INFLUENCE OF MUSIC TRAINING ON ACADEMIC EXAMINATIONS AND STRESS IN ADOLESCENTS

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INFLUENCE OF MUSIC TRAINING ON ACADEMIC EXAMINATIONS AND STRESS IN ADOLESCENTS

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ABSTRACT

Several pieces of evidence suggested that academic examinations fulfill the classical requirement of a psychological stressor. Academic examinations represent a stressful challenge to many students, and studies on the examination-dependent corticosteroid response, a sensitive physiological indicator of a stress response, are inconsistent. In addition, several studies showed that music can also decrease cortisol and adrenocorticotropic hormone (ACTH) levels, and other studies have found that music may also enhance a variety of cognitive functions, such as attention, learning, communication, and memory. This study aims to investigate the salivary cortisol response upon the examination in Thai adolescents and analyze the discrepancy of the stress response between musician and control subjects. Then, we observed whether the academic examination-dependent corticosteroid response could affect learning and memory in Thai adolescents. There were 30 musician and 30 control students participating in this study. The age range of all subjects was 15 – 17 years. Mathematical examinations were used as a familiar stressful condition. Baseline, pre- and post-examination salivary cortisol responses were quantified and correlated with a general questionnaire and a stress inventory questionnaire, including self-estimated stress levels. Results showed that the saliva cortisol concentration at the pre-examination stage of the musician group was significantly lower than that of the control group ($p < 0.001$), whereas there was no difference in the stress inventory scores. Interestingly, at the GPA 3.50-4.00 range, pre-examination cortisol levels differed between the musician and control groups, the musicians having significantly lower cortisol levels than the control group ($p < 0.001$). This study suggests that, under stress, the effects of music may be associated with reduction of saliva cortisol levels and academic examination stress.

KEY WORDS: ACADEMIC STRESS/ SALIVA CORTISOL/ MUSIC/
RADIOIMMUNOASSAY

116 pages

อิทธิพลของการฝึกดนตรีต่อการสอบของโรงเรียนและความเครียดในเด็กวัยรุ่น
INFLUENCE OF MUSIC TRAINING ON ACADEMIC EXAMINATIONS AND STRESS IN ADOLESCENTS

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บทคัดย่อ

ข้อมูลจากหลายหลักฐานการศึกษาแสดงให้เห็นว่าการสอบของโรงเรียนเป็นสภาวะที่ทำให้เกิดความตึงเครียดต่อสิ่งท้าทายของเด็กนักเรียนจำนวนมาก ปัจจุบันการศึกษาการตอบสนองต่อความเครียดจากการสอบโดยการวัดฮอร์โมนคอร์ติโคสเตอรอยด์นั้นยังเป็นที่ถกเถียงกันอยู่ นอกจากนี้ยังมีหลายการศึกษาพบว่าดนตรีสามารถลดระดับฮอร์โมนคอร์ติซอลและฮอร์โมน adrenocorticotropic และดนตรีอาจจะเพิ่มการทำงานที่เกี่ยวกับกระบวนการเรียนรู้หลายชนิด เช่น ความตั้งใจ การเรียน การสื่อสาร และความจำ อีกด้วย การศึกษาวิจัยครั้งนี้มีวัตถุประสงค์เพื่อตรวจสอบการตอบสนองต่อการสอบโดยการวัดระดับฮอร์โมนคอร์ติซอลในเด็กวัยรุ่นไทย และวิเคราะห์ความแตกต่างต่อการตอบสนองต่อความเครียดระหว่างกลุ่มนักเรียนที่เล่นดนตรีและกลุ่มควบคุม โดยสังเกตผลกระทบต่อการเรียนรู้และความจำ ผู้เข้าร่วมการทดลองเป็นนักเรียนที่เล่นดนตรี จำนวน 30 คนและนักเรียนที่ไม่เล่นดนตรีหรือกลุ่มควบคุม จำนวน 30 คน ซึ่งมีช่วงอายุระหว่าง 15-17 ปี การศึกษานี้ทดลองในช่วงที่มีสภาวะตึงเครียดจากการสอบวิชาคณิตศาสตร์ โดยการเก็บตัวอย่างน้ำลายจากนักเรียนทุกคนในช่วงเวลาปกติ ก่อนสอบและหลังสอบ พร้อมกับกรอกข้อมูลในแบบสอบถามข้อมูลทั่วไปและแบบประเมินความเครียดด้วยตนเอง ผลการทดลองพบว่าระดับความเข้มข้นของฮอร์โมนคอร์ติซอลก่อนสอบของกลุ่มที่เล่นดนตรีมีค่าต่ำกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$) ในขณะที่ไม่พบความแตกต่างของระดับความเครียดที่ได้จากแบบประเมินความเครียดด้วยตนเอง และเป็นที่น่าสนใจว่า กลุ่มที่เล่นดนตรีที่มีผลการเรียนเฉลี่ยอยู่ในช่วง 3.50-4.00 นั้นมีระดับฮอร์โมนคอร์ติซอลที่ต่ำกว่ากลุ่มควบคุมอย่างมีนัยสำคัญทางสถิติ ($p < 0.001$) ผลจากการศึกษาวิจัยครั้งนี้แสดงให้เห็นว่า ภายใต้ภาวะตึงเครียด ดนตรีอาจมีความเกี่ยวข้องกับการลดลงของระดับฮอร์โมนคอร์ติซอลในน้ำลายและความเครียดที่เกิดจากการสอบได้

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LIST OF ABBREVIATIONS

µCi	microcurie
µl	microlitre
^{125}I	iodine-125
^{3}H	tritium (hydrogen-3)
ABL	amygdala basolateral region
ACM	amygdala centromedial region
ACTH	adrenocorticotropin/ adrenocorticotropic hormone/ corticotropin
AL	amygdala lateral nuclei
AM	ante meridiem (Latin: "before noon")
AMPA	α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate
ANS	autonomic nervous system
ATP	adenosine-5'-triphosphate
AVP	arginine-vasopressin
BDNF	brain-derived neurotrophic factor
BMI	body mass index
BSA	bovine serum albumin
BST	bed nucleus of the stria terminalis
CA1	cornu ammonis 1
Ca^{2+}	calcium
CaMKII	calmodulin-dependent protein kinase II
cAMP	cyclic adenosine monophosphate
CBG	corticosteroid-binding globulin
Cl^{-}	chloride ion
CNS	central nervous system
CORT	corticosterone
CR	conditioned response

LIST OF ABBREVIATIONS (cont.)

CREB	cAMP response element binding
CRH	corticotropin-releasing hormone
CS	conditioned stimulus
CYP11B1	cytochrome P450 11β-hydroxylase
EEG	electroencephalography
E-LTP	early phase of long-term potentiation
eNOS	endothelial nitric oxide synthase
ERα	estrogen receptor -α
FAD	flavin adenine dinucleotide
GABA	γ-Aminobutyric acid
GAD	glutamate decarboxylase
GH	growth hormone
GPA	grade point average
GRs	glucocorticoid receptors
HCl	hydrogen chloride
HCO_3^-	hydrogen carbonate
HF	hippocampal formation
HPA	hypothalamic-pituitary-adrenal axis
IgA	immunoglobulin A
IGF-1	insulin-like growth factor-1
IL-6	interleukin-6
IR	insulin receptor
KBq	kilobecquerel
LC	locus ceruleus
LFS	low-frequency stimulation
LH	luteinizing hormone
L-LTP	late-phase long-term potentiation
LTD	long term depression

LIST OF ABBREVIATIONS (cont.)

LTP	long-term potentiation
MAP	mitogen-activated protein
MAPK	mitogen-activated protein kinases
mBDNF	mature BDNF
Mg^{2+}	magnesium
mRNA	messenger ribonucleic acid
MRs	mineralocorticoid receptors
NA	noradrenaline
Na^{+}	sodium ion
NaN3	sodium azide
NE	norepinephrine
NGF	nerve growth factor
NMDA	N-methyl-D-aspartate glutamate
NO	nitric oxide
NT3	neurotrophin 3
NTS	nucleus of the solitary tract
p75NTR	p75 neurotrophin receptor
PI3K	phosphoinositide 3-kinases
PKA	protein kinase A
PKB	protein kinase B
PKC	protein kinase C
PLC	phospholipase C
PM	post meridiem (Latin: “after noon”)
PP1	protein phosphatase 1
PP2B	calcineurin
PR	progestin receptors
PVN	paraventricular nucleus
SD	standard deviation

LIST OF ABBREVIATIONS (cont.)

T3	triiodothyronine
TBS	theta-burst stimulation
tPA	tissue plasminogen activator
Tris	tris (hydroxymethyl) aminomethane
TSH	thyroid stimulating hormone
UR	unconditioned responses
US	unconditioned stimulus

CHAPTER I
INTRODUCTION

Stress is a process in which environmental demands tax or exceeds individuals' adaptive capacities, contributing to biological and psychological changes that may place them at risk for illness (Cohen et al., 1997). Stressful life experience can have significant effects on a variety of physiological systems, including the autonomic nervous system, the hypothalamic-pituitary-adrenal axis, and the immune system. Stressors, or stressful life experiences, are defined as circumstances that threaten a major goal, including the maintenance of one's physical integrity (physical stressors) or one's psychological well-being (psychological stressors) (Kemeny, 2003; Lazarus and Folkman, 1984). Several studies showed that exposure to a variety of acute psychological stressors (e.g. giving a speech, doing difficult cognitive tasks, taking exams), for short durations, can cause an increase in the levels of the hormone cortisol in the plasma, urine, and saliva. This increase is due to activation of the hypothalamic-pituitary-adrenal (HPA) axis. The activation of the HPA axis increases occurring in release of corticotropin-releasing hormone (CRH) from the hypothalamus. CRH stimulates the anterior pituitary to release adrenocorticotropin releasing hormone (ACTH) into the bloodstream, which stimulates the adrenal cortex to release the cortisol (Baum and Grunberg, 1997).

The acute stress is the most common form of stress. It comes from demands and pressures of the recent past and anticipated demands and pressures of the near future. Acute stress is thrilling and exciting. Although cortisol activation in response to stress is protective in the short term, chronic or extreme activation may have long term negative consequences (McEwen and Magarinos, 1997). Chronic or extreme activation can lead to changes in HPA axis activity, as evidenced by abnormal cortisol levels, which may in turn increase vulnerability to developing health problems (e.g. hippocampus damage and permanent memory impairment).

Smyth et al. (1998) examined the effects of past, current, and anticipated naturalistic daily stressors and of affect on salivary cortisol levels. The 120 participants were reported on stressors and of affect 6 times per day in response to a preprogrammed wristwatch. Twenty minutes after each assessment they took a sample of saliva for cortisol analysis. The result showed that both the experience of a current stressor and anticipating a stressor, were associated with increased salivary cortisol levels. This result suggested that the increase of cortisol levels have been observed not only with an acute stressor but also in anticipation of a stressful experience.

Academic examinations represent stressful challenges to many students, studies on the examination-dependent adrenocortisol response, a sensitive physiological indicator for a stress response. Academic examinations fulfill classical requirement for a psychological stressor, including non-controllable conditions and shortage of time. Martinek et al. (2003) studied the cortisol response upon two written and one oral examination in adolescents, analyzed the consistency of the response within subjects, and evaluated the association between cortisol response and biometric measurements, including sensation seeking according to Zuckerman and self-reported stress level. The results shown that salivary cortisol response (average of all subjects) have transient increase upon examination but when comparing individual cortisol responses revealed three distinct cortisol profiles, including a transient increase (Type 1), a transient decline (Type 2), or no response (Type 3). These results suggest that upon academic examinations the cortisol response varies among subjects.

Although some studies reported that academic examination leads to an increase of cortisol, whereas other studies describe either no effect or a decrease of cortisol levels upon examinations. These controversies surrounding the impact of academic examinations on the cortisol response may be due to the variety of age, sex, time of day, coffee consumption, sleep duration, music performance, examinations conditions and individual differences on personality traits. In addition, several studies showed that music can also decrease cortisol and ACTH levels (Mockel et al., 1994; Khalfa et al., 2003). Supportive evidence can be found in the observation that the concentration of saliva cortisol decreased more rapidly in the subjects exposed to music than the subjects who exposed to silence. Khalfa et al (2003) suggested that relaxing music after a stressor can decreasing the post stress response of the HPA axis.

Suda and her colleague reported that salivary cortisol levels were reduced by major mode music than by minor mode (Suda et al., 2008). In this study, we examined the academic-dependent corticosteroid response could affect the learning and memory, when one doing the music performance in adequate time, cortisol levels can be decreased as well as the improvement of learning and memory. The stress inventory questionnaire and salivary cortisol response were used to investigate the stress response of Thai adolescences upon academic examination, examined correlation between the stress inventory questionnaire and saliva cortisol response within subjects, and studied the relationship between school performance (as measured by GPA) and stress responses upon academic. This study was expected to explain effects of music on academic examination stress of Thai adolescences.

CHAPTER II
OBJECTIVES

Several evidences suggested that academic examinations fulfill classical requirement for a psychological stressor. Academic examinations represent stressful challenges to many students, studies on the examination-dependent corticosteroid response, a sensitive physiological indicator for a stress response are inconsistent (Martinek et al., 2003). However, the effects of academic examination stress on learning and memory in Thai adolescences had not yet been determined in detail. Therefore, this study aims to investigate the stress response upon the academic examination in Thai adolescences. Then, we observed whether academic examination-dependent corticosteroid response could affect learning and memory in Thai adolescences. The objectives are as follows:

1. To investigate the stress response of Thai adolescences upon academic examination.
2. To examine correlation between the stress inventory questionnaire and saliva cortisol response within subjects.
3. To study the relationship between school performance (as measured by GPA) and stress responses upon academic examination.

CHAPTER III
LITERATURE REVIEW

3.1 Stress

3.1.1 Definition

Stress is process in which environment demands tax or exceeds individuals' adaptive capacities, contributing to biological and psychological changes that may place them at risk for illness (Cohen et al., 1997). Stressful life experience can have significant effects on a variety of physiological systems, including the autonomic nervous system, the hypothalamic-pituitary-adrenal (HPA) axis, and the immune system (Figure 3.1).

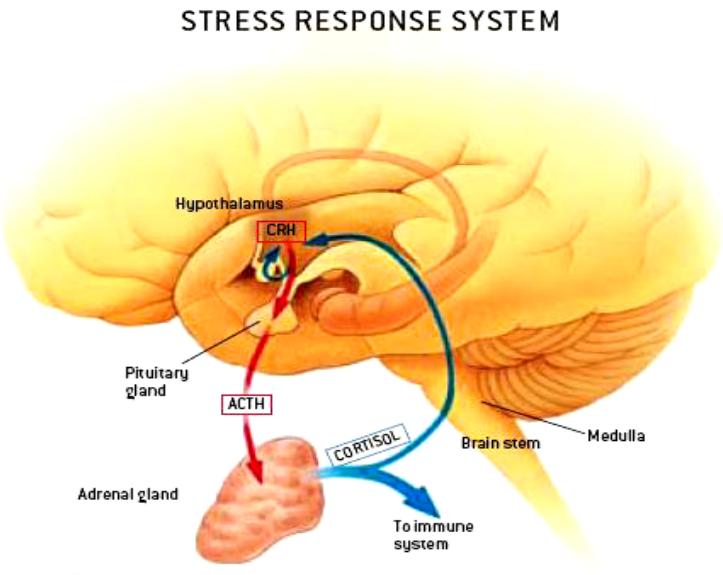

Figure 3.1 The stress response system**.**

3.1.2 Type of stress

3.1.2.1 Eustress

This is the good type of stress and refers to the optimal amount of stress which helps promote health and growth. Many times stressful events push us to perform to higher levels and excel (Figure 3.2).

3.1.2.2 Distress

Opposite to eustress, distress is the bad type of stress and occurs when we have excessive adaptive demands placed upon us and can include a variety of affective and cognitive states, such as anxiety, sadness, frustration, the sense of being overwhelmed, or helplessness. A number of properties of stressful circumstances can influence the severity of the psychological and physiological response. These properties include the stressor's controllability, ambiguity, level of demand placed on the individual, novelty, and duration (Kemeny, 2003).

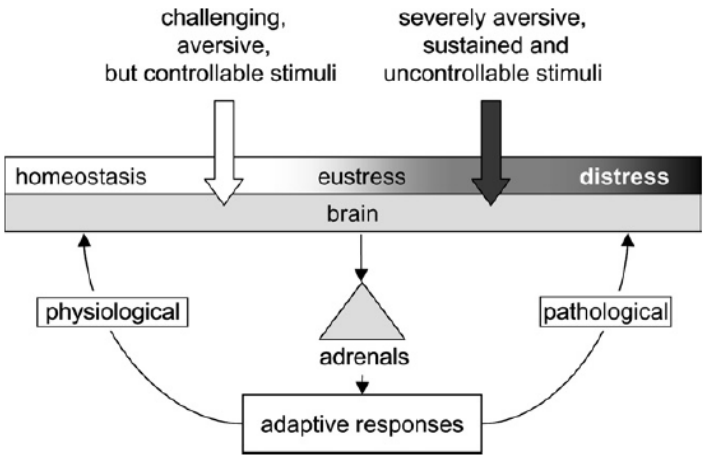

Figure 3.2 The concept of eustress and distress. Challenging but controllable aversive stimuli (white arrow) can induce a state of eustress, which is characterised by adaptive responses aimed at restoring homeostasis. The transient activation of the HPA axis during eustress is beneficial for this process. Various feedback loops ensure that the activated physiological systems are turned off after a stressor has ceased. If the activation of the neuroendocrine systems is not appropriately terminated, chronically elevated neurotransmitter/neuromodulator/hormone levels might become dangerous for

an organism. This is the reason why severe aversive, sustained, and uncontrollable stimuli (black arrow) may lead to distress. In this state, a variety of autonomic, endocrine, and behavioural parameters are permanently changed to secure proper functioning of important body functions. The chronic HPA axis activation during distress becomes maladaptive when it leads to the development of pathologies. The incidence of eustress versus distress critically depends on the subjects genetic predisposition, life history, and ontogenetic stage (Engelmann et al., 2004).

3.1.3 Class of stress

3.1.3.1 Acute stress

Acute stress is the most common form of stress or positive stress that comes from demands and pressures of the recent past and anticipated demands and pressures of the near future. Acute stress is thrilling and exciting. Cortisol activation in response to stress is protective in the short term.

3.1.3.2 Chronic stress

Chronic stress is the negative stress or long duration of stress that can lead to changes in HPA axis activity, as evidenced by abnormal cortisol levels, which may in turn increase vulnerability to developing health problems such as digestive disorders, cardiovascular problems, autoimmune disease and muscular-skeletal problem.

3.1.4 Physiological effects of exposure to stressful

3.1.4.1 Impact on the autonomic nervous system

Since the American physiologist Walter B. Cannon first proposed that the parasympathetic and sympathetic divisions have distinctly different functions. The parasympathetic nervous system is responsible for rest and digest, maintaining basal heart rate, respiration, and metabolism under normal conditions. The sympathetic nervous system governs the emergency reaction, or fight-or-flight reaction. In an emergency the body needs to respond to sudden changes in the external or internal environment, be it emotional stress, combat, athletic competition, severe change in temperature, or blood loss. Cannon correctly proposed that exposure to

emergency situations results in the release of the hormone epinephrine (or adrenaline) from the adrenal medulla. This extremely rapid response system can be activated within seconds and results in the "adrenaline rush" that occurs after an encounter with an unexpected threat.

3.1.4.2 Impact on the hypothalamic-pituitary-adrenal axis

Several studies suggested that exposure to a variety of acute psychological stressors (e.g. giving a speech, doing difficult cognitive tasks, taking exams), for short durations, can cause an increase in the levels of the hormone cortisol in the plasma, urine and saliva. This hormone increase when activate the HPA axis. The neural pathways link perception of stressful experience stimulus to an integrated response in the hypothalamus, which results in the release of corticotrophin-releasing hormone (CRH). The CRH stimulates the anterior part of the pituitary gland to release adrenocorticotropic hormone, which then pass through the bloodstream to the adrenal glands and causes the adrenal cortex to release cortisol hormone (Figure 3.3).

3.1.4.3 Impact on the immune system

The acute stress is the most common form of stress. It comes from demands and pressures of the recent past and anticipated demands and pressures of the near future. Acute stress is thrilling and exciting. Although cortisol activation in response to stress is protective in the short term, chronic or extreme activation may have long term negative consequences (McEwen and Magarinos, 1997). Chronic or extreme activation can lead to changes in HPA axis activity, as evidenced by abnormal cortisol levels, which may in turn increase vulnerability to developing health problems (e.g. hippocampus damage and permanent memory impairment).

Cortisol stimulates the immune system and counteracts inflammatory and allergic reactions at normal levels, but can suppress the immune system at excessive levels. The stressful experiences, such as bereavement, job loss, and even taking exams, can reduce circulating levels of classes of immunological cells called lymphocytes; inhibit various lymphocytes functions and slow integrated immune responses (Ader et al., 2001). In addition, some of the immunological effects of stressors are due to the potent suppressive effects of cortisol on immunological cells. Cortisol can inhibit the production of cytokines, is the chemical mediators

released by immune cells to regulate the activities of other immune cells, and suppress a variety of immune functions.

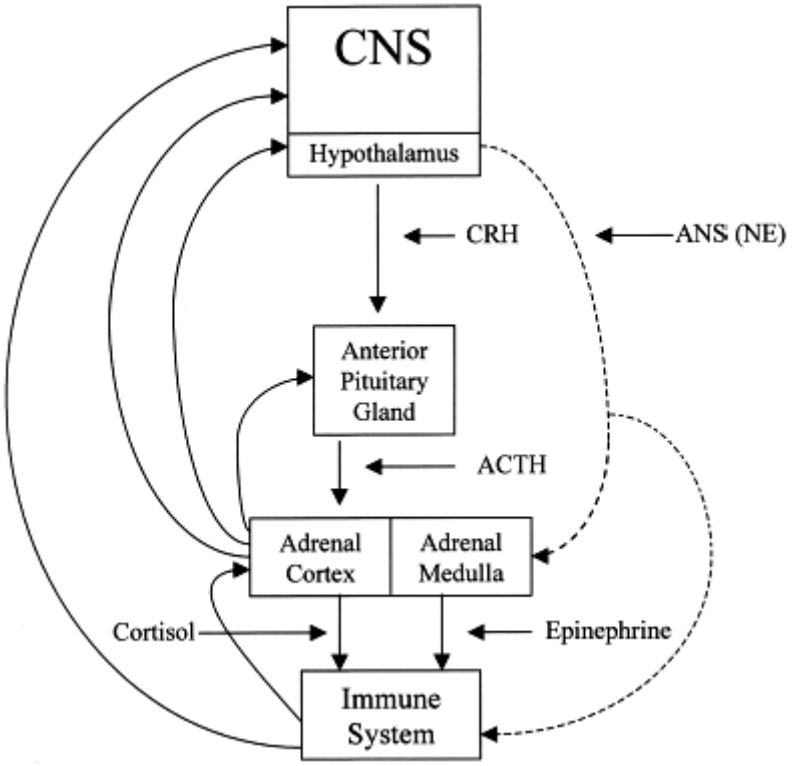

Figure 3.3 Schematic representation of interrelationships among the central nervous system (CNS), the hypothalamic-pituitary-adrenal (HPA) axis, the autonomic nervous system (ANS), and the immune system. Dashed lines indicate ANS neural pathways, and solid lines indicate hormonal pathways. ACTH = adrenocorticotropic hormone; CRH = corticotrophin-releasing hormone; NE = norepinephrine (Kemeny, 2003).

3.1.5 Stress pathways in the central nervous system

Stress can be produced by environmental (external) or psychical (internal) events. Thus, a number of pathways carrying information about stressors have been identified in the central nervous system (CNS), some that carry information from the periphery to the brain and others that originate in the brain. Because the hypothalamus organizes the body's response to homeostatic disruptions, it is the ultimate recipient of information about stress. Many axons that relay information about stressors terminate in a particular region of the hypothalamus known as the paraventricular nucleus (PVN). A number of structures in the hindbrain, midbrain, and forebrain serve as relay

stations, gathering information about stressors from numerous inputs and then sending that information on to the PVN (Wilson, 2003).

3.1.5.1 Processing internal stressors : the nucleus of the solitary tract

Information about stressors coming from the gut or other internal receptors is relayed via the vagus and other cranial nerves to the nucleus of the solitary tract in the medulla. This nucleus sorts out the stress-inducing stimuli from the other signals and sends information about these stressors to the PVN. Axons from the nucleus of the solitary tract excite neurons in the PVN by releasing norepinephrine and other catecholamines (Wilson, 2003).

3.1.5.2 Processing somatosensory stressors : tegmentum and reticular formation

Somatosensory information from the skin and stretch receptors in nucleus is sent to relay stations in the pons and midbrain. These relay areas include the tegmentum and particular regions of the reticular formation. The tegmentum is involved in attentional processes, and the reticular formation is responsible for arousing the nervous system in response to novel or important stimuli. The tegmentum appears to relay visual and auditory information to the PVN. In addition, the tegmentum and reticular formation have direct projections to the PVN. Like the axons coming from the nucleus of solitary tract, axons from the tegmentum and reticular formation also release catecholamines, stimulating the PVN. Acetylcholine, which also has excitatory effects on the PVN, is released by some axons coming from these structures (Wilson, 2003).

3.1.5.3 Processing painful stressors : the periaqueductal gray and central gray areas

The periaqueductal gray area of the midbrain and central gray area of the pons play an important role in the response to pain. Pathways have been identified between these areas and the PVN of the hypothalamus. Axons in the pathways between the periaqueductal gray and central gray areas and the PVN release acetylcholine and substance P, both of which have excitatory effects on the PVN.

3.1.5.4 Processing homeostatic challenges : the locus coeruleus

The cells of the locus coeruleus produce almost all of the norepinephrine found in the brain. This structure is located in the pons along the ventral aspect of the fourth ventricle in an area that is considered to be part of the reticular formation. Many of the pathways going to and from the locus coeruleus are feedback loops that allow this structure to play a role in homeostatic regulation. The locus coeruleus is also sensitive to physical sensations and changes in heart rate and blood pressure. As further evidence that the locus coeruleus is involved in initiating stress responses in the brain, a direct pathway from the locus coeruleus to the PVN has been identified (Bremner et al., 1996).

