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評估 amantadine 對巴金森氏症神經免疫功能之影響：麩胺酸神經系統在神經退化性失智症之角色
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Effects of escapable and inescapable stressors on behavior and interleukin-2 in the brain

Yen-Ti Lee[1], Wen-Fu Wang[2], Chun-Wen Cheng [3], Shey-Lin Wu[2,4], Cornelius R. Pawlak[5], **Ying-Jui Ho**[6#]

[1] Institute of Behavioral Medicine, Cheng Kung University, Taiwan, ROC
[2] Department of Neurology, Chang-Hua Christian Hospital, Taiwan, ROC
[3] Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taiwan, ROC
[4] Department of Bioindustry Technology, Dayeh University, Taiwan, ROC
[5] Department of Psychopharmacology, Central Institute of Mental Health, Mannheim, Germany
[6] School of Psychology, Chung Shan Medical University, Taiwan, ROC

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[#]Corresponding author:

Dr. **Ying-Jui Ho**

School of Psychology, Chung Shan Medical University, Taiwan, ROC

Address: No. 110, Sec. 1, Jianguo N. Rd., Taichung City 402, Taiwan, ROC

e-mail: yjho@csmu.edu.tw; joshuayjho@yahoo.com.tw

Tel: +886-4-24730022 ext. 11858

Fax: +886-4-23248191

Abstract

This study aimed to clarify the effects of inescapable and escapable stressors on behavior and interleukin-2 (IL-2) levels in the brain. Inescapable trials, consisting of pairings of conditioned (CS) and unconditioned stimuli (UCS), were used to induce fear-conditioned stress, while trials of escapable pairings of CS and UCS in an active avoidance test were used as acute and conditioned stressors. IL-2 levels in the brain were analyzed by enzyme-linked immunoadsorbent assays. Inescapable and escapable stressors had different effects on behavior in the modified active avoidance test and on IL-2 levels in brain areas that are known to be involved in emotional processes. These data provide insight into the pathophysiological role of IL-2 in stress-related disorders.

Keywords: stress; cytokine; interleukin-2; active avoidance test; emotion; psychoneuroimmunology; immune system; conditioning

Introduction

Cytokines have been linked to stress-related behavioral responses. For example, the function of immune cells and the production of interleukin-2 (IL-2), a T lymphocyte-associated cytokine, are reduced in subclinical anxiety and in patients with anxiety disorders [1]. Furthermore, IL-2 can modulate the activity of the central nervous system [2] and its levels in different tissues show relationships with various emotional behaviors [2-5]. Specifically, our previous study on ovariectomized rats showed that IL-2 is differentially distributed in the brain and this distribution shows relationships between anxiolytic-like activity in the cerebral cortex and anxiogenic-like activity in the prefrontal cortex [3]. IL-2 mRNA levels in the striatum and prefrontal cortex are related to avoidance behavior in the elevated plus-maze test [4,5]. In addition, a single striatal microinjection of IL-2 can have one of two effects on the avoidance response: a high dose (25 ng) results in a trend to anxiolytic-like behaviour in the elevated plus-maze [6], while a low dose (0.1 ng) induces an anxiogenic-like effect in rats, which spend less time in the centre of an open field test [7]. Finally, chronic irregular mild foot shock and restraint stress increases IL-2 levels in the brain [8]. Although all of the above data indicate that IL-2 is involved in stress and anxiety responses, the effect of physical/psychological stressors on IL-2 levels in the brain is still unclear.

When animals face an escapable stressor, for example, electrical foot shock, they show an active coping response to avoid, or escape from, the stressor [9]. The typical paradigm is the active avoidance test which employs pairing of a conditioned (CS) (tone plus light) with an unconditioned stimulus (UCS) (foot shock) in a shuttle box. This test is commonly used to assess learning ability, because, with repeated trials, animals can learn to avoid, or escape from, the aversive stimulus by crossing to the opposite side of the testing apparatus. However, when rats undergo an inescapable CS and UCS pairing on day 1 and are then tested in the active avoidance paradigm on day 2, the behavior during testing is largely different from that of rats receiving only active avoidance test [10]. Thus, the aim of this study was to clarify the effects of inescapable and escapable stressors on behavior and IL-2 levels in different brain areas that are known to be critical in emotional behaviors.

Materials and Methods

Animals

Forty 12-week-old male Wistar rats (National Laboratory Animal Center, ROC) were used and housed in groups of five in acrylic cages (35 × 56 × 19 cm) on a 12 hr light-dark cycle (lights on at 07:00 hr) with food and water provided *ad libitum*. Each animal was handled for 5 min in the morning and for 5 min in the afternoon on the day before the experiment to reduce defensive behavior towards the experimenter. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University.

General procedure

The shuttle box (AccuSan, USA) consisted of two equal compartments (25 × 25 × 45 cm) with a grid floor made of stainless steel bars separated by a wall with a central door (6 × 6 cm). The central door was either closed to separate the two compartments or opened to allow the animals to cross to the opposite compartment. The animals were tested on 2 consecutive days, undergoing 40 1-min trials on day 1 and 20 on day 2. In the day 1 session, the rats

were randomly placed in one of the compartments with the central door open, allowing them to explore the compartments for 1 min, then the door was closed and the rat remained in one compartment to receive the treatment (inescapable trial). In the day 2 session, the rats were again placed in the shuttle box with the door open to receive the treatment (escapable trial).

