6.99)	25	
Cytotoxic T cells (%)	MA	27.75(4.37)	27.45(4.35)	28.01(3.85)	25	n.s
	CP	28.10(4.11)	27.99(4.39)	28.04(4.19)	25	

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

[b] Mental arithmetic, [c]Cold pressor.

**:p<.01, *:p<.05

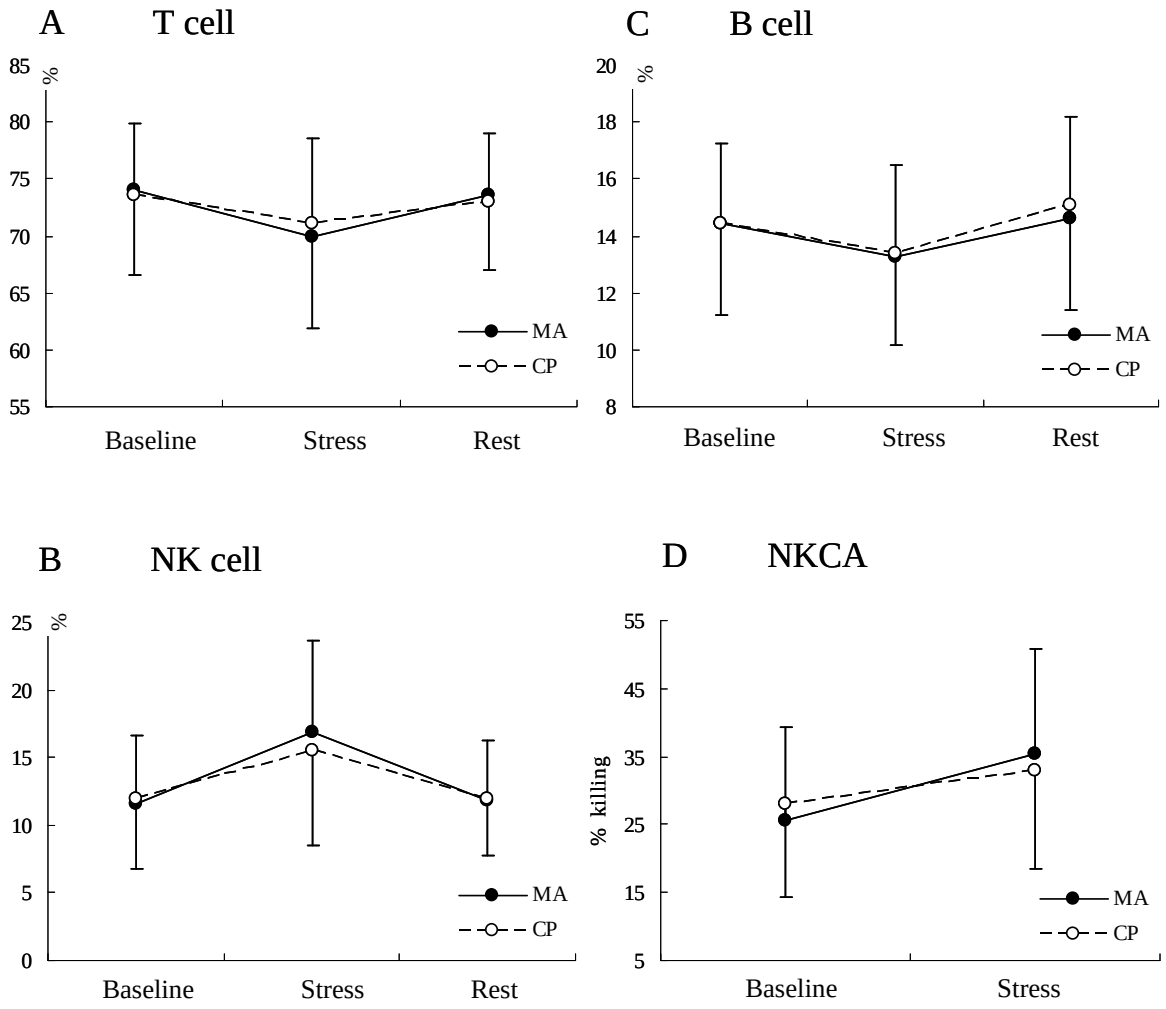

Figure 2.1 Changes in percentages of T cells (A), NK cells (B), B cells (C), and NKCA (D) at three measurement points. Error bars indicate standard deviations. MA = mental arithmetic; CP = cold pressor

2.3.2 Cardiovascular measures

Changes in cardiovascular indexes are illustrated in Figure 2.2. ANOVAs yielded a significant main effect of Task for HR ($F(1,23) = 7.34$, $p < .01$), and significant main effects of Period for HR, SBP, and DBP ($F(3,69) = 11.79$, $p < .01$; $F(3,69) = 22.86$, $p < .01$; $F(3,69) = 22.51$, $p < .01$). These results indicate increased cardiovascular activity during both stress tasks from at baseline or in the rest periods. Interactions between Task and Period were significant for HR and DBP ($F(3,69) = 23.43$, $p < .01$; $F(3,69) = 6.27$, $p < .01$). Further analyses ($p < .05$) indicated that HR increased during the mental arithmetic, and recovered to the baseline in the rest period. For the cold pressor task, on the other hand, HR was lower during the task than at the baseline, and it remained below baseline through the rest period. HR was higher during the mental arithmetic task as active coping condition than during the cold pressor task as passive coping condition at stress $_{5min}$, stress$_{10min}$ periods. Additionally, the degree to which DBP increased was significantly larger during the mental arithmetic than during the cold pressor.

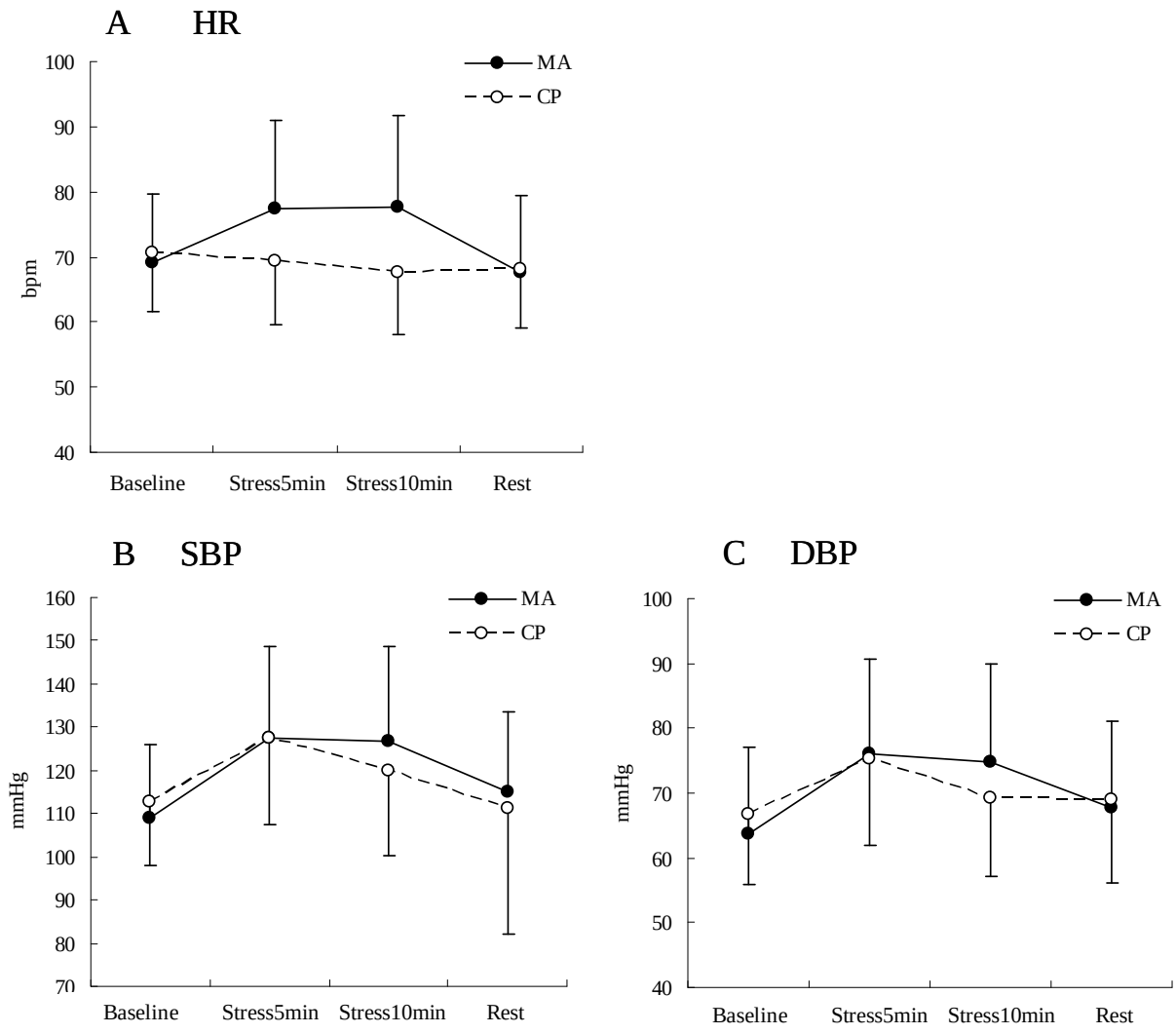

Figure 2.2 Changes of HR (A), SBP (B), and DBP (C) at four measurement points. Error bars indicate standard deviations. MA = mental arithmetic; CP = cold pressor

2.3.3 Psychological measures

The psychological data at the baseline, stress, and rest periods are presented in Table 2.2. ANOVAs yielded a significant main effect of Task for state anxiety ($F(1,23) = 6.27$, $p < .05$). In addition, the main effects of Period were significant for physical fatigue, mental fatigue, perception of stress, and state anxiety ($F(2,46) = 25.23$, $p < .01$; $F(2,46) = 25.03$, $p < .01$; $F(2,46) = 35.00$, $p < .01$; $F(2,46) = 25.84$, $p < .01$). Additionally, significant main effect of Period were observed in the Tension-Anxiety, Anger-Hostility, Vigor, and Confusion of POMS ($F(2,46) = 9.01$, $p < .01$, , $F(2,46) = 14.60$, $p < .01$, , $F(2,46) = 12.43$, $p < .01$, and, $F(2,46) = 9.56$, $p < .01$). A significant Task and Period interaction was observed only for mental fatigue ($F(2,44) = 3.29$, $p < .05$). Post hoc analyses ($p < .05$) indicated that mental fatigue was higher during the mental arithmetic task than during the cold pressor task. However, except for mental fatigue, both the tasks showed the same influences on the psychological measures. That is, physical fatigue, perception of stress, trait anxiety, and Tension-Anxiety and Confusion of POMS increased, whereas Vigor of POMS decreased after the tasks.

Table 2.2 Means (SDs) of psychological measures and results of ANOVAs

	Task	Baseline	Stress	Rest	N	[a]Effect
Perception of stress	[b]MA	20.43(17.92)	45.39(22.23)	34.30(20.10)	24	Period**
	[c]CP	21.39(17.29)	38.52(20.76)	35.39(20.83)	24	
Mental fatigue	MA	25.70(19.03)	44.52(19.13)	39.43(20.81)	24	Period**,
	CP	24.48(17.47)	35.91(18.09)	39.48(19.37)	24	Task×Period*
Physical fatigue	MA	28.30(19.56)	40.61(18.30)	40.65(18.49)	24	Period**
	CP	26.96(16.36)	35.83(15.39)	42.00(18.12)	24	
STAI						
State-anxiety	MA	40.00(8.91)	48.10(10.29)	41.05(8.74)	24	Task*, Period**
	CP	37.71(7.26)	43.81(7.28)	40.86(7.48)	24	
Trait-anxiety	MA	44.20(12.00)	—	—	24	n.s
	CP	43.44(9.70)	—	—	24	
POMS						
Tension-Anxiety	MA	12.70(7.84)	14.30(7.56)	11.39(8.06)	24	Period**
	CP	11.09(6.94)	13.83(7.24)	10.69(6.46)	24	
Depression-Dejection	MA	12.47(10.37)	11.70(11.44)	10.78(11.03)	24	n.s
	CP	10.65(10.17)	11.52(9.96)	10.39(9.62)	24	
Anger-Hostility	MA	9.41(9.27)	8.41(8.58)	6.14(7.35)	24	Period*
	CP	7.95(8.73)	8.05(8.81)	6.86(8.20)	24	
Vigor	MA	13.09(6.05)	10.35(6.18)	10.48(6.76)	24	Period**
	CP	13.00(5.47)	10.57(6.75)	10.65(6.05)	24	
Fatigue	MA	9.30(6.09)	10.04(5.42)	9.17(6.68)	24	n.s
	CP	8.87(5.52)	9.91(6.06)	9.30(5.71)	24	
Confusion	MA	9.82(4.74)	11.48(4.90)	9.52(5.42)	24	Period**
	CP	10.22(4.67)	11.26(5.05)	10.00(5.34)	24	

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

[b] Mental arithmetic, [c]Cold pressor.

**:p<.01, *:p<.05

2.3.4 Associations between immune and cardiovascular reactivity

Correlations between changes in immune and cardiovascular parameters were computed for the whole sample (see Table 2.3). The results indicated that, for the mental arithmetic task, measures of blood pressure correlated negatively with change in the proportion of T cells as acquired immunity but positively with change in the proportion of NK cells as innate immunity. For the cold pressor task, measures of blood pressure correlated positively with changes in the proportion of B cells as acquired immunity.