3.1.5.5 Processing emotional stressors : the raphe system

The raphe system in the brainstem is the sole source of serotonin in the brain. Researchers have discovered a pathway carrying axons from the raphe system to the PVN of the hypothalamus. Therefore, stressors impacting the raphe system are believed to stimulate stress responses in the PVN. Lesioning studies have indicated that the serotonergic pathway from the raphe to the PVN relays information about only certain types of stressors, especially those emotional stressors that activate the cerebral cortex and limbic system (Wilson, 2003).

3.1.5.6 Processing homeostatic challenges: the hypothalamus

The hypothalamus is composed of many clusters of neurons, called nuclei. The PVN is just one of those nuclei. All hypothalamus nuclei send axons to the PVN. This means that diverse nuclei in the hypothalamus, most of which monitor homeostatic processes such as blood glucose or osmotic pressure, relay information about changes in homeostasis to the PVN. In addition, axons from numerous in the limbic system carry information from the limbic system to other hypothalamic nuclei, which in turn relay that information in to the PVN. These nuclei have been demonstrated to have both excitatory and inhibitory effects on the PVN.

3.1.5.7 Processing cognitive and emotional stressors: the limbic system

Structures in the limbic system, including the hippocampus, the septum, and the amygdala, have been shown experimentally to play a role in stress responses. Cognitive and emotional stressors stimulate these limbic system structures, which in turn excite the PVN. However, only a few areas of the amydala have direct projections to the PVN. Most information from the limbic system arrives at the PVN indirectly. One forebrain structure, the bed nucleus of the stria terminalis (BST), appears to receive inputs from numerous areas of the limbic system (Wang, Cen and Lu, 2001). Thus, the BST is believed to be a major relay station between the limbic system and the PVN.

3.1.5.8 Processing cognitive and emotional stressors: the cerebral cortex

Emotional and cognitive stressors can certainly be generated or moderated by the cerebrum, particularly the prefrontal cortex. The cerebral cortex undoubtedly plays an important role in monitoring and interpreting stimuli, determining which are stressors and which are benign. But there are no direct projections from the cerebral cortex to the PVN. The BST is thought to be a relay station between the cerebrum and the hypothalamus.

3.2 Cortisol hormone

3.2.1 Control of cortisol secretion and circadian rhythm

Cortisol (hydrocortisone, 11, 17, 21-trihydroxy-4ene-3, 20 dione), is the most important glucocorticoid that synthesized in the adrenal cortex from cholesterol by a series of enzyme-catalyzed reaction, the last of which involves hydroxylation of 11-deoxycortisol by an 11-B-hydroxylase (Figure 3.4 and 3.5).

Cortisol (corticosterone in rats) is the most potent glucocorticoid hormones secreted by the adrenal cortex into the bloodstream. The secretion of glucocorticoids is controlled by the HPA axis. The neural pathways link perception of a stressful

stimulus to an integrated response in the hypothalamus, which results in the release of corticotropin-releasing hormone (CRH), a 41 amino acid peptide. This hormone stimulates the anterior pituitary to release adrenocorticotropic hormone, which then flows through the bloodstream to adrenal cortex to release cortisol.

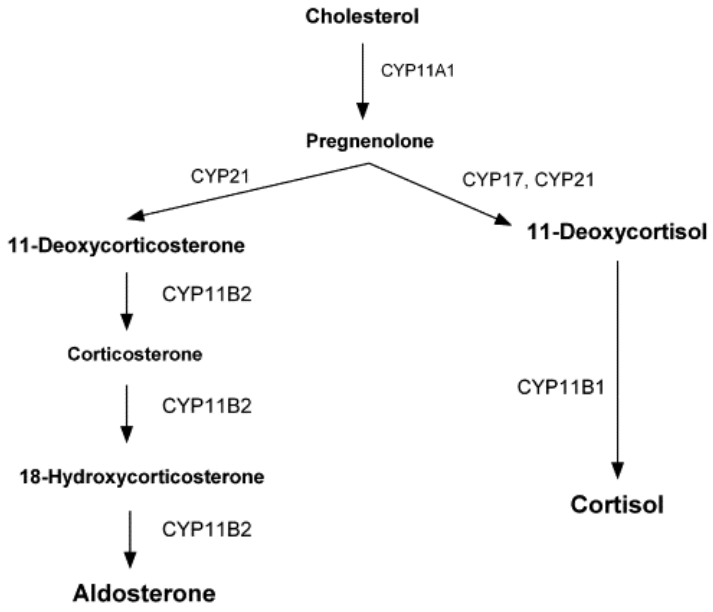

Figure 3.4 Pathway of cortisol synthesis (Hakki and Bernhardt, 2006).

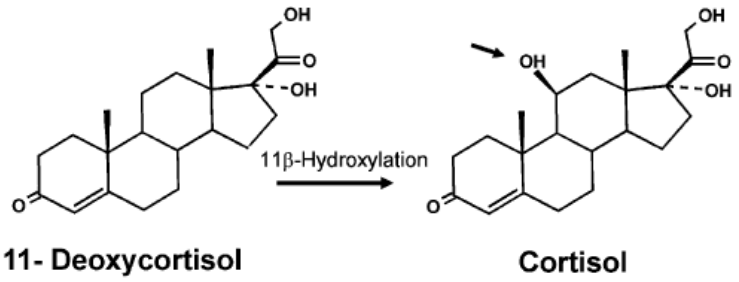

Figure 3.5 Cytochrome P450 11β-hydroxylase (CYP11B1) catalyzes the 11β-hydroxylation reaction that produces cortisol from 11-deoxycortisol (Hakki and Bernhardt, 2006).

The central control stations of the stress system are located in the hypothalamus and the brain stem and include the parvocellular corticotropin releasing hormone (CRH) and arginine-vasopressin (AVP) neurons of the paraventricular nuclei

of the hypothalamus, and the locus ceruleus (LC)-norepinephrine system (central sympathetic system). The HPA axis represents the effector limbs, via which the brain influences all body organs during exposure to threatening stimuli (Figure 3.6).

In basal conditions, both CRH and AVP (is a potent synergistic factor with CRH in stimulating ACTH secretion) are secreted in the portal system in a circadian, pulsatile fashion, with a frequency of about two to three secretory episodes per hour. In resting conditions, the amplitude of the CRH and AVP pulses increase in the early morning hours, resulting in ACTH and cortisol secretory bursts in the general circulation (Tsigos and Chrousos, 2002). In stress conditions, the amplitude of the CRH and AVP pulsations increase resulting in increases of ACTH and cortisol secretory episodes.

The circadian (pertaining to the 24-hour clock) and diurnal (pertaining to daylight hours or daylight to dark) rhythms of cortisol are clear. Normally, about 15 and more pulsatile bursts of cortisol are related in a 24-hour period in children and adults. Cortisol levels peak about half and hour after awakening and reach to the lowest levels around midnight (Kirschbaum and Hellhammer, 2000) as shown in Figure 3.7. In addition, the normal values for salivary cortisol concentrations in early morning samples were showed in the Table 3.1.

Table 3.1 Normal values for salivary cortisol concentrations in early morning samples

Subjects	**Ethnic group**	**Saliva cortisol (nmol/l)**		**References**
		Mean ± SD	**Range**	
Male Female Male + Female (9-11 a.m.)	Asian	11.07 ± 5.36 12.37 ± 6.93 11.81 ± 6.28	5.51 - 28.43 4.52 - 32 4.52 - 52	Chearskul, 1995
Male Female (morning)	Asian	12.9 ± 5.0 13.7 ± 6.3	-	Al-Ansari et al., 1982
Male + Female (8 a.m.)	Caucasian	-	13 - 17	Read et al., 1990

3.2.2 Physiological effects of cortisol

Cortisol (corticosterone in rats) is the most potent glucocorticoid hormones secreted by the adrenal gland into the bloodstream and have actions on almost all cell types of the body. In the central nervous system, glucocorticoids influence protein synthesis, neuronal excitability, and neurotransmitter metabolism (McCornick and Mathews, 2006). In some regions of the brain glucocorticoids have inhibitory effects, such as restraint of the HPA axis and suppression of hippocampal glucose metabolism and blood flow (Erickson et al., 2003) but in some other areas of the brain, glucocorticoids increase activation, such as amygdala, this suggesting site-specific effects of glucocorticoid activation that have implications for behavioral and cognitive functions. In addition, glucocorticoids are important regulators of brain development and neuronal plasticity. McEwen (2000) reported the glucocorticoids can influence all aspects of neural development, including neurogenesis, synaptogenesis and dendritic morphology, and cell death.

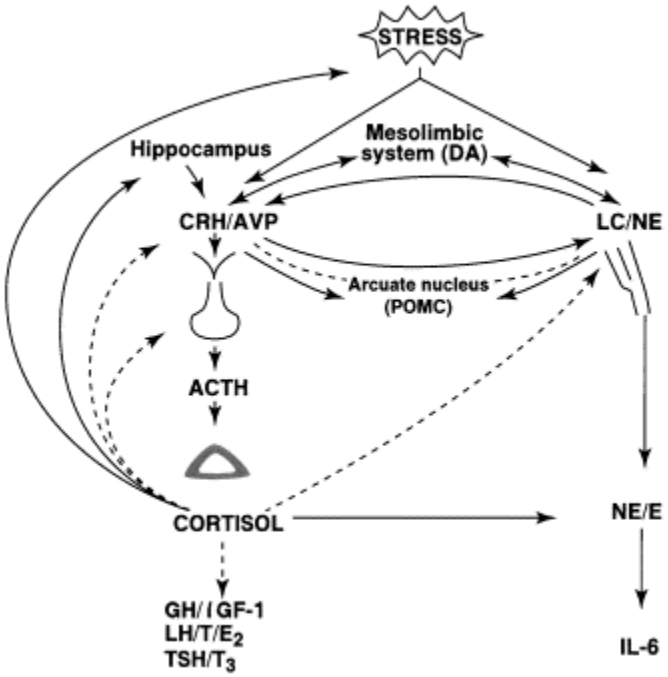

Figure 3.6 A simplified schematic representation of the central and peripheral components of the stress system, their functional interrelations and their relations to other central systems involved in the stress response. The CRH/AVP neurons and

central catecholaminergic neurons of the LC/NE system reciprocally innervate and activate each other. The HPA axis is controlled by several feedback loops that tend to normalize the time-integrated secretion of cortisol, yet glucocorticoids stimulate the fear centers in the amygdala. Activation of the HPA axis leads to suppression of the Growth hormone/Insuline-like growth factor-1 (GH/IGF-1), Luteinizing hormone/ testosterone/E2 (LH/testosterone/E2) and Thyroid stimulating hormone /Triiodothyronine (TSH/T3) axes; activation of the sympathetic system increases interleukin-6 (IL-6) secretion. There are the interaction affect and anticipatory phenomena (mesocortical/mesolimbic systems); the initiation, propagation and termination of stress system activity (amygdala/hippocampus complex); and the setting of the pain sensation (arcuate nucleus). Solid lines indicate stimulation; dashed lines indicate inhibition (Tsigos and Chrousos, 2002).

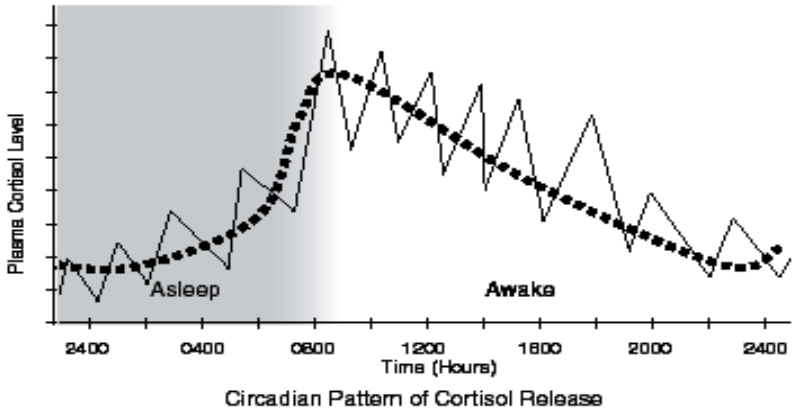

Figure 3.7 An example of an episodic "burst" pattern that is characteristic of the circadian release of cortisol. The largest burst during the 24-hour period occurs about 30 minutes after awakening for the day, and the nadir occurs around midnight.

The secretion of glucocorticoids is controlled by the HPA axis. This axis is involved in the regulation of threats to homeostasis and it can be activated by a variety of stressors. Some of the most potent stressors are psychological stressors (McEwen, 2000). Many psychological stressors are anticipatory in nature based on expectations as the result of learning and memory (e.g., conditioned stimuli) or species-specific predispositions (Herman et al., 2003; McCornick and Mathews, 2006).

The actions of corticosteroids are mediated by two types of the intracellular receptors. Distribution of corticosteroid receptors in the brain is different

by its type. Mineralocorticoid receptors (MRs) located primarily in lateral septum, hippocampus, medial amygdala and brain stem nuclei and glucocorticoid receptors (GRs) more widespread and higher concentrations in the hippocampus, parvocellular paraventricular nucleus (PVN), and frontal cortex (McCornick and Mathews, 2006; Erickson et al., 2003).

Mineralocorticoid receptors have a 6 to 10 time higher affinity for corticosteroids than glucocorticoid receptors. In the normal conditions or at low basal levels of corticosteroids, at least 80 % of hippocampal mineralocorticoid receptors are occupied, whereas only 10-15 % of hippocampal glucocorticoid receptors are occupied (Maheu et al., 2005). In human, corticosteroid levels follow a circadian rhythm, with higher levels in the morning after waking (or AM phase), and slightly declining to lower levels in the evening (or PM phase). These variations in corticosteroid levels thus lead to a differential activation of MR and GR corticosteroid receptors in the AM versus PM phase. During the circadian peak of corticosteroid secretion or AM phase, MRs are saturated and there is a 67-74 % occupation of GRs and in the PM phase, 90 % of MRs are occupied and only 10 % of GRs are occupied (De Kloet et al., 1999; Maheu et al., 2005).

In the stress conditions or at high levels of corticosteroids, glucocorticoid receptors increase in the occupation than in the normal conditions. However, percent occupation of corticosteroid receptors under normal and stress conditions varies among brain areas and among locations in the periphery (McCornick and Mathews, 2006). Unlike the case of the ANS, The activation of HPA axis occurs over minutes. The peak cortisol response occurs 20 to 40 minutes from the onset of acute stressors and recovery to baseline levels, occurs 40 to 60 minutes following the end of the stressors on average (Kemeny, 2003).

In numerous studies suggested that in humans, the main glucocorticoid is cortisol. Cortisol is predominantly (90-95 %) bound to binding proteins in blood, only 5-10 % of the total plasma cortisol circulates as biologically active (or unbound) free cortisol. While in plasma both bound and free cortisol can be measured, only free cortisol appears in saliva. Cortisol levels measured in saliva agree very well with the amount of the free cortisol in plasma. However, the collection of salivary cortisol

provides a noninvasive and relatively inexpensive means to obtain an index of the biologically active fraction of this corticosteroid hormone.

3.3 Learning and memory

3.3.1 Definition

Learning is the process by which are acquire knowledge. Memory is the process by which that knowledge is encoded, stored, and later retrieved. In 1940s Wilder Penfield, the neurosurgeon, studied in the methods of electrical stimulation to map the motor, sensory, and language functions in the cerebral cortex of patients undergoing brain surgery for relief of focal epilepsy. Since the brain does not have pain receptors, brain surgery is painless and can be carried out under local anesthesia in patients that are fully awake. Thus, patients undergoing brain surgery are able to describe what they experience in response to electrical stimuli applied to different cortical areas. This studied suggested that the memory processes might be localized to specific regions of the human brain (Kandel et al., 2000).

3.3.2 The different forms of memory and the brain regions involved in learning and memory

Cognitive psychologists had distinguished these two types of memory in normal subjects. They refer to information about how to perform something as implicit memory and they refer to factual knowledge of people, places and things as explicit memory (Figure 3.8).

3.3.2.1 Implicit memory (nondeclarative memory)

Implicit memory (nondeclarative memory) is typically involved in training reflexive motor or perceptual skills that are recalled unconsciously. Implicit memory is more rigid and tightly connected to the original stimulus conditions under which the learning occurred. This type builds up slowly, through repetition over many trials, and is expressed primarily in performance.

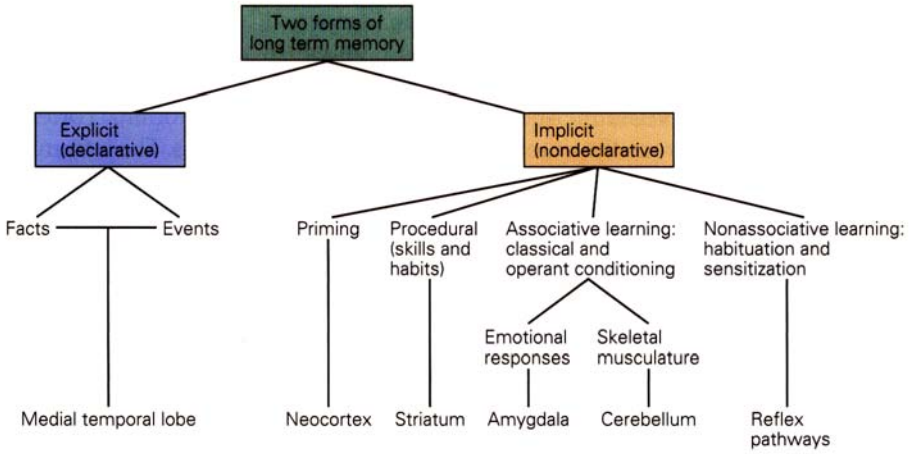

Figure 3.8 Various forms of memory can be classified as either explicit or implicit (Kandel et al., 2000).

Different forms of implicit memory are acquired through different forms of learning and involve different brain regions. The memory acquired through fear conditioning is thought to involve the amygdala region. The memory acquired through operant conditioning requires the striatum and cerebellum and the memory acquired through classical conditioning, sensitization, and habituation (three simple forms of learning) involves in the sensory and motor systems.

Psychologists have identified two major subclasses of implicit memory as non-associative and associative. In non-associative learning the subject learns about the properties of a single stimulus. In associative learning the subject learns about the relationship between two stimuli or between a stimulus and a behavior (Kandel et al., 2000).

3.3.2.1.1 Three forms of nonassociative learning

Habituation is a decrease in response to a benign stimulus when that stimulus is presented repeatedly. Habituation was first investigated by Ivan Pavlov and Charles Sherrington. While studying posture and locomotion, Sherrington observed a decrease in the intensity of reflexes in response to repeated stimulation. He suggested that habituation results from diminished synaptic effectiveness within the pathways to the motor neurons that had been repeatedly activated (Kandel et al., 2000).

Sensitization or pseudo conditioning is an increase in response to a wide variety of stimuli after the presentation of an intense or noxious stimulus (Figure 3.10). In contrast, with a harmful stimulus the animal typically learns to respond more vigorously not only to that stimulus but also to other stimuli, even harmless one. Defensive reflexes for withdrawal and escape become heightened. This enhancement of reflexes responses is called sensitization (Kandel et al., 2000).

Imprinting or imitation learning is a key factor in the acquisition of language, has no obvious associational element. Konrad Lorenz (1965) examined the imprinting, a form of learning in birds (Figure 3.11). Just after birth, birds become indelibly attached, or imprinted, to almost any prominent moving object in their environment, typically their mother. The process of imprinting is important for the protection of the hatchling. Although the attachment is acquired rapidly and persists, such imprinting can occur only during a critical period soon after hatchling. The clearest way to show that certain social or perceptual experiences are important for human development is to study children who have been deprived of these stimuli early in life. Reliable histories of infants who were abandoned in the wild and who later returned to human society describe children without language who are socially maladjusted, usually in an irreversible way (Kandel et al., 2000).

3.3.2.1.2 Two forms of associative learning

Classical conditioning involves learning a relationship between two stimuli. Classical conditioning was introduced into the study of learning by Russian physiologist Ivan Pavlov who established a procedure from which reasonable inferences could be made about the relationship between changes in behavior (learning) and the environment (stimuli). The conditioned stimulus (CS), such as a light, tone, or tactile stimulus, is used because of it produces either no overt response or a weak response usually unrelated to the response that will be learned. The unconditioned stimulus (US), such as food or electrical shock to the leg, is used because it normally produces a strong, consistent, overt response (the unconditioned response), such as salivation or withdrawal of the leg. The unconditioned responses (UR) are innate that produced without learning.

The experiment starts by presenting a CS, and then presents the US, which elicits the UR. After some pairings of the CS followed by the US, the individual begins responding to the CS, produced a conditioned response (CR). In original experiment, Pavlov presented a dog with a sound (CS) followed by meat (US), that stimulated the dog to salivate (UR) and after many such pairings, the sound alone would stimulate the dog to salivate (Figure 3.12).

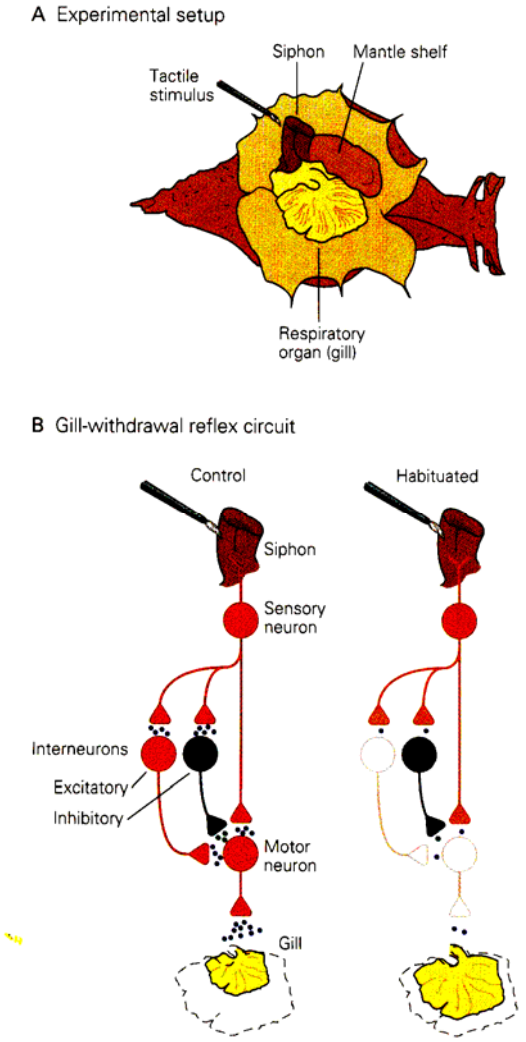

Figure 3.9 The cellular mechanisms of habituation have been investigated in the gill-withdrawal reflex of the marine snail Aplysia (Kandel et al., 2000).

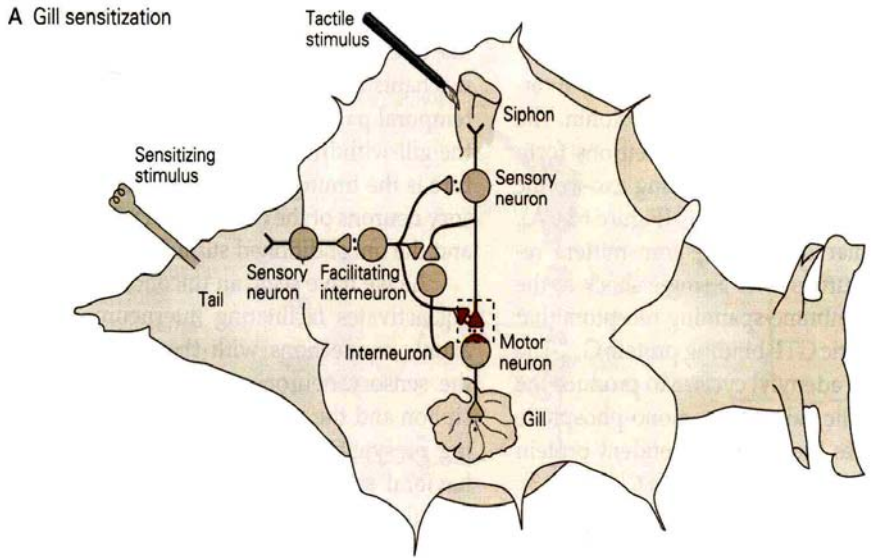

Figure 3.10 Sensitization of the gill is produced by applying a noxious stimulus to another part of the body, such as the tail. Stimuli to the tail activate sensory neurons in the tail that excite facilitating interneurons, which form synapses on the terminals of the sensory neurons innervating interneurons enhance transmitter release from the sensory neurons (presynaptic facilitation) (Kandel et al., 2000).

Figure 3.11 Konrad Lorenz (1903 - 1989) who first called the phenomenon "stamping in" in German, which has been translated to English as imprinting.