The cues [tone (75 db, 3 s) plus-light (250 lux, 3 s)] and scrambled foot shock (0.5 mA, 10 s) were applied as conditioned (CS) and unconditioned stimuli (UCS), respectively, and were delivered and controlled by a computer. The rats were randomly assigned to four groups: (1) the inescapable-escapable group undergoing 40 trials of inescapable CS-UCS pairings on day 1, followed by 20 trials of escapable CS-UCS pairings on day 2; (2) the inescapable-CS group undergoing 40 trials of inescapable CS-UCS pairings on day 1, followed by 20 trials of the cues on day 2; (3) the CS-escapable group undergoing 40 trials of cues on day 1, followed by 20 trials of escapable CS-UCS pairings on day 2; and (4) the control group undergoing 40 trials of cues on day 1 and 20 trials of cues on day 2. The day 1 inescapable CS-UCS pairings and the day 2 escapable CS-UCS pairings were used to induce fear-conditioning stress and acute conditioned stress, respectively. The day 2 session was an active avoidance test. In a "normal" active avoidance test, rats do not receive pre-treatment in the shuttle box before the test day. However, in this study, the rats received CS alone or inescapable CS-UCS pairings the day before. This modified procedure allowed us to examine the effect of inescapable stress on the response to subsequent escapable stressors. The total time of the day 1 session was 40 min and that of the day 2 session was at most 20 min. All behavioral tests were performed during the period of 10:00 – 16:00 hr.

Behavioral test

The active avoidance/escape behavioral data were recorded from rats in the inescapable-escapable and CS-escapable groups (groups 1 and 3, respectively). Both groups underwent 20 trials of escapable CS-UCS pairings in the shuttle box in the day 2 session. Briefly, the rats needed to cross from one compartment of the shuttle box to the opposite compartment to avoid or escape the shock. Each trial began with a 3-second CS, which was followed by the UCS of foot shock. If the animal passed through the door during the CS, the CS was terminated and no shock was delivered, and an avoidance response was recorded. If the animal passed through the door during shock delivery, the shock was terminated, and an escape response was recorded. If the rat did not go through the door, the shock was terminated after 10 s, and a failure response was recorded. After an inter-trial interval of 47-57 s controlled by a computer, the next trial was initiated. The latency to avoid or escape the shock and the number of avoidances, escapes, and failures were recorded.

Measurement of IL-2

After the day 2 session, the rats were immediately sacrificed by exposure to CO_2. After cardiac perfusion using phosphate-buffered saline (PBS) at 4℃, the brain was immediately removed. The prefrontal cortex (the rostral part of the cortex about 12 mm anterior of the coronal plane passing through the interaural line [11]), was dissected out on an ice-cold plate. The rest of the cortex (here, termed the "cerebral cortex"), amygdala, striatum (ventral and dorsal part), hippocampus, and pituitary gland were dissected out for detecting the IL-2 levels using commercial enzyme-linked immunoadsorbent assays (ELISA) kits with monoclonal anti-rat IL-2 antibody (CytoSets™, BioSource, CA, USA) according to the manufacturer's instructions, as described previously [3].

Data analysis

Behavioral responses in the day 2 test were compared using the *t*-test or analysis of variance (ANOVA) with repeated measures. IL-2 analyses were carried out by one-way ANOVA, followed by the least-significant difference post hoc test. All results are expressed as the mean ± SEM. The level of significance was defined as $P < 0.05$ (two-tailed).

Results

Behavior

Behavior in our modified version of the active avoidance test could only be assessed in the inescapable-escapable and CS-escapable groups, as only those animals could show successful avoidance, escape, and failure behaviors. The numbers of avoidances (df = 18, t = -1.16, P = 0.260) and escapes (df = 14.02, t = -1.31, P = 0.208) were not different between these two groups. However, the inescapable-escapable group tended to have a lower number of failure responses (df = 10.40, t = 2.02, P = 0.058) than the CS-escapable group (Table 1). ANOVA with repeated measures showed that the escape latency decreased gradually as the trial number increased ($F(19,323) = 5.02$, $P < 0.001$) and showed a group effect ($F(1,17) = 5.03$, $P = 0.039$) (Fig. 1): The mean escape latency in the inescapable-escapable group (5.3 ± 0.4 s) was significantly lower than that in the CS-escapable group (7.6 ± 0.3 s)

(df = 38, $t = 4.32$, $P < 0.001$). A similar profile was also observed in the mean duration of shocks received, which was 2.9 ± 0.3 s and 5.3 ± 0.2 s (df = 38, $t = 6.20$, $P < 0.001$) for the inescapable-escapable and CS-escapable group, respectively.

Endogenous IL-2 levels

IL-2 levels were in the range given in our previous report [3]. Basal IL-2 levels were different in the analyzed brain areas ($F(5,52) = 32.65$, $P < 0.001$), levels being highest in the striatum and lowest in the pituitary gland (Table 2). In the CS-escapable group, IL-2 levels in the amygdala, cerebral cortex, and pituitary gland were decreased compared to the CS-CS (control) group ($F(3,36) \geq 2.51$, P values < 0.05), while, in the inescapable-CS group, IL-2 levels in the amygdala and cerebral cortex were also decreased ($F(3,36) \geq 6.62$, P values < 0.001). In the inescapable-escapable group, IL-2 levels in the prefrontal cortex, hippocampus, amygdala, cerebral cortex, and pituitary gland were decreased compared to the control group ($F(3,36) \geq 2.35$, P values < 0.05). However, IL-2 levels in the striatum showed no difference between the groups (Table 2).