Table 2.3 Correlations between changes in immune and cardiovascular measures

Mental arithmetic	HR_{5min}	HR_{10min}	SBP_{5min}	SBP_{10min}	DBP_{5min}	DBP_{10min}
WBC						
Lymphocyte						
Monocyte						
Granulocyte						
T cells			－.44*	－.46*	－.40*	－.41*
Helper T cells						
Cytotoxic T cells						
B cells						
NKcells			.40*	.44*		
NKCA						
Cold pressor	HR_{5min}	HR_{10min}	SBP_{5min}	SBP_{10min}	DBP_{5min}	DBP_{10min}
WBC						
Lymphocyte						
Monocyte						
Granulocyte						
T cells						
Helper T cells						
Cytotoxic T cells						
B cells			.46*	.37*	.51**	.49**
NKcells						
NKCA						

**: p<.01, *:p<.05

2.4 Discussion

Supporting the hypothesis, both stress tasks induced typical acute stress responses in the circulating lymphocytes such as facilitation of innate immunity represented by the proportion of peripheral blood NK cells and NKCA, and the suppression of acquired immunity represented by the proportion of peripheral blood helper T cells. Furthermore, the decrease of CD3＋ T cells and increases of NK cells were more remarkable in the mental arithmetic task than in the cold pressor task, indicating that active coping situation should have a greater impact on immunity than passive coping situation (Figure 2.1).

According to the literatures, redistribution of peripheral lymphocytes to acute stressor is mainly mediated by increased blood flow and adrenaline and

noradrenaline through surface receptors (mainly, β2- and α-adrenoreceptors) (Benschop et al., 1993, 1996; Landmann et al., 1984; Mills et al., 2000). Thus, it was suggested that differences of lymphocytes responses under both tasks were explained by differences of blood flow and secretion of catecholamine depending on both coping situations. In this experiment, HR and BP were increased in both tasks. Especially, both of HR and BP remarkably increased during mental arithmetic task, whereas only BP increased by cold pressor (Figure 2.2). The increased BP with unaffected HR in the cold pressor task might be explained by peripheral vasoconstriction by noradrenaline and inhibition of sympathetic nervous system associated with increase of cardiac vagus reaction (Bandler et al., 2000; Bosch et al., 2003; Keay and Bandler, 2001; LeBlanc et al., 1979; Willemsen et al., 2002).

To sum, in the active coping situation, innate immunity as NK cells was increased and acquired immunity as CD3 + T cells was decreased. Additionally, these immune responses in the passive coping situation were remarkably lower than those in the active coping situation. It suggested that immune responses under the active coping situation were evoked by increase of HR and BR triggered by adrenaline and noradrenaline, while immune responses in the passive situation were elicited by mainly increase of only BP cased by noradrenaline. Furthermore, it suggested that HR under the passive situation was inhibited by reactivity of cardiac vagus system.

Chapter 3

Effects of controllability on immune and autonomic nervous responses to acute stressor (Experiment 2)

In the previous chapter 2, peripheral immune responses to active and passive coping situations were demonstrated. The next aim of this study is an experimental examination of effects of stressor controllability on immune responses to acute stressor. According to previous evidences, it was predicted that active coping is usually evoked under controllable stressors while passive coping is evoked under uncontrollable stressors (Bandler et al., 2000; Keay and Bandler, 2001).

However, it seems that active stress coping is evoked at the beginning of exposure to a new stressor, even if the situation was uncontrollable (Obrist, 1976). They examined acute stress responses in three conditions with different difficulty (easy, hard, and impossible conditions), and further examined time course changes of the responses. Acute cardiovascular stress responses in the three conditions evoked equally at the beginning of the task, however responses of the easy and impossible conditions decreased gradually as time course while HR and blood pressure in the hard condition maintained high levels. This is because an organism continues to more actively challenge the stressor and seek an appropriate coping strategy. Only after active stress coping and over continuance of uncontrollable situation should the strategy shift to passive coping type (Obrist, 1976). Therefore, at the beginning of exposure to acute stress task, acute stressor should evoke rapidly response of active coping type, regardless of controllable or uncontrollable. Subsequently, stress responses in only uncontrollable condition should be inhibited gradually over continuance of stress task.

In this chapter, the effect of controllability over acute stressor on peripheral immune responses under active coping situation will be addressed.

Data in experiment 2 was published in Isowa, Ohira, and Murashima (2006). Additionally, this study was provided in 45th Annual Meeting of Society for Psychophysiological Research (Portugal, 2005).

3.1 Introduction

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; Peters et al., 2003, 1999, 1998; Sieber et al., 1992; Weisse et al., 1990). The effects of stressor controllability have been widely studied in animal models using a triadic-yoked design (Seligman, 1975). Some previous studies using animals such as rats found that controllability over stress task affects physiological responses (Laudenslager et al., 1983; Nakata et al., 1996). Several researchers attempted to extend these findings to human subjects (Weisse et al., 1990; Sieber et al., 1992; Gomez et al., 1994; Peters et al., 2003, 1999, 1998), though their results have been inconsistent.

The aim of this chapter is to examine effects of controllability over acute stressor on immune responses. For the purpose, in this experiment 2 I used the triadic-yoked design: controllable, uncontrollable, and control conditions. According to discussion in section 1.5.1, type and physical load of stress task in the controllable and the uncontrollable conditions must be equivalent to examine effects of stressor controllability. Furthermore, mental arithmetic task as active coping established in experiment 1 was used in this experiment 2, since it was predicted that uncontrollable stressor should inhibit typical acute stress responses as active coping (Bandler et al., 2000; Keay and Bandler, 2001). As manipulation of controllability, I used a correct/error feedback to participants' answers. In the controllable condition, correct feedback corresponding to the participants' answers was presented, but bogus and yoked feedback was given in the uncontrollable condition.

Following previous studies described above, I hypothesized that mental arithmetic task should elicit typical acute stress responses such as an increase of NK cells and a decrease of helper T cells and B cells in the controllable and the uncontrollable condition at the beginning of task. Subsequently, stress responses in only uncontrollable condition should make a shift from active coping type to passive coping type gradually over continuance of stress task. Thus, immune responses in the uncontrollable condition should be inhibited in the later time course of the stress task.

3.2 Methods

3.2.1 Participants

Forty-three female undergraduates (age range, 19 to 34 years; mean age = 21.51 years) participated in the present study. The mean BMI of the subjects was 21.03 kg/m^2 (SD = 2.22). None of the subjects were suffering from any chronic illness, and none were taking medication known to influence immunity. In addition, no participant was using oral contraceptives. They participated in the experiments during the late luteal and early follicular phases. The mean value of estradiol was 50.87 pg/ml (SD = 55.31), and the mean value of progesterone was 0.96 ng/ml (SD = 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.

3.2.2 Acute stress task and manipulation of controllability

Each participant was randomly assigned to one of three groups: a controllable stressor group (C), an uncontrollable stressor group (UC), or no stressor group (control). Eighteen subjects were assigned to the C and UC groups, and seven to the control group. The subjects in the C and the UC groups performed mental arithmetic as active coping condition for 15 min. The subjects were instructed 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 100dB) 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 maintain 90% accuracy in the task, otherwise their data would be excluded from the dataset.

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 even 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.

3.2.3 Experimental procedure

Subjects were instructed to eat light breakfast and avoid drinking caffeine-containing beverages. Also, 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 one hour before the experiment to reduce pain. Subjects suffering from infectious illness in last two weeks were rescheduled.

The experimental sessions were composed of a mental arithmetic task and four rest periods. Subjects were tested individually between 9:00 AM and 12:00 PM in a temperature- and humidity-controlled room. First, 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, subjects filled out psychological questionnaires. After 15 minutes of rest period, the first blood sample (for assays of female sex hormones and immunological parameters) was taken. Next, instructions were given for the mental arithmetic task, and the subjects were allowed to practice it for two minutes. Subjects then performed the mental arithmetic task for 15 minutes. Immediately after the task, the second blood sample was taken, and each subject filled out the questionnaire again. After each rest period (15 min, 30 min, and 60 min), the third, fourth, and fifth blood sample was 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. Two thousand and four hundred Japanese yen was paid to subjects for reward.

3.2.4 Measures

3.2.4.1 Immunological measures

Blood samples for immunological determinations were collected in heparinized

tubes. The immune parameters (the numbers of total white blood cells (WBC), lymphocytes, monocytes, and granulocytes per sample, proportion of lymphocyte subsets (CD3+ indicating T cells (T cell), CD3+ / CD4+ indicating helper T cells (helper T cell), CD3+ / CD8+ indicating cytotoxic T cells (cytotoxic T cell), CD3- / CD19＋ indicating B cells (B cell), and CD3- / CD16＋ / CD56＋ indicating Natural Killer cells (NK cell), and NKCA were determined by same standard means as first experiment. Percentages of lymphocyte subsets were determined by flow cytometry (FACS Calibur; Becton-Dickinson, San Jose, CA), and a chromium release assay was used to determine Natural Killer cell activity (NKCA).

3.2.4.2 Cardiovascular measures

As in the first experiment, 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. Analyses of ECGs and FINAP waveforms were performed using the software package AcqKnowledge for the MP 100.

3.2.4.3 Psychological measures

The subjects were asked to evaluate subjectively the intensity of their stress, physical fatigue, and mental fatigue on visual–analog scales (0% to 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).

3.2.5 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 to 5 min, 5 to 10 min, and 10 to 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) x Period (baseline, $stress_{5min}$, $stress_{10min}$, $stress_{15min}$, $rest_{15min}$, $rest_{30min}$, $rest_{60min}$). The immune and psychological measures were analyzed using repeated-measures ANOVAs: Group x Period (baseline, stress, $rest_{15min}$, $rest_{30min}$, $rest_{60min}$). The Greenhouse-Geisser epsilon correction factor, ε (Jennings & 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 and cardiovascular 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.

3.3 Results

3.3.1 Immune measures

The immune data at the baseline, task, and rest periods are summarized in Table 3.1. Figure 3.1 illustrates changes in the percentages of T cells, NK cells, B cells, and NKCA. The main effects of Period were significant for WBCs, lymphocytes, NK cells, NKCA, T cells, B cells, helper T cells, and cytotoxic T cells ($Fs(1 - 2,34 - 113) = 9.47 - 50.11$, $ps < .001$).

In the C and the UC groups, indices of innate immunity (percentages of NK cells

and NKCA) were significantly higher, and indices of acquired immunity (percentages of T cells, B cells, helper T cells and cytotoxic T cells) were significantly lower, after the acute stress task than at the respective baseline (see Figure 3.1). 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* < .001 - .05) but not during the rest periods (ps > .01). Notably, there was no significant difference between the C and the UC groups in any indices.

Table 3.1 Means (SDs) of immunological measures and results of ANOVAs

	Group	Baseline	Stress	Rest 15min.	Rest 30min.	Rest 60min.	N	[a]Effect
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	Period**
	UC	4.45(1.10)	4.73(1.06)	4.62(1.03)	4.71(1.03)	4.78(1.04)	18	
	Control	3.85(.45)	3.95(.48)	4.08(.46)	4.13(.50)	4.17(.46)	6	
Lymphocyte（$\times 10^3\mu/l$）	C	1.59(.45)	1.76(.51)	1.61(.46)	1.72(.50)	1.77(.55)	17	Period**
	UC	1.44(.23)	1.66(.33)	1.57(.25)	1.59(.28)	1.69(.33)	18	
	Control	1.15(.36)	1.23(.12)	1.33(.15)	1.38(.12)	1.69(.42)	6	
Monocyte ($\times 10^3\mu/l$)	C	.46(.21)	.51(.20)	.47(.19)	.45(.18)	.47(.16)	17	n.s.
	UC	.44(.18)	.43(.19)	.40(.15)	.46(.19)	.41(.16)	18	
	Control	.43(.12)	.43(.15)	.42(.13)	.40(.06)	.30(.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(.98)	2.66(.91)	2.67(.86)	18	
	Control	2.27(.42)	2.28(.41)	2.33(.48)	2.35(.49)	2.3(.43)	6	
helper T cells（%）	C	49.68(7.53)	42.47(10.48)	47.09(7.55)	45.90(7.21)	44.85(6.32)	17	Period** Group×Period*
	UC	48.29(6.68)	39.57(7.78)	44.45(7.03)	44.58(8.07)	43.81(6.98)	18	
	Control	46.73(3.45)	45.45(4.28)	44.62(3.33)	43.86(3.68)	42.64(4.34)	6	
cytotoxic T cells（%）	C	28.66(6.85)	27.25(6.16)	28.52(6.21)	28.11(5.84)	27.87(5.72)	17	Period*
	UC	30.41(5.74)	29.09(5.53)	30.20(5.58)	29.59(5.26)	29.40(5.33)	18	
	Control	28.69(2.94)	27.38(3.42)	27.92(3.50)	27.58(3.69)	27.40(3.44)	6	