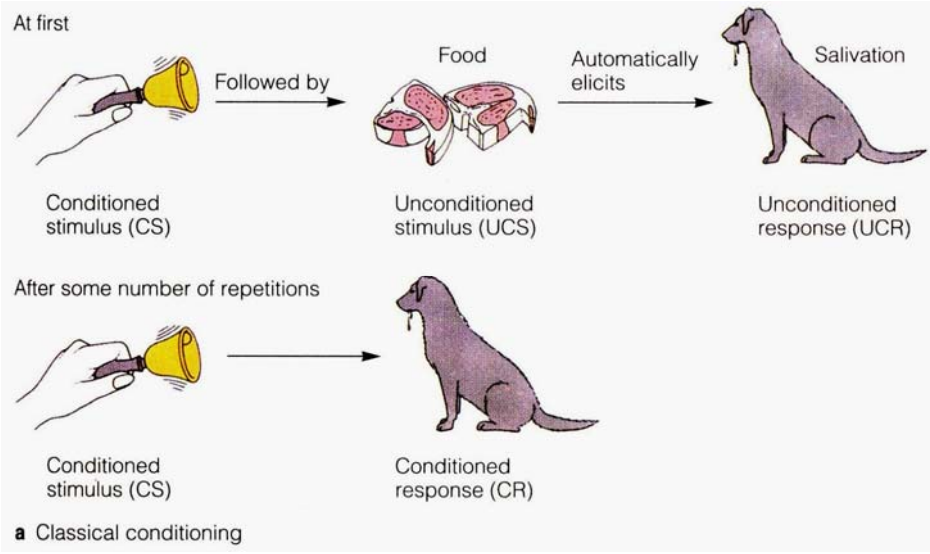

Figure 3.12 Classical conditioning (Kalat, 1995)

Operant conditioning involves learning a relationship between the organism's behavior and the consequences of that behavior. By opposite way, in operant conditioning, an individual's response is followed by a reinforcement or punishment. Reinforcement is any event that increases the future probability of the response and a punishment is an event that decreases the future probability of the response (Figure 3.13).

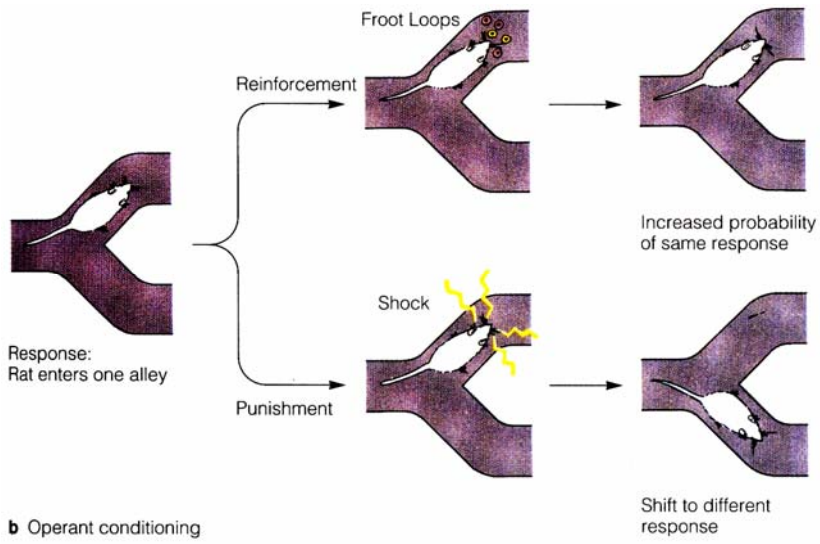

Figure 3.13 Operant conditioning (Kalat, 1995)

3.3.2.2 Explicit memory (declarative memory)

Explicit memory (declarative memory) is involved in the factual knowledge of people, places, and things that is recalled by deliberate, conscious effort. Explicit memory is highly flexible and involves the association of multiple bits and pieces of information. The psychologist Endel Tulving who classified the explicit memory in to two types as episodic (a memory for events and personal experience) and semantic (a memory for facts). Explicit memory knowledge involved at least four distinct processes such as encoding, consolidation, storage, and retrieval.

Encoding refers to the processes by which newly learned information is attended to and processed when first encountered. The nature and extent of this process are important for determining how well the learned material will be remembered at later times. The persistent or be well remembered memory that needed the information encoded thoroughly and deeply.

Consolidation refers to the processes that alter the newly stored and still labile information to make it more stable for long-term storage.

Storage refers to the mechanism and sites by which memory is retained over time.

Retrieval refers to the processes that permit the recall and use of the stored information.

However, both the initial encoding and the ultimate recall of explicit knowledge are requiring recruitment of stored information into a special short-term memory store called working memory. In 1974, the cognitive psychologist Alan Baddeley reported that working memory refer to the active maintenance of information relevant to an ongoing behavior. Working memory has three components: for verbal memories, a parallel component for visual memories, and a third component that functions as a central executive, coordinating the flow of attention from one component of working memory to another. Therefore the prefrontal association cortex is involved in short-term memory. The lesion of this region is specific for working memory.

3.3.3 Long-term potentiation and long-term depression

What mechanisms are used to store explicit memory? The important component of the medial temporal system involved in the storage of explicit memory is the hippocampus. Per Anderson showed the major pathways of hippocampus: the first is the perforant pathway, which projects from the entorhinal cortex to the granule cells of the dentate gyrus, the mossy fiber pathway, which contains the axons of the granule cells and projects to the pyramidal cells in the CA3 region of the hippocampus, and the Schaffer collateral pathways, which consists of the excitatory collaterals of the pyramidal cells in the CA3 region and runs to the pyramidal cells in the CA1 region (Figure 3.14).

In 1973, Timothy Bliss and Terje Lomo found that if they stimulated the perforant pathway with a train of electrical impulses and then recorded from the hippocampal cells that received this input. They showed increased electrical activity that persisted for up to 10 hours after the previous stimulation had occurred. Other studies found that long-term potentiation could be produced in slices of hippocampal tissue in a saline bath. A high-frequency train of stimuli or tetanus to any of the three major synaptic pathways increases the amplitude of the excitatory postsynaptic potentials in the target hippocampal neurons. This facilitation is called long-term potentiation (LTP).

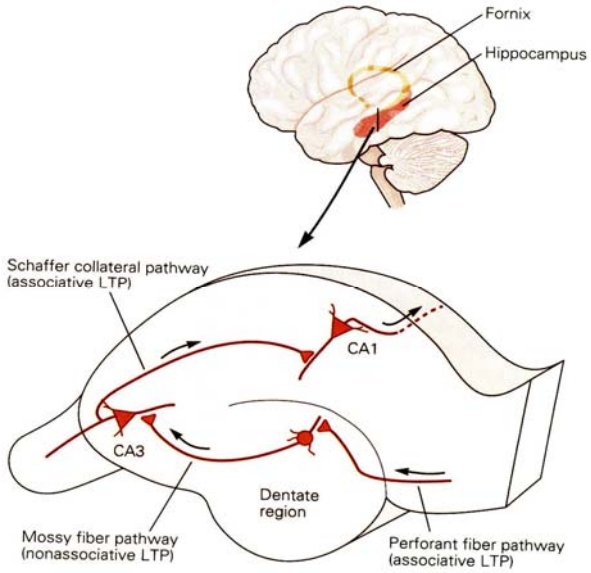

Figure 3.14 The three major afferent pathways in the hippocampus (Kandel et al., 2000).

The mossy fiber pathway consists of the axons of the granule cells of the dentate gyrus. The mossy fiber terminals release glutamate as a neurotransmitter, which binds to both N-methyl-D-aspartate glutamate (NMDA) and non-NMDA receptors on the target pyramidal cells. However, LTP in the mossy fiber pathway region has been found to depend on Calcium (Ca^{2+}) influx into the presynaptic cell after the tetanus. The Ca^{2+} influx appears to activate Ca^{2+}/calmodulin-dependent adenylyl cyclase that cause of increasing the level of cyclic adenosine monophosphate (cAMP) and activating protein kinase A (PKA) in the presynaptic neuron.

The Schaffer collateral pathway connects the pyramidal cells of the CA3 region to pyramidal cells of the CA1 region of the hippocampus. Similarly to the mossy fiber terminals, the terminals of the Schaffer collateral also use glutamate as transmitter. In contrast, LTP in the Schaffer collateral pathway requires activation of the NMDA receptor. Initial LTP in the Schaffer collateral pathway requires activation of several afferent axons together, a feature called cooperativity. The NMDA receptor-channel becomes function and conducts Ca^{2+} only when glutamate must bind to the postsynaptic NMDA receptor and the membrane potential of the postsynaptic cell must be sufficiently depolarized by the cooperative firing of several afferent axons to expel magnesium (Mg^{2+}) from the channel (Figure 3.15). Only when Mg^{2+} is expelled can Ca^{2+} influx into the postsynaptic cell occur. Calcium influx initiates the persistent enhancement of synaptic transmission by activating calcium-dependent serine-threonine protein kinases and protein kinase C or PKA and the tyrosine protein kinase fyn. Second, LTP in the Schaffer collateral pathway requires concomitant activity in both the presynaptic and postsynaptic cells to adequately depolarize the postsynaptic cell, a feature called associativity. In summary, the induction of LTP in the CA1 region of the hippocampus depends on four postsynaptic factors such as postsynaptic depolarization, activation of NMDA receptors, influx of Ca^{2+}, and activation by Ca^{2+} of several second-messenger systems in the postsynaptic cell (Kandel et al., 2000).

There is evidence that calcium-activated second messenger because the postsynaptic cell to release more retrograde messengers from its active dendritic spines. Nitric oxide (NO) is formed through the oxidation of the amino acid arginine by the enzyme nitric oxide synthase together with an electron donor such as flavin

adenine dinucleotide (FAD). NO is a gas that diffuses readily from cell to cell, as one of the possible candidate retrograde messengers involved in LTP.

Homosynaptic long term depression (LTD) can occur at synapses that are activated, normally at low frequencies (Bear and Abraham, 1996). Like LTP, it may be NMDA-receptor dependent or independent. LTD is also observed in the amygdala and cortex. Depotentiation, the reversal of LTP, is also observed in vivo and in vitro (see Figure 3.16) (Martin et al., 2000).

Willshaw and Dayan (1990) reported that learning may occur through LTP-like processes alone; having LTD-like modifications adds flexibility and information storage capacity to the system. The most neural network models now include both up- and down-regulation of synaptic efficacy as storage mechanisms. This suggested that LTP as a learning mechanism and LTD as a forgetting mechanism.

John Lisman showed the model of kinase–phosphatase signalling in LTD and LTP that the concentrations of intracellular Ca^{2+} achieved by the activation of NMDA receptors have a critical role in determining whether long-term potentiation (LTP) or long-term depression (LTD) is elicited (Figure 3.17). In vitro, calcineurin (PP2B) has a much higher affinity for Ca^{2+} than do calcium/calmodulin-dependent protein kinase II (CaMKII) and protein kinase C (PKC). The weak activation of the NMDA receptor would elicit low-level increases in Ca^{2+}, resulting in the preferential activation of PP2B over CaMKII, and the dephosphorylation of substrates, leading to LTD. In contrast, the strong activation of the NMDA receptor would give rises in Ca^{2+} sufficient to recruit CaMKII and PKC, resulting in the induction of LTP. According to this model, PP2B would be activated by the same stimulus that recruits CaMKII and PKC unless a feedback inhibition mechanism was in place. So, it was proposed that the activation of calcium-sensitive adenylyl cyclases by strong NMDA receptor activation would lead to the activation of cAMP-dependent protein kinase (PKA) and the subsequent phosphorylation of inhibitor-1 (I-1) or an I-1-like protein, which would then inhibit the downstream protein phosphatase 1 (PP1). Although this model has been modified to some extent over time due in large part to the realization that, in addition to the concentration of Ca^{2+}, the localization and duration of changes in Ca^{2+} are critical many of its predictions have been borne out, and this model still serves as a

useful construct in considering experimental design for studies aimed at examining the roles of phosphatases in synaptic plasticity (Winder and Sweatt, 2001).

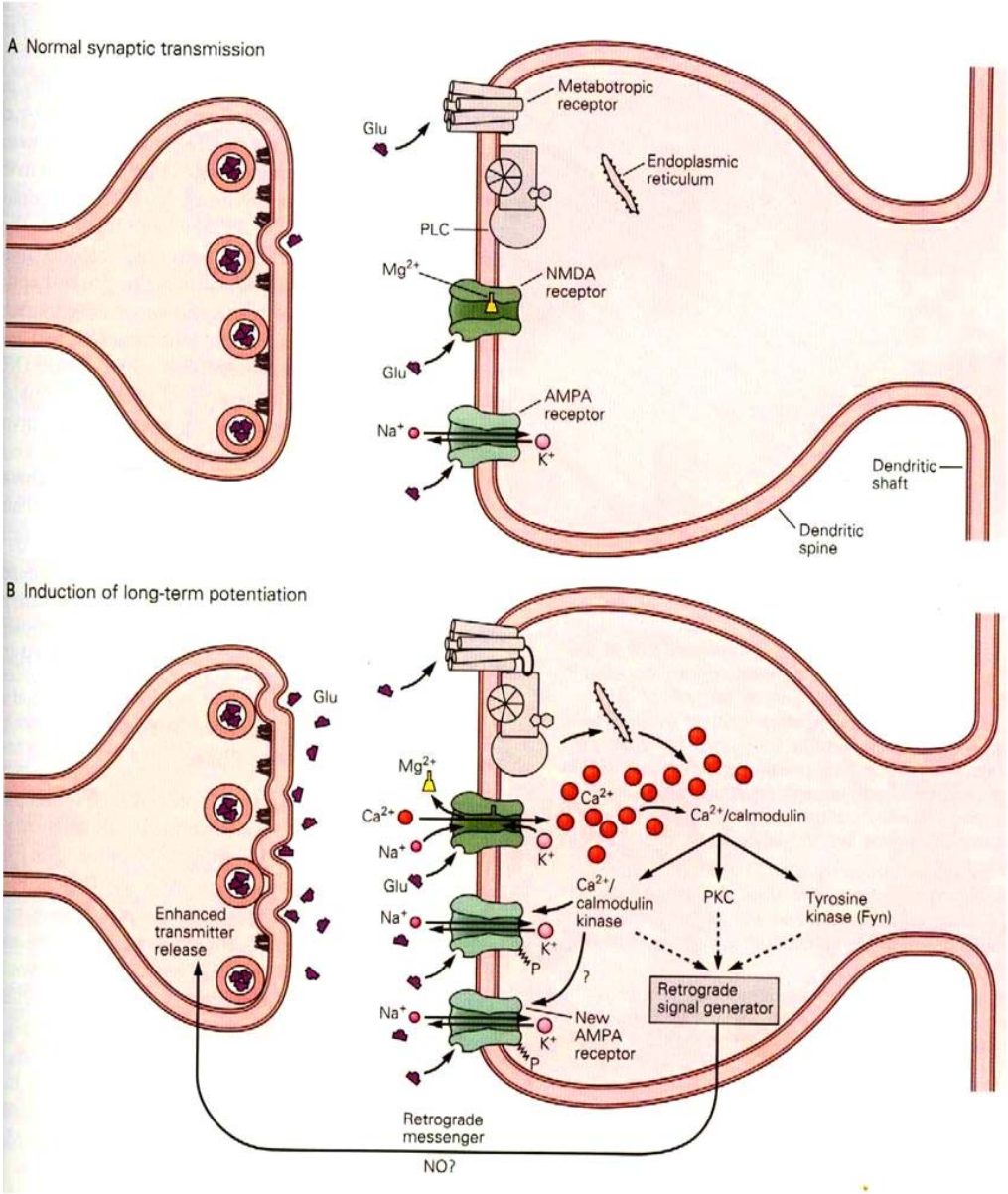

Figure 3.15 A model for the induction of the early phase of long-term potentiation (Kandel et al., 2000).

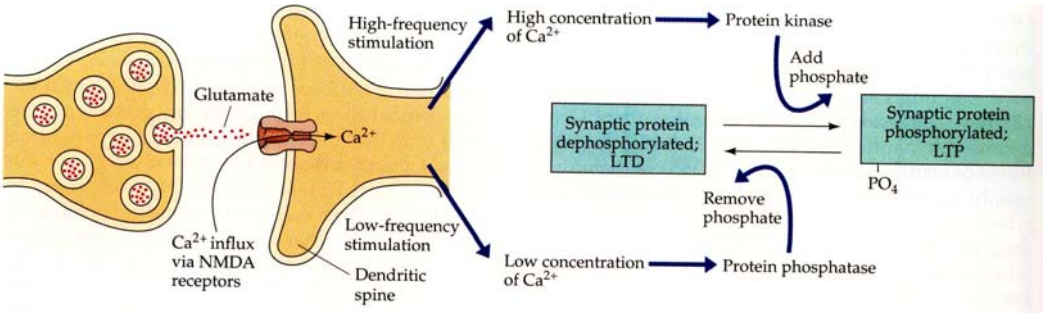

Figure 3.16 Model of the induction LTP and LTD (Rosenzweig et al., 1999).

3.4 Hormone involved in learning and memory

The endocrine system consists of a number of glands scattered throughout the body that secrete chemicals called hormones which released into the bloodstream for transported to various parts of the body. In human, all of several glands come under the control of a single master gland situated on the underside of the brain and attached to the hypothalamus. This important master gland, known as the pituitary gland, which releases a number of trophic hormones whose role is to regulate the other endocrine glands of the body. The pituitary consists of two glands such as anterior pituitary, which connected to the hypothalamus via a complex series of blood vessels and the posterior pituitary, which has neural connections with the hypothalamus.

The control of hormonal release by the pituitary gland generally works on the basis of negative feedback that is occur when hormone levels begin to increase, the pituitary gland will detect this change and respond by decreasing the output of its controlling hormone.

Research on the effects of hormones on memory has focused on hormones such as corticotropin-releasing hormone (CRH), vasopressin, adrenocorticotropic hormone (ACTH), glucocorticoid, epinephrine, estrogen, and insulin that are released into the blood and brain following arousing or stressful experiences.

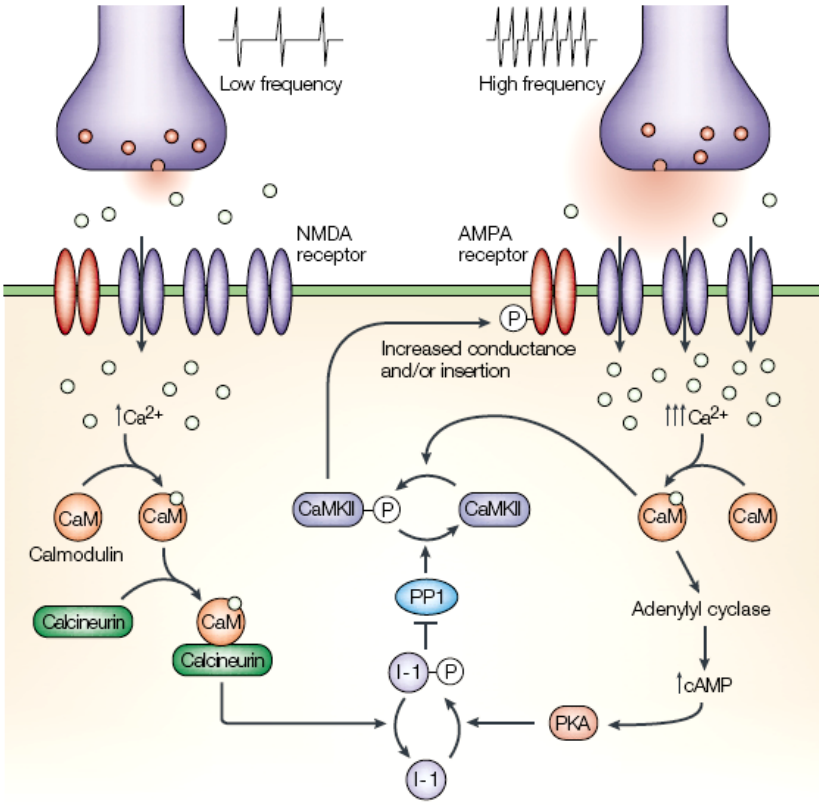

Figure 3.17 The Lisman model of kinase–phosphatase signalling in LTD and LTP (Winder and Sweatt, 2001).

3.4.1 Corticotropin-releasing hormone (CRH), vasopressin, adrenocorticotropic hormone (ACTH) and glucocorticoid

Most of CRH cell bodies are found within the amygdala, hypothalamus and bed nucleus of the stria terminalis. Vasopressin- and CRH-containing parvocellular neurons from the nucleus paraventricularis project to the median eminence where the hormones are released into the portal system and transported to the anterior pituitary to stimulate ACTH release. CRH is the primary activator of the pituitary adrenal system. Vasopressin has a powerful synergistic action on CRH-induced ACTH secretion. During stress (insulin-induced hypoglycemia or immobilization), the vasopressin-containing subset of CRH neurons in the external zone of the median eminence is activated, resulting in increases of ACTH and cortisol secretory.

Chronic stress has been associated with neuropsychiatric changes, neurophysiological changes, neurotransmitter effects, and neuroanatomical changes,

such as hippocampal degeneration, cortical atrophy and ventricular enlargement. The ventricular enlargement and the cortical atrophy are associated with the cognitive impairment (Belanoff et al., 2001).

The hippocampal formation (HF) is an important site for cortisol in the central nervous system (CNS). The hippocampus is essential for explicit memory. Several studies reported that there are many inhibitory effects of cortisol that are site-preferential to the hippocampus, such as inhibition of glucose transport into hippocampal neurons and glia at high concentration of cortisol, involution of dendritic processes of hippocampal neurons, and inhibition of long-term potentiation in an experiment using stress (Belanoff et al., 2001).

3.4.2 Epinephrine

Cahill and Alkire (2003) examined the enhanced memory consolidation in humans produced by adrenergic hormones. In this study, healthy subjects viewed a series of 21 slides, and immediately after received an intravenous infusion of either saline or epinephrine. Memory for first three (primacy) and last three (recency) slides viewed was assessed with an incident free recall test one week later. Epinephrine dose-dependently increased memory for the primacy slides, but did not affect memory of the recency slides. This result suggested that adrenergic hormones can produce retrograde enhancement of long-term memory in humans (Figure 3.18). Because epinephrine was administered after learning, its enhancing effect on memory cannot be attributed to actions on attentional, emotionally, perceptual, or encoding processes during slide presentation. This findings can supported by hypothesis that epinephrine released by emotionally stressful events, modulate memory consolidation of the events (McGaugh et al., 1996; Roozendaal, 2002).

3.4.3 Estrogen

Estrogens affect multiple brain regions and do so via multiple mechanisms that have different consequences for cell function and survival. The decline of ovarian activity after surgical and natural menopause appears to affect a number of brain functions, including mood and certain types of memory. However, these effects appear

to be largely reversible and distinguishable from the long-term degenerative changes associated with Alzheimer's disease.

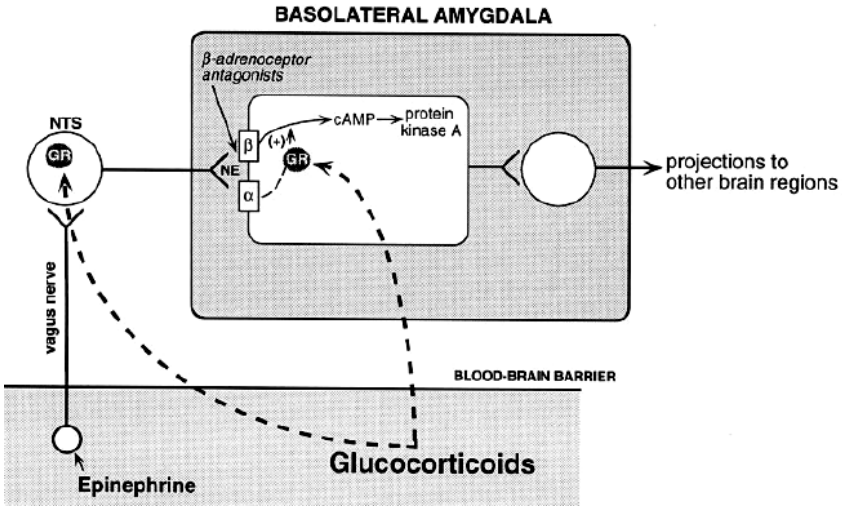

Figure 3.18 Schematic summarizing the interactions of glucocorticoids with the noradrenergic system of the basolateral amygdala at both presynaptic and postsynaptic sites. Norepinephrine (NE) is released following training in aversively motivated tasks and binds to both β-adrenoceptors and α1-adrenoceptors at postsynaptic sites. The β-adrenoceptor is coupled directly to adenylate cyclase to stimulate cAMP formation. The α1-adrenoceptor modulates the response induced by β-adrenoceptor stimulation. Glucocorticoids may influence the β-adrenoceptor–cAMP system via a coupling with α1-adrenoceptors. In addition, glucocorticoids may activate the noradrenergic system by activation of GRs in brain-stem noradrenergic cell groups. α, α-adrenoceptor; β, β-adrenoceptor; cAMP, adenosine 3',5'-cyclic monophosphate; GR, glucocorticoid receptor; NTS, nucleus of the solitary tract (Roozendaal, 2002).

In human subjects, there is also some evidence that estrogen treatment has a negative effect on performance of spatial tasks in women while enhancing verbal performance. The inhibitory effects of estrogen on spatial memory, while estrogen also appears to have positive effects on declarative memory, may indicate that spatial memory is affected differently from declarative memory. McEwen and Alves (1999) showed estrogen effects are the regulation of synapse turnover in the CA1 region of the hippocampus during the 4- to 5-day estrous cycle of the female rat (Figure 3.19). Formation of new excitatory synapses is induced by estrogen and involves NMDA

receptors, whereas down-regulation of these synapses involves intracellular progestin receptors (PR).

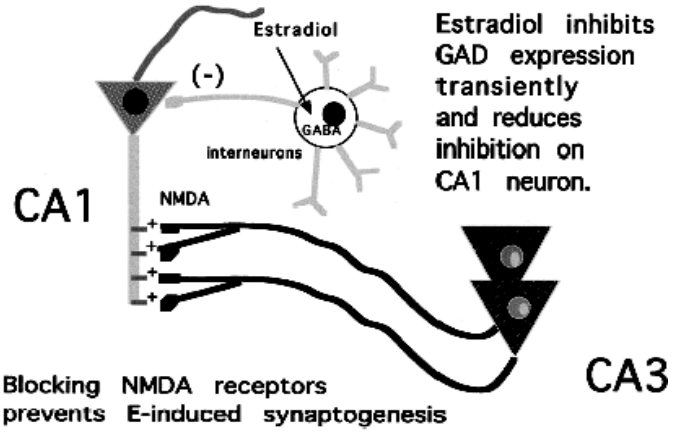

Figure 3.19 This model of synaptogenesis in the hippocampus emphasizes the role of NMDA receptors and the key role of inhibitory GABA interneurons. Estrogen receptor -α (ERα) is present in interneurons, and its presence coincides with the distribution of ER-binding sites from *in vivo* [^{3}H] estradiol autoradiography. According to the best evidence to date, based upon immunocytochemistry of hippocampus and cell culture studies, estrogens suppress GABA function transiently and lead to disinhibition of a large number of innervated CA1 neurons resulting in up-regulation of NMDA receptors and synapse formation. Blocking NMDA receptors prevents estrogen-induced synapse formation. GAD, glutamate decarboxylase (McEwen and Alves, 1999).