Discussion

In the active avoidance test, there were no significant differences between the CS-escapable and inescapable-escapable groups in the number of avoidance and escape responses. However, the inescapable-escapable group tended to have a lower number of failure responses and had a significantly decreased escape latency, and so received shorter periods of shock than the CS-escapable group. IL-2 levels in the prefrontal cortex and hippocampus were decreased in rats in the inescapable-escapable group. A reduction in IL-2 levels in the amygdala and cerebral cortex was observed in all three experimental groups (inescapable-CS, CS-escapable, and inescapable-escapable groups). The acute stress caused by the escapable CS-UCS pairings may be an important factor leading to the decrease in IL-2 levels in the pituitary gland. Finally, no change in IL-2 levels in the striatum was seen. These results suggest that escapable and inescapable shock treatments have different effects on behavior in a modified active avoidance test and modulate IL-2 levels in critical brain areas that may participate in emotional processes.

Rats in the CS-escapable group received CS-UCS pairings on the test day, which resulted in a decrease in IL-2 levels in the amygdala, cerebral cortex, and pituitary gland. Since the decrease in IL-2 levels in the amygdala and cerebral cortex was also observed in the inescapable-CS group, which received cues without shocks on the test day, the change in IL-2 levels in the pituitary gland in the CS-escapable group may have been a response to the acute shock. In addition to having the same IL-2 change profile in the amygdala, cerebral cortex, and pituitary gland to the CS-escapable group, the inescapable-escapable group also showed a decrease in IL-2 levels in the prefrontal cortex and hippocampus, which may result from the combination of psychological stress experiences, activated by conditioned fear cues, and acute stress from CS-UCS conditioning. One might argue that the total number of foot shocks received in the inescapable-escapable group was higher than that in the other group. However, the mean duration of foot shock received in the inescapable-escapable group on the test day was lower than that in the CS-escapable group. Thus, the duration of foot shocks could not be the only factor accounting for the observed differences in IL-2 in the brain. However, an additional CS-escapable vs. CS-inescapable design could provide direct comparison and strengthen this study.

Classical fear conditioning caused by pairings of UCS (e.g., shock) and CS (e.g., tone and/or illumination) is a typical stress paradigm [12]. When re-exposed to the CS, a state of anxiety-like or fear-like behavior will be induced and has been shown to have endocrine and immunological effects [13]. Thus, the rats would experience psychological stress when they had to re-enter the shuttle box and receive the CS in the day 2 session. Although foot shocks in the escapable CS-UCS pairings, for example, in the active avoidance test, provide acute physical stress, psychological factors are also involved. Furthermore, a conditioned aversive stimulus can be considered as a predominantly psychological stressor, which causes fear and/or anxiety [12]. In accordance with a recent study showing that acute stress accompanied by fear context cues has significant effects on neuronal activity in the rat hippocampus [14], decreased IL-2 levels in the hippocampus and prefrontal cortex were observed in the inescapable-escapable group which encountered the conditioned fear cues on the test day. We suggest that IL-2 in the hippocampus and prefrontal cortex may be involved in responses to acute stress accompanied by psychological stress experiences.

IL-2 levels in the amygdala and cerebral cortex were decreased not only in the CS-escapable and inescapable-escapable groups, but also in the inescapable-CS group, which was exposed to acute shocks and aversive conditioned cues. This suggests that IL-2 in the amygdala and cerebral cortex may be involved in the regulation of responses to physical and/or psychological stress. Since IL-2 can directly inhibit N-methyl-D-aspartate receptor-mediated currents [15], the decrease in IL-2 levels seen during stress provides a possible neuronal mechanism underlying the function of IL-2 in emotional behavior in various stress conditions [4]. Although it has been found that IL-2 mRNA levels in the striatum correlate with unconditioned anxiety-like/avoidance levels [5,6],

there was no relationship between IL-2 levels in the striatum and the conditioned stressor used in the present study.

As for the pituitary gland, IL-2 levels decreased in the CS-escapable and inescapable-escapable groups, but not the inescapable-CS group, suggesting that acute stress may be the factor that caused the reduction in IL-2. The pituitary gland has an important neuroendocrine function in the hypothalamic-pituitary-adrenal (HPA) axis, which plays a critical role in the regulation of responses to physical and psychological stress, in which cytokines are known to be involved [16]. Physical stress, such as restraint and electrical shock, and psychological stress caused by conditioned aversive stimulus can activate the HPA axis and facilitate the release of glucocorticoids into the blood, which can reduce plasma IL-2 levels [17]. Our findings are in line with the view that IL-2 in neuroendocrine tissues takes part in stress responses [18].