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

C; controllable group, UC; uncontrollable group, Control; control group

**:p<.01, *:p<.05

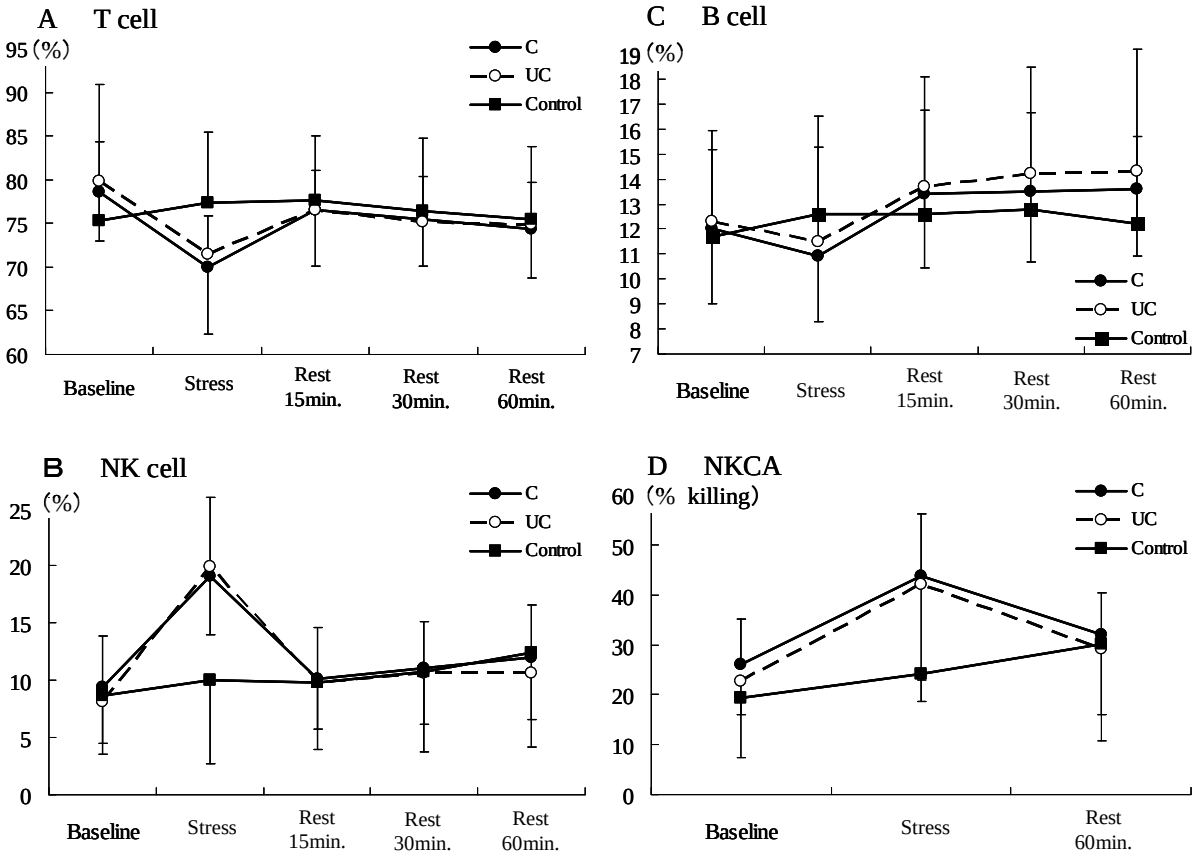

Figure 3.1 Percentage of T cells（A）, NK cells（B）, and B cells（C）, and NKCA （D） at the five measurement points. Vertical bars indicate standard deviations. C = Controllable group, UC = Uncontrollable group, Control = Control group

3.3.2 Cardiovascular measures

Changes in cardiovascular indices are illustrated in Figure 3.2. ANOVAs yielded significant main effects of Group for HR and DBP ($F(2,39) = 4.09$, $p < .05$, $F(2,39) = 4.90$, $p < .05$). There were significant main effects of Period for HR, SBP, and DBP ($Fs(1 - 2,64 - 83) = 25.62 - 38.85$, $p < .01$). In the C and UC groups, all cardiovascular parameters were significantly higher during the stress task than at the respective baseline. These parameters remained elevated and did not show 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 ($Fs(12,234) = 4.33 - 20.93$, $p < .01$) but not during the rest periods ($ps > .01$). For the cardiovascular measures, there were no significant differences between the C and the UC groups in any indices.

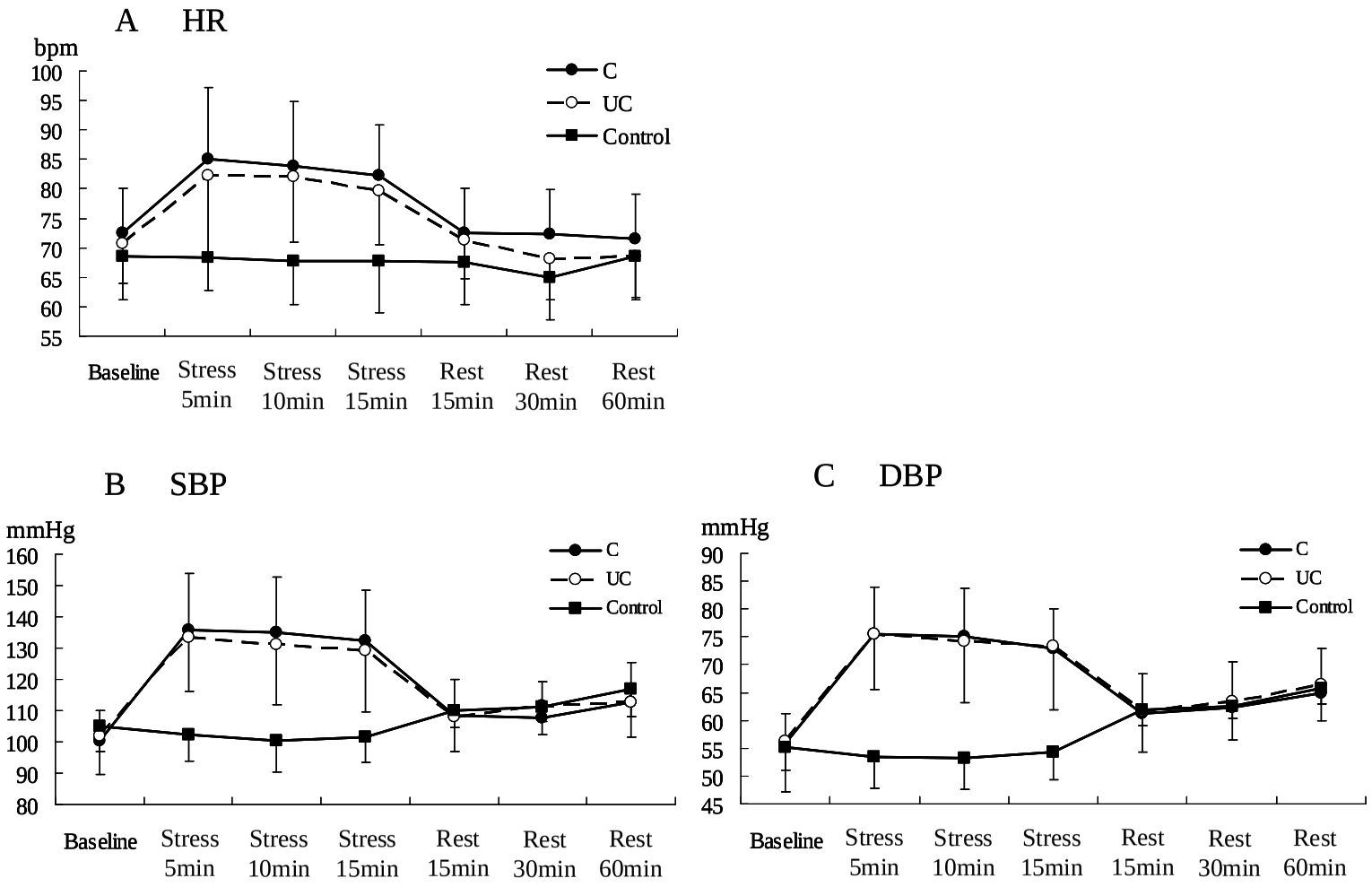

Figure 3.2 HR (A), SBP (B), and DBP (C) at the seven measurement points during baseline and task periods. Vertical bars indicate standard deviations. C = Controllable group, UC = Uncontrollable group, Control = Control group

3.3.3 Psychological measures

The psychological data at baseline, after the stress task, and during the rest periods are presented in Table 3.2. Concerning the perceived controllability of the groups, the mean value of this parameter was 49.22 (SD = 17.20) in the C group, and 38.56 (SD = 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 revealed significant main effects of Group for perception of stress, state anxiety, and T-A, D, A-H, C of POMS ($Fs(2,34 - 39) = 3.53 - 6.72$, $ps < .01 - .05$). 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 ($Fs(1 - 2,54 - 109) = 3.83 - 18.44$, $ps < .01 - .05$). Significant Group×Period interactions were observed for mental fatigue, perception of stress, state anxiety, and T-A, V, C of POMS, ($Fs(4 - 8,68 - 156) = 2.72 - 4.92$, $ps < .01 - .05$). Post hoc analyses ($p < .05$) indicated that mental 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.

Table 3.2 Means (SDs) of psychological measures and results of ANOVAs

	Group	Baseline	Stress	Rest 15min.	Rest 30min.	Rest 60min.	N	[a]Effect
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** Group×Period**
	UC	26.06(21.53)	53.83(27.89)	26.22(15.44)	20.94(14.74)	20.72(17.83)	18	
	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** Group×Period**
	UC	33.67(24.15)	53.33(23.93)	33.22(18.27)	25.83(16.88)	25.39(19.01)	18	
	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** Group×Period**
	UC	38.83(5.26)	50.83(10.06)	37.78(4.81)	37.17(4.63)	34.72(5.49)	18	
	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** Group×Period**
	UC	13.19(4.83)	18.13(8.34)	—	—	7.75(3.40)	16	
	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** Group×Period*
	UC	13.76(4.28)	8.35(5.20)	—	—	12.41(5.36)	17	
	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 parameters that showed interaction are shown in table 3.2. The main effect of periods was found in the following parameters: Physical fatigue** and Depression-Dejection**, Confusion** and Anger-Hostility** of POMS. The main effect of group was found in the following parameters: Depression-Dejection* and Anger-Hostility* of POMS. There was no significant effect not interaction in Trait-anxiety. a Main effects and interactions revealed by ANOVAs.

C; controllable group, UC; uncontrollable group, Control; control group

**:p<.01, *:p<.05

3.3.4 Associations between immune and cardiovascular reactivity

Stressor controllability did not affect cardiovascular or immune parameters. Therefore, to further examine effects of controllability on functional associations among the immune and cardiovascular systems during the acute stress task, I performed correlation analyses among changes in immune and cardiovascular parameters in each experimental group separately. Furthermore, to examine the temporal characteristics of the influences of autonomic activity on the immune functions, I 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 of the C and UC groups are presented in Table 3.3. There was no significant correlation 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 3.3). To examine whether these correlations in the UC group were artifacts, I performed a scatterplotting of the SBP and immune parameters in the UC and the C group, respectively, as shown in Figure 3.3. Namely, I certificated whether such correlations are actually liner or not, by observing the patterns of the scatterplotting. 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 T cells and 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 T cells, helper T cells, and 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.

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 T cells, helper T cells, or NK cells in the UC group were significantly larger than those in the C group (zs = 1.99 - 2.39, $p < .05$).

Table 3.3 Correlations between changes in immune and cardiovascular measures (each group)

Controllable	HR_{5min}	HR_{10min}	HR_{15min}	SBP_{5min}	SBP_{10min}	SBP_{15min}	DBP_{5min}	DBP_{10min}	DBP_{15min}
Granulocyte									
T cells	.01	-.15		-.04	-.16	-.14	.00	-.04	-.04
helper T cells				-.24	-.28	-.28	-.14	-.19	-.16
cytotoxic T cells									
B cells	-.46	-.43	-.41	-.35	-.39	-.42	-.19	-.12	
NK cells	.19	.33	.32	.19	.33	.32	.08	.10	.15
NKCA	.78*								
Uncontrollable	HR_{5min}	HR_{10min}	HR_{15min}	SBP_{5min}	SBP_{10min}	SBP_{15min}	DBP_{5min}	DBP_{10min}	DBP_{15min}
Granulocyte									
T cells	-.54*	-.51*		-.63**	-.66**	-.64**	-.55**	-.69**	-.68**
helper T cells				-.58**	-.74**	-.70**	-.51**	-.70**	-.73**
cytotoxic T cells									
B cells	-.46**	-.44*	-.42**	-.43**	-.45**	-.42**	-.32*	-.31*	
NK cells	.64**	.58**	.52**	.65**	.69**	.68**	.49*	.62**	.60**
NKCA	.33								

**: p<.01, *:p<.05

Correlation coefficients of controllable versus uncontrollable groups are shown. The underlined scores in Uncontrollable indicate significant differences from those in Controllable.

In control group, there were significant correlations between SBP10min and Monocyte (r = .80*), B cells (r = -.77*), or NKCA (r = .91*).