3.4.4 Insulin

In 1920, several studies reported that the cognitive deficits in both Type 1 and Type 2 diabetic patients. In adult Type 1 diabetic patients, modest reductions in mental efficiency have been reported repeatedly, involving learning and memory, problem solving and mental and motor speed (Ryan, 1988). In addition, the exposure to the two extremes of blood glucose levels, severe hypoglycaemia on the one hand and chronic hyperglycaemia on the other, varies between patients. Chronic hyperglycaemia and repeated episodes of severe hypoglycaemia may both adversely affect the brain by different mechanisms, leading to different cerebral deficits.

In the other studies, Zhao et al. (2004) showed that the insulin/insulin receptor associated with the hypothalamus plays important roles in regulation of the body energy homeostasis, the hippocampus- and cerebral cortex-distributed insulin/insulin receptor has been shown to be involved in brain cognitive functions. In the higher limbic system including the hippocampus, piryform cortex and amygdala, the insulin receptor (IR) has been demonstrated to play an important role in spatial and emotional memory via effects on a variety of signaling pathways (Figure 3.20). By activating the signaling cascade involving Shc, Grb-2/SOS and Ras/MAPK shortly after training, the IR appears to mediate long-term memory storage processes requiring activation of gene expression leading to new protein synthesis. Activation of other IR-associated pathways involving PI3K, PKC and Akt/PKB may also participate in modulation of memory formation processes and neuronal survival by interacting with the Ras/MEK/MAPK cascades. Finally, via cross-talk with other protein tyrosine kinases, such as pp60c-src, the IR may regulate the formation of an earlier stage of memory (Zhao and Alkon 2001; Zhao et al., 2004).

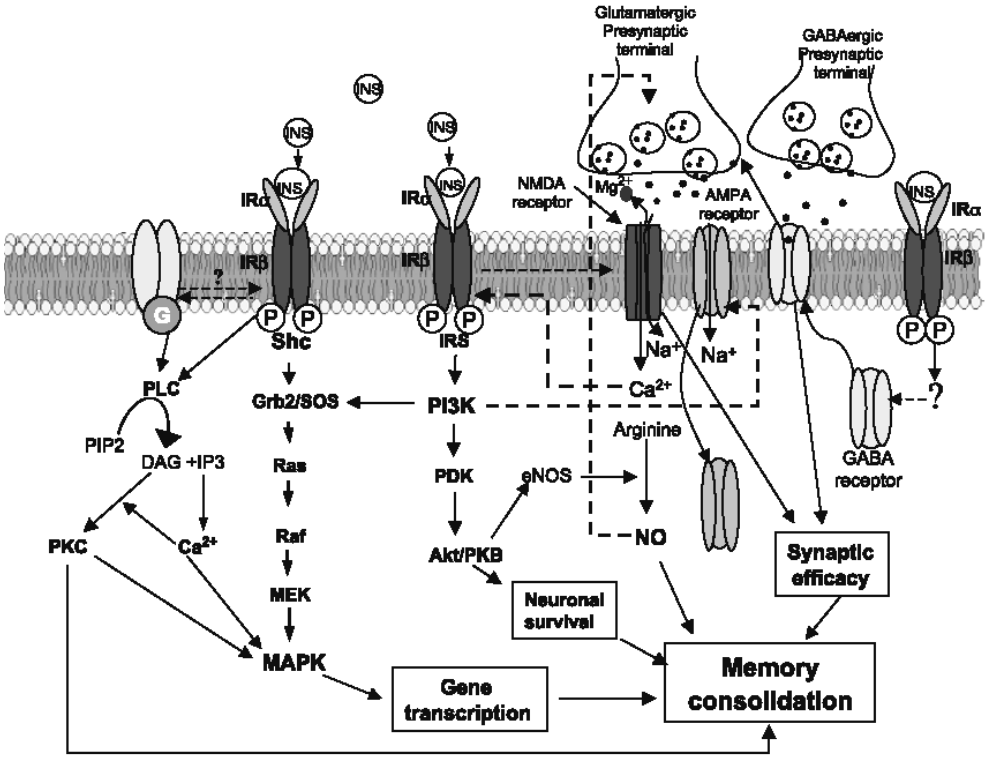

Figure 3.20 Hypothetic schema for insulin/insulin receptor modulation of memory-associated neuronal activities. Activated insulin receptor may be involved memory formation via several mechanisms:

(1) via modulation of glutamatergic and GABAergic transmission. Insulin/insulin receptor potentiates NMDA channel activity, functions of which depend on the presence and activation of AMPA receptor that cause synaptic membrane depolarization and removal of the Mg^{2+} blockage of the NMDA receptor leading to LTP. Increased Ca^{2+} influx via the NMDA receptor and neuronal activities may inhibit tyrosine phosphorylation of insulin receptor via a feedback mechanism. Depending on spatial and temporal specificity of information processing, insulin receptor signaling through PI3 kinase may be involved in LTD via internalization of AMPA receptors. Insulin receptor may also modulate GABA transmission by recruiting functional GABA receptor to the postsynaptic membrane. GABAergic neurons sense the excitatory transmission and regulate synaptic strength by sending feedforward and/or feedback inhibitory inputs to the principal neurons. Regulation of synaptic efficacy by integrated excitatory and inhibitory transmissions within specific neuronal network is thought to underlie memory encoding and retrieval in the hippocampus.

(2) Activation of insulin receptor-Shc-MAP kinase pathway after learning may lead to regulation of gene expression that is required for long-term memory storage.

(3) Insulin receptor may interact with G-protein coupled receptor and PLC to activate PKC leading to facilitation of short-term memory encoding. (4) The insulin receptor/IRS/PI3 kinase pathway may trigger synthesis of NO via eNOS activity. NO acts as a retrograde messenger for neurotransmitter release, and may also act intracellularly on memory processing. Furthermore, insulin receptor signaling with the same pathway may promote neuronal survival that is certainly beneficial for long-term memory consolidation (Zhao et al., 2004).

3.5 Cortisol hormone involved in learning and memory

Stress and cortisol have specific effects on cognitive function in humans and in animal models. Cortisol and stressful experiences produce short-term and reversible deficits in episodic and spatial memory in humans and in animal models, whereas repeated stress also impairs cognitive function in animal models and repeated

cortisol elevation in humans is accompanied by cognitive dysfunction (McEwen, 2000).

Acute effects of stress or cortisol administration are within a time span ranging from a few hours to a day and are generally reversible and quite selective to the task or situation. Cortisol effects are implicated in both selective attention as well as in memory consolidation, and such actions are consistent with the effects of cortisol on the modulation of long-term potentiation and primed-burst potentiation. McEwen (2000) demonstrated that rats that received 21 days of restraint stress were impaired in performance on an eight-arm radial maze when they were trained starting one day after the end of stress but not when trained 18 days later. Chronic restraint stress for 21 days produced apical dendrites of CA3 pyramidal neurons to atrophy but dendritic atrophy is reversible within 7-10 days after the end of stress (McEwen 2000; Conrad et al., 1999).

Cortisol treatment causes dendritic atrophy. There are several ways in which cortisol affect the excitatory amino acid system. First, adrenal steroids modulate expression of NMDA receptors in hippocampus, with chronic cortisol exposure leading to increased expression of NMDA receptor binding and both NR2A and NR2B subunit mRNA levels. Second, there are cortisol effects on the expression of mRNA levels for specific subunits of GABAa receptor in CA3 and the dentate gyrus. Third, adrenal steroids regulate the release of glutamate. Mossy fiber terminals in the stratum lucidum contain presynaptic kainite receptors that positively regulate glutamate release (McEwen, 2000).

Granule neurons are replaced in adult life, and neurogenesis, as well as apoptotic neuronal death, are regulated by stress as well as by seizure-like activity. Granule neurons send mossy fibers to both the CA3 pyramidal neurons and to interneurons in the hilus, which, in turn, send inhibitory projections to the CA3 pyramidal neurons. The balance between the excitatory input and the inhibitory tone from the interneurons is presumed to be very important to the excitability of CA3 neurons (Figure 3.21). Evidence summarized in the text indicates that excitatory amino acid release during repeated stress, aided by circulating glucocorticoids, leads to a reversible remodeling of apical dendrites over 3- 4 weeks in rats and tree shrews. Serotonin also participates, possibly by aiding the excitatory amino acid activity at the

NMDA receptor, and reduced GABA-benzodiazepine-mediated inhibitory activity at synapse from the interneurons on CA3 pyramidal neurons may also exacerbate the remodeling. Excitatory input to the dentate granule neurons from the entorhinal cortex acts via NMDA receptors in concert with circulationg adrenal steroids to regulate the rate of neurogenesis and apoptotic cell death, and both acute and chronic stress appear to be capable of inhibiting neurogenesis in the dentate gyrus (McEwen, 2000).

Joëls et al. (2006) proposed a unifying theory of the effects of stress on learning and memory, which states that stress will only facilitate learning and memory processes: (i) when stress is experienced in the context and around the time of the event that needs to be remembered, and (ii) when the hormones and transmitters released in response to stress exert their actions on the same circuits as those activated by the situation, that is, when convergence in time and space take place.

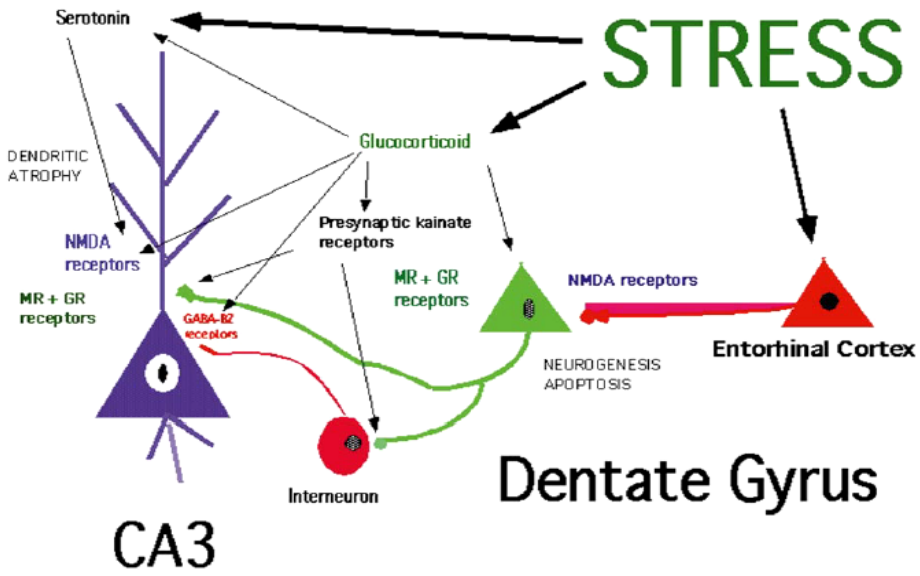

Figure 3.21 Schematic diagram of the role of neurotransmitters and glucocorticoids in regulating neurogenesis and dendritic remodeling in the dentate gyrus CA3 system of the hippocampal formation (McEwen, 2000).

Convergence in time seems to be crucial for the nature of the effects. Thus, although stress hormones generally act in a facilitatory way when they are present around the time of learning, they have opposite effects when present in high amounts either before or a considerable time after a learning task. Clearly, stress has differential

effects on distinct phases of the learning and memory processes: consolidation can be facilitated when stress is experienced at the time and within the context of the event to be remembered, whereas retention seems to be impaired by exposure to stress shortly before a retrieval test. The latter results are sometimes interpreted as a specific, negative effect of corticosteroid hormones on retrieval of information, but they could also signify a facilitated new process of learning, in competition with or overwriting earlier learned information. The nature of the stressor and the learning task itself also determine how stress affects memory. This relates to the brain circuits that are activated by the stressful situation. Physical stressors will activate lower brain regions that are implicated, for example, in pain responses, whereas psychological stressors are more likely to activate limbic regions. They propose that facilitation will only occur when stress hormones (corticosteroids, noradrenaline, corticotropin releasing hormone) exert their actions in the same areas as those activated by the particular stressful situation; that is, when convergence in space takes place (Joëls et al., 2006).

In summary, they propose that in the short term, stress induced hormones will facilitate the strengthening of contacts involved in the formation of memories of the event by which they are released. But at the same time, corticosterone initiates a gene-mediated signal that will suppress any information unrelated to the event reaching the same areas hours later. This is a very efficient strategy to preserve an appropriate priority in the reaction to challenges. The proposed mechanism also explains why the timing of stress application and learning is so important. If corticosterone is released by a stressor one hour before training of a learning task starts, the genomic action will have developed already by the time input related to the learning event reaches the circuit, so this input will encounter an elevated threshold for synaptic strengthening (Figure 3.22). If, however, corticosteroid levels rise some time (e.g. one hour) before noradrenaline is active, the memory-facilitating action by noradrenaline is suppressed and dose dependently desensitized. In this respect it is revealing that, at the cellular level, corticosterone given several hours before noradrenaline indeed suppresses the effectiveness of the latter, via a gene-mediated pathway (Joëls et al., 2006).

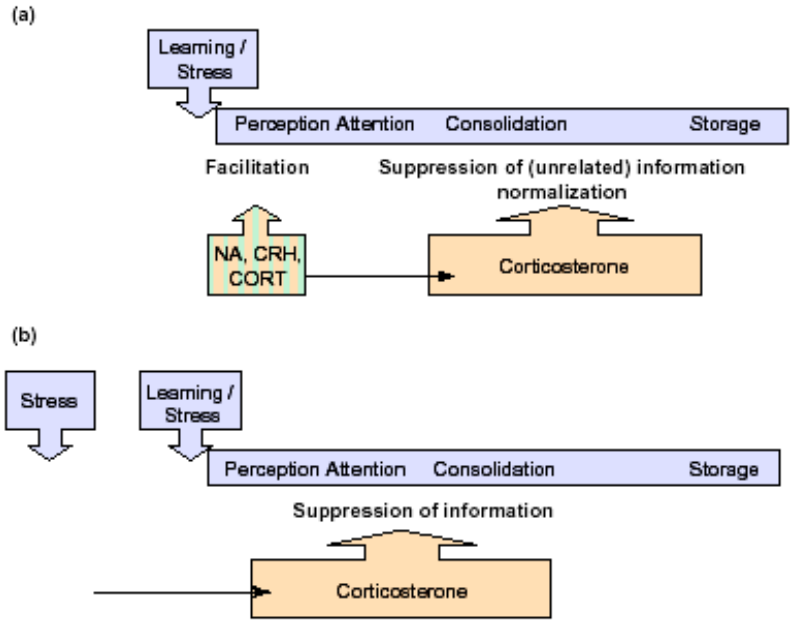

Figure 3.22 Opposing effects of stress on learning depend on the timing of the events. (a) Stress within the context of a learning situation leads to the release of noradrenaline: NA, corticotropin releasing hormone: CRH and corticosterone: CORT, all of which are active in the brain at the time that the initial phases of learning take place. At this stage the neurotransmitters and hormones facilitate the ongoing process. Corticosterone, however, also initiates a gene-mediated pathway, which will elevate the threshold for input unrelated to the initial event and restore neuronal activity (normalization), with a delay of more than an hour. (b) If an organism has been exposed to a stressor some time before the learning process takes place, the gene mediated suppression of activity will have developed by the time that acquisition occurs. Under these conditions corticosterone will impair learning processes (Joëls et al., 2006).

3.6 Neurotrophic factor involved in learning and memory

The neurotrophins are a family of proteins that are essential for the development of the vertebrate nervous system. In the mammalian brain, four neurotrophins have been identified: nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin 3 (NT3) and neurotrophin 4 (NT4). These closely related molecules act by binding to two distinct classes of transmembrane

receptor: the p75 neurotrophin receptor (p75NTR) and the Trk family of receptor tyrosine kinases, which includes TrkA, TrkB and TrkC. Different neurotrophins show binding specificity for particular receptors such as NGF binds preferentially to TrkA, BDNF and NT4 to TrkB, and NT3 to TrkC. These interactions have been considered to be of high affinity. The p75 receptor can bind to each neurotrophin, and also act as a co-receptor for Trk receptors. The expression of p75 can increase the affinity of TrkA for NGF and can enhance its specificity for cognate neurotrophins (Chao, 2003; Lu et al., 2005).

In the adult brain, neurotrophins have a key role in synaptic plasticity. Of all the neurotrophins, BDNF is by far the best characterized for its role in regulating LTP, particularly the early phase of LTP (E-LTP). Acute application of mature BDNF facilitates E-LTP in the hippocampus and visual cortex. Because proBDNF can be cleaved by the extracellular protease plasmin, and the plasminogen activator (tPA) is known to be involved in late-phase LTP (L-LTP), it is reasonable to propose that an important function of the tPA/plasmin system is to convert proBDNF to mature BDNF at hippocampal synapses, and that this conversion is involved in the expression of L-LTP (Figure 3.23). Indeed, proBDNF is expressed in the hippocampal CA1 area and its expression is increased in mice that lack tPA and plasminogen. tPA, through the activation of plasmin, converts proBDNF to mature BDNF *in vitro* (Lu et al., 2005).

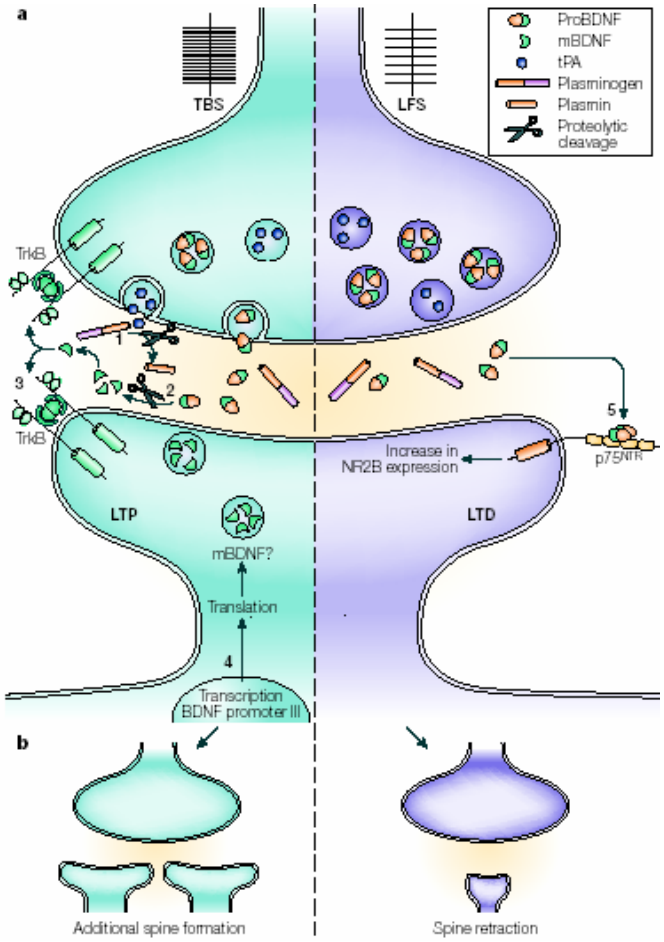

Figure 3.23 The yin and yang of long-term synaptic regulation by pro- and mature BDNF.

(a) Molecular cascade of brain-derived neurotrophic factor (BDNF) processing in late-phase long-term potentiation (L-LTP). In response to theta-burst stimulation (TBS), tissue plasminogen activator (tPA) is secreted into the synaptic cleft and cleaves the extracellular protease plasminogen to yield plasmin (1). Plasmin then cleaves proBDNF (the precursor of BDNF, which is released in an activity-dependent manner), yielding mature BDNF (mBDNF) (2). mBDNF binds to TrkB and triggers a series of downstream signalling pathways to induce LTP (3). During the maintenance stage of LTP, mBDNF might be generated by intracellular cleavage after postsynaptic transcription and translation (4). By contrast, proBDNF secreted extracellularly remains uncleaved after low-frequency stimulation (LFS). Uncleaved proBDNF binds to the p75 neurotrophin receptor (p75NTR) (5). to facilitate the induction of long-term depression (LTD), possibly through the regulation of NMDA (*N*-methyl-D-aspartate) receptor NR2B subunit expression.

(b) Morphological alterations in synapses induced by pro- and mature BDNF. Left, BDNF–Trk signalling might be an active mechanism that converts activity-induced molecular signals into structural plasticity, contributing to synapse formation. Right, proBDNF–p75NTR signaling might be important in translating activity-dependent signals into negative modulation of structural plasticity, contributing to synapse retraction (Lu et al., 2005).

3.7 Stress hormone measurement

3.7.1 Saliva and its physiology

3.7.1.1 Salivary glands

Three pairs of salivary glands secrete into the oral cavity (Figure 3.24). Each pair of salivary glands has a distinctive cellular organization and produces saliva with slightly different properties:

1) The large **parotid salivary glands** lie inferior to the zygomatic arch beneath the skin that covers the lateral and posterior surface of the mandible. The parotid salivary glands produce a thick, serous secretion containing large amounts of salivary amylase, an enzyme that breaks down starches (complex carbohydrates). The secretion of each parotid gland are drained by a parotid duct (Stensen's duct), which empties into the vestibule at the level of the second upper molar.

2) The **sublingual salivary glands** are covered by the mucous membrane of the floor of the mouth. These glands produce a watery, mucous secretion that acts as a buffer and lubricant. Numerous sublingual salivary ducts (Rivinus'ducts) open along either side of the lingual frenulum.

3) The **submandibular salivary glands** are situated in the floor of the mouth along the inner surfaces of the mandible within a depression called the mandibular groove. The submandibular glands secrete a mixture of buffers, glycoproteins called mucins, and salivary amylase. The submandibular ducts (Wharton's duct) open into the mouth on either side of the lingual frenulum immediately posterior to the teeth (Martini et al., 1998).

The tissue of the salivary glands consists of a system of blind ducts surrounded by webs of capillary vessels and embedded in connective tissue. In the extremity of these ducts the primary saliva is produced by filtering the blood of the capillaries through the membranes of the acinar cells. The cell membrane of the acinar cells in the distal end of the salivary glands consists of a duplicate layer of lipids with a hydrophil end at the outer side and lipophil end at the inner part. There are many mechanism exists for blood components to pass the membrane barrier into the salivary ducts (Figure 3.25).

Mechanism exists for blood components to pass the membrane barrier into the salivary ducts

1) Passing through the space between the acinar cells. Because of barriers in the intercellular space, called tight junctions, only molecules with a relative small molecular weight less than 1,900 may pass through (e.g. H_2O = 18, Na^+= 23).

2) Filtration through pores of the cell membranes. The transfer is only possible for substances of a molecular weight less than 400 (e.g. water, electrolytes).

3) Selected transport across the cell membrane

3.1) Passive diffusion of lipophilic molecules (e.g. steroids)

3.2) Active transport through protein channels (e.g. peptides)

3.3) Pinocytosis: passing into the cell by taking along a part of the cell; on the other side of the cell the vacuole membrane is reintegrated into the cell membrane and the contents of the vacuole are released in the duct of the glands (e.g. larger proteins such as enzymes)

4) Sodium ions are actively pumped into the acinar cells and therefore build up an osmotic gradient so that water and small molecules will flow into the cell. In the ductal cells the sodium in the saliva is exchanged with potassium ions. This process is dependent on the saliva flow rate. So during a great flow the sodium concentration in saliva is relative greater than that of potassium compared to a reduced flow rate.

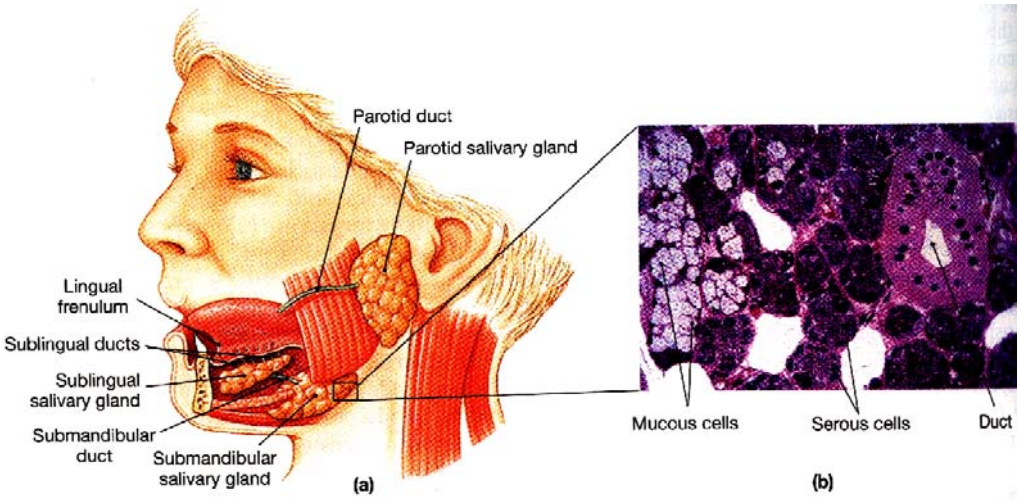

Figure 3.24 The salivary glands. (a) Lateral view, showing the relative positions of the salivary glands and ducts on the left side of the head. (b) The submandibular gland (Light microscope x 300) secretes a mixture of mucins, produced by mucous cells, and enzymes, produced by serous cells (Martini et al., 1998).