Stress, e.g. electric foot shock, restraint, and conditioned aversive stimuli, is known to increase the production of plasma cytokines [16]. Repeated restraint stress [19] and the psychological stress of behavioral conditioning [20] reduce IL-2 production by lymphocytes and splenocytes in animals. Chronic stress in caregivers of dementia patients results in an increased concentration of cortisol in the saliva and a decrease in IL-2 levels in the blood [21]. All the above reports show the relationship between stress and cytokines; however, they mainly focused on the effects of stress on IL-2 in the periphery. Although some cytokines secreted by immune cells in the blood can actively pass through the blood-brain-barrier [22], astrocytes and microglia in the brain can also produce cytokines locally [23].

Interestingly, chronic irregular mild foot shock in mice causes an increase in IL-2 levels in brain tissue which is correlated with the activation of the HPA axis [8]. Pijlman et al. have reported that the physical stress of foot shock and the psychological stress of passively perceiving foot shocks performed on other animals result in different behavioral responses [24]. Chronic mild stress decreases IL-2 levels in serum and this is reversed by anti-depressants [25], suggesting a relationship between IL-2 and neuronal activity. Furthermore, peripheral administration of IL-2 increases locomotor activity and the turnover of monoaminergic neurotransmitters in the hypothalamus and prefrontal cortex [2]**,** suggesting that IL-2 may affect neurochemical activity and therefore mediate emotional behavior.

Conclusion

The results suggest that inescapable and escapable stressors may have different effects on behavior in a modified active avoidance test and on IL-2 levels in specific brain areas that are known to be involved in emotional processes. These data provide insight into the pathophysiological role of IL-2 in stress and suggest that targeting the immune system by modulating the actions of cytokines may impact on stress-related disorders.

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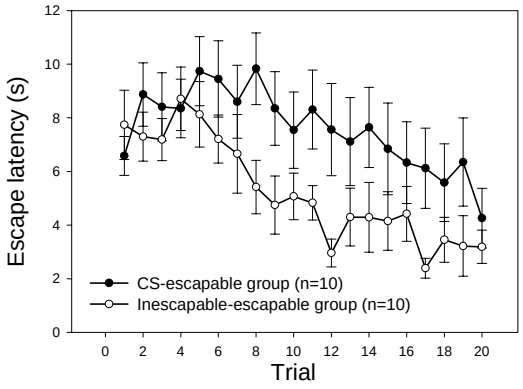
Figure legend

Fig. 1. Effects of escapable and inescapable stressors on escape latency in the modified active avoidance test. The data are expressed as the mean ± SEM.

Fig. 1.



Tab. 1. Effects of escapable and inescapable stressors on behavior in the modified active avoidance test.

	CS-escapable group (n=10)	Inescapable-escapable group (n=10)
Avoidance number	3.90 ± 1.38	6.00 ± 1.16
Escape number	9.60 ± 1.87	12.40 ± 1.03
Failure number	6.50 ± 2.33	1.60 ± 0.65 #

Data are expressed as the mean ± SEM. # $P = 0.058$, compared to the CS-escapable group.

Table. 2. Effects of escapable and inescapable stressors on IL-2 levels in different brain areas.

	CS-CS group (n=10)	CS-escapable group (n=10)	Inescapable-CS group (n=10)	Inescapable-escapable group (n=10)
Prefrontal cortex	1.72 ± 0.09	1.58 ± 0.32	1.46 ± 0.07	1.20 ± 0.07 *
Hippocampus	1.25 ± 0.06	1.22 ± 0.05	1.14 ± 0.04	1.06 ± 0.07 *
Amygdala	1.41 ± 0.04	1.16 ± 0.04 ***	1.17 ± 0.06 ***	1.16 ± 0.05 ***
Cerebral cortex	1.58 ± 0.06	1.33 ± 0.06 **	1.09 ± 0.05 ***	1.33 ± 0.07 **
Striatum	1.81 ± 0.08	1.61 ± 0.08	1.68 ± 0.07	1.77 ± 0.10
Pituitary gland	0.71 ± 0.09	0.52 ± 0.02 *	0.68 ± 0.08	0.52 ± 0.03 *

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared to the control CS-CS group. The IL-2 concentration is expressed as pg/μg total protein. Data are expressed as the mean ± SEM.

出席國際會議 報告

發表論文摘要

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投稿摘要

Physical and psychological stressors differently affect the level of interleukin-2 in the brain

Yen-Ti Lee [1], An-Li Wang [2], Yu-Han Wu [3], Chuen-Hui Fan [3], Cornelius R Pawlak [4], **Ying-Jui Ho** [5]

[1] Institute of Behavioral Medicine, Cheng Kung University; [2]Department of Life Sciences, National Chung Hsing University; [3]School of Medical Laboratory and Biotechnology, [5]School of Psychology, Chung Shan Medical University, Taiwan, ROC; [4]Psychopharmacology, Central Institute of Mental Health, Germany

Abstract

This study detected the effects of physical and psychological stressors on the levels of interleukin-2 (IL-2) in the rat brain. A two-day session of inescapable and escapable conditioning was administered for inducing psychological and physical stressors, respectively. IL-2 level in the prefrontal cortex and hippocampus was decreased when the rats encountered physical and psychological stressors simultaneously. All the physical, psychological, and the combination of theses two stressors were able to reduce the IL-2 level in the amygdala and cerebral cortex. Acute physical stress caused the reduction of IL-2 in the pituitary gland. These results showed that physical and psychological stressors differently affect the IL-2 levels in the brain.