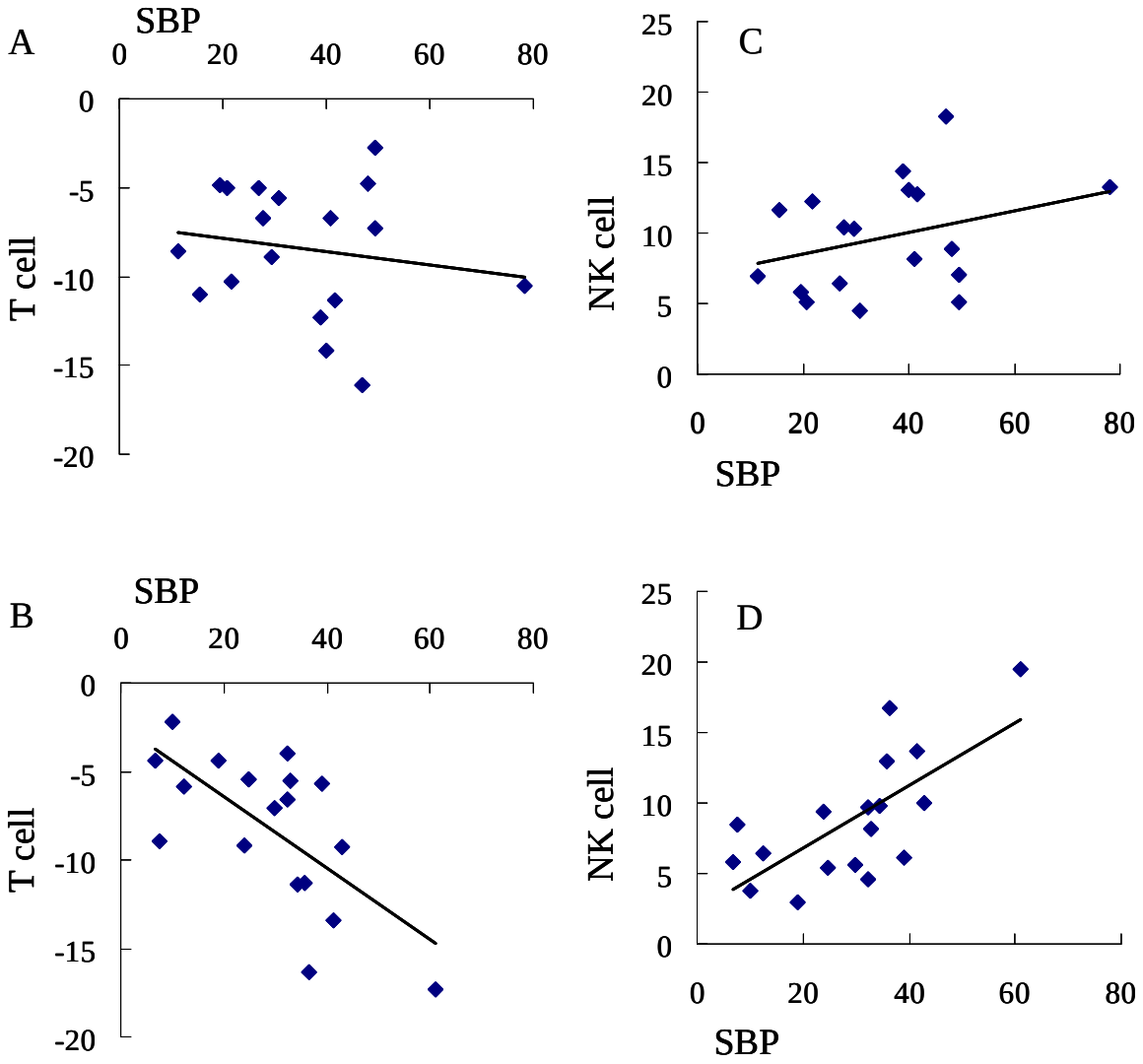

Figure 3.3 Scatterplotting of SBP and immune parameters in controllable and uncontrollable groups. A and C = Controllable group, B and D = Uncontrollable group.

3.4 Discussion

During the mental arithmetic task, the proportion of NK cells increased while proportions of T cells, helper T cells, and B cells decreased, thus the present result replicated findings in experiment 1. Enhanced cardiovascular responses (HR, SBP, and DBP) were observed in both groups, which mediate redistribution of these lymphocytes. These are typical acute stress response as active coping, and 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 is 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; Noha et al., 2002).

Contrary to the prediction, no effects of controllability over the acute stressor were observed in any immune parameters, whereas the experimental manipulation of controllability was valid. ANOVA revealed there was no significant difference in immune and cardiovascular responses under the task and rest periods in both groups. Thus, acute stress responses of uncontrollable group didn't shift from responses of the active coping type to that of passive coping type for 15 minutes of the task (Figure 3.1, 3.2).

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 3.3). Uncontrollability served to consistently strengthen the association between cardiovascular and immune parameters which began from 5 min after task start and continued until the end of the task. The scatterplotting of blood pressure and immune parameters (see Figure 3.3) suggested that those effects were not just artifacts. Taken together, the findings in the present study for the first time suggested that uncontrollability of acute stress, at least in some situations, might strengthen the correspondence between the autonomic nervous systems and immune systems rather than increasing the reactivity in each system.

Strong correlations between immune and cardiovascular reactivity in the UC group reflected individual differences under the uncontrollable stressor. In the UC group, some subjects showed high reactivity of immune and cardiovascular system, while other subjects showed low reactivity under the uncontrollable task (Figure

3.3). The high reactivity is associated with active coping, and low reactivity is associated with passive coping. Under controllable situation, organism takes active coping, and under uncontrollable situation organism takes passive coping. Thus, though participants in the UC group were exposed to uncontrollable acute stressor, responses to acute stressor should vary depending on evaluation of the situation at the time whether it is controllable or uncontrollable.

The two types of physiological stress responses can be interpreted as adaptive for survival in terms of the efficient allocation and usage of energy under variable environments. In other words, when a stressor is controllable, it would be adaptive to invest vast energy to overcome the stressful situation. On the other hand, when a stressor is uncontrollable, such a strategy might lead to waste energy, and thus another strategy to partially suspend investment of energy and seeks the most appropriate ways to cope with the stressor might be more adaptive (Blascovich et al., 1999; Maier et al., 2003). Therefore, low immune and cardiovascular reactivity observed in the UC group might be adaptive down-regulation of physiological responses to stressor for energy saving.

In the present experiment, no effect of controllability was observed in any immune and cardiovascular parameters for 15 minute of stress task. This is probably because at the beginning of the new stress task, all subjects continued to actively challenge the stressor and seek an appropriate coping strategy, regardless of groups. Similarly, absence of the main effect of GROUP in any immune and cardiovascular parameters might be due to mixed responses of active coping and passive coping.

3.5 Conclusion

At the beginning of exposure to a new stressor, active coping was evoked regardless of C or UC groups. In the situation of uncontrollable acute stress, active and passive coping was mixed due to individual differences. Finally, effects of uncontrollability appeared as strong correlations between immune and cardiovascular reactivity, but not as difference of mean value.

Chapter 4

The time course of effects of controllability on acute stress responses (Experiment 3)

In the chapter three, strong correlations between immune and cardiovascular reactivity were demonstrated as effects of the short-duration uncontrollable stressor. Active coping reaction should shift to passive coping reaction over longer continuance of uncontrollable situation. However, during for 15 minutes uncontrollable task in the experiment 2, the shift was not observed.

In the previous studies on the effects of stressor controllability on immune response to acute stressor in human, the timing of measurement was different and the results were inconsistent (Brosschot et al., 1998; Gomez et al., 1994; Peters et al., 1999, 2003; Weisse et al., 1990). In human studies, the effects of stressor controllability have been measured during the first block of an acute stress task or just after that. On the other hand, in typical animal studies, there was 24 hours interval between the first inescapable stressor and the second escapable stressor (Laudenslager et al., 1983; for a review, Maier and Watkins, 2005). Thus, there was interval length discrepancy between human and animal studies.

In this chapter, I changed part of procedures of experiment 2, and examined the time course effects of an uncontrollable acute stressor on immune responses up to 24 hours after exposure to the stressor.

4.1 Introduction

The aim of experiment 3 three is to examine effects of stressor controllability on peripheral immune responses when re-exposed to the same, but controllable stressor 24 hours after exposure to the first uncontrollable stressor.

It was already demonstrated in the experiment 2 that effects of stressor controllability for short-duration acute stress task was not observed due to individual difference of situation evaluation. In typical experimental procedures in

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Data in this study was provided in American Psychosomatic Society 64th Annual Meeting (Denver, Colorado-March, 2006).

animal studies on stressor controllability, the animals were first exposed to an inescapable stressor, and then re-exposed to the same, but escapable stressor, over 24 hours after exposure to the first stressor (Laudenslager et al., 1983; for a review, Maier and Watkins, 2005). The effects of stressor controllability were measured 24 hours after the stressor exposure rather than during, or soon after, exposure. Additionally, some previous studies on stressor controllability reported that rats exposed to escapable or inescapable shock presented the same response just after the shock, but that both groups exhibited different reactions when re-exposed to the same, but escapable stressor 24 hours after exposure to the first stressor (Drugan et al., 1984; Jackson et al., 1978; Maier et al., 1990; Weiss et al., 1981).

Because of this past disparity in methods, in the present study I examined the time course effects of an uncontrollable acute stressor on physiological responses up to 24 hours after exposure to the stressor. For this purpose, participants conducted an acute stress task (mental arithmetic) under a controllable or uncontrollable situation, and then re-conducted the same acute stress task several times, but in a controllable situation. I measured the parameters of the immune (proportions of NK cells, T cells, helper T cells, cytotoxic T cells, and B cells in blood) responses during the task. Furthermore, I measured the cardiovascular (heart rate: HR and blood pressure: BP) and neuroendocrine (adrenaline and noradrenaline) activities as indices of typical physiological responses to acute stress and as potential mediators in such modulation of peripheral immune responses.

Based on abovementioned studies, I hypothesized that mental arithmetic should elicit typical acute stress responses such as an increase of NK cells and a decrease of helper T cells and B cells. Additionally, the effects of uncontrollable stress would not dominantly emerge during the first exposure to the stress task, but instead it would emerge in the later time course as the down-regulation of immune reactivity via inhibited cardiovascular and autonomic nervous responses.

4.2 Methods

4.2.1 Participants

Twenty healthy female undergraduates (age range, 20 to 22 years; mean = 21.05) participated in the present study. The mean BMI of the participants was 20.27

kg/m^2 (SD = 1.39). None of the participants suffered from any chronic illness, and none of them was taking medication known to influence immunity. In addition, none of them was using oral contraceptives. They participated in the experimental sessions during their late luteal and early follicular phases. For confirmation of the periods of the menstrual cycle, the participants reported information about both their current menstrual cycles and basal body temperature. Our previous studies proved that the hormone levels of participants matched the levels expected based on the each participant's self report (Isowa et al., 2004, 2006; Kimura et al., 2005). All participants provided written informed consent to participate in the study. The Ethics Committee of the Mie Prefectural College of Nursing approved the present study.

4.2.2 Acute stress task, and manipulation of controllability

Participants were randomly assigned to one of two groups: a controllable stressor group (C) and an uncontrollable stressor group (UC). Each group consisted of ten participants. They performed a mental arithmetic task of the active coping condition under time pressure in four sessions (three sessions on the first day and one session on the second day) spread over two days. The first session lasted 30 min., and the second, third, and fourth sessions lasted six minutes for each. The participants were required to add the currently displayed number (from 2 to 9) to the next number shown on a PC monitor, and to indicate only one digit of the current answer by pressing a key (from 0 to 9). Each number was displayed for 500 ms followed by a 1500 ms interval. To maintain the motivation of both groups of participants to perform the task, they were instructed that in order to obtain a reward for participating in the study, they had to get over 90 % of the answers correct in total in each session.

Stressor controllability was manipulated by correct-error feedback to participants' answers. To the C group, correct feedback corresponding to the participants' answers was presented, but bogus and yoked feedback were given to the UC group. Feedback of correct or wrong was presented by visual signs displaying a circle, or a cross with an accompanying aversive noise (approximately 100 dB), respectively. For the purposes of this study, a typical experimental protocol that has been used in many animal studies on learned helplessness was adopted. In other words, the manipulation of controllability was introduced only in

the first session on the first day, and the effects of such manipulation of controllability were tested in the second, third, and fourth sessions. Thus, the participants in both the C and the UC groups commonly conducted the mental arithmetic task with feedback that accurately corresponded to their answers in the second, third, and fourth sessions.

4.2.3 Experimental procedure

The participants were instructed to eat light breakfast and avoid drinking caffeine-containing beverages. Participants suffering from an infectious illness within two weeks of the experiment were rescheduled.

A protocol of the experiment is presented in Figure 4.1. The experiment was composed of four sessions of stress tasks with rest periods, and was conducted over two days in both the C and UC groups. Before and after each session, and at the end of the final rest period of each day, the participants were asked to rate their subjective sense of stress and anxiety. Their subjective sense of control and effort was rated immediately at the end of each session. Blood samples were taken from a cannula inserted in the left forearm vein to measure neuroendocrine and immune indices at the same points as the psychological indices. Blood samples were taken every 10 min. during the first session. Cardiovascular indices were measured continuously throughout the experimental periods.

The participants stayed in accommodations at the university so that they could spend time in the same environment after the end of the experiment on the first day. The second day's experiment started at the same time as the first day‘s. Participants were tested individually between 12:45 PM and 17:00 PM on the first day, and between 12:45 PM and 14:00 PM on the second day in a temperature- and humidity-controlled room. At the end of the experimental period, the participants were fully debriefed about the purpose and manipulation of the experiment and thanked. All participants were paid with 5,000 yen for participation.

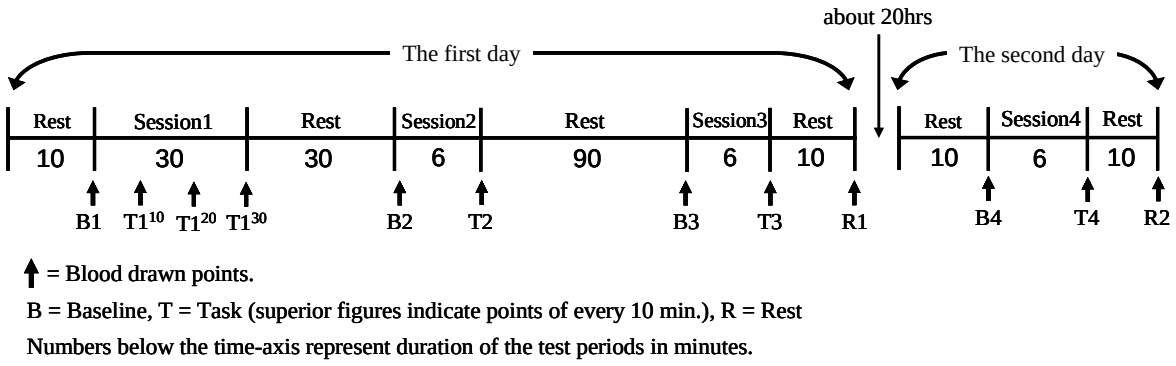


Figure 4.1 Time line for experimental sessions for two days. Each participant completed successive 4 experimental sessions which lasted for 2 days. The manipulation of controllability was performed only in the first session of the first day of the experiment.