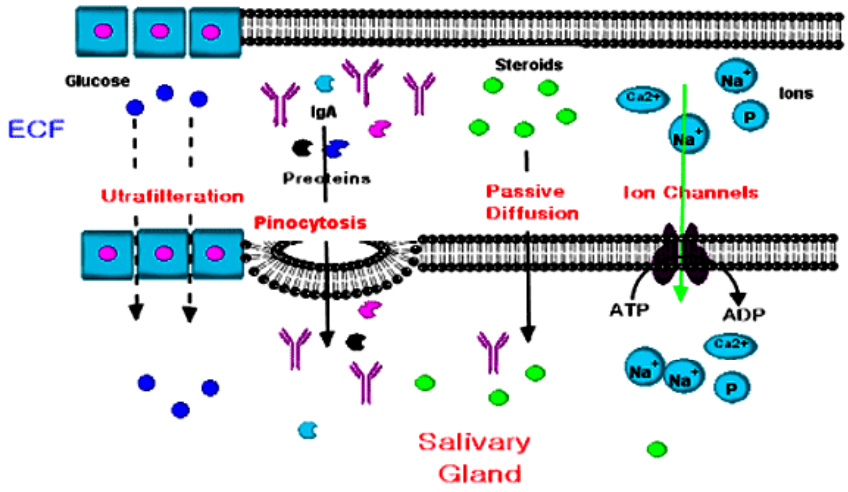

Figure 3.25 The mechanism exists for blood components to pass the membrane barrier into the salivary ducts (Heaekel and Hanecke, 1996).

3.7.1.2 Saliva

Salivary glands produce 1.0 - 1.5 liters of saliva each day. Saliva is 99.4 percent water, and the remaining 0.6 percent includes an assortment of electrolytes (principally Na^+, Cl^-, and HCO_3^-), buffers, glycoproteins, antibodies, enzymes, and waste products. The glycoproteins, called mucins, are primarily

responsible for the lubricating action of saliva. Saliva is a mixture of glandular secretions; about 70 percent of the saliva originates in the submandibular salivary glands, 25 percent in the parotids, and the remaining 5 percent in the sublingual salivary glands.

A continuous background level of secretion flushes the oral surfaces, helping keep them clean. Buffers within the saliva keep the pH of the mouth near 7.0 and prevent the buildup of acids produced through bacterial action. In addition, saliva contains immunoglobulins (IgA) and lysozymes that help control populations of oral bacteria. A reduction or elimination of salivary secretions, caused by radiation exposure, emotional distress, or other factors, triggers a bacterial population explosion in the oral cavity. This proliferation rapidly leads to recurring infections and the progressive erosion of the teeth and gums (Martini et al., 1998).

3.7.1.3 Control of salivary secretion

Salivary secretions are normally controlled by the autonomic nervous system (ANS). Each gland receives parasympathetic and sympathetic innervation. The parasympathetic outflow originates in the salivatory nuclei of the medulla oblongata and synapses within the submandibular and otic ganglia. Any object placed in your mouth can trigger a salivary reflex by stimulating receptors monitored by the trigeminal nerve or by stimulating taste buds innervated by N VII, IX, or X. Parasympathetic stimulation accelerates secretion by all the salivary glands, resulting in the production of large amounts of saliva. In contrast, the role of the sympathetic innervation remains uncertain; evidence suggests that it provokes the secretion of small amounts of very thick saliva.

The salivatory nuclei are also influenced by other brain stem nuclei as well as by the activities of higher centers. For example, chewing with an empty mouth, the smell of food, or even thinking about food will initiate an increase in salivary secretion rates. The presence of irritating stimuli in the esophagus, stomach, or intestines will also accelerate saliva production, as will the sensation of nausea. In functional terms, increased saliva production in response to unpleasant stimuli helps reduce the magnitude of the stimulus by dilution, a rinsing action, or by buffering strong acids or bases (Martini et al., 1998).

3.7.2 Advantages of salivary measurement

Cortisol can be measured in urine, plasma and saliva. Salivary cortisol appears to be as sensitive a measure of stress reactivity as urinary and plasma cortisol (Weinstein, 1999; King and Hegadoren, 2002). Saliva testing is the most reliable way to measure free, bioavailable hormone activity at a cellular level. Free steroid hormones passively traverse into the cells in the salivary gland and flow with the fluid that passively accompanies Na^+ that is pumped by the sodium/potassium ATPase mechanism. Thus, there is no change in hormone concentration with change in flow rate. Bound steroids are too large to diffuse freely through the salivary cells into the salivary gland lumen as they have a large molecular weight. Total blood hormone levels are not comparable to saliva levels and can only be loosely compared using the approximate ratio (1-10%) of free to bound hormones.

Benefits of salivary hormone testing

- Testing measures free, bioavailable hormones levels
- Painless, non-invasive and economical and can be done at home
- Multiple saliva collections can be taken in a single day or over a number of weeks to evaluate levels
- Less expensive/more convenient for health care provider and patient
- Hormones stable in saliva for prolonged period of time
- More representative than serum of total bioavailable steroid hormone levels

However salivary cortisol is not without disadvantages. Home testing often suffers from major problems with compliance and subjects may provide insufficient saliva or deviate from instructions. Saliva provided after eating or drinking substances with low pH (i.e. fruit juices) as well as the presence of blood in saliva due to oral lesion may artificially raise cortisol levels. Some disadvantages may be resolved by sound planning, rigorous follow-up and other strategies (Levine et al., 2006).

3.7.3 Sampling cortisol from blood or urine

In plasma, cortisol is present in unbound (free) and bound quantities. At least 90% of cortisol is bound to plasma proteins, with the largest portion being bound

to corticosteroid-binding globulin (CBG) and a lesser portion to albumin (Schimmer and Parker, 1996; King and Hegadoren, 2002). As a result, normally less than 10% of cortisol is unbound and biologically active. Total cortisol level is the sum of the bound and the unbound cortisol. Total plasma cortisol levels can be misleading if there are individual differences in level or activity of CBG (e.g. decreased CBG in women with major depressive episodes or fluctuations in CBG occur during pregnancy) (Nicolson, 1997; Heim, 2000; Haourigui et al., 1995; Meulenberg and Hofman, 1990).

In urine, because cortisol is expensively metabolized to tetrahydrocortisone in the liver, a small amount of free cortisol and larger quantities of its metabolite are excreted in urine (Baum and Grunberg, 1997). Thus, the metabolite is more readily available and measurable in urine samples than is free cortisol. However, urinary excretion does not directly reflect adrenal activity because it is dependent on cortisol metabolism and urine excretion. For these reasons, most studies measure either plasma or salivary cortisol (King and Hegadoren, 2002).

3.7.4 Disadvantages of plasma cortisol testing

Sampling cortisol in blood may have insurmountable drawbacks for researchers: it requires medical staff and specialized equipment, is costly and it may be considered invasive by some populations. Although cortisol is a stable molecule at room temperature, plasma may require special handling as it could be considered a biohazard.

In addition, there is considerable variability in reported reference range intervals not only for total plasma cortisol but also for free cortisol and CBG. Normally free cortisol is less than 6% of total cortisol but as the total cortisol exceeds saturation of CBG the percentage of free cortisol increases. In the case of CBG deficiency, the free cortisol fraction is maximal, around 30%, since remaining cortisol is sequestered by serum albumin which is in vast molar excess. The percentage of free cortisol within an individual can fluctuate due to both endogenous and exogenous factors, which may include illness, stress and trauma (Levine et al., 2006).

3.8 Factors influencing cortisol levels

A review of the literature indicated that a number of factors may influence cortisol levels. Many of these must be considered in the design, the selection of variables, and the interpretation of results of studies measuring cortisol. Important factors include age, gender, menstrual phase, physical activity, sleep, diet, obesity, and anticipatory stress.

3.8.1 Age

Age-related differences in HPA axis functioning are often explained by assumptions derived from the Sapolsky's hypothesis that so called glucocorticoid cascade hypothesis (Sapolsky et al., 1986). They suggested that age-related alterations in HPA axis regulation emerge due to a decrease in the ability of hippocampal neurons to maintain a sufficient negative feedback function. Sapolsky (2000) found that the aged have approximately the same size bursts of the cortisol secretion as younger adults, it may take longer for their cortisol levels to return to baseline after a stressor and they may secrete more stress-related hormones in their normal non-stressed states. In the other hand, De Kloet et al. (1991, 1998) formulated the corticosteroid receptor balance theory, proposing that even with older age homeostatic control could be maintained by a new balance between glucocorticoid and mineralocorticoid receptors, resulting in similar endocrine responses to stress in young and old subjects. A study examining the effect of age on levels of cortisol in response to a psychosocial stressor found that although basal cortisol levels were moderately increased, cortisol responses to the stressor did not increase in either magnitude or duration in the elderly (Nicolson et al., 1997).

3.8.2 Gender

Investigations regarding potential gender differences have produced equivocal results. The previous studies found that men and women have equivalent daily cortisol secretion rates, despite the finding that men secrete more ACTH than women to maintain this equivalency (Roelfsema, 1993). In addition, King and coworkers (2000) found no gender differences in morning or evening basal salivary cortisol levels in 147 healthy volunteers sampled 4 times in 1 year. However,

menstrual phase and estradiol use were not controlled. Significant differences in cortisol levels in relation to menstrual phase and estradiol levels in women have been reported, particularly when conducting challenge tests. Other previous studies determined that gender, menstrual cycle phase, and oral contraceptive use bore important effects on HPA axis responsiveness to psychosocial stress in healthy subjects, with salivary cortisol being greatly reduced in oral contraceptive users after HPA axis challenge (Kirschbaum et al., 1999).

3.8.3 Music

3.8.3.1 Definition

The word *music* comes from the Greek *mousikê (tekhnê)* by way of the Latin *musica*. It is ultimately derived from *mousa*, the Greek word for muse. In ancient Greece, the word *mousike* was used to mean any of the arts or sciences governed by the Muses.

3.8.3.2 Physiological and psychological effects of music on stress

The positive effects of music have been affecting to physiological parameters such as heart rate, blood pressure (Camara et al., 2008; Lai et al., 2008), electroencephalography (EEG), PET scan and neural hormone. The several studies showed that music can also decrease cortisol and ACTH levels (Mockel et al., 1994; Khalfa et al., 2003). Supportive evidence can be found in the observation that the concentration of saliva cortisol decreased more rapidly in the subjects exposed to music than the subjects who exposed to silence. Khalfa et al (2003) suggested that relaxing music after a stressor can decreasing the post stress response of the HPA axis. Suda and her colleague reported that salivary cortisol levels were reduced by major mode music than by minor mode (Suda et al., 2008).

In addition, other studies provide promising support for the use of music as a powerful anxiolytic treatment. The health benefits of preventing moderate reactions to cognitive stress are considerable, particularly in situations in which a patient's health is already vulnerable or compromised, such as prior to surgery (Miluk-Kolasa et al., 1996), or waiting for their cardiac catheterization (Hamel, 2001), in acute and coronary care settings (Guzzetta, 1989), during postsurgical recovery

(Good, 1995), or during painful medical procedures such as chemotherapy (Weber et al., 1997). In contrast, Wang and colleagues (2002) reported that adult patients undergoing anesthesia and surgery, who listened to music before surgery were found lower levels of state anxiety whereas physiological outcomes did not differ between the two study groups. Chlan and colleagues (2007) found the stress response did not differ significantly between subjects receiving music therapy and who not.

Takahashi and Matsushita (2006) found that the lasting effect of once-a-week continuous music therapy. Even the elderly with moderate or severe dementia were able to participate in the group music therapy and these results suggest that enjoying singing and playing musical instruments in a concert was effective in preventing cardiac and cerebral diseases.

Previous studies have shown that projections from the amygdala to the hypothalamus in the rat (Watkins, 1997). Projections from various amygdaloid nuclei to the hypothalamic nuclei result in excitation or inhibition of endocrine function. Neuroanatomical and histochemical studies have identified two main amygdaloid regions: basolateral and centromedial. Endocrine function is inhibited by stimulation of the basolateral region and enhanced by stimulation of the centromedial region. Auditory stimuli project to the amygdala lateral (AL) nucleus, located in the basolateral region; research is needed to determine if a neural pathway exists between the AL nucleus and amygdaloid nuclei projecting to the hypothalamus. Stimulation of the basolateral amygdaloid region may decrease CRH release directly by inhibition of hypothalamic nuclei and indirectly by preventing stimulation of the centromedial amygdaloid region (Figure 3.26).

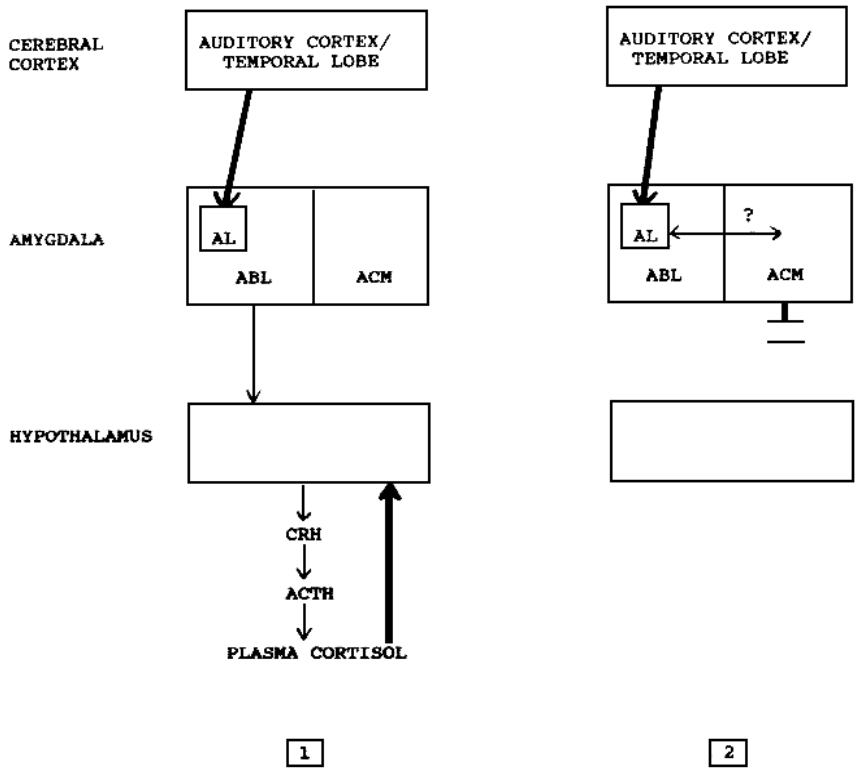

Figure 3.26 Proposed mechanisms for the effect of anxiolytic music on the release of corticotropin-releasing hormone (CRH). Auditory stimuli may decrease CRH: (1) directly by stimulation of the amygdala basolateral region (ABL), which results in inhibition of hypothalamic nuclei, or (2) indirectly by preventing stimulation of the amygdala centromedial region (ACM), which, if stimulated, results in stimulation of hypothalamic nuclei. Inhibition of hypothalamic nuclei decreases CRH release, which decreases adrenocorticotrophic hormone (ACTH) release from the anterior pituitary and results in decreased plasma cortisol levels; a feedback loop allows plasma cortisol levels to also influence the rate of CRH release. Research is needed to confirm the existence of a connecting neural pathway between the amygdala lateral nuclei (AL) and amygdala nuclei projecting to the hypothalamus. Symbols and abbreviations used: bold line, stimulation/excitation; thin line, inhibition (Watkins, 1997).

3.8.3.3 Music and learning and memory

3.8.3.3.1 Music listening

A sound reaching the eardrum sets into motion a complex cascade of mechanical, chemical, and neural events in the cochlea, brain stem, midbrain nuclei, and cortex that eventually results in a percept (Peretz and Zatorre, 2005). Music has strong connections to both attention and memory systems. Brain imaging studies have shown that listening to real polyphonic music calls for rule based analysis and combination of sound patterns from multiple auditory streams, which naturally recruits bilateral temporal, frontal and parietal neural circuits underlying multiple forms of attention, working memory, semantic and syntactic processing, and imagery (Janata et al., 2002; Peretz and Zatorre, 2005).

The Mozart effect refers to an enhancement of performance or change in neurophysiological activity associated with listening to Mozart's music. Rauscher, Shaw, and Ky (1993) reported that college students perform better on standardized tests of spatial abilities after listening to 10 minutes of a Mozart sonata than after listening to relaxation instructions or sitting in silence. Thompson and colleagues (2001) found that the performance on the spatial task was better following the music than the silence condition, but only for participants who heard Mozart. In addition, Jausovec and colleagues (2006) reported that Mozart's music enhances the learning of spatio-temporal rotation tasks by activating task-relevant brain areas. However, just as many, if not even more studies have failed to replicate the Mozart effect (Newman et al., 1995; Steele et al., 1997; Steele et al., 1999; Rauscher and Shaw, 1998).

Särkämö and colleagues (2008) found that recovery in the domains of verbal memory and focused attention improved significantly more in the music group than in the language and control groups. The music group also experienced less depressed and confused mood than the control group. Music that is pleasant and enjoyed by a particular listener is the most likely to have positive impacts on the listeners' emotional states, and positive influences on emotional state can improve cognitive performance (Schellenberg and Hallam, 2005). In addition, Chikahisa and colleagues (2006) reported that perinatal exposure of mice to music has an influence on BDNF/TrkB signaling and its intracellular signaling

pathway targets, including PDK1, and thus may induce improved learning and memory functions.

3.8.3.3.2 Music training

Music performance includes a variety of tasks, such as playing or singing well learned pieces from memory, sight-reading, and improvisation. They were all combined rapid motor skills and relatively elaborated cognitive operations in addition to the perceptual, memory, and emotion components (Peretz and Zatorre, 2005). Playing an instrument also depends on the brain interpreting somatosensory information from the fingers and lips in contact with the instrument. Musicians represent a unique model in which to study plastic changes in the human brain (Münte et al. 2002). As suggested by animal research, experience can shape the size of cortical networks either by expansion or by reduction, depending on stimuli and on the structural levels examined (i.e., synaptic or macroscopic). The prime areas to look for differences are the motor areas. Indeed, there is clear evidence that the motor cortex of musicians is enhanced structurally (Gaser and Schlaug, 2003) and functionally (Krings et al., 2000). Anatomical changes also have been seen in other motor-related structures, including cerebellum and corpus callosum (Schlaug, 2003). Schellenberg (2004) found that the children in the music lesson groups (keyboard or voice lessons) exhibited greater increases in full-scale IQ than the children in the control groups who received drama lessons or no lessons. Schlaug and colleagues (2005) has also demonstrated that music training in children results in long-term enhancement of visual–spatial, verbal, and mathematical performance.

Blood and Zatorre (2001) found that strong emotional responses to music, leading to shivers down the spine and changes in heart rate, are accompanied by the activation of a brain network that includes the ventral striatum, midbrain, amygdala, orbitofrontal cortex and ventral medial prefrontal cortex areas that are thought to be involved in reward, emotion and motivation.

3.8.4 Physical activity

Exercise represents a physical stress that challenges homeostasis. In response to this stressor, the autonomic nervous system and hypothalamus-pituitary adrenal axis are known to react and participate in the maintenance of homeostasis and

the development of physical fitness. This includes elevation of cortisol and catecholamines in plasma. However, physical conditioning is associated with a reduction in pituitary-adrenal activation in response to exercise. Therefore, they can assume regular moderate exercise as the mild, repeated "stressful" stimulation (which is good for health). While excessive and prolonged stress (as in heavy exercise) can lead to depression, mild and irregular (nonlinearly applied, hormetic) stress can actually improve depression.

Some studies reported that salivary cortisol levels increased when healthy volunteers were standing, but not when they were sitting or lying, during sample collection (Hennig et al., 2000). However, plasma cortisol levels did not change appreciably as a result of exercise in convenience samples of pre-, peri-, and postmenopausal women (Clearlock and Nuzzo, 2001). Specific to exercise, Vaynman and coworkers (2004) found an association between CREB and BDNF expression and cognitive function, such that animals that were the fastest learners and had the best recall showed the highest expression of BDNF and associated CREB mRNA levels (Figure 3.27).

Furthermore, exposure to stress causes a decrease in BDNF mRNA levels in the hippocampus, which may be linked to depression. Cotman and Engesser-Cesar (2002) found that antidepressant administration or exercise alone prevented the BDNF decrease caused by the acute stress. The combination of exercise with antidepressant led to significantly greater increases in hippocampal BDNF mRNA levels than did either treatment alone. It is possible that select antidepressants and exercise converge at a cellular level to promote brain health.

3.8.5 Sleep

Cortisol provides an important link between the immune system, sleep and psychological stress. The previous studies showed that significant increases in salivary cortisol in response to both nocturnal and morning awakening (Hucklebridge et al., 2000) and after 1 night of sleep deprivation, especially at 1:30 PM the next day (Goh et al., 2001; Leproult et al., 1997; Spiegel K et al., 1999). In addition, Caufriez (2002) found that sleep onset was consistently followed by a decrease in plasma cortisol

concentrations, while both sleep-wake and dark-light transitions were consistently associated with heightened cortisol secretion in a small group of subjects.

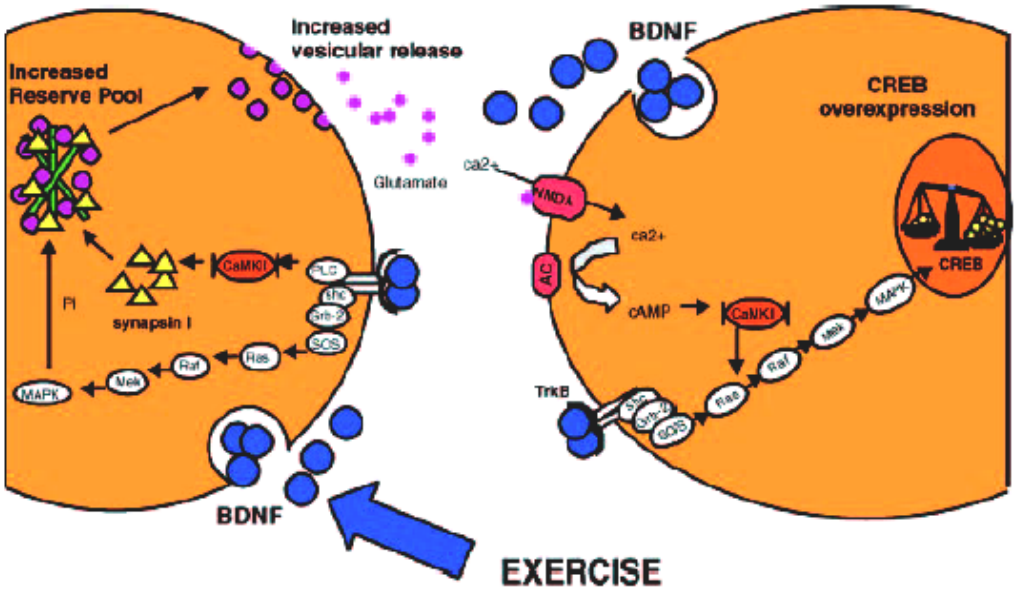

Figure 3.27 Potential mechanism through which BDNF may enhance learning and memory in the hippocampus under the action of exercise. Exercise-induced BDNF levels are depicted activating signal transduction cascades that lead to synapsin I and CREB mediated plasticity. BDNF activation of its TrkB receptor may increase the synaptic reserve pool by activating a PLC-CAMK-II cascade to increase synapsin I levels. Additionally, BDNF may increase neurotransmitter release by phosphorylating synapsin I via the MAP-kinase cascade. Postsynaptically, BDNF activation of the MAP-Kinase cascade, supplemented by additionally interaction (Vaynman et al., 2004).

3.8.6 Diet

Feeding and digestion patterns may also influence cortisol levels and have implications for scheduling collection times. Gibson and coworkers (1999) found that salivary cortisol was found to increase more after the midday meal than after other meals and after a high protein meal. On the other hand, some previous studies have also demonstrated that caffeine can intensify both cardiovascular and humoral responses to experimental stressors, amplifying the increases in cardiac output and

skeletal muscle blood flow and the increases in plasma levels of epinephrine and cortisol elicited by challenging or threatening tasks (Lane et al., 1990; Quinlan et al., 1997). In addition, Quinlan and coworkers (1997) found that caffeine ingestion was also associated with a significant decrease in salivary cortisol levels and anxiety index, and an increase in hedonic tone and energetic arousal. These positive responses may be due to alleviation of mild caffeine withdrawal after an overnight abstinence. However, the moderate intakes of caffeine had beneficial effects on mood/anxiety, an excessive intakes of caffeine in particular individuals can be associated with increases in anxiety (Quinlan et al., 1997; James, 1990).

A cup of coffee or tea represents the start of the day for hundreds of millions of people, and caffeinated drinks are used throughout the day as a stimulant by both adults and children. The caffeine content of these drinks is summarized in the Figure 3.28.

Table 1 | **The caffeine content of various beverages, foods and drugs**

Beverages, foods and drugs	*Caffeine content (mg)
Sprite or Fanta (12 oz/360 ml)	0
Decaffeinated coffee (8 oz/240 ml)	1–5
Milk chocolate (1 oz/28 g)	6
Green tea (8 oz/240 ml)	15–20
Dark chocolate (1 oz/28 g)	20
Pepsi Cola (12 oz/360 ml)	38
Dr Pepper (12 oz/360 ml)	40
Coca-Cola (12 oz/360 ml)	46
Black tea (8 oz/240 ml)	40–60
Espresso (2 oz/60 ml)	50–120
Red Bull (8.2 oz/246 ml)	80
Instant coffee (8 oz/240 ml)	65–100
Brewed coffee (8 oz/240ml)	80–135
Drip coffee (8 oz/240ml)	115–175
Typical caffeine pill	200

*Values supplied by the US Food and Drug Administration.

Figure 3.28 The caffeine content of these drinks (Foster and Wulff, 2005).

The alerting effects of caffeine occur within 15 – 30 min. This stimulant modulates performance, learning and memory, and muscular strength, and reduces overall sleepiness. Caffeine seems to advance the time of REM sleep, produce an

overall reduction in SWS and interrupt consolidated sleep. There is considerable individual variation in the speed at which caffeine is metabolized, having a half-life of between 3 h and 7 h, with an average of 4 h. So, an afternoon or evening cup of coffee can still result in a significant amount of caffeine in the body at bedtime, which will delay sleep. Caffeine might act by competitively binding to an adenosine receptor subtype, thereby blocking the mood-depressing and sleep-inducing effects of adenosine (Foster and Wulff, 2005).