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報告內容

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Effects of escapable and inescapable stressors on behavior and interleukin-2 in the brain

Yen-Ti Lee[1], Wen-Fu Wang[2], Chun-Wen Cheng [3], Shey-Lin Wu[2,4], Cornelius R. Pawlak[5], **Ying-Jui Ho**[6#]

[1] Institute of Behavioral Medicine, Cheng Kung University, Taiwan, ROC
[2] Department of Neurology, Chang-Hua Christian Hospital, Taiwan, ROC
[3] Institute of Biochemistry and Biotechnology, Chung Shan Medical University, Taiwan, ROC
[4] Department of Bioindustry Technology, Dayeh University, Taiwan, ROC
[5] Department of Psychopharmacology, Central Institute of Mental Health, Mannheim, Germany
[6] School of Psychology, Chung Shan Medical University, Taiwan, ROC

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[#]Corresponding author:

Dr. **Ying-Jui Ho**

School of Psychology, Chung Shan Medical University, Taiwan, ROC

Address: No. 110, Sec. 1, Jianguo N. Rd., Taichung City 402, Taiwan, ROC

e-mail: yjho@csmu.edu.tw; joshuayjho@yahoo.com.tw

Tel: +886-4-24730022 ext. 11858

Fax: +886-4-23248191

Abstract

This study aimed to clarify the effects of inescapable and escapable stressors on behavior and interleukin-2 (IL-2) levels in the brain. Inescapable trials, consisting of pairings of conditioned (CS) and unconditioned stimuli (UCS), were used to induce fear-conditioned stress, while trials of escapable pairings of CS and UCS in an active avoidance test were used as acute and conditioned stressors. IL-2 levels in the brain were analyzed by enzyme-linked immunoadsorbent assays. Inescapable and escapable stressors had different effects on behavior in the modified active avoidance test and on IL-2 levels in brain areas that are known to be involved in emotional processes. These data provide insight into the pathophysiological role of IL-2 in stress-related disorders.

Keywords: stress; cytokine; interleukin-2; active avoidance test; emotion; psychoneuroimmunology; immune system; conditioning

Introduction

Cytokines have been linked to stress-related behavioral responses. For example, the function of immune cells and the production of interleukin-2 (IL-2), a T lymphocyte-associated cytokine, are reduced in subclinical anxiety and in patients with anxiety disorders [1]. Furthermore, IL-2 can modulate the activity of the central nervous system [2] and its levels in different tissues show relationships with various emotional behaviors [2-5]. Specifically, our previous study on ovariectomized rats showed that IL-2 is differentially distributed in the brain and this distribution shows relationships between anxiolytic-like activity in the cerebral cortex and anxiogenic-like activity in the prefrontal cortex [3]. IL-2 mRNA levels in the striatum and prefrontal cortex are related to avoidance behavior in the elevated plus-maze test [4,5]. In addition, a single striatal microinjection of IL-2 can have one of two effects on the avoidance response: a high dose (25 ng) results in a trend to anxiolytic-like behaviour in the elevated plus-maze [6], while a low dose (0.1 ng) induces an anxiogenic-like effect in rats, which spend less time in the centre of an open field test [7]. Finally, chronic irregular mild foot shock and restraint stress increases IL-2 levels in the brain [8]. Although all of the above data indicate that IL-2 is involved in stress and anxiety responses, the effect of physical/psychological stressors on IL-2 levels in the brain is still unclear.

When animals face an escapable stressor, for example, electrical foot shock, they show an active coping response to avoid, or escape from, the stressor [9]. The typical paradigm is the active avoidance test which employs pairing of a conditioned (CS) (tone plus light) with an unconditioned stimulus (UCS) (foot shock) in a shuttle box. This test is commonly used to assess learning ability, because, with repeated trials, animals can learn to avoid, or escape from, the aversive stimulus by crossing to the opposite side of the testing apparatus. However, when rats undergo an inescapable CS and UCS pairing on day 1 and are then tested in the active avoidance paradigm on day 2, the behavior during testing is largely different from that of rats receiving only active avoidance test [10]. Thus, the aim of this study was to clarify the effects of inescapable and escapable stressors on behavior and IL-2 levels in different brain areas that are known to be critical in emotional behaviors.

Materials and Methods

Animals

Forty 12-week-old male Wistar rats (National Laboratory Animal Center, ROC) were used and housed in groups of five in acrylic cages (35 × 56 × 19 cm) on a 12 hr light-dark cycle (lights on at 07:00 hr) with food and water provided *ad libitum*. Each animal was handled for 5 min in the morning and for 5 min in the afternoon on the day before the experiment to reduce defensive behavior towards the experimenter. All experimental procedures were performed according to the NIH Guide for the Care and Use of Laboratory Animals and were approved by the Animal Care Committee of Chung Shan Medical University.

General procedure

The shuttle box (AccuSan, USA) consisted of two equal compartments (25 × 25 × 45 cm) with a grid floor made of stainless steel bars separated by a wall with a central door (6 × 6 cm). The central door was either closed to separate the two compartments or opened to allow the animals to cross to the opposite compartment. The animals were tested on 2 consecutive days, undergoing 40 1-min trials on day 1 and 20 on day 2. In the day 1 session, the rats

were randomly placed in one of the compartments with the central door open, allowing them to explore the compartments for 1 min, then the door was closed and the rat remained in one compartment to receive the treatment (inescapable trial). In the day 2 session, the rats were again placed in the shuttle box with the door open to receive the treatment (escapable trial).