4.2.4 Measures

4.2.4.1 Immunological measures

Blood samples for immunological determinations were collected in heparinized tubes. The immune parameters (the numbers of total white blood cells (WBC), lymphocytes, monocytes, and granulocytes per sample, proportion of lymphocyte subsets (CD3+ indicating T cells (T cell), CD3+ / CD4+ indicating helper T cells (helper T cell), CD3+ / CD8+ indicating cytotoxic T cells (cytotoxic T cell), CD3- / CD19＋ indicating B cells (B cell), and CD3- / CD16＋ / CD56＋ indicating Natural Killer cells (NK cell) were determined by same standard means as first experiment. Percentages of lymphocyte subsets were determined by flow cytometry (FACS Calibur; Becton-Dickinson, San Jose, CA).

4.2.4.2 Cardiovascular measures

As in first and second experiments, cardiodynamic activity was recorded using non-invasive finger blood pressure (FINAP) measurements. HR, 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 participant. Each indicator was recorded continuously during the task and the rest periods. The HR, SBP, and DBP were calculated for the last 3 minutes of

the pre-experimental baseline period, for 3 minutes period during each task, and for the last 3 minutes of the rest period.

4.2.4.3 Neuroendocrine measures

Blood samples for plasma catecholamine were anticoagulated with ethylenediamine tetra-acetate, chilled, and centrifuged; the plasma was then removed and frozen at -80 ^{o}C until analysis. Plasma adrenaline and noradrenaline were determined by high performance liquid chromatography. Alumina was used for extraction; the recovery rate for all amines, evaluated with a dihydroxybenzylamine (DHBA) standard, was between 60% and 70%. The intra-assay coefficient of variation was less than 5%, and the inter-assay variations were less than 6% for the measurement of adrenaline and noradrenaline.

4.2.4.4 Psychological measures

The participants were asked to rate the strength of the stress they experienced on a visual–analog scale (VAS) (0% to 100%), and they completed a Japanese version (Katsuharu and Tadanobu, 1982) of the State-Trait Anxiety Inventory (STAI) (Speilberger et al., 1970) consisting of 40 items. To evaluate their subjective sense of control over the task, I asked them to estimate what percentage of their correct answers could be attributed to their own ability and effort rather than to incidental factors (0% to 100%).

4.2.5 Statistical analysis

Prior to statistical analysis, mean values of HR, SBP, and DBP were calculated for the last three minutes of the baseline period, for the last three minutes of the periods during the stress task, and for the last three minutes of each rest period. In the first task session for 30 minutes, these were calculated every 10 minutes to examine the temporal effects of controllability. The cardiovascular, immune, and neuroendocrine data and perceived stress and state-anxiety were analyzed using repeated-measures analyses of variance (ANOVAs) in the following three patterns: a two-way mixed ANOVA (Group (C vs. UC) x Period (baseline 1, task 1_{10min}, task 1_{20min}, and task 1_{30min})) was conducted for the first session, a three-way mixed

ANOVA (Group x Session (session 2, session 3, and session 4) x Period (baseline 2-4 and task 2-4)) was conducted for the second, third, and fourth sessions, and a two-way mixed ANOVA (Group x Period (baseline 4 and task 4)) was conducted for the fourth session. The psychological measures were analyzed using repeated-measures ANOVAs: a two-way mixed ANOVA (Group x Period (baseline 1 and task 1)) was conducted for the first task, a three-way mixed ANOVA (Group x Session (session 2, session 3, and session 4) x Period (baseline and task)) was conducted for the second, third, and fourth sessions, and a two-way mixed ANOVA (Group x Period (baseline 4 and task 4)) was conducted for the fourth session. The Greenhouse-Geisser epsilon correction factor, ε (Jennings & Wood, 1976), was used where appropriate. In cases where significant interactions were found in the ANOVAs, post hoc analyses using LSD tests ($p < .05$) were conducted to examine which combinations of data points differed significantly. A Student's *t*-test was used for the sense of subjective control and effort.

4.3 Results

4.3.1 Immune measures

The immune data at the baseline, task, and rest periods during the two day test period are summarized in Table 4.1. Figure 4.2 illustrates changes in the percentages of T cells and NK cells. For the first session, significant main effects of Period were observed in the numbers of WBCs and lymphocytes, and in the proportion of NK cells, T cells, helper T cells, and cytotoxic T cells (*Fs* (3,27) = 4.32-15.26, *p*s < .01-.05), indicating an increase of WBCs, lymphocytes, NK cells, and cytotoxic T cells and a decrease of T cells and helper T cells. There was no main effect of Group or interaction in the first session. Significant main effects of Period were found in the numbers of WBCs, granulocytes, lymphocytes, and monocytes and in the proportions of T cells, helper T cells, and NK cells (*Fs* (1,9) = 20.53-5.65, *ps* < .01-.05) in the second to fourth sessions, as were seen in the first session. In addition, the proportion of helper T cells in the UC group was higher than that in the C group (F (1,9) = 5.28, $p < .05$). Furthermore, changes in the numbers of WBCs and granulocytes and the proportions of T cells, helper T cells, B cells, and NK cells (*Fs* (2,11-18) = 12.91-49.42, *ps* < .01) in the fourth session were lower than those in the second and third sessions. Significant Group

and Period interaction in NK cells (F (1,9) = 5.32, p < .05), Group and Session interaction in cytotoxic T cells (F (2,18) = 5.51, p < .05), and Session and Period interaction in helper T cells and B cells (F (2,12) = 9.36, p < .01; F (2,18) = 6.63, p < .01) were observed. Post hoc comparisons (p < .05) indicated that there was a greater increase in the proportion of NK cells in the C group than that in the UC group. Similarly, the numbers of WBCs, granulocytes, lymphocytes, and monocytes, and the proportions of T cells, helper T cells, and B cells (Fs (1,9) = 6.12-43.96, ps < .01-.05) in the fourth session showed significant changes after the task. There was also a significant main effect of Group in monocytes (F (1,9) = 7.42, p < .05). A significant Group and Period interaction in NK cells (F (1,9) = 6.62, p < .05) was observed. LSD tests (p < .05) revealed that the increase in the proportion of NK cells in the UC group was lower than in the C group.

These results indicate that active coping with acute stress increased innate immunity represented by NK cells, while it decreased acquired immunity represented by helper T cells in both groups. However, although changes of NK cells and helper T cells to an acute stressor occurred to the same degree during the first session in both groups, these responses in the UC group became lower than in the C group as the sessions continued,.

Table 4.1 Means (SDs) of immune measures and results of ANOVAs.

	Group	Baseline1	Task1^{10min}	Task1^{20min}	Task1^{30min}	N	[a]Effect
WBC	C	5.87(0.37)	6.14(0.56)	6.10(0.36)	6.05(0.35)	10	Period**
(×10^3/μl)	UC	5.63(0.42)	6.12(0.43)	6.00(0.39)	6.05(0.40)	10	
Lymphocyte	C	1.52(.15)	1.68(.16)	1.61(.13)	1.56(.14)	10	Period**
(×10^{3}μll)	UC	1.64(.19)	1.91(.25)	1.82(.23)	1.84(.22)	10	
Monocyte	C	.41(.05)	.43(.06)	.42(.04)	.42(.05)	10	n.s
(×10^{3}μl)	UC	.33(.05)	.36(.06)	.36(05)	.36(.05)	10	
Granulocyte	C	3.82(.42)	3.88(.44)	3.94(.40)	3.92(.38)	10	n.s
(×10^{3}μl)	UC	3.56(.40)	3.72(.41)	3.70(.40)	3.74(.41)	10	
Helper T cells	C	41.58(1.19)	38.39(1.25)	39.14(1.19)	39.91(1.27)	10	Period**
(%)	UC	41.63(2.03)	42.71(2.21)	43.58(2.04)	44.67(1.89)	10	
Cytotoxic T	C	30.28(1.79)	30.25(1.70)	30.71(1.57)	31.10(1.46)	10	Period*
cells (%)	UC	26.24(1.88)	26.77(2.11)	26.95(2.09)	27.26(1.88)	10	
B cells	C	10.79(1.26)	10.74(0.67)	10.10(0.88)	10.25(1.07)	10	n.s
(%)	UC	12.07(0.87)	11.57(0.94)	12.06(0.74)	12.39(1.04)	10	

The table above shows data in the first session, and that in the bottom shows data in the second, third and fourth session. [a] Main effects and interactions in the first session revealed by ANOVAs. [b] Main effects and interactions in the second, third, and fourth session revealed by ANOVAs. [c] Main effects and interactions in the fourth session revealed by ANOVAs .
C; controllable group, UC; uncontrollable group
**:p<.01, *:p<.05

	Group	Baseleine2	Task2	Baseline3	Task3	Rest1	Baseline4	Task4	Rest2	N	Effect
WBC	C	5.92(0.41)	6.10(0.36)	6.61(0.55)	6.97(0.69)	6.74(0.65)	5.47(0.46)	5.84(0.49)	5.45(0.38)	10	[b]Session**, [b]Period**,
(×10^3/μl)	UC	5.85(0.38)	6.03(0.36)	6.11(0.33)	6.14(0.31)	6.03(0.35)	4.64(0.28)	4.92(0.25)	4.71(0.29)	10	[c]Period**
Lymphocyte	C	1.54(.13)	1.61(.16)	1.72(.15)	1.75(.12)	1.69(.15)	1.46(.13)	1.57(.11)	1.37(.13)	10	[b]Period*, [c]Period*
(×10^{3}μll)	UC	1.74(.16)	1.81(.20)	1.87(.17)	1.85(.16)	1.83(.16)	1.60(.09)	1.68(.10)	1.54(.11)	10	
Monocyte	C	.39(.04)	.42(.05)	.40(.04)	.42(.04)	.40(.03)	.39(.04)	.41(.04)	.37(.03)	10	[b]Period*, [c]Group*, [c]Period*
(×10^{3}μl)	UC	.33(.05)	.34(.05)	.34(.05)	.33(.04)	.34(.05)	.26(.03)	.28(.03)	.27(.03)	10	
Granulocyte	C	3.88(.43)	3.92(.39)	4.37(.56)	4.64(.66)	4.49(.68)	3.56(.47)	369(.50)	3.65(.42)	10	[b]Session**, [b]Period**, [c]Period**
(×10^{3}μl)	UC	3.69(.39)	3.78(.37)	3.79(.29)	3.85(.29)	3.74(.31)	2.68(.28)	2.86(.30)	2.81(.31)	10	
Helper T cells	C	43.57(1.25)	41.70(1.25)	41.36(1.23)	41.09(1.14)	41.94(1.25)	40.62(1.59)	38.27(1.71)	40.52(1.33)	10	[b]Group*, [b]Session**, [b]Period*,
(%)	UC	47.23(5.47)	46.42(1.73)	46.16(1.69)	46.19(1.67)	45.80(1.67)	43.48(1.66)	42.53(1.96)	44.08(1.83)	10	[b]Session x Period**, [c]Period*
Cytotoxic T	C	32.63(4.11)	32.28(1.30)	32.58(1.77)	32.23(1.48)	32.88(1.60)	31.83(1.35)	31.34(1.63)	32.54(1.52)	10	[b]Group x Session*
cells (%)	UC	27.77(6.19)	27.23(1.96)	28.31(2.09)	28.49(2.06)	28.62(2.13)	28.33(1.81)	28.26(1.92)	28.17(2.21)	10	
B cells	C	11.33(3.04)	10.63(0.96)	10.44(0.75)	10.20(0.91)	11.69(0.67)	8.50(0.66)	9.00(0.73)	9.96(0.73)	10	[b]Session**,
(%)	UC	12.66(3.09)	12.20(0.98)	11.90(0.87)	12.35(0.82)	12.22(0.76)	8.84(0.48)	10.43(0.80)	10.21(0.80)	10	[b]Session x Period**, [c]Period*