3.8.7 Anticipatory stress

Academic examinations have often been used in stress research as early as 1914 because they are "predictable, standardized, and discrete examples of real-life stressors" (Stowell, 2003). Undergoing academic examinations has been associated with changes in mental and physical health including increased anxiety, increased negative mood, changed in hormone levels such as cortisol, immune function, and wound healing. In school, adolescents often see themselves as being evaluated in terms of their academic performance and the pressure to excel is an important measure of their success in school. Specifically, in an Asian context, academic stress arising from adolescents' self-expectations and expectations of others (e.g., parents and teachers) are particularly salient. Academic achievement is highly valued by Asians because it is perceived as one of the few avenues for upward mobility and expanded options, thus the significance that individuals and families attribute to academic success is intensified (Ang and Huan, 2006).

When the cortisol measurement design includes multiple sampling, the potential physiological and psychological responses of subjects to repeated collection should be considered. King and coworkers (2000) found a significant decrement in HPA axis response and stabilization in cortisol levels over time that they attributed to habituation to repeated saliva collection with a subsequent blunting of anticipatory stress. Other studies have reported similar finding (e.g., Weinstein, 1999; Martinek et al., 2003). However, anticipation of the novelty, discomfort or inconvenience of collection may activate the HPA axis and increase cortisol levels (Weinstein, 1999).

CHAPTER IV
MATERIALS AND METHODS

4.1 Subjects

Sixty normal male (n = 19) and female (n = 41) healthy volunteers aged between 15 - 17 years were the students from Triam Udom Suksa Pattanakarn Bangyai School, Nonthaburi, Thailand**.** Subjects were classified into 2 groups: amateur musician (n = 30) and control (n = 30).

Inclusion criteria

- The subjects were classified as amateur musicians had to be regularly played a musical instrument.
- The subjects were classified as control or non-musicians had never received formal musical training or played a musical instrument for any reasonable period of time.
- Signed consent form.

Exclusion criteria

- The subjects were had personal history of psychiatric or chronic illness and take any medications with known hormonal effect or receive hormone medications for at least 6 months prior to the study.
- The female subjects who have irregular menstrual cycle or current menstruation at experimental days.

All subjects filled out a general questionnaire to record the age of commencement of musical training, and intensity of lifelong practice (self estimates of the hours of practice per day and the days of practice per week). All subjects,

musicians and non-musicians, selected for this analysis were consistently right-handed according to general questionnaires. The experimental procedure, objective, nature and risks of the study were explained in details before the subjects gave their inform consent. This study protocol has been ethically approved by the Ethical Clearance Committee on Human Rights Related to Human Experimentation, Mahidol University, Nakhon Pathom, Thailand (No. Mu 2007-143).

4.2 General and stress inventory questionnaires

Information about age, weight, height, body mass index, medical history, activity in free time, grade point average (GPA), mathematic grade, sleep pattern and menstrual history were obtained by general questionnaire. In addition, subjects were asked to indicate stress level by the stress inventory of Department of Mental Health, Ministry of Public Health, Thailand, on days without and with an anticipated examination at similar time points (summarized in Figure 4.1).

4.3 Radioimmunoassay of salivary cortisol

4.3.1 Saliva collection and storage

Before the beginning of the study, subjects were instructed about harvesting saliva samples. Five minutes before providing saliva, all subjects should rinse their mouth with clean water. Subjects chewed the swab for up to three minutes, and put the soaked swab back into the tube. After the collection, the samples were kept in an icebox and immediately were transported to the laboratory, where the saliva samples were centrifuged at 4,500 rpm for 15 minutes and the saliva samples were stored at - 80°C until quantification for cortisol. Saliva samples were taken approximately 30-60 minutes before and 30-60 minutes after the examination. On day without announced examination, saliva samples were harvested at similar time points (Figure 4.2).

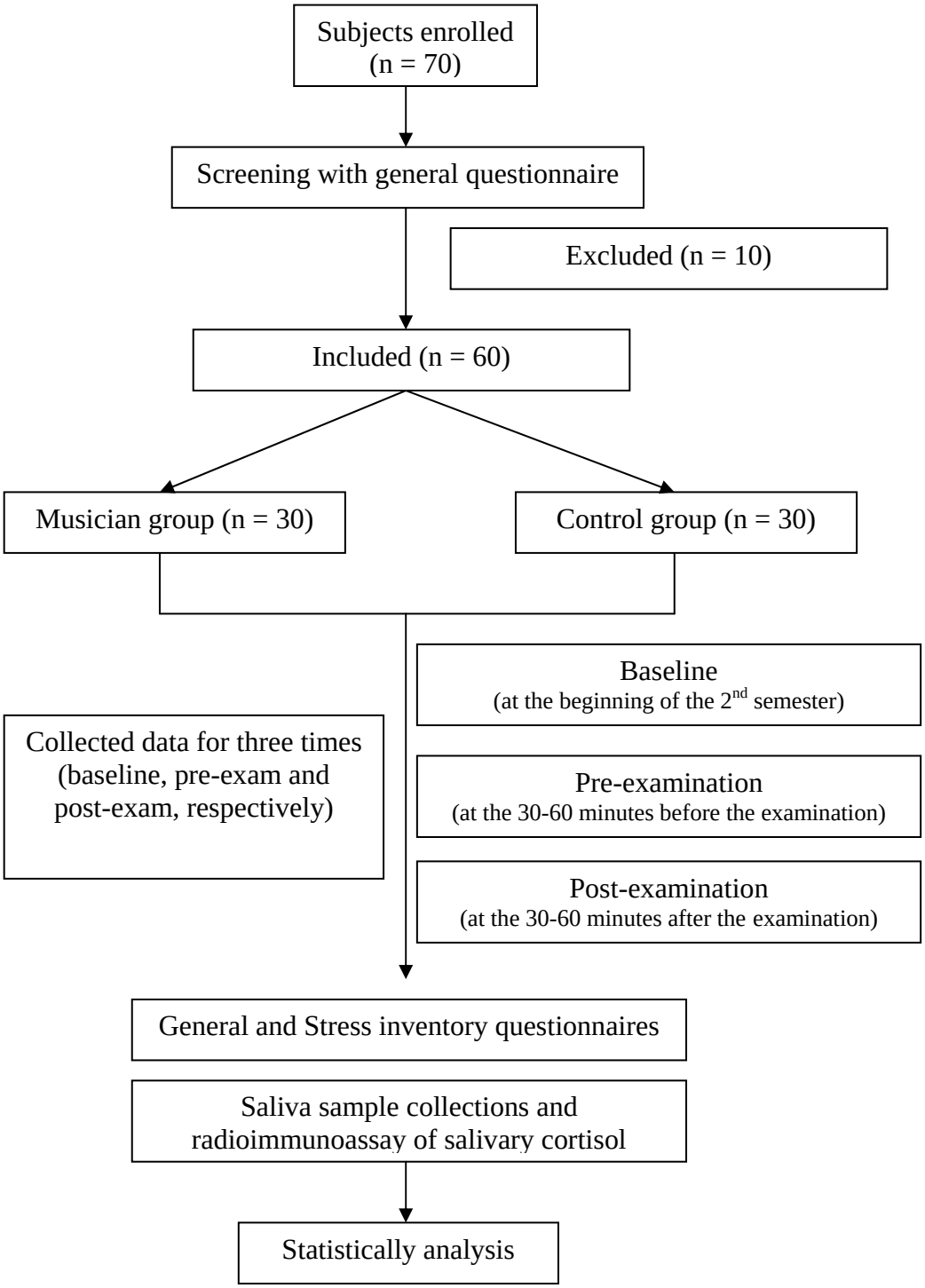

Figure 4.1 Summarized method

4.3.2 Salivary cortisol measurement

Cortisol will be quantified using the CORT-CT2, is a radioimmunoassay kit for the quantitative determination of cortisol in human serum, plasma, urine and saliva (CIS Bio International, Gif-sur-Yvette, France). The sensitivity of the method is approximately 0.8 nmol/l with salivary cortisol measurement.

4.3.3 Principle of the test

The principle of the assay is based on the competition between the labeled cortisol and cortisol contained in standards or specimens to be assayed for a fixed and limited number of antibody binding sites bound to the solid phase (coated tubes). After incubation, the unbound tracer is easily removed by a washing step. The amount of labeled cortisol bound to the antibody is inversely related to the amount of unlabeled cortisol initially present in the sample.

4.3.4 Reagents

1) Coated tubes: the polyclonal rabbit cortisol antibodies were coated onto the bottom of the tube. They were stored at 2-8°C until the expiry date (in their original package).

2) ^{125}I-Cortisol: This solution was supplied in liquid form and red color additive, consisting of ^{125}I labeled cortisol in tris buffer < 0.1% Thimerosal, NaN3, at concentration less than 250 KBq (6.75 µCi) per vial. Radioactive cortisol was stable at 2-8°C until the expiry date.

3) Standards: The vials of standard cortisol consisting of human serum and 0.1% sodium azide and Kathon, at concentration 2000 nmol/l. These standards were stable at 2-8°C until the expiry date and for 8 weeks after reconstitution.

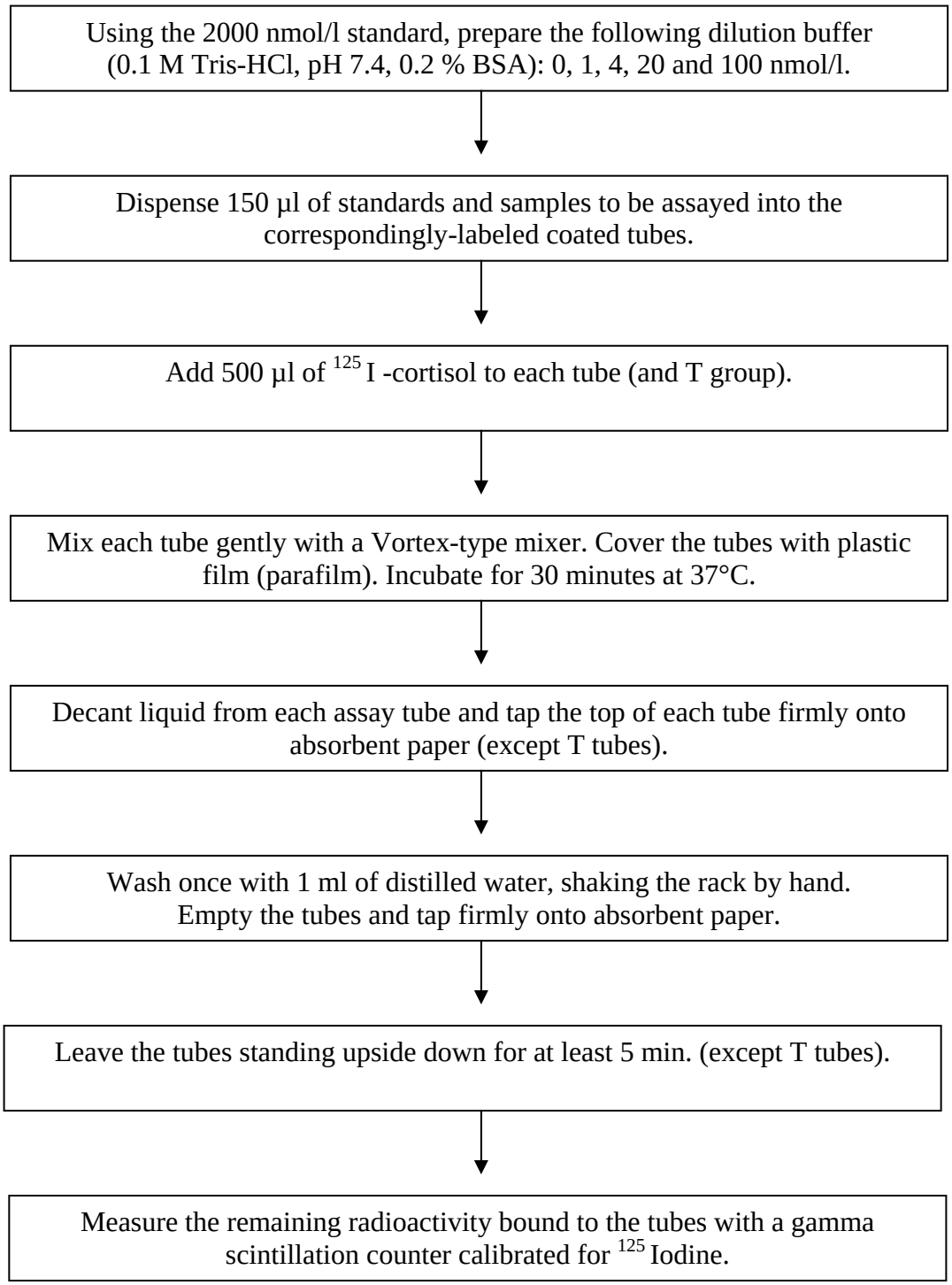

Figure 4.2 Summary of radioimmunoassay of salivary cortisol

4.3.5 Assay procedure

4.3.5.1 Reconstitution of the standards

Reconstitute the standards with 0.5 ml of distilled water. Recap the vial. Mix gently to ensure complete dissolution of the lyophilized material. The reconstituted standards should stand at least 30 minutes after reconstitution before proceeding.

4.3.5.2 Protocol

All reagents should be brought to room temperature (18-25°C) at least 30 minutes before their use. Dispensing of reagents is also carried out at room temperature.

1) Plain tubes were labeled for T group for the total activity determination and antibody coated tubes for standard groups (to establish the standard curve) and samples in duplicate.

2) Using the 2000 nmol/l standard, prepare the following dilution buffer (0.1 M Tris-HCl, pH 7.4, 0.2 % BSA): 0, 1, 4, 20 and 100 nmol/l. Prepare the 0 standard with the buffer.

3) Dispense 150 µl of standards, controls and samples to be assayed into the correspondingly-labeled coated tubes.

4) Add 500 µl of ^{125}I -cortisol to each tube (and T group)

5) Mix each tube gently with a Vortex-type mixer. Cover the tubes with plastic film (parafilm). Incubate for 30 minutes at 37°C.

6) Decant liquid from each assay tube and tap the top of each tube firmly onto absorbent paper (except T tubes).

7) Wash once with 1 ml of distilled water, shaking the rack by hand.

8) Empty the tubes and tap firmly onto absorbent paper. Leave the tubes standing upside down for at least 5 min. (except T tubes).

9) Measure the remaining radioactivity bound to the tubes with a gamma scintillation counter (Figure 4.3) calibrated for ^{125} Iodine.

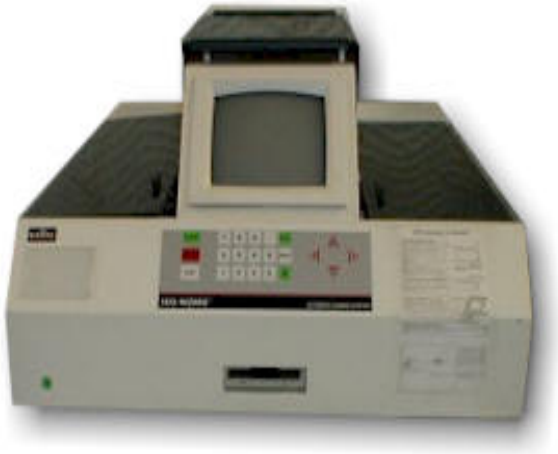

Figure 4.3 Wallac 1470 gamma scintillation counter (Perkin Elmer)

4.4 Statistical analysis

Data were expressed as mean ± standard deviation of the mean (mean ± SD) values derived from experiments indicated. Significant level was set at $p < 0.05$ for all parameters.

Comparison of age, body weight, height and body mass index (BMI) between male and female groups was done using unpaired Student's t-test.

Single factor repeated measures One-way ANOVA was used to examine the alterations of salivary cortisol concentrations. Post-hoc analysis was performed using Tukey's HSD. All statistical analyses were conducted using SPSS 11.0 statistical software.

CHAPTER V
RESULTS

5.1 Physiological characteristics

Physiological characteristics of the musician and control groups were summarized in Table 5.1 and the general information of subjects showed in Table 5.2. The musician and control groups showed insignificant difference on physiological variables in terms of age, weight, height and body mass index (BMI).

5.2 Stress inventory

All subjects were asked to answer the stress inventory on days with and without an anticipated examination at similar time points. The stress inventory (Department of Mental Health, Ministry of Public Health, Thailand) was used to measure the level of stress in the subjects, there contains 20 items. Each item is scored from 0 to 3. Higher scores indicate higher levels of stress. The results of stress inventory scores were separated into 5 groups; less than normal, normal, higher than normal, moderately higher than normal and extremely higher than normal (Table 5.3). At the higher than normal level of post-examination, the stress level of the musician group was less than the control group [4 (13.33%) and 6 (20%), respectively], but not significantly different. The result of Spearman correlation coefficients between saliva cortisol concentration and stress inventory (Table 5.4) showed a significant association between saliva cortisol level and stress inventory scales in the post-examination of musician group ($p < 0.05$).

Table 5.1 Physiological characteristics (mean ± SD) of all subjects.

Parameter	Control group			Musician group		
	Male	**Female**	**Total**	**Male**	**Female**	**Total**
Sample size (number)	9	21	30	13	17	30
Age (years)	15.89 ± 0.33	15.33 ± 0.48	15.5 ± 0.51	15.46 ± 0.66	15.88 ± 0.49	15.70 ± 0.60
Weight (kg)	59.22 ± 13.03	51.45 ± 10.03	53.78 ± 11.37	57.69 ± 9.10	51.71 ± 7.39	54.30 ± 8.57
Height (m)	1.70 ± 0.07	1.60 ± 0.04	1.63 ± 0.07	1.71 ± 0.06	1.61 ± 0.05	1.66 ± 0.08
BMI (kg/m^2)	20.32 ± 3.17	20.07 ± 3.68	20.14 ± 3.48	19.59 ± 2.79	19.91 ± 2.36	19.77 ± 2.51

Table 5.2 The general information of all subjects.

No.	Sex	Age (Y)	Wt (kg)	Ht (m)	BMI (kg/m^2)	Age of onset (Y)	Musical instr	Stress level (scores)	
								Baseline	Post-exam
C1	F	16	44.5	1.63	16.75	-	-	17	8
C2	F	15	48	1.59	18.99	-	-	7	15
C3	F	15	46	1.60	17.97	-	-	8	8
C4	F	16	53	1.55	22.06	-	-	15	17
C5	F	16	43	1.60	16.80	-	-	19	18
C6	M	16	55	1.66	19.96	-	-	14	19
C7	M	16	52	1.75	16.98	-	-	8	4
C8	M	16	55	1.72	18.59	-	-	21	23
C9	F	15	52	1.64	19.33	-	-	15	20
C10	F	16	48	1.60	18.75	-	-	8	7
C11	F	15	48	1.60	18.75	-	-	13	13
C12	F	15	72	1.67	25.82	-	-	24	18
C13	F	15	50	1.63	18.82	-	-	21	13
C14	F	15	49	1.56	20.13	-	-	7	1
C15	F	15	41	1.56	16.85	-	-	13	14
C16	F	16	56	1.61	21.60	-	-	21	4
C17	M	16	90	1.80	27.78	-	-	10	2
C18	M	16	61	1.68	21.61	-	-	9	11
C19	M	15	45	1.60	17.58	-	-	13	11
C20	M	16	55	1.66	19.96	-	-	16	18

Y = years kg = kilograms m = meters BMI = body mass index
Wt = weight Ht = height F = female M = male
Musical instr = musical instrument

Table 5.2 The general information of all subjects (cont.)

No.	Sex	Age (Y)	Wt (kg)	Ht (m)	BMI (kg/m^2)	Age of onset (Y)	Musical instr.	Stress level (scores)	
								Baseline	Post-exam
C21	F	15	40	1.58	16.02	-	-	7	8
C22	F	15	49	1.64	18.22	-	-	15	14
C23	F	15	83	1.59	32.83	-	-	13	14
C24	F	15	50	1.57	20.28	-	-	13	9
C25	F	15	56	1.61	21.60	-	-	12	8
C26	F	16	59	1.68	20.90	-	-	13	12
C27	F	16	48	1.54	20.24	-	-	19	17
C28	F	15	45	1.55	18.73	-	-	8	7
C29	M	16	53	1.65	19.47	-	-	14	6
C30	M	16	67	1.79	20.91	-	-	8	6
M1	M	16	60	1.80	18.52	14	piano	14	11
M2	F	16	40	1.57	16.23	12	brass	22	19
M3	F	15	49	1.55	20.53	12	brass	18	15
M4	F	16	57	1.62	21.72	5	piano	9	8
M5	F	16	45	1.61	17.47	10	keyboard	12	19
M6	M	16	53	1.70	18.34	6	brass	21	16
M7	F	15	55	1.60	21.48	6	brass	6	8
M8	F	16	58	1.61	22.52	3	bowed string	25	15
M9	F	16	64	1.68	22.68	9	khim	24	19
M10	F	16	48	1.58	19.23	10	brass	24	17

Y = years kg = kilograms m = meters BMI = body mass index
Wt = weight Ht = height F = female M = male
Musical instr = musical instrument

Table 5.2 The general information of all subjects (cont.)

No.	Sex	Age (Y)	Wt (kg)	Ht (m)	BMI (kg/m^2)	Age of onset (Y)	Musical instr.	Stress level (scores)	
								Baseline	Post-exam
M11	F	17	65	1.69	22.76	10	brass	4	6
M12	M	15	63	1.67	22.59	12	brass	7	16
M13	M	15	67	1.80	20.68	10	brass	7	4
M14	M	16	58	1.75	18.94	10	brass	19	11
M15	F	16	56	1.56	23.01	13	guitar	2	6
M16	M	17	60	1.70	20.76	14	guitar	11	6
M17	M	15	60	1.70	20.76	12	brass	11	7
M18	M	15	55	1.78	17.36	10	keyboard	12	12
M19	F	16	50	1.56	20.55	12	brass	24	23
M20	F	16	48	1.59	18.99	11	brass	12	9
M21	F	15	52	1.59	20.57	3	keyboard	18	16
M22	F	16	45	1.66	16.43	13	guitar	24	22
M23	M	16	41	1.56	16.85	14	drum	14	13
M24	M	15	48	1.70	16.61	13	guitar	11	11
M25	F	16	58	1.70	20.07	10	guitar	10	8
M26	F	16	49	1.65	18.00	11	ranat	16	14
M27	F	16	40	1.57	16.23	5	piano	19	16
M28	M	15	56	1.70	19.38	10	guitar	11	10
M29	M	15	78	1.71	26.67	14	guitar	4	9
M30	M	15	51	1.72	17.24	4	piano	17	13

Y = years kg = kilograms m = meters BMI = body mass index
Wt = weight Ht = height F = female M = male
Musical instr = musical instrument

Table 5.3 Stress level compare between musician and control groups on day with or without academic examination.

Stress level (Scores)	**Control**				**Musician**			
	Baseline		**Post-exam**		**Baseline**		**Post-exam**	
	N	%	N	%	N	%	N	%
Less than normal (0-5)	0	0	4	13.33	2	6.67	2	6.67
Normal (6-17)	23	76.67	20	66.67	18	60.00	24	80.00
Higher than normal (18-25)	7	23.33	6	20	10	33.33	4	13.33
Moderately higher than normal (26-29)	-	0	-	0	-	0	-	0
Extremely higher than normal (30-60)	-	0	-	0	-	0	-	0

Table 5.4 Spearman correlation coefficients between saliva cortisol concentration and stress inventory

Group	**Correlation coefficients**			
	Baseline	P value	Post-examination	P value
Control	-0.045	0.813	0.106	0.578
Musician	-0.147	0.437	-0.405 *	0.026

** Correlation is significant at the 0.05 level (2-tailed).*

The result showed a significant association between saliva cortisol level and stress inventory scales in the post-examination of musician group ($p < 0.05$).

5.3 Saliva cortisol concentration

The comparability of cortisol levels between baseline, pre-and post-examination was ensured by matching the time of harvesting salivary samples between musician and control groups (Figure 5.1, Table 5.5). The mean saliva cortisol concentration of pre-examination of musician group was significantly lower than the control group ($p < 0.001$). In control group, mean saliva cortisol concentration significantly increased from baseline to pre-examination ($p < 0.001$) and significantly fell from pre-examination to post-examination ($p < 0.001$) whereas in musician group had no significant change. From the results (Table 5.5), saliva cortisol concentration response by gender upon academic examination stress. Pre-examination cortisol levels differed between musician and control, both female and male musicians had significantly lower cortisol levels than female control ($p < 0.001$). Neither baseline cortisol nor post-examination cortisol levels showed significant gender differences between musician and control groups (see Figure 5.2). In female control, mean saliva cortisol concentration significantly increased from baseline to pre-examination ($p < 0.001$) and significantly declined from pre-examination to post-examination ($p < 0.001$) whereas in the other group had no significant change. Nevertheless, no gender differences in the saliva cortisol responses among group.