The cues [tone (75 db, 3 s) plus-light (250 lux, 3 s)] and scrambled foot shock (0.5 mA, 10 s) were applied as conditioned (CS) and unconditioned stimuli (UCS), respectively, and were delivered and controlled by a computer. The rats were randomly assigned to four groups: (1) the inescapable-escapable group undergoing 40 trials of inescapable CS-UCS pairings on day 1, followed by 20 trials of escapable CS-UCS pairings on day 2; (2) the inescapable-CS group undergoing 40 trials of inescapable CS-UCS pairings on day 1, followed by 20 trials of the cues on day 2; (3) the CS-escapable group undergoing 40 trials of cues on day 1, followed by 20 trials of escapable CS-UCS pairings on day 2; and (4) the control group undergoing 40 trials of cues on day 1 and 20 trials of cues on day 2. The day 1 inescapable CS-UCS pairings and the day 2 escapable CS-UCS pairings were used to induce fear-conditioning stress and acute conditioned stress, respectively. The day 2 session was an active avoidance test. In a "normal" active avoidance test, rats do not receive pre-treatment in the shuttle box before the test day. However, in this study, the rats received CS alone or inescapable CS-UCS pairings the day before. This modified procedure allowed us to examine the effect of inescapable stress on the response to subsequent escapable stressors. The total time of the day 1 session was 40 min and that of the day 2 session was at most 20 min. All behavioral tests were performed during the period of 10:00 – 16:00 hr.

Behavioral test

The active avoidance/escape behavioral data were recorded from rats in the inescapable-escapable and CS-escapable groups (groups 1 and 3, respectively). Both groups underwent 20 trials of escapable CS-UCS pairings in the shuttle box in the day 2 session. Briefly, the rats needed to cross from one compartment of the shuttle box to the opposite compartment to avoid or escape the shock. Each trial began with a 3-second CS, which was followed by the UCS of foot shock. If the animal passed through the door during the CS, the CS was terminated and no shock was delivered, and an avoidance response was recorded. If the animal passed through the door during shock delivery, the shock was terminated, and an escape response was recorded. If the rat did not go through the door, the shock was terminated after 10 s, and a failure response was recorded. After an inter-trial interval of 47-57 s controlled by a computer, the next trial was initiated. The latency to avoid or escape the shock and the number of avoidances, escapes, and failures were recorded.

Measurement of IL-2

After the day 2 session, the rats were immediately sacrificed by exposure to CO_2. After cardiac perfusion using phosphate-buffered saline (PBS) at 4℃, the brain was immediately removed. The prefrontal cortex (the rostral part of the cortex about 12 mm anterior of the coronal plane passing through the interaural line [11]), was dissected out on an ice-cold plate. The rest of the cortex (here, termed the "cerebral cortex"), amygdala, striatum (ventral and dorsal part), hippocampus, and pituitary gland were dissected out for detecting the IL-2 levels using commercial enzyme-linked immunoadsorbent assays (ELISA) kits with monoclonal anti-rat IL-2 antibody (CytoSets™, BioSource, CA, USA) according to the manufacturer's instructions, as described previously [3].

Data analysis

Behavioral responses in the day 2 test were compared using the *t*-test or analysis of variance (ANOVA) with repeated measures. IL-2 analyses were carried out by one-way ANOVA, followed by the least-significant difference post hoc test. All results are expressed as the mean ± SEM. The level of significance was defined as $P < 0.05$ (two-tailed).

Results

Behavior

Behavior in our modified version of the active avoidance test could only be assessed in the inescapable-escapable and CS-escapable groups, as only those animals could show successful avoidance, escape, and failure behaviors. The numbers of avoidances (df = 18, t = -1.16, P = 0.260) and escapes (df = 14.02, t = -1.31, P = 0.208) were not different between these two groups. However, the inescapable-escapable group tended to have a lower number of failure responses (df = 10.40, t = 2.02, P = 0.058) than the CS-escapable group (Table 1). ANOVA with repeated measures showed that the escape latency decreased gradually as the trial number increased ($F(19,323) = 5.02$, $P < 0.001$) and showed a group effect ($F(1,17) = 5.03$, $P = 0.039$) (Fig. 1): The mean escape latency in the inescapable-escapable group (5.3 ± 0.4 s) was significantly lower than that in the CS-escapable group (7.6 ± 0.3 s)

(df = 38, $t = 4.32$, $P < 0.001$). A similar profile was also observed in the mean duration of shocks received, which was 2.9 ± 0.3 s and 5.3 ± 0.2 s (df = 38, $t = 6.20$, $P < 0.001$) for the inescapable-escapable and CS-escapable group, respectively.

Endogenous IL-2 levels

IL-2 levels were in the range given in our previous report [3]. Basal IL-2 levels were different in the analyzed brain areas ($F(5,52) = 32.65$, $P < 0.001$), levels being highest in the striatum and lowest in the pituitary gland (Table 2). In the CS-escapable group, IL-2 levels in the amygdala, cerebral cortex, and pituitary gland were decreased compared to the CS-CS (control) group ($F(3,36) \geq 2.51$, P values < 0.05), while, in the inescapable-CS group, IL-2 levels in the amygdala and cerebral cortex were also decreased ($F(3,36) \geq 6.62$, P values < 0.001). In the inescapable-escapable group, IL-2 levels in the prefrontal cortex, hippocampus, amygdala, cerebral cortex, and pituitary gland were decreased compared to the control group ($F(3,36) \geq 2.35$, P values < 0.05). However, IL-2 levels in the striatum showed no difference between the groups (Table 2).