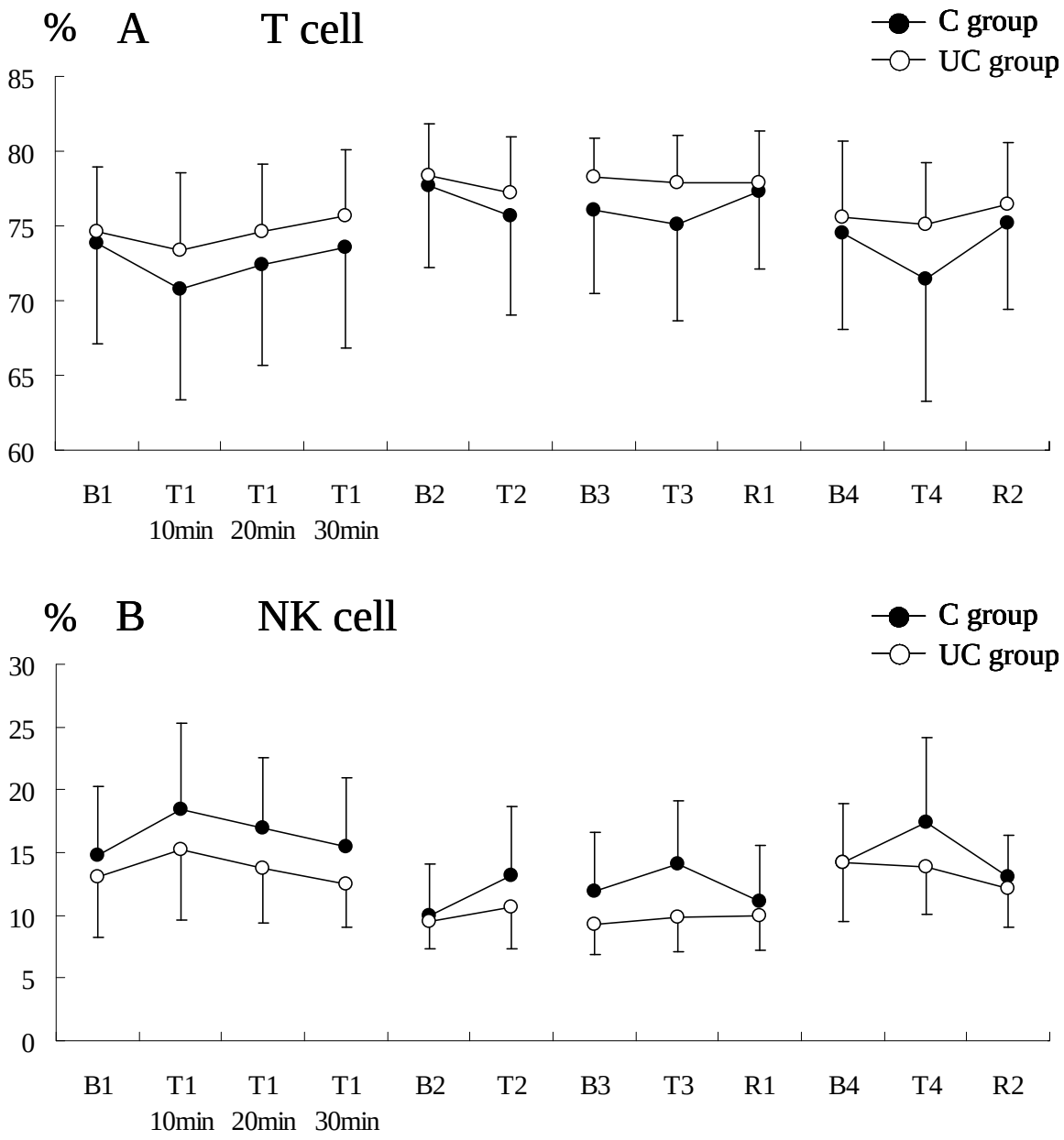


Figure 4.2 Change of percentage of T cells (A) and NK (B) from baseline periods in the both groups. Vertical bars indicate standard deviations. Black circles indicate the controllable group, and white circles indicate the uncontrollable group. B: Baseline, T: Task, R: Rest.

4.3.2 Cardiovascular measures

Changes in the cardiovascular indices are illustrated in Figure 4.3. For the first session, significant main effects of Period were observed in HR, SBP, and DBP (*Fs* (3,27) = 35.33-82.50, *ps* < .001-.01), but there was no main effect of Group or interactions of Group and Period. For the second to fourth sessions, HR, SBP, and DBP significantly increased (*Fs* (1,9) = 24.67-62.24, *ps* < .001-.01) during the acute stress task compared to the baseline. In addition, SBP and DBP (*F* (2,8) = 8.91, *p* < .01; *F* (2,8) = 34.06, *p* < .01) in the fourth session were lower than those in the other sessions. Furthermore, significant interactions of Group and Period in DBP were found (*Fs* (1,9) = 10.10, *ps* < .01). The LSD tests did not show any significant difference. Significant interactions were observed between Session and Period in SBP and DBP (*F* (2,18) = 10.71, *p* < .01; *F* (2,18) = 10.71, *p* < .01). Further analysis (*p* < .01) indicated that increases of DBP were lower in the fourth session than those in the second and third sessions in both groups. Following a similar pattern, HR, SBP, and DBP (*Fs* (1,9) = 20.93-66.55, *ps* < .001-.01) increased by the acute stressor in the fourth session. In addition, significant Group and Period interactions for HR (*F* (1,9) = 9.84, *p* < .05) were found, and post hoc comparisons confirmed that the increments of HR during acute stress in the UC group were lower than that in the C group.

In summary, HR and BP were remarkably elevated during the mental arithmetic task as active coping condition compared to the baseline during all sessions. However, the changes by acute stress were lower during the fourth session than during the other sessions, especially in the UC group.

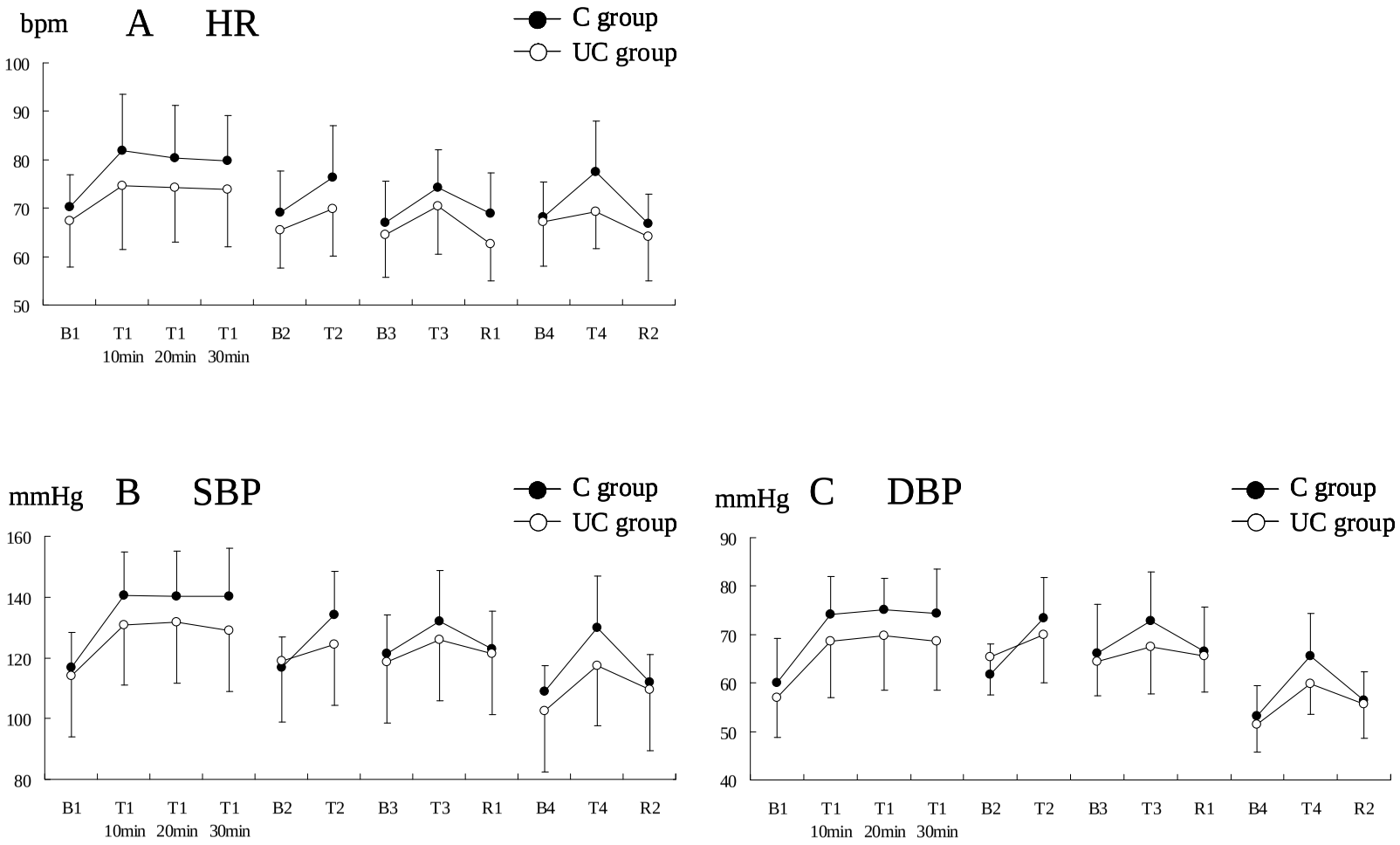
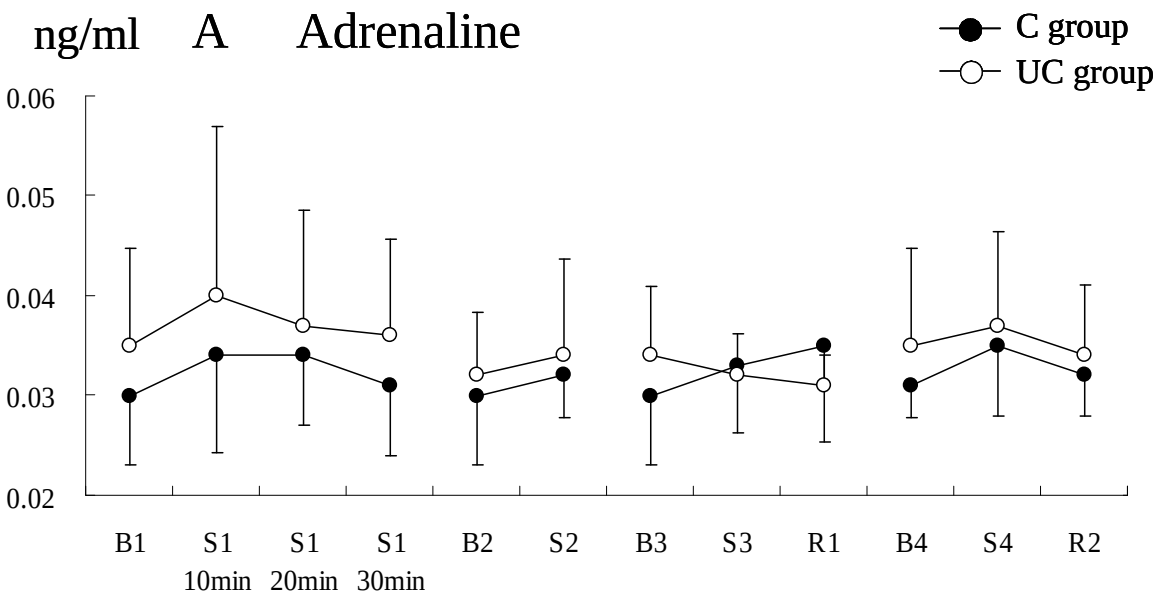


Figure 4.3 Change of the HR (A), DBP (B), and SBP (C) from baseline periods in the both groups. Vertical bars indicate standard deviations. Black circle indicates the controllable group while white circle indicates the uncontrollable group. T: Task, B: Baseline.

4.3.3 Neuroendocrine measures

The adrenaline and noradrenaline levels at the baseline, task, and rest periods in the two days are presented in Figure 4.4. The concentration of noradrenaline in the first session and adrenaline from the second to fourth sessions increased significantly by acute stress (F (3,27) = 2.98, $p < .05$; F (1,19) = 9.99, $p < .05$). Similarly, main effects of Period in the fourth session were found for adrenaline and noradrenaline (F (1,9) = 7.36, $p < .05$; F (1,9) = 6.45, $p < .05$). In addition, the concentration of noradrenaline in the second session was higher than in the fourth session (F (2,18) = 6.04, $p < .01$). For only noradrenaline in only the fourth session, there was a main effect of Group (F (1,9) = 6.70, $p < .05$), indicating that the level of noradrenaline in the UC group was lower than that in the C group, and that there was significant Group and Period interaction (F (1,9) = 15.84, $p < .01$). LSD tests ($p < .05$) showed that the increase in the concentration of noradrenaline after the task from the baseline in the UC group was lower than the increase in the U group.

The concentrations of adrenaline and noradrenaline as part of autonomic nervous activation were increased by the acute stressor. However, in only the fourth session, although the concentration of noradrenaline in the C group increased after the task, it was not affected by the acute stressor in the U group (Figure 4.4).