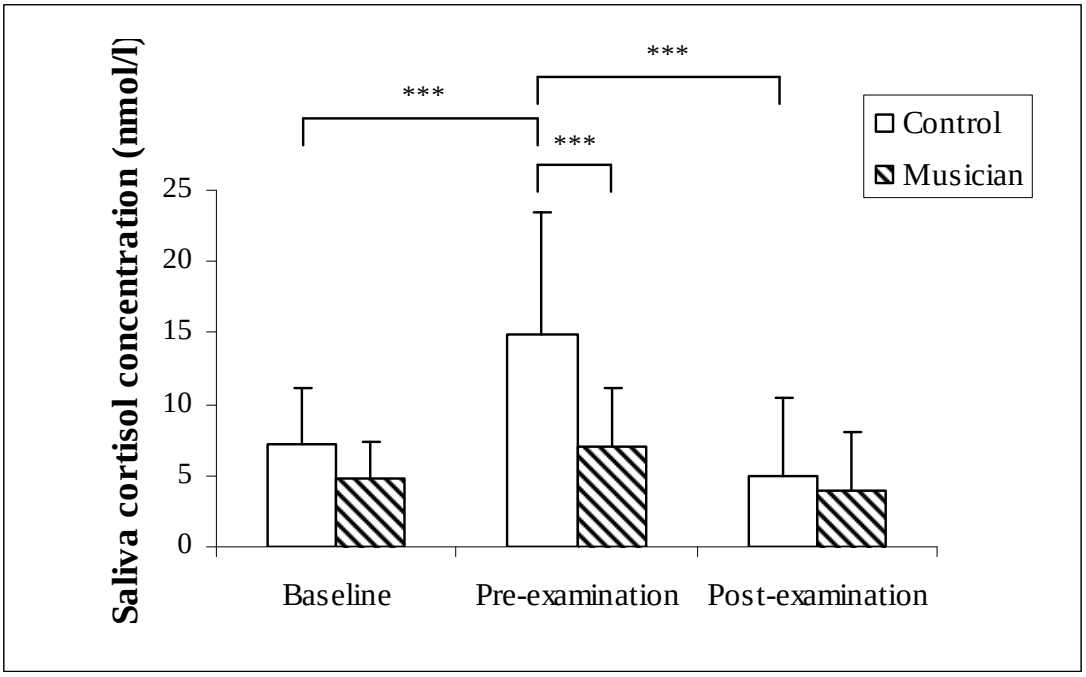

Figure 5.1 Saliva cortisol concentration (mean ± SD) was compared between musician and control groups. The mean saliva cortisol concentration of pre-examination of musician group was significantly lower than the control group (***$p <$ 0.001). In control group, mean saliva cortisol concentration significantly increased from baseline to pre-examination and significantly fell from pre-examination to post-examination whereas in musician group had no significant change.

Table 5.5 Saliva cortisol concentration (mean ± SD) was compared between musician and control groups at baseline, pre- and post- examination.

Group	N	Baseline (nmol/l)	Pre-exam (nmol/l)	Post-exam (nmol/l)
Total control	30	7.23 ± 3.85 [a,***]	14.91 ± 8.63	4.90 ± 5.46 [a,***]
Female control	24	7.52 ± 3.97 [a,***]	15.89 ± 9.12	5.10 ± 6.05 [a,***]
Male control	6	6.06 ± 3.39	10.99 ± 5.12	4.09 ± 1.92
Total musician	30	4.74 ± 2.62	7.03 ± 4.14 [b,***]	3.92 ± 4.11
Female musician	17	5.03 ± 2.98	6.14 ± 3.79 [b,***]	3.52 ± 3.83
Male musician	13	4.35 ± 2.09	8.18 ± 4.44 [b,***]	4.44 ± 4.56

*** $p < 0.001$

[a] Significantly different from value at pre-examination period within group

[b] Significantly different between musician and control groups at pre-examination period

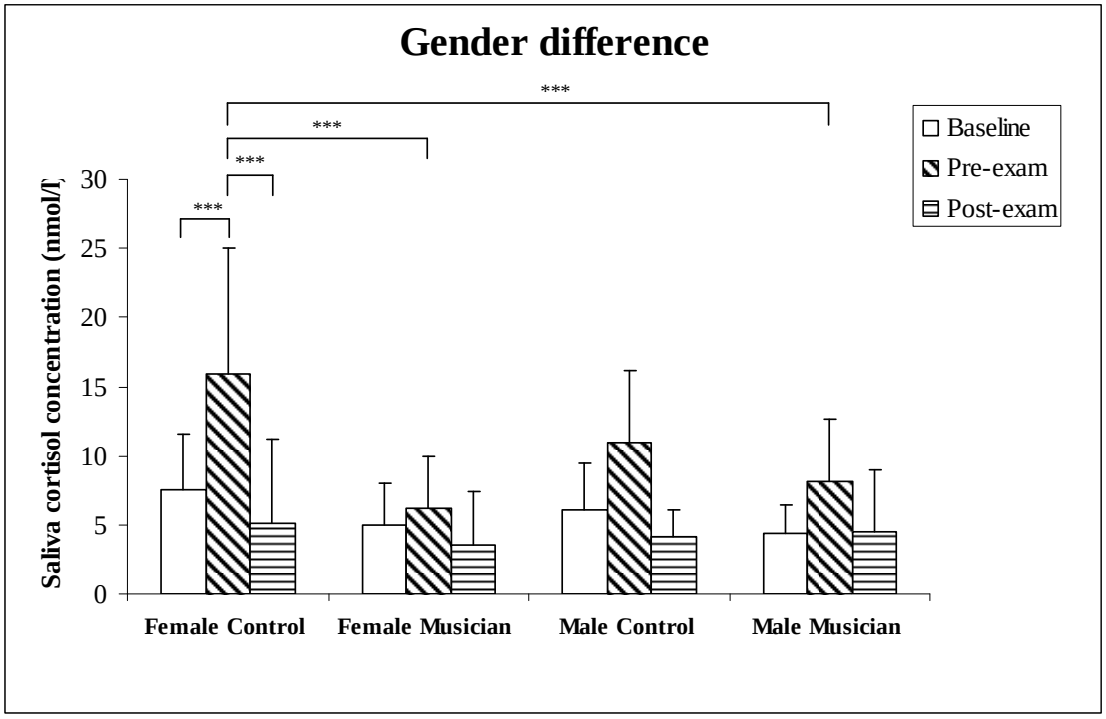

Figure 5.2 The saliva cortisol concentration response (mean ± SD) by gender upon academic examination stress. Pre-examination cortisol levels differed between musician and control, both female and male musicians had significantly lower cortisol levels than female control (*** $p < 0.001$). In female control, mean saliva cortisol concentration significantly increased from baseline to pre-examination (*** $p < 0.001$) and significantly declined from pre-examination to post-examination (*** $p < 0.001$) whereas in the other group had no significant change. Nevertheless, no gender differences in the saliva cortisol responses among group.

5.4 Grade point average (GPA) and cortisol levels

The stress level, saliva cortisol concentration and grade point average (GPA) of all subjects showed in the Table 5.6. The results of saliva cortisol responses among GPA rank are shown in Figure 5.3 and Table 5.7. From the results, saliva cortisol responses (mean ± SD) among grade point average (GPA) rank of all subjects. At GPA 3.50-4.00 range, pre-examination cortisol levels differed between musician and control, with musician having significantly lower cortisol levels than control ($p < 0.001$). Interestingly, control had statistically higher mean value of cortisol at pre-examination than baseline and post-examination, respectively ($p < 0.001$), whereas the musician group had no significantly different. Moreover, at GPA 3.00-3.49 range, the control group had significantly higher pre-examination cortisol levels than post-examination ($p < 0.001$), no differences between pre-examination and post-examination cortisol levels in the musician group were observed.

Table 5.6 Stress level, saliva cortisol concentration and grade point average (GPA) of all subjects.

No.	GPA (total = 4)	Saliva cortisol (nmol/l)			Stress level (scores)	
		Baseline	Pre-exam	Post-exam	Baseline	Post-exam
C1	3.52	8.46	48.97	2.98	17	8
C2	3.37	16.82	28.02	8.53	7	15
C3	3.95	5.83	22.92	3.30	8	8
C4	3.92	8.85	22.22	1.92	15	17
C5	3.90	3.16	20.81	14.84	19	18
C6	3.87	6.09	18.89	4.62	14	19
C7	3.05	11.87	18.08	6.91	8	4
C8	3.38	11.27	17.77	3.41	21	23
C9	3.49	11.15	17.01	3.92	15	20
C10	3.70	5.57	16.99	3.72	8	7
C11	3.31	7.29	16.87	1.28	13	13
C12	3.52	3.93	16.19	11.38	24	18
C13	3.57	12.44	15.37	1.37	21	13
C14	3.69	10.56	15.34	2.85	7	1
C15	2.98	9.74	15.25	4.41	13	14
C16	2.42	4.52	13.60	5.41	21	4
C17	3.06	4.96	12.19	1.32	10	2
C18	3.65	8.34	12.03	3.14	9	11
C19	3.29	2.24	11.67	3.53	13	11
C20	3.21	5.67	11.11	3.42	16	18

Table 5.6 Stress level, saliva cortisol concentration and grade point average (GPA) of all subjects (cont.)

No.	GPA (total = 4)	Saliva cortisol (nmol/l)			Stress level (scores)	
		Baseline	Pre-exam	Post-exam	Baseline	Post-exam
C21	2.91	4.43	10.84	1.00	7	8
C22	3.16	3.64	10.31	3.52	15	14
C23	3.26	4.23	9.95	2.59	13	14
C24	3.77	4.38	8.84	2.42	13	9
C25	3.68	6.27	7.88	29.18	12	8
C26	3.71	3.76	7.22	1.65	13	12
C27	3.24	16.14	6.30	2.83	19	17
C28	2.81	3.16	5.24	4.20	8	7
C29	2.86	3.52	4.79	3.59	14	6
C30	3.98	8.60	4.59	3.85	8	6
M1	3.96	7.12	2.43	14.36	14	11
M2	3.89	7.13	2.63	2.04	22	19
M3	3.96	2.86	2.66	0.60	18	15
M4	3.91	5.72	2.67	2.17	9	8
M5	4.00	3.36	3.49	2.25	12	19
M6	3.97	4.92	3.52	6.31	21	16
M7	3.92	12.16	3.79	16.12	6	8
M8	3.90	4.25	3.80	4.51	25	15
M9	3.10	3.08	4.01	2.80	24	19
M10	2.91	5.21	4.04	3.73	24	17

Table 5.6 Stress level, saliva cortisol concentration and grade point average (GPA) of all subjects (cont.)

No.	GPA (total = 4)	Saliva cortisol (nmol/l)			Stress level (scores)	
		Baseline	Pre-exam	Post-exam	Baseline	Post-exam
M11	3.12	3.73	4.46	4.39	4	6
M12	2.84	1.55	4.61	0.85	7	16
M13	2.78	5.03	5.03	13.27	7	4
M14	3.09	2.79	5.49	1.91	19	11
M15	2.96	5.49	5.49	3.83	2	6
M16	3.02	4.62	5.74	6.44	11	6
M17	3.86	1.89	5.79	1.40	11	7
M18	3.52	1.89	6.99	0.16	12	12
M19	3.06	1.71	7.39	0.10	24	23
M20	3.63	3.84	7.54	1.06	12	9
M21	3.20	3.70	8.07	2.85	18	16
M22	2.61	2.60	8.76	0.70	24	22
M23	2.39	1.50	9.25	1.89	14	13
M24	3.20	4.14	9.31	1.30	11	11
M25	3.33	4.02	9.36	4.95	10	8
M26	3.25	7.70	13.41	1.25	16	14
M27	3.62	8.26	15.02	1.84	19	16
M28	3.08	4.99	15.10	1.74	11	10
M29	2.92	5.64	15.32	5.76	4	9
M30	3.85	11.17	15.51	7.10	17	13

Table 5.7 Saliva cortisol concentration (mean ± SD) among grade point average (GPA) rank of all subjects.

Group	GPA rank	N	Baseline (nmol/l)	Pre-exam (nmol/l)	Post-exam (nmol/l)
Control	3.50 - 4.00	14	6.87 ± 2.75 [a,***]	17.02 ± 10.86	6.23 ± 7.64 [a,***]
	3.00 - 3.49	11	8.66 ± 5.06	14.48 ± 5.90	3.75 ± 2.18 [a,***]
	2.00 - 2.99	5	5.07 ± 2.67	9.95 ± 4.77	3.72 ± 1.66
Musician	3.50 - 4.00	13	5.74 ± 3.30	5.83 ± 4.50 [b,***]	4.61 ± 5.18
	3.00 - 3.49	10	4.05 ± 1.59	8.23 ± 3.70	2.77 ± 1.95
	2.00 - 2.99	7	3.86 ± 1.89	7.50 ± 4.01	4.29 ± 4.36

*** $p < 0.001$

[a] Significantly different from value at pre-examination period among GPA rank.

[b] Significantly different between musician and control groups at pre-examination period.

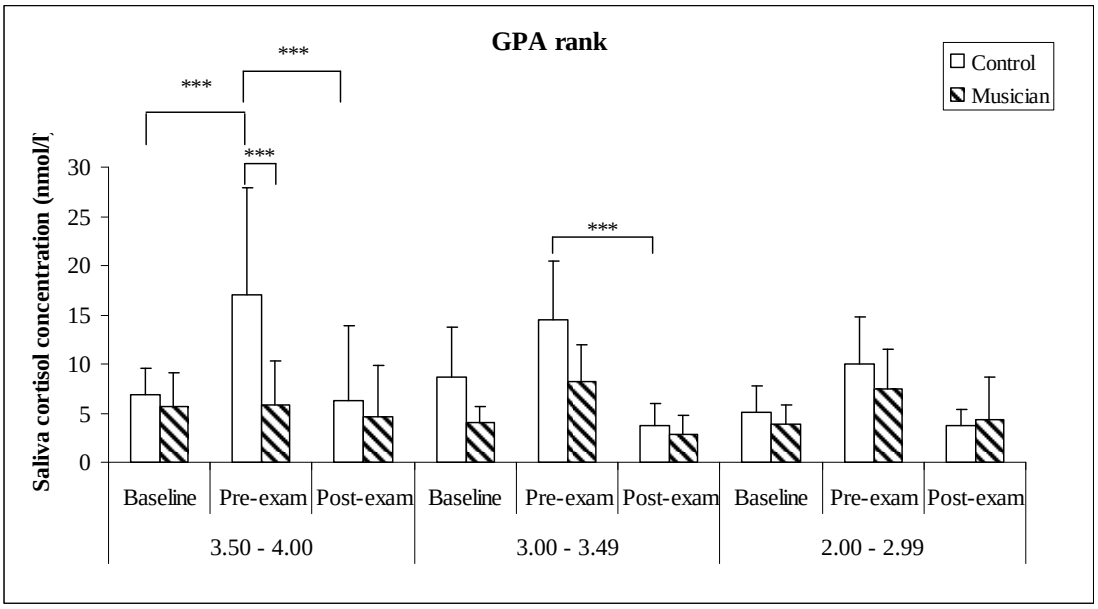

Figure 5.3 Saliva cortisol responses (mean ± SD) among grade point average (GPA) rank of all subjects. At GPA 3.50-4.00 range, pre-examination cortisol levels differed between musician and control, with musician having significantly lower cortisol levels than control (***$p < 0.001$). Interestingly, control had statistically higher mean value of cortisol at pre-examination than baseline and post-examination, respectively (***$p < 0.001$). Moreover, at GPA 3.00-3.49 range, the control group had significantly higher pre-examination cortisol levels than post-examination (***$p < 0.001$), whereas the musician group had no statistically significant.

5.5 Factors influencing cortisol levels

The results showed the effects of factors on the salivary cortisol response. Important factors include sleep and coffee consumption. Table 5.8 showed sleep duration and coffee consumption. In the sleep duration effects, a repeated measures ANOVA was used to analyze sleep duration on the salivary cortisol responses within and between musician and control groups at baseline and post-examination (see Figure 5.4, Table 5.9 Results revealed no significant difference between musician and control groups. In addition, the effects of coffee consumption on salivary cortisol responses also showed no significant difference between musician and control groups (Figure 5.5, Table 5.10).

Table 5.8 Sleep duration and coffee consumption

No.	Sleep duration of last night (hours)		Coffee consumption	
	Baseline	**Post-exam**	**Baseline**	**Post-exam**
C1	5 - 6	5 - 6	not drink	1-2 cups/day
C2	5 - 6	5 - 6	not drink	not drink
C3	7 - 8	7 - 8	not drink	not drink
C4	3 - 4	3 - 4	not drink	not drink
C5	5 - 6	7 - 8	not drink	not drink
C6	5 - 6	5 - 6	not drink	not drink
C7	7 - 8	7 - 8	not drink	not drink
C8	7 - 8	7 - 8	not drink	not drink
C9	5 - 6	3 - 4	1-2 cups/day	1-2 cups/day
C10	7 - 8	3 - 4	not drink	not drink
C11	7 - 8	5 - 6	not drink	not drink
C12	5 - 6	5 - 6	not drink	not drink
C13	5 - 6	5 - 6	1-2 cups/day	1-2 cups/day
C14	7 - 8	7 - 8	not drink	not drink
C15	5 - 6	5 - 6	not drink	not drink
C16	3 - 4	5 - 6	not drink	not drink
C17	5 - 6	7 - 8	not drink	3 cups/day
C18	5 - 6	3 - 4	not drink	not drink
C19	7 - 8	7 - 8	not drink	not drink
C20	7 - 8	5 - 6	not drink	not drink

Table 5.8 Sleep duration and coffee consumption (cont.)

No.	Sleep duration of last night (hours)		Coffee consumption	
	Baseline	**Post-exam**	**Baseline**	**Post-exam**
C21	7 - 8	5 - 6	not drink	not drink
C22	> 8 hours	7 - 8	not drink	not drink
C23	5 - 6	3 - 4	not drink	not drink
C24	7 - 8	7 - 8	not drink	not drink
C25	5 - 6	5 - 6	1-2 cups/day	not drink
C26	5 - 6	5 - 6	not drink	not drink
C27	7 - 8	7 - 8	not drink	1-2 cups/day
C28	5 - 6	5 - 6	not drink	not drink
C29	7 - 8	7 - 8	not drink	not drink
C30	7 - 8	7 - 8	not drink	not drink
M1	5 - 6	5 - 6	not drink	not drink
M2	5 - 6	7 - 8	not drink	not drink
M3	3 - 4	3 - 4	not drink	not drink
M4	3 - 4	3 - 4	not drink	not drink
M5	3 - 4	3 - 4	not drink	not drink
M6	5 - 6	5 - 6	not drink	not drink
M7	7 - 8	7 - 8	not drink	not drink
M8	5 - 6	3 - 4	not drink	not drink
M9	3 - 4	5 - 6	not drink	not drink
M10	5 - 6	5 - 6	not drink	not drink

Table 5.8 Sleep duration and coffee consumption (cont.)

No.	Sleep duration of last night (hours)		Coffee consumption	
	Baseline	**Post-exam**	**Baseline**	**Post-exam**
M11	7 - 8	7 - 8	not drink	not drink
M12	5 - 6	5 - 6	not drink	not drink
M13	> 8 hours	> 8 hours	1-2 cups/day	1-2 cups/day
M14	3 - 4	5 - 6	not drink	not drink
M15	5 - 6	7 - 8	not drink	not drink
M16	5 - 6	3 - 4	not drink	not drink
M17	5 - 6	3 - 4	1-2 cups/day	1-2 cups/day
M18	7 - 8	5 - 6	not drink	not drink
M19	5 - 6	5 - 6	not drink	not drink
M20	7 - 8	7 - 8	not drink	not drink
M21	7 - 8	5 - 6	not drink	not drink
M22	5 - 6	7 - 8	1-2 cups/day	not drink
M23	7 - 8	5 - 6	not drink	not drink
M24	7 - 8	3 - 4	not drink	not drink
M25	5 - 6	7 - 8	not drink	not drink
M26	5 - 6	5 - 6	not drink	not drink
M27	5 - 6	7 - 8	not drink	not drink
M28	5 - 6	5 - 6	not drink	not drink
M29	7 - 8	> 8 hours	not drink	not drink
M30	5 - 6	5 - 6	not drink	not drink

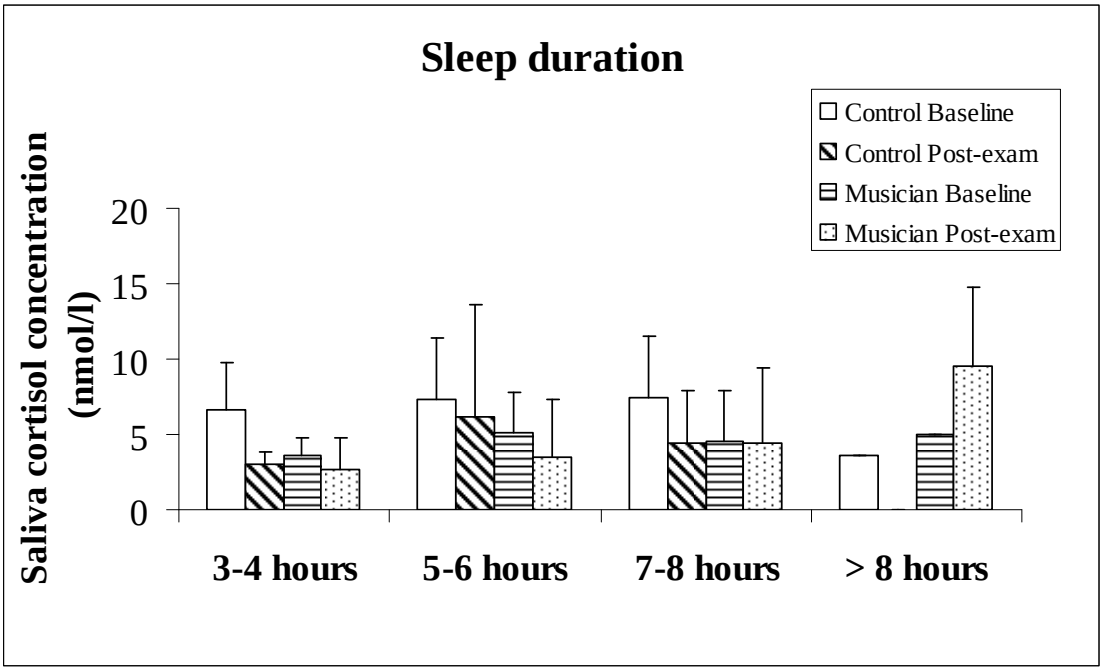

Figure 5.4 Effects of the sleep duration (hours of sleep last night) on the mean of saliva cortisol concentration (nmol/l). A repeated measures ANOVA was used to analyze sleep duration on the salivary cortisol responses within and between musician and control groups at baseline and post-examination. Results revealed no significant difference between musician and control groups.

Table 5.9 Effects of the sleep duration (hours of last night sleep) on the mean saliva cortisol concentration (nmol/l). Data presented as group mean ± SD.

Group	Sleep duration (hours)							
	N	**3 - 4 hours**	**N**	**5 - 6 hours**	**N**	**7 - 8 hours**	**N**	**> 8 hours**
Control baseline	2	6.68 ± 3.06	14	7.32 ± 4.07	13	7.49 ± 3.99	1	3.64 ± 0.00
Control post-exam	5	3.06 ± 0.82	13	6.11 ± 7.55	12	4.36 ± 3.55	-	-
Musician baseline	5	3.56 ± 1.23	16	5.16 ± 2.64	8	4.58 ± 3.33	1	5.03 ± 0.00
Musician post-exam	7	2.67 ± 2.07	13	3.46 ± 3.90	8	4.37 ± 5.00	2	9.52 ± 5.31

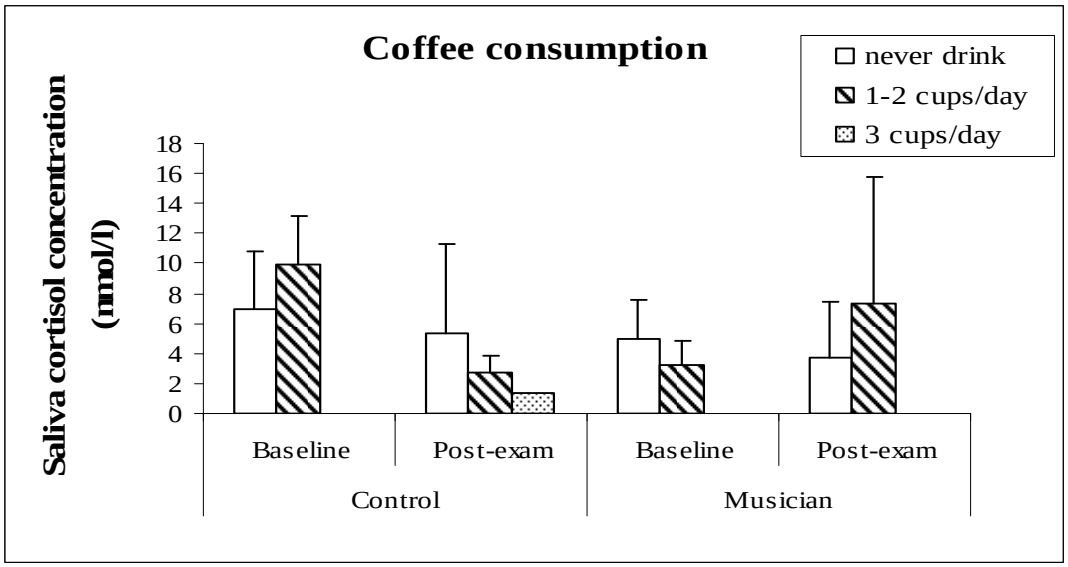

Figure 5.5 Effects of the coffee consumption on the mean of saliva cortisol concentration (nmol/l). Effects of coffee consumption on salivary cortisol responses showed no significant difference between musician and control groups.

Table 5.10 Effects of the coffee consumption on the mean of saliva cortisol concentration (nmol/l). Data presented as group mean ± SD.

Group	Coffee consumption					
	N	not drink	N	1 - 2 cups/day	N	3 cups/day
Control baseline	27	6.93 ± 3.85	3	9.95 ± 3.25	-	-
Control post-exam	25	5.39 ± 5.86	4	2.77 ± 1.05	1	1.32 ± 0.00
Musician baseline	30	4.91 ± 2.67	-	-	-	-
Musician post-exam	28	3.68 ± 3.82	2	7.33 ± 8.39	-	-

CHAPTER VI
DISCUSSION

6.1 Academic examination and stress responses

Academic examinations have often been used in stress research as early as 1914 because they are “predictable, standardized, and discrete examples of real-life stressors” (Stowell, 2003). Undergoing academic examinations has been associated with changes in mental and physical health including increased anxiety, increased negative mood, changed in hormone levels such as cortisol, immune function, and wound healing. In school, adolescents often see themselves as being evaluated in terms of their academic performance and the pressure to excel is an important measure of their success in school. Specifically, in an Asian context, academic stress arising from adolescents’ self-expectations and expectations of others (e.g., parents and teachers) are particularly salient. Academic achievement is highly valued by Asians because it is perceived as one of the few avenues for upward mobility and expanded options, thus the significance that individuals and families attribute to academic success is intensified (Ang and Huan, 2006).