Discussion

In the active avoidance test, there were no significant differences between the CS-escapable and inescapable-escapable groups in the number of avoidance and escape responses. However, the inescapable-escapable group tended to have a lower number of failure responses and had a significantly decreased escape latency, and so received shorter periods of shock than the CS-escapable group. IL-2 levels in the prefrontal cortex and hippocampus were decreased in rats in the inescapable-escapable group. A reduction in IL-2 levels in the amygdala and cerebral cortex was observed in all three experimental groups (inescapable-CS, CS-escapable, and inescapable-escapable groups). The acute stress caused by the escapable CS-UCS pairings may be an important factor leading to the decrease in IL-2 levels in the pituitary gland. Finally, no change in IL-2 levels in the striatum was seen. These results suggest that escapable and inescapable shock treatments have different effects on behavior in a modified active avoidance test and modulate IL-2 levels in critical brain areas that may participate in emotional processes.

Rats in the CS-escapable group received CS-UCS pairings on the test day, which resulted in a decrease in IL-2 levels in the amygdala, cerebral cortex, and pituitary gland. Since the decrease in IL-2 levels in the amygdala and cerebral cortex was also observed in the inescapable-CS group, which received cues without shocks on the test day, the change in IL-2 levels in the pituitary gland in the CS-escapable group may have been a response to the acute shock. In addition to having the same IL-2 change profile in the amygdala, cerebral cortex, and pituitary gland to the CS-escapable group, the inescapable-escapable group also showed a decrease in IL-2 levels in the prefrontal cortex and hippocampus, which may result from the combination of psychological stress experiences, activated by conditioned fear cues, and acute stress from CS-UCS conditioning. One might argue that the total number of foot shocks received in the inescapable-escapable group was higher than that in the other group. However, the mean duration of foot shock received in the inescapable-escapable group on the test day was lower than that in the CS-escapable group. Thus, the duration of foot shocks could not be the only factor accounting for the observed differences in IL-2 in the brain. However, an additional CS-escapable vs. CS-inescapable design could provide direct comparison and strengthen this study.

Classical fear conditioning caused by pairings of UCS (e.g., shock) and CS (e.g., tone and/or illumination) is a typical stress paradigm [12]. When re-exposed to the CS, a state of anxiety-like or fear-like behavior will be induced and has been shown to have endocrine and immunological effects [13]. Thus, the rats would experience psychological stress when they had to re-enter the shuttle box and receive the CS in the day 2 session. Although foot shocks in the escapable CS-UCS pairings, for example, in the active avoidance test, provide acute physical stress, psychological factors are also involved. Furthermore, a conditioned aversive stimulus can be considered as a predominantly psychological stressor, which causes fear and/or anxiety [12]. In accordance with a recent study showing that acute stress accompanied by fear context cues has significant effects on neuronal activity in the rat hippocampus [14], decreased IL-2 levels in the hippocampus and prefrontal cortex were observed in the inescapable-escapable group which encountered the conditioned fear cues on the test day. We suggest that IL-2 in the hippocampus and prefrontal cortex may be involved in responses to acute stress accompanied by psychological stress experiences.

IL-2 levels in the amygdala and cerebral cortex were decreased not only in the CS-escapable and inescapable-escapable groups, but also in the inescapable-CS group, which was exposed to acute shocks and aversive conditioned cues. This suggests that IL-2 in the amygdala and cerebral cortex may be involved in the regulation of responses to physical and/or psychological stress. Since IL-2 can directly inhibit N-methyl-D-aspartate receptor-mediated currents [15], the decrease in IL-2 levels seen during stress provides a possible neuronal mechanism underlying the function of IL-2 in emotional behavior in various stress conditions [4]. Although it has been found that IL-2 mRNA levels in the striatum correlate with unconditioned anxiety-like/avoidance levels [5,6],

there was no relationship between IL-2 levels in the striatum and the conditioned stressor used in the present study.

As for the pituitary gland, IL-2 levels decreased in the CS-escapable and inescapable-escapable groups, but not the inescapable-CS group, suggesting that acute stress may be the factor that caused the reduction in IL-2. The pituitary gland has an important neuroendocrine function in the hypothalamic-pituitary-adrenal (HPA) axis, which plays a critical role in the regulation of responses to physical and psychological stress, in which cytokines are known to be involved [16]. Physical stress, such as restraint and electrical shock, and psychological stress caused by conditioned aversive stimulus can activate the HPA axis and facilitate the release of glucocorticoids into the blood, which can reduce plasma IL-2 levels [17]. Our findings are in line with the view that IL-2 in neuroendocrine tissues takes part in stress responses [18].