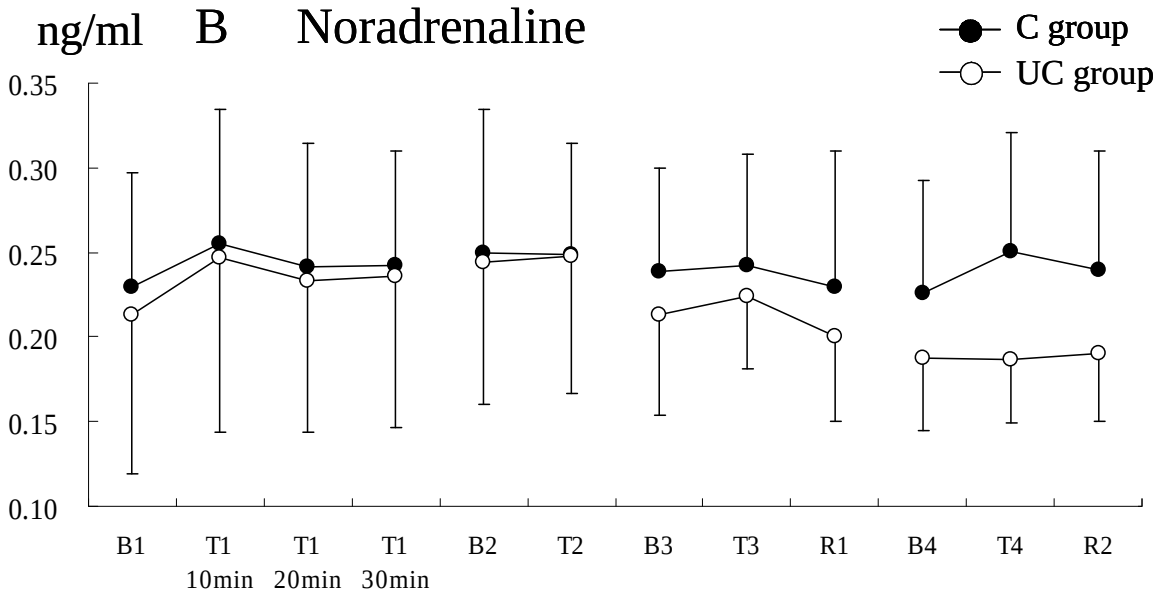


Figure 4.4 Change of the adrenaline (A) and noradrenaline (B) from baseline periods in the both groups. Vertical bars indicate standard deviations. Black circle indicates the controllable group while white circle indicates the uncontrollable group. T: Task, B: Baseline.

4.3.4 Psychological measures

The subjective stress and state anxiety data at baseline, after the task, and during the rest periods are presented in Table 4.2. The mean value of perceived controllability in session 1, session 2, session 3, and session 4 were 48.40, 71.50, 69.80, and 80.60 (SD = 18.36, 17.19, 15.98, and 12.54) in the C group, and 41.00, 59.00, 62.30, and 76.80 (SD = 17.15, 20.32, 21.58, and 17.56) in the UC group, respectively. The sense of subjective control during the task in the C group was higher than that of the UC group (t (9) = 3.46, $p < .01$) during the second session.

For subjective stress and state anxiety, there was no significant main effect of Group, Session, Period, or interaction in the first to fourth sessions. The subjective stress increased in a statistically marginal fashion in response to acute stress in both groups, and the degree of response gradually decreased as the sessions continued.

The mean rates of correct answers in the first to fourth session in the C and UC groups were calculated. The mean value of correct answers in the first to fourth sessions were 79.86, 89.39, 87.22, and 91.73(SD = 7.69, 6.85, 7.16, and 4.60) in the C group, and 74.64, 82.69, 84.54, and 89.92 (SD = 12.37, 15.20, 12.86, and 10.83) in the UC group, respectively. There was no significant difference in the total number of correct answers in the two groups.

Table 4.2 Means (SDs) of psychological measures and results of ANOVAs

	Group	Baseline1	Task1	N	[a]Effect
Perception of stress	C	32.20(7.11)	42.30(5.25)	10	n.s
	UC	33.30(5.89)	42.60(5.95)	10	
State-anxiety	C	42.80(2.38)	44.00(3.28)	10	n.s
	UC	46.10(2.64)	48.10(2.54)	10	

	Group	Baseline2	Task2	Baseline3	Task3	Rest1	Baseline4	Task4	Rest2	N	Effect
Perception of stress	C	28.70(5.79)	36.60(5.00)	23.60(6.66)	42.70(7.77)	30.00(6.59)	24.10(7.49)	30.70(5.17)	18.50(4.53)	10	[b]Session x Period*
	UC	32.90(5.59)	35.40(7.72)	24.60(5.98)	32.00(6.28)	28.20(6.50)	36.00(6.52)	35.60(7.98)	28.20(6.68)	10	
State-anxiety	C	39.20(3.17)	38.00(2.79)	36.10(2.39)	38.50(3.21)	35.90(3.10)	37.10(3.01)	36.30(1.96)	33.00(2.44)	10	[c]Group x Period*
	UC	43.40(2.15)	42.50(1.78)	40.90(1.49)	40.80(1.48)	39.40(1.95)	44.40(2.47)	39.20(2.29)	35.90(1.64)	10	

The table above shows data in the first session, and that in the bottom shows data in the second, third, and fourth session.
a Main effects and interactions in the first session revealed by ANOVAs. [b] Main effects and interactions in the second, third, and fourth session revealed by ANOVAs . [c] Main effects and interactions in the fourth session revealed by ANOVAs .
C; controllable group, UC; uncontrollable group
**:p<.01, *:p<.05

4.4 Discussion

The mental arithmetic task in both the C and UC groups robustly elicited typical physiological stress responses, that is, the elevation of HR, SBP, and DBP, an increase in the concentration of adrenaline and noradrenaline, facilitation of innate immunity represented by the proportion of peripheral blood NK cells, and the suppression of acquired immunity represented by the proportion of peripheral blood helper T cells. This response pattern was precisely consistent with the results of our previous studies using the same acute stress task (Isowa et al., 2004; Kimura et al., 2005). The result could be interpreted as typical acute stress physiological responses (Burleson et al., 1998; Delahanty et al., 1996; Pike et al., 1997). Taken together, these results show that the task used in this study was valid as a method for inducing a typical acute stress response. The hypothesis that the effects of an uncontrollable acute stressor would be shown as the down-regulation of immune reactivity based on inhibited cardiovascular and autonomic nervous responses seemed to be confirmed (Figure 4.2-4.4). The increase of NK cells was lower among participants exposed to the uncontrollable stressor in the first session than those exposed to the controllable stressor. In particular, it should be noted that these effects of controllability differed in magnitude among the sessions. Although the inhibition of immune activation in the UC group did not appear in the first session, it appeared after the second session and was especially remarkable in the fourth session (Figure 4.2). Furthermore, cardiovascular and neuroendocrine parameters also showed a comparable controllability-dependent pattern. The increases of HR, SBP, DBP, and noradrenaline in the UC group were demonstrably lower than in the C group, and the attenuated responses of these parameters were gradually strengthened as the sessions progressed, but not in the first session (Figure 4.3, 4.4).

The elevation of HR, SBP, and DBP to the acute stressor was elicited by the activation of the autonomic nervous system on the basis of α- and β-adrenergic activity, and the subsequent increase of NK cells was caused by both catecholamine-induced changes in hemodynamics and adhesion via adrenoreceptors. Thus, the down-regulated activation of NK cells in the UC group seemed to be mediated by the attenuated autonomic activity. Such down-regulation of immune responses in uncontrollable stress situations was consistent with the results of our previous study as well as those of some animal studies (Isowa et al.,

2006; Laudenslager et al., 1983; Maier and Laudenslager, 1988; Weiss et al., 1990). In the previous chapters, the experiment 2 using the same mental arithmetic task and manipulation of controllability as the experiment 3 reported a strengthened association between immune and cardiovascular activity in a UC group compared to a C group, and these results were explained by synchronized attenuation of immune and cardiovascular responses in the UC group. Furthermore, our another study using a stochastic learning task showed that increases of NK cells to an acute stress were suppressed with decreased HR and BP in a yoked uncontrollable group compared to a controllable group (Kimura et al., in press).

The stress responses of an organism are affected not only by the physical aspects of a stressor (e.g., intensity and difficulty) but also by evaluation of the controllability over the stressor based on experiences of success or failure as consequences of coping effort (Isowa et al., 2006; Maier and Watkins, 2005). To date, it has been shown that two distinct coping strategies, namely active coping and passive coping, elicit different patterns of physiological responses (Bosch et al., 2003; Isowa et al., 2004; Hartley et al., 1999; Keay and Bandler, 2001; LeBlanc et al., 1979; Willemsen et al., 2002). Active coping is usually evoked when organisms evaluate the current stress situation as controllable, and is accompanied by an elevation of cardiac reactivity and reduction of vascular resistance. In contrast, passive coping is elicited when the stress situation is evaluated as uncontrollable, and leads to the reduction of cardiac reactivity and increase of vascular resistance (Bandler et al., 2000; Bosch et al., 2003; Isowa et al., 2004; Keay and Bandler, 2001; LeBlanc et al., 1979; Willemsen et al., 2002). It seems that these dissociated physiological responses according to the evaluation of controllability over the stress situation are adaptive reactions in terms of the efficient use of energy. Namely, it is more adaptive to invest vast energy to overcome the stress situation when an organism can deal with the stressor. On the other hand, it is more adaptive to avoid a waste of energy when an organism cannot deal with the stressor, and thus the organism partially suspends investment of energy and seeks the most appropriate ways to cope with the stressor. (Blascovich et al., 1999; Maier et al., 2003). In line with this reasoning, attenuation of an increase of NK cells and elevation of cardiovascular responses in the UC group can be interpreted as consequences of the adoption of a passive coping strategy once the stressor was evaluated as uncontrollable.

The experiment 3 elucidated that under uncontrollable stress situation,

behavioral and physiological responses were not suppressed only at the initiation of an uncontrollable stress situation. This is probably because coping strategies were changed by temporally progressive evaluation of controllability over the stressor from trial and error processes (Kimura et al., in press; Maier and Watkins, 2005). Particularly at the beginning of exposure to a new stressor, an organism actively challenges the stressor to seek an appropriate coping strategy. Given that, participants in the UC group might have continued to make efforts to cope with, and pay attention to, the uncontrollable stressor initially, and, as a result, simultaneous activation of the autonomic nervous system in the first session was observed in both groups. This result was consistent with experiment 2 in the previous chapter (Isowa et al., 2006). In the present experiment, the effects of uncontrollable stressor were present after the second session and persisted for 24 hours. By the fourth session, in particular, the differences between the two groups were remarkable. Although the biological mechanisms underlying such a phenomenon are not yet clear, this is the first report detailing the physiological residual effects of an uncontrollable stressor in humans that is consistent with results reported in previous animal studies (Drugan et al., 1984; Jackson et al., 1978; Maier et al., 1990; Weiss et al., 1981).

Chapter 5

Conclusion

This study includes three empirical examinations in order to elucidate the effects of stressor controllability on physiological and psychological responses to an acute stressor. In this chapter, first, the key findings of these three experiments are summarized. Next, I address the contribution of the current work and present a new model. Furthermore, the brain substrata modulating the effects of stressor controllability on peripheral immune responses are discussed. Finally, some of limitations involved in the present study and future tasks are discussed.

5.1 Summary of empirical findings in this thesis

The aim of this study was to investigate the effects of stressor controllability on physiological and psychological responses to an acute stressor. For the aim, first, an acute-stress task evoking typical acute-stress responses was established, and induced peripheral immune responses and active and passive coping response mechanisms. Then, effects of stressor controllability on the stress responses were examined under the typical acute stress task.

In Chapter 2, immune responses and cardiovascular reactivity as a mediator of the immune responses against two acute stressors (mental arithmetic as the active coping situation and cold pressor tasks as the passive coping situation) were investigated. As the hypothesis predicted, both stress tasks induced typical acute-stress responses in the circulating lymphocytes such as facilitation of innate immunity represented by the proportion of peripheral blood NK cells and NKCA, and the suppression of acquired immunity represented by the proportion of peripheral blood helper T cells. In parallel, the decrease of CD3+ T cells and increase of NK cells were more remarkable in the mental arithmetic task than in the cold pressor task, indicating that the active coping situation had a greater impact on immunity than the passive coping situation. Thus, it was confirmed that the mental arithmetic task evoked active coping responses (increase of BP and NK cells with the increased HR) while the cold pressor task elicited passive coping responses (increase of BP and NK cells with the increased total peripheral resistance).

In Chapter 3, effects of stressor controllability on immune responses to an acute stressor were examined. The mental arithmetic task established in the experiment 1 was employed as an active-coping stress task. Correct-error feedbacks to participants' answers were used as manipulation of controllability. From the results, it was suggested that stressor controllability was successfully manipulated. Contrary to the prediction, however, no effect of stressor controllability over the acute-stress task was observed in any immune parameters. Instead, strong correlations between cardiovascular and immune parameters were observed only in the UC condition. The results reflected individual differences depending on different evaluations of the uncontrollable situation. During exposure to the uncontrollable acute stressor for 15 minutes, reactivities of active and passive coping were mixed according to individual difference. As a result, effects of uncontrollability appeared as strong correlations between immune and cardiovascular reactivity, but not as a difference in mean values.

In Chapter 4, to examine the time course of the effect of stressor controllability, part of the procedure of experiment 2 was changed. Specifically, immune, cardiovascular, and endocrine parameters were measured when subjects were re-exposed to the same, but controllable stressor 24 hours after exposure to the first uncontrollable stressor. The hypothesis was confirmed by the results that the effects of the uncontrollable acute stressor were observed as down-regulation of immune reactivity based on inhibited cardiovascular and autonomic nervous responses. The effects of uncontrollable stress appeared after the second session, and persisted for 24 hours. These dissociated physiological responses may show adaptation to two types of stressful situations by efficient allocation of energy.

5.2 Primary contributions of this thesis

5.2.1 Biological model of adaptation based on evaluation of stressor controllability and stress responses.

Here, based on previous adaptation models and the findings of this study, I propose a hypothetical new model in regard to effects of stressor controllability on acute-stress responses for adaptation.

The environments in which organisms live change from hour to hour, and not always mildly. The organisms need to survive these changes by changing their physiological responses based on their evaluation of their environment to maintain organism stability. Such organism stability is called “allostasis” (McEwen, 1998). Primary mediators of allostasis include, but are not confined to, the hormones of the HPA axis, catecholamines, and cytokines. Allostasis clarifies the inherent ambiguity of the term “homeostasis” by distinguishing between the systems that are essential for life (homeostasis) and those that maintain these systems in balance (allostasis).