Stress, e.g. electric foot shock, restraint, and conditioned aversive stimuli, is known to increase the production of plasma cytokines [16]. Repeated restraint stress [19] and the psychological stress of behavioral conditioning [20] reduce IL-2 production by lymphocytes and splenocytes in animals. Chronic stress in caregivers of dementia patients results in an increased concentration of cortisol in the saliva and a decrease in IL-2 levels in the blood [21]. All the above reports show the relationship between stress and cytokines; however, they mainly focused on the effects of stress on IL-2 in the periphery. Although some cytokines secreted by immune cells in the blood can actively pass through the blood-brain-barrier [22], astrocytes and microglia in the brain can also produce cytokines locally [23].

Interestingly, chronic irregular mild foot shock in mice causes an increase in IL-2 levels in brain tissue which is correlated with the activation of the HPA axis [8]. Pijlman et al. have reported that the physical stress of foot shock and the psychological stress of passively perceiving foot shocks performed on other animals result in different behavioral responses [24]. Chronic mild stress decreases IL-2 levels in serum and this is reversed by anti-depressants [25], suggesting a relationship between IL-2 and neuronal activity. Furthermore, peripheral administration of IL-2 increases locomotor activity and the turnover of monoaminergic neurotransmitters in the hypothalamus and prefrontal cortex [2]**,** suggesting that IL-2 may affect neurochemical activity and therefore mediate emotional behavior.

Conclusion

The results suggest that inescapable and escapable stressors may have different effects on behavior in a modified active avoidance test and on IL-2 levels in specific brain areas that are known to be involved in emotional processes. These data provide insight into the pathophysiological role of IL-2 in stress and suggest that targeting the immune system by modulating the actions of cytokines may impact on stress-related disorders.

Acknowledgements

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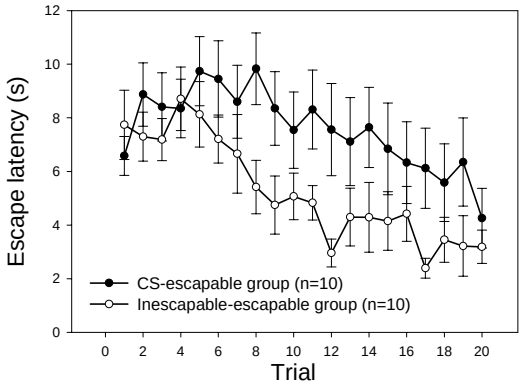
Figure legend

Fig. 1. Effects of escapable and inescapable stressors on escape latency in the modified active avoidance test. The data are expressed as the mean ± SEM.

Fig. 1.



Tab. 1. Effects of escapable and inescapable stressors on behavior in the modified active avoidance test.

	CS-escapable group (n=10)	Inescapable-escapable group (n=10)
Avoidance number	3.90 ± 1.38	6.00 ± 1.16
Escape number	9.60 ± 1.87	12.40 ± 1.03
Failure number	6.50 ± 2.33	1.60 ± 0.65 #

Data are expressed as the mean ± SEM. # $P = 0.058$, compared to the CS-escapable group.

Table. 2. Effects of escapable and inescapable stressors on IL-2 levels in different brain areas.

	CS-CS group (n=10)	CS-escapable group (n=10)	Inescapable-CS group (n=10)	Inescapable-escapable group (n=10)
Prefrontal cortex	1.72 ± 0.09	1.58 ± 0.32	1.46 ± 0.07	1.20 ± 0.07 *
Hippocampus	1.25 ± 0.06	1.22 ± 0.05	1.14 ± 0.04	1.06 ± 0.07 *
Amygdala	1.41 ± 0.04	1.16 ± 0.04 ***	1.17 ± 0.06 ***	1.16 ± 0.05 ***
Cerebral cortex	1.58 ± 0.06	1.33 ± 0.06 **	1.09 ± 0.05 ***	1.33 ± 0.07 **
Striatum	1.81 ± 0.08	1.61 ± 0.08	1.68 ± 0.07	1.77 ± 0.10
Pituitary gland	0.71 ± 0.09	0.52 ± 0.02 *	0.68 ± 0.08	0.52 ± 0.03 *

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, compared to the control CS-CS group. The IL-2 concentration is expressed as pg/μg total protein. Data are expressed as the mean ± SEM.

出席國際會議 報告

發表論文摘要

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投稿摘要

Physical and psychological stressors differently affect the level of interleukin-2 in the brain

Yen-Ti Lee [1], An-Li Wang [2], Yu-Han Wu [3], Chuen-Hui Fan [3], Cornelius R Pawlak [4], **Ying-Jui Ho** [5]

[1] Institute of Behavioral Medicine, Cheng Kung University; [2]Department of Life Sciences, National Chung Hsing University; [3]School of Medical Laboratory and Biotechnology, [5]School of Psychology, Chung Shan Medical University, Taiwan, ROC; [4]Psychopharmacology, Central Institute of Mental Health, Germany

Abstract

This study detected the effects of physical and psychological stressors on the levels of interleukin-2 (IL-2) in the rat brain. A two-day session of inescapable and escapable conditioning was administered for inducing psychological and physical stressors, respectively. IL-2 level in the prefrontal cortex and hippocampus was decreased when the rats encountered physical and psychological stressors simultaneously. All the physical, psychological, and the combination of theses two stressors were able to reduce the IL-2 level in the amygdala and cerebral cortex. Acute physical stress caused the reduction of IL-2 in the pituitary gland. These results showed that physical and psychological stressors differently affect the IL-2 levels in the brain.