Acute stress, the focus of this study, produces a situation in which biological systems of organisms must change rapidly to maintain organism stability. When an organism is exposed to a new stressor or intimidating situation, the organism recognizes it as an acute-stress situation, and the endocrine system (mainly adrenal gland) is activated to excite the organism while the immune system is mobilized via the autonomic nervous system to defend against invaders. In this study, a mental arithmetic task served as an active coping condition which rapidly increased HR and BP via activation of the autonomic nervous system, and subsequently increased NK cells. The series of reactions could be understood as rapid mobilization of energy under a fight-or-flight situation (Figure 5.1 (A); finding obtained in experiment 1).

Lazarus and Folkman (1984) argued that psychological as well as behavioral reactions (coping) are determined by the results of cognitive appraisals of comparisons between individual resources and environmental demands. Thus, stressor controllability is one of the environmental factors that alter the direction of biological responses (arrows on the Figure 5.1; activation or inhibition). The direction of responses, in other words, the degree of energy supply, is modulated by evaluation of whether the organism can take control over the stressor. When the

stressor is evaluated as controllable, resources are greater than the demands, and an active coping strategy can be chosen, leading to allocation of energy to take control over the stressor. By contrast, when the stressor is evaluated as uncontrollable, resources are less than demands, and an active coping strategy would end up as waste of energy. Instead, the passive coping strategy is chosen to save energy for survival.

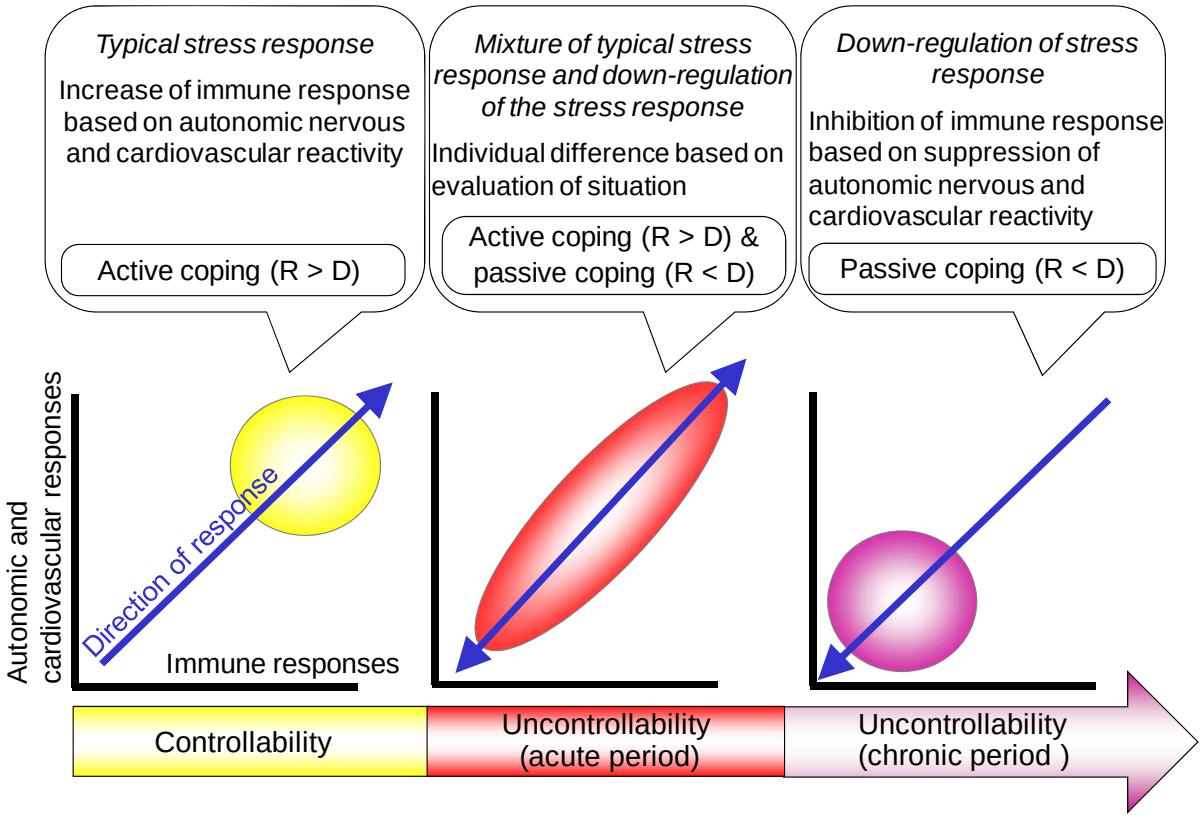


Figure 5.1 Effects of controllability on acute stress responses (energy adjustment for adaptation). R, individual resources; D, environmental demands

At the beginning of exposure to a new stressor, organisms try to actively challenge the stressor and thus seek for an appropriate coping strategy, regardless of stressor controllability. If the new stressor is uncontrollable, for the short initial duration, reactivities of the active and passive coping mechanisms would be mixed. In this stage, there would be wide individual differences in coping behavior depending on individuals' evaluations of resources and demands. Subsequently, the

effects of uncontrollability would become dominant as strong correlations between immune and cardiovascular reactivity (Figure 5.1 (B); finding obtained in experiment 2). In the case of a long-lasting uncontrollable situation, the organism's responses to the stressor would move toward passive coping as the stressor cannot be overcome by individual effort. Thus, energy would be now minimally allocated for the sake of its conservation (Figure 5.1 (C); finding obtained in experiment 3).

In summary, this model postulates that all organisms including humans have an innate system to modulate energy consumption efficiently by adapting to a changing environment in a way that maximizes the possibility of survival. Under acute-stress situations, the system mobilizes energy rapidly to prepare for fight-or-flight behaviors. The system continues to cope actively with the stressor as long as the organism evaluates the stressor as controllable. The system for energy control mobilization is such that when individual resources are greater than the environmental demand, it allocates sufficient energy to take control of the stressor. On the other hand, when the environmental demand surpasses the individual resource, the organism suppresses energy consumption so as to save energy rather than to waste it. Thus, for organisms to survive, controllability is one of the key factors when organisms determine coping behavior and biological reactions to environmental stressors.

5.2.2 The central role of the brain under an uncontrollable acute-stress situation

In an acute-stress situation, an organism assesses environmental demands to modulate biological responses dynamically to cope with it. The three experiments in this study have shown that autonomic nervous, cardiovascular, and immune parameters change during an acute-stress task, and these responses are affected by controllability of the stressor (Chapter 2, 3, 4). These results suggest activations in several nuclei of the hypothalamus and the midbrain, both of which control peripheral physiological systems to maintain homeostasis. In this section, I discuss the association between the brain and immune system in terms of cognitive appraisal of the acute stressor by referring to findings from another study of ours (Ohira et al., 2008). This purpose of that study was to examine the neural basis of corticolimbic modulations upon peripheral redistribution of lymphocytes by

appraisal of controllability over an acute stressor. For this purpose, we measured simultaneously regional cerebral blood flow (rCBF) using ^{15}O-water positron emission tomography (PET) and physiological parameters such as cardiovascular, neuroendocrine, and immune activities during the same mental arithmetic task I used in the three experiments in this thesis (Chapter 2, 3, 4). Task controllability was also manipulated by feedback to subjects' performance in each trial: under the high-controllability condition (HC), correct feedback was given; under the low-controllability condition (LC), bogus feedback was given from time to time. Under the LC condition, participants were expected to notice the gap between subjectively monitored performance and actually given feedback; thus, they were expected to experience less controllability over the task.

Subjective reports from the experiment indicated that manipulation of the controllability was successful. Neuroimaging results showed that the orbitofrontal cortex (OFC), medial prefrontal cortex (MPFC), anterior cingulated cortex (ACC), and lateral prefrontal cortex (LPFC) were involved in evaluating controllability under the LC condition. Furthermore, the prefrontal cortex (PFC) including the OFC and MPFC were commonly associated with peripheral immune responses, i.e., redistribution of lymphocytes, and with cardiovascular and neuroendocrine activities which probably mediated changes in the immune functions, accompanying appraisal of stressor controllability. The PFC does not directly regulate peripheral immune cells but does so indirectly via autonomic and neuroendocrine pathways. Specifically, the OFC and MPFC might first affect activities in stress-related brain structures such as the thalamus, hypothalamus, and midbrain through direct neural projections. Then, such centers of autonomic and endocrine systems might affect the immune functions through modulation of cardiovascular parameters and secretion of catecholamines and acetylcholine (Maier and Watkins, 1998; Tracey, 2002). In this study, observed activations within the thalamus, pulvinar, and midbrain correlating with HR, SBP, and epinephrine, respectively, are suggested to be the key areas to explain the effect of controllability over the stressor.

In spite of common correlations between activation in the OFC and MPFC and peripheral physiological responses in the LC condition, self-reported data of controllability and subjective stress showed no significant correlation in those regions. This result also suggests that observed correlations between brain activation and the peripheral responses might not mean conscious modulation of

bodily responses by higher-order cortical brain regions but reflect complex and possibly bi-directional functional associations between the brain and body. Furthermore, in the present experiment 3 (Chapter 4) using the same manipulation of controllability as in the PET study, down-regulation of physiological parameters appeared in the third and fourth sessions, so that there were no longer significant differences in subjective controllability between the controllable and uncontrollable groups. Thus, these results suggest that the adaptation processes determined by controllability appraisal can work, at least partly, automatically and unconsciously via the bi-directional brain and body systems. This finding seems to raise a question about Lazarus's theory saying that behavioral and physiological responses to stressors are evoked by consciously cognitive appraisal.

This PET study as well as the second and third experiments here showed that acute-stress responses in peripheral immune functions and possibly mediating cardiovascular, and neuroendocrine activities are neither rigid nor stereotyped, but are regulated flexibly and dynamically on the basis of the evaluation of current environmental factors such as stressor controllability. We clarified that physiological stress responses are strengthened when a stressor is well controlled, whereas, at least, a part of those responses (i.e. HR, epinephrine, NK cells and helper T cells) was, to some degree, attenuated or regulated downward when the stressor was less controllable and reappraisal of the stressor was required. Furthermore, in this PET study, the combination of increased involvement of the PFC and attenuated elicitation of physiological responses in the less controllable situation were shown. This might indicate search mechanisms for appropriate strategies for coping and for the prevention of energy expenditure by cutting off provided energy to ongoing behaviors and physiological responses which have become inappropriate. Since considerable energy is necessary for the immune response cascade, down-regulation of immune responses might be appropriate under a stressful but uncertain situation. For adapting to constantly changing environmental demands, patterns of organized variability rather than static levels are required in the central brain and peripheral physiological systems (Thayer and Brosschot, 2005).

5.3 Limitations and future tasks

In this study, the effects of controllability on acute-stress responses were

examined from results of three experiments as well as previous studies. In experiment 2, the effects of uncontrollability appeared as individual differences in stress responses (Chapter 3). Additionally, in experiment 3, experience of an uncontrollable stressor elicited down-regulation of physiological responses to the acute stressor. These results are consistent with previous reports that uncontrollable stressors elicit suppression of physiological and behavioral responses (Lauendlager, 1983; Shavit, 1983; Sieber, 1992; Weiss, 1990).

However, at the same time, some limitations should be noted. One of the limitations in the series of experiments is the large individual differences in physiological responses. Because sample sizes in all three experiments were rather small, a greater number of participants might help clarify or diminish this variation. The related issue is that I examined only female participants in the three experiments, whereas a previous study (Willemsen et al., 2002) reported sex differences in immune cell counts during a stress task. Thus, the generalizability of the present findings awaits further tests using a larger sample composed of both sexes. Furthermore, effects of controllability seem to relate to factors such as the kind of experimental task, manipulation of controllability, personality, behavioral patterns, and past experiences. The relationship between effects of controllability and these factors should be examined in future works.

Many studies have suggested that clinical states such as posttraumatic stress syndrome, depression, chronic fatigue, or burnout are caused by chronic stress situations that cannot be controlled or by one-time events that are traumatic or shocking (Foa, et al., 1992; Maier, 1984; Riley, 2004). Empirical findings for the mechanisms of down-regulation in biological systems in humans depending on the controllability of the stressor may have important implications for understanding the development of clinical states by stressors. Thus, it would be of further interest to examine the medium- and long-term health effects of an uncontrollable situation.

Another issue raised by the present study is the effect of the subjective sense of controllability. It was statistically marginal in experiment 2, and in experiment 3 it was significant, but only in the second session in which controllability wasn't manipulated. Despite this discrepancy, effects of controllability were present as down-regulation of acute stress responses. This might be because the effects of controllability are automatic and unconscious. Alternatively, it might be due to the different nature of indices of psychological and physiological parameters. These

ostensibly inconsistent results between subjective and physiological measures have also been indicated in some previous studies. For example, Bechara et al (1997) demonstrated that in a gambling task, participants showed differentiated physiological reactivity, specifically in skin conductance levels, to a stimulus that was associated with a stochastically larger monetary risk without explicit knowledge about the contingency between the stimulus and the risk. The authors argued that such physiological responses can work as “hunches” to guide appropriate behaviors. In line with this report, Kimura et al. (2007) indicated that immune and cardiovascular reactivity are down-regulated in an uncontrollable stochastic learning task without explicit conscious perception of uncontrollability. The results from the present study are in harmony with these findings. Thus, although one should be careful in interpreting the present results, we speculated that the appraisal of stressor controllability can work at the subconscious level (Ohira, 2001). Further studies are awaited to elucidate this issue theoretically and empirically. Despite the above limitations, this study, for the first time, elucidated that evaluation of stressor controllability is one of the determinants of coping strategies and biological responses for energy control.

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