In the present studies, the analysis of stress response included 30 musician and 30 control groups. All subjects were asked to answer the stress inventory on days with and without an anticipated examination at similar time points. The stress inventory (Department of Mental Health, Ministry of Public Health, Thailand) was used to measure the level of stress in the subjects, there contains 20 items. Each item is scored from 0 to 3. Higher scores indicate higher levels of stress. Our results found that both groups showed similar responses with respect to stress scores to academic examination stress. According to our finding self-report questionnaires offered valuable information on individual response to academic examination stress. Pruessner and colleague (1999) found that stress responses to psychological challenges are associated with levels of self-esteem and positive and/or negative affect in individuals. Therefore, to reduce susceptibility from bias, less reliance on self-report measures of

stress and more direct markers of neuroendocrinological agents (e.g., saliva cortisol) are necessary. Although academic examinations represent stressful challenges to many students, studies on the examination-dependent cortisol response, a sensitive physiological indicator for a stress response, are inconsistent. Some studies report that examinations lead to an increase of cortisol (Malarkey et al., 1995; Armario et al., 1996; Lacey et al., 2000), whereas other studies describe either no effect (Malarkey et al., 1995; Dobbin et al., 1991) or a decrease of cortisol levels upon examinations (Vedhara et al., 2000). In the present studies, the pre-examination period shows an increase saliva cortisol levels. Interestingly, the mean saliva cortisol concentration of pre-examination of musician group was significantly lower than the control group ($p < 0.001$).

The cortisol concentrations before anticipated examinations were higher than those on days without an examination (Lacey et al. 2000; Martinek et al., 2003; Spangler 1997; Vedhara et al. 2000). This finding was commensurate with our observation that the magnitude of increase of stress response correlate with the examination and non-examination periods. Our finding indicates that pre-academic examination period directly affects stress levels in all subjects.

6.2 Music and stress responses

Music listening and playing altered steroid levels agrees with the results of various previous studies that have documented strong correlations between steroids (Suda et al, 2008), spatial perception and cognition (Schellenberg, 2004). Music can elicit not only psychological mood changes, but also physiological changes, for example in heart rate and respiration. Music-induced emotion has been shown to recruit the reward–motivational circuit, including the basal forebrain, midbrain and orbitofrontal regions, as well as the amygdala (Zatorre et al., 2007; Levitin and Tirovolas, 2009). In this behavioral results showed that at the higher than normal level of post-examination, the stress level of the musician group was less than the control group [4 (13.33%) and 6 (20%), respectively], but not significantly different. This finding supported by music may also be a means through which people are able to

cope with emotional conflicts, increase their self-awareness, and express their unspoken and often unconscious concern (Boso et al., 2006).

From previous studies, Fukui and Toyoshima (2008) reported that the effects of music on steroids are unclear, but music appears to be involved with steroid production via the pathway from the auditory system to the auditory area, particularly the neural pathway (emotion circuits) mediated by the cerebral limbic system (hypothalamic-pituitary- adrenal axis and amygdaloid complex). In this research, musician's salivary cortisol levels demonstrated a most significant lower than the control group at baseline, pre- and post-examination. This is similar to Watkins (1997) finding that music may facilitate a reduction in the stress response include decreased anxiety levels, decreased blood pressure and heart rate, and changes in plasma stress hormone levels. These results suggested that music was affecting the reduction of the saliva cortisol concentration.

6.3 School performance and stress responses

School achievement has been measured by using different parameters: grade point average (GPA), self-reported average grades, teacher comments/ behavior ratings, parent reports, and school behavior (Curcio et al., 2006). In this study, at GPA 3.50-4.00 range, the pre-examination cortisol levels of musician having significantly lower than control ($p < 0.001$). Additionally, control had statistically higher mean value of salivary cortisol at pre-examination than baseline and post-examination, respectively ($p < 0.001$), whereas musician had no significantly different. Moreover, at GPA 3.00-3.49 range, control had significantly higher pre-examination cortisol levels than post-examination ($p < 0.001$), no differences between pre-examination and post-examination cortisol levels in musician were observed. These results indicated that at the similar GPA range, music had significant effect on the stress response.

These results supported by Fröjd and colleague (2008) reported that while an improvement in the GPA per se might be associated with depression because of a possible loss of popularity among peers or overwhelming stress and tiredness resulting from the process of getting to that higher GPA. Previous studies suggest that self-esteem might play a significant role in the regulation of the HPA axis. The individuals

scoring high on self-esteem scales have lower free cortisol responses to an experimental stressor (Seeman et al., 1995; Zorrilla et al., 1995; Pruessner et al., 1999). Additionally, Buttsworth and Smith (1995) found that the musicians were more emotionally stable, more sensitive and less anxious than the non musician comparison group.

6.4 Coffee consumption and stress responses

Caffeine is a widely consumed pharmacologic agent found in coffee, tea, and soft drinks. Its popularity is attributable to its effects in the nervous system, including its ability to increase rates of dopamine release in the anterior cingulate gyrus. Caffeine also activates the stress axis, elevating glucocorticoid and catecholamine, output along with increases in blood pressure. As such, caffeine intake during times of stress may contribute to the duration and magnitude of blood pressure and stress endocrine responses. Caffeine in dietary doses increases both ACTH and cortisol secretion in humans. Caffeine's effect on glucocorticoid regulation therefore has the potential to alter circadian rhythms and to interact with stress reactions (Lovallo et al., 2005).

In these studies found that the effects of coffee consumption on salivary cortisol responses also showed no significant difference between musician and control groups. These results supported by some previous studies have not observe increases in neither basal free nor total cortisol levels after consumption of coffee or tea (Quinlan et al., 1997; Lane et al., 2002; Lovallo et al., 2006; MacKenzie et al., 2007; Steptoe et al., 2007), while other studies have found that caffeine may elevate cortisol secretion in humans at rest and during mental stress (Lovallo et al., 1996; Lane et al., 2002; Lovallo et al., 2005).

6.5 Sleep duration and stress responses

Sleep is an active, repetitive and reversible behavior serving several different functions, such as repair and growth, learning or memory consolidation, and restorative processes: all these occur throughout the brain and the body. Thus, during

sleep behavioral, physiological and neurocognitive processes occur: these very processes are susceptible to be impaired by the absence of sleep (Curcio et al., 2006). In addition, cortisol provides an important link between the immune system, sleep and psychological stress. Sleep disruption and sustained psychological stress increase cortisol concentrations in the blood. Indeed, one night of lost sleep can raise cortisol concentrations by almost 50 % by the following evening (Foster and Wulff, 2005). In these study compared the sleep duration effect on the salivary cortisol responses between musician and control groups at baseline and post-examination. Results revealed no significant difference between musician and control groups. The majority of subjects in this study indicated that high school students should get between 5 and 8 hours of sleep each night. However, both groups had similar number of sleeping hours, the cortisol concentration was not found difference between groups.

6.6 Gender difference and stress responses

One of the most consistent findings employing psychological stress tasks (e.g., free speech, mental arithmetic, experimental harassment) is the significantly larger salivary cortisol response in healthy adult men compared to women following short-term laboratory stress (Kudielka et al., 2009). In consistency, Stroud and colleague (2002) reported that men showed significantly greater cortisol responses to the achievement challenges, but women showed greater cortisol responses to the social rejection challenges.

In these studies were found both female and male musicians had significantly lower cortisol levels than female control ($p < 0.001$) at pre-examination period, whereas no gender differences between female and male controls were found. However, we did not find sex differences in saliva cortisol responses among group. Association between personality traits and cortisol response imply two major physiological consequences: (1) individual consistency of the cortisol response upon exposure to stressors and (2) exposure of subjects, differing in their personality traits, to identical stressors show individual differences in the physiological response towards identical stressors (Martinek et al., 2003).

Limitations

The present study had several limitations. First, the subjects were recruited from a single school, to reduce confounding factor regarding school environment. The number of subjects was not large, making it statistically impractical to perform subgroup analyses. Second, only self-report of general and stress inventory questionnaires were used. By doing so, we assumed that the subjects were able to accurately report on their personal data and stress level. Third, the subjects were not willing to answer the stress inventory questionnaire at pre-examination period. The stress inventory was measured at baseline and post-examination (2 months after baseline) because the interval of measure stress level with stress inventory recommended 2 months for accurately. Fourth, the stress inventory was used to measure the chronic stress (within 2 months) and the saliva cortisol concentration was used to measure the acute stress in this situation, brought the correlation between the stress inventory questionnaire and the saliva cortisol concentration in this study was inconclusive.

CHAPTER VII
CONCLUSIONS

This study demonstrated influence of music on academic examination stress in adolescences using general questionnaire, stress inventory and salivary cortisol radioimmunoassay. The results were concluded as follows:

1. The results of stress inventory scores were separated into 5 groups; less than normal, normal, higher than normal, moderately higher than normal and extremely higher than normal. At higher than normal level of post-examination, the stress level of musician group was less than the control group, but not significantly different.
2. The saliva cortisol concentration of pre-examination of musician group was significantly lower than the control group. In control group, saliva cortisol concentration significantly increased from baseline to pre-examination and significantly fell from pre-examination to post-examination whereas in musician group had no significant change.
3. The saliva cortisol response by gender upon academic examination stress. Pre-examination cortisol levels differed between musician and control, both female and male musicians had significantly lower cortisol levels than female control. Neither baseline cortisol nor post-examination cortisol levels showed significant gender differences between musician and control groups. In female control, saliva cortisol concentration significantly increased from baseline to pre-examination and significantly declined from pre-examination to post-examination whereas in the other group had no significant change.
4. At the GPA 3.50-4.00 range, pre-examination cortisol levels differed between musician and control, with musician having significantly lower cortisol levels than control.

5. The sleep duration effects, a repeated measure ANOVA was used to analyze sleep duration on the salivary cortisol responses between musician and control groups at baseline and post-examination. Results revealed no significant difference between musician and control groups.

6. The effects of coffee consumption on salivary cortisol responses also showed no significant difference between musician and control groups.

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APPENDIX

General questionnaire

วันที่.....รหัส.....

แบบสอบถามข้อมูลทั่วไป

โปรดเติมข้อมูลในช่องว่างและทำเครื่องหมาย √ ในช่องที่ต้องการเลือก

1. ชื่อ.....นามสกุล.....อายุ.....ปี
2. เพศ ☐ ชาย ☐ หญิง โรงเรียน.....ชั้นปีที่.....เบอร์โทรศัพท์.....
3. ขณะนี้ท่านมีน้ำหนัก…… กก. ส่วนสูง.....ซม. มือข้างถนัดคือ.....
4. ขณะกำลังศึกษา ท่านพักอยู่ที่ ☐ บ้าน ☐ หอพัก ☐ บ้านญาติ ☐ อื่นๆระบุ.....
5. กรุณา<u>เรียงลำดับ</u>งานอดิเรกที่ทำในยามว่างจากมากที่สุดไปน้อยที่สุด ☐ เล่นกีฬา ☐ เล่นดนตรี ☐ ฟังเพลง ☐ งานศิลปะ ☐ ดูหนัง/ทีวี ☐ อ่านหนังสือ ☐ เล่นเกม ☐ อื่นๆ ระบุ.....
6. ท่านใช้เวลาในการทำงานอดิเรก.....ชั่วโมง/สัปดาห์
7. ท่านเล่นเครื่องดนตรีชนิดใด<u>เป็นประจำ</u>
   ☐ ไม่มี ☐ เปียโน ☐ กีตาร์ ☐ กลอง ☐ เครื่องดนตรีชนิดเป่า ☐ อื่นๆระบุ.....
8. ท่านเริ่มเล่นดนตรีตั้งแต่อายุ.....ปี ท่านซ้อมดนตรีวันละ.....ชั่วโมง ซ้อมจำนวน.....วัน/สัปดาห์
9. ท่านดื่มกาแฟหรือไม่
   ☐ ไม่ดื่ม ☐ ดื่ม 1-2 แก้ว/วัน ☐ ดื่ม 3 แก้ว/วัน ☐ ดื่มมากกว่า 3 แก้ว/วัน
10. ท่านสูบบุหรี่หรือไม่
    ☐ ไม่สูบ ☐ สูบน้อยกว่าครึ่งซอง/วัน ☐ สูบน้อยกว่าหนึ่งซอง/วัน ☐ สูบมากกว่าหนึ่งซอง/วัน
    ☐ เคยสูบแต่เลิกแล้วเป็นเวลา.....เดือน
11. ท่านมีโรคประจำตัวทางด้านร่างกายหรือไม่
    ☐ ไม่มี ☐ โรคหัวใจ ☐ โรคไต ☐ โรคตับ ☐ โรคเบาหวาน ☐ โรคอื่นๆ .....
12. ท่านมีโรคประจำตัวทางด้านจิตใจหรือไม่ ☐ ไม่มี ☐ มี คือ.....
13. ท่านมียาที่รับประทานเป็นประจำหรือไม่ ☐ ไม่มี ☐ มี คือ.....
14. ท่านมีปัญหาเรื่องการนอนหรือไม่ ☐ ไม่มี ☐ มี คือ.....
15. เมื่อ<u>คืนที่ผ่านมา</u>ท่านนอนเป็นเวลา
    ☐ ไม่ได้นอน ☐ 3-4 ชั่วโมง ☐ 5-6 ชั่วโมง ☐ 7-8 ชั่วโมง ☐ มากกว่า 8 ชั่วโมง
16. ในระหว่างนี้ ท่านมีปัญหาเรื่องน้ำหนักหรือไม่ ☐ น้ำหนักเพิ่มขึ้น ☐ น้ำหนักลดลง
17. วิชาที่ท่านถนัด คือ..... วิชาที่ท่านไม่ถนัด คือ.....เกรดเฉลี่ยของปีที่ผ่านมา.....
18. เกรดเฉลี่ยของวิชาคณิตศาสตร์ ☐ 3.50 – 4.00 ☐ 3.00–3.49 ☐ ต่ำกว่า 3.00
19. สำหรับนักเรียนหญิง ท่านมีประจำเดือนมาสม่ำเสมอหรือไม่ ☐ สม่ำเสมอ ☐ ไม่สม่ำเสมอ คือ.....
    ท่านมีประจำเดือนครั้งสุดท้ายวันที่ (วัน/เดือน/ปี).....

Stress inventory questionnaire

รหัส..... วันที่.....

แบบประเมินและวิเคราะห์ความเครียดด้วยตนเอง

กรมสุขภาพจิต กระทรวงสาธารณสุข

ในระยะเวลา 2 เดือนที่ผ่านมานี้ ท่านมีอาการ พฤติกรรม หรือ ความรู้สึก ต่อไปนี้มากน้อยเพียงใด

โปรดขีดเครื่องหมาย √ ลงในช่องแสดงระดับอาการที่เกิดขึ้นกับตัวท่านตามความเป็นจริงมากที่สุด

อาการ พฤติกรรม หรือความรู้สึก	ระดับอาการ			
	ไม่เคยเลย	เป็นครั้งคราว	เป็นบ่อยๆ	เป็นประจำ
1. นอนไม่หลับเพราะคิดมากหรือกังวลใจ				
2. รู้สึกหงุดหงิด รำคาญใจ				
3. ทำอะไรไม่ได้เลย เพราะประสาทตึงเครียด				
4. มีความวุ่นวายใจ				
5. ไม่อยากพบปะผู้คน				
6. ปวดหัวข้างเดียว หรือปวดบริเวณขมับทั้ง 2 ข้าง				
7. รู้สึกไม่มีความสุขและเศร้าหมอง				
8. รู้สึกหมดหวังในชีวิต				
9. รู้สึกชีวิตไม่มีคุณค่า				
10.กระวนกระวายอยู่ตลอดเวลา				
11.รู้สึกว่าตนเองไม่มีคุณค่า				
12.รู้สึกอ่อนเพลียไม่มีแรงจะทำอะไร				
13.รู้สึกเหนื่อยไม่อยากจะทำอะไร				
14. มีอาการหัวใจเต้นแรง				
15.เสียงสั่น ปากสั่น หรือมือสั่นเวลาไม่พอใจ				
16.รู้สึกกลัวผิดพลาดในการทำสิ่งต่างๆ				
17.ปวดหรือเกร็งกล้ามเนื้อบริเวณท้ายทอย หลังหรือไหล่				
18. ตื่นเต้นง่ายกับเหตุการณ์ที่ไม่คุ้นเคย				
19. มึนงงหรือเวียนศีรษะ				
20. ความรู้สึกทางเพศลดลง				
รวม				

Informed consent form

แบบฟอร์มใบยินยอมให้ทำการวิจัย

โดยได้รับการบอกกล่าวและเต็มใจ (Informed Consent Form)

โครงการวิจัยเรื่อง

การศึกษาอิทธิพลของดนตรีต่อความเครียดจากการสอบในเด็กวัยรุ่น

วันให้คำยินยอม วันที่ ..... เดือน ..... พ.ศ. .....

ก่อนที่จะลงนามในใบยินยอมให้ทำการวิจัยนี้ ข้าพเจ้าได้รับการอธิบายจากผู้วิจัยถึง วัตถุประสงค์ของการวิจัย วิธีการวิจัย รวมทั้งประโยชน์ที่จะเกิดขึ้นจากการวิจัยอย่างละเอียด และมีความเข้าใจดีแล้ว

ผู้วิจัยรับรองว่าจะตอบคำถามต่าง ๆ ที่ข้าพเจ้าสงสัยด้วยความเต็มใจ ไม่ปิดบังซ่อนเร้น จนข้าพเจ้าพอใจและข้าพเจ้ามีสิทธิที่จะบอกเลิกการเข้าร่วมโครงการวิจัยนี้เมื่อใดก็ได้ และเข้าร่วมโครงการวิจัยนี้ โดยสมัครใจ และการบอกเลิกการเข้าร่วมการวิจัยนี้ จะไม่มีผลต่อการรักษาโรคที่ข้าพเจ้าจะพึงได้รับ ต่อไป หากข้าพเจ้ามีข้อสงสัยหรือปัญหาประการใดเกี่ยวกับโครงการวิจัยนี้ ผู้วิจัยรับรองว่าจะตอบคำถามต่างๆ ด้วยความเต็มใจ ไม่ปิดบังซ่อนเร้น จนข้าพเจ้าพอใจโดยติดต่อได้ตลอดเวลาที่เบอร์โทร 08-6884-xxxx

ผู้วิจัยรับรองว่าจะเก็บข้อมูลเฉพาะที่เกี่ยวกับตัวข้าพเจ้าเป็นความลับ และจะเปิดเผยได้เฉพาะ ในรูปที่สรุปผลการวิจัย การเปิดเผยข้อมูลเกี่ยวกับตัวข้าพเจ้าต่อหน่วยงานต่าง ๆ ที่เกี่ยวข้องกระทำ ได้เฉพาะกรณีจำเป็นด้วยเหตุผลทางวิชาการเท่านั้น

ผู้วิจัยรับรองว่าหากมีข้อมูลเพิ่มเติมที่ส่งผลกระทบต่อการวิจัย ข้าพเจ้าจะได้รับการแจ้งให้ทราบ โดยไม่ปิดบังซ่อนเร้น

ข้าพเจ้าได้อ่านข้อความข้างต้นแล้ว และมีความเข้าใจดีทุกประการ และได้ลงนามในใบยินยอมนี้ด้วยความเต็มใจ

ในกรณีที่ผู้ถูกทดลองยังไม่บรรลุนิติภาวะจะต้องได้รับการยินยอมจากผู้ปกครองหรือผู้อุปการะโดยชอบด้วยกฎหมาย

ลงนาม ..... ผู้ยินยอม

(.....)

ลงนาม.....ผู้ปกครอง/

ผู้อุปการะโดยชอบ ด้วยกฎหมาย

(.....)

ลงนาม ..... พยาน

(.....)

ลงนาม ..... พยาน

(.....)

Information sheet

คำอธิบายโครงการวิจัย

1. ชื่อโครงการวิจัย

การศึกษาอิทธิพลของดนตรีต่อความเครียดจากการสอบในเด็กวัยรุ่น

2. วัตถุประสงค์และวิธีการวิจัย

<u>วัตถุประสงค์</u>

1. เพื่อศึกษาระดับความเครียดของนักเรียนชั้นมัธยมศึกษาตอนปลายของโรงเรียนเตรียมอุดมศึกษาพัฒนาการบางใหญ่ในช่วงเวลาที่ไม่มีการสอบ, ช่วงเวลาก่อนสอบและช่วงเวลาหลังสอบ
2. เพื่อศึกษาเปรียบเทียบระดับความเครียดในนักเรียนทั้ง 2 กลุ่ม ได้แก่ กลุ่มนักเรียนที่เล่นดนตรีกับกลุ่มนักเรียนที่ไม่ได้เล่นดนตรี
3. เพื่อศึกษาอิทธิพลของดนตรีที่มีผลต่อการเรียนรู้และความเครียดของนักเรียน

<u>วิธีการวิจัย</u>

1. ผู้ยินยอมตนจะได้รับฟังคำอธิบายรายละเอียดต่างๆ ของงานวิจัยนี้ พร้อมทั้งได้รับการตอบคำถามอย่างชัดเจนจนเป็นที่พอใจ
2. ผู้ยินยอมตนเซ็นชื่อในใบแสดงความยินยอมพร้อมทั้งกรอกประวัติส่วนตัวในแบบสอบถามข้อมูลทั่วไป
3. ผู้ยินยอมตนจะได้รับการทดสอบด้วยแบบประเมินและวิเคราะห์ความเครียดด้วยตนเอง (Stress inventory questionnaire) ของกรมสุขภาพจิต กระทรวงสาธารณสุข เป็นจำนวน 2 ครั้ง ได้แก่ ในวันที่ไม่มีการสอบของโรงเรียน และในช่วงเวลา 30-60 นาทีก่อนสอบในวันที่มีการสอบของโรงเรียน
4. ผู้ยินยอมตนจะได้รับการเก็บตัวอย่างน้ำลายเป็นจำนวน 3 ครั้ง ได้แก่ 1. ในวันที่ไม่มีการสอบของโรงเรียน, 2. ในวันที่มีการสอบของโรงเรียนคือ ช่วงเวลา 30-60 นาทีก่อนสอบ และ 3.ในช่วงเวลา 30-60 นาทีหลังสอบ
5. ผู้ยินยอมตนจะได้รับเอกสารคำอธิบายโครงการวิจัย และสำเนาแบบฟอร์มแสดงความยินยอม
6. หลังจากโครงการวิจัยสิ้นสุดลง ผู้ยินยอมตนสามารถติดต่อขอสอบถามข้อมูลจากการวิจัยทางด้านการประเมินระดับความเครียดของผู้ยินยอมตน รวมทั้งสามารถรับคำแนะนำจากคณะวิจัยได้โดยตรง

3. เหตุผลที่เชิญชวนให้ผู้ยินยอมตนให้ทำการวิจัยเข้าร่วมโครงการวิจัย

เนื่องจากต้องการศึกษาอิทธิพลของดนตรีที่มีต่อการเรียนรู้ในเด็กวัยรุ่นไทย ซึ่งอยู่ในภาวะที่มีความเครียดจากการสอบของทางโรงเรียนโดยการเปรียบเทียบระหว่างกลุ่มนักเรียนที่เล่นดนตรีเป็นประจำกับกลุ่มนักเรียนที่ไม่ได้เล่นดนตรี ซึ่งความรู้ที่ได้จะนำมาเพิ่มประสิทธิภาพในการเรียน การสอนของทางโรงเรียน

และอาจนำมาประยุกต์ใช้ในการบำบัดรักษาทางการแพทย์ในผู้ป่วยที่มีความเครียดได้ นอกจากนี้ยังมีแพทย์บางกลุ่มที่ใช้วิธีการรักษาทางเลือกอื่นเพิ่มเติมนอกเหนือจากการใช้ยา แต่การรักษาเช่นนี้ยังไม่มีแบบแผนที่เป็นมาตรฐานและข้อสนับสนุนทางวิทยาศาสตร์ที่ชัดเจน ดังนั้นโครงการวิจัยนี้อาจช่วยเพิ่มความชัดเจนในเรื่องอิทธิพลของดนตรีที่มีผลต่อการเรียนรู้และความเครียดได้ และเนื่องจากมีการดำเนินการวิจัยในกลุ่มที่นักเรียนที่มีสุขภาพร่างกายแข็งแรง ดังนั้นจึงสามารถนำผลการศึกษานี้มาประยุกต์ใช้เพื่อเพิ่มประสิทธิภาพในด้านการเรียนและความจำของเยาวชนทั่วไปได้อีกด้วย

4. ระยะเวลาที่ต้องทำการทดสอบผู้ยินยอมตนให้ทำการวิจัย

การทดสอบจะแบ่งเป็น 3 ครั้ง ครั้งแรก คือในช่วงเวลาที่ไม่มีการสอบของทางโรงเรียน (วันที่ไม่มีการสอบ) ครั้งที่สองในช่วงเวลา 30-60 นาทีก่อนสอบของทางโรงเรียนและครั้งสุดท้ายในช่วงเวลา 30-60 นาทีหลังสอบของทางโรงเรียน โดยผู้ยินยอมตนจะต้องได้รับการทดสอบด้วยแบบประเมินและวิเคราะห์ความเครียดด้วยตนเอง (Stress inventory questionnaire) ของกรมสุขภาพจิต กระทรวงสาธารณสุข พร้อมทั้งได้รับการเก็บตัวอย่างน้ำลายในวันเดียวกันซึ่งจะใช้เวลาโดยรวมครั้งละประมาณ 30-60 นาที

5. ประโยชน์ที่คาดว่าจะเกิดขึ้นทั้งต่อผู้ยินยอมตนให้ทำการวิจัยและผู้อื่น

1. เพื่อเก็บข้อมูลเกี่ยวกับปัจจัยที่มีผลต่อความเครียดของนักเรียนชั้นมัธยมศึกษาตอนปลาย
2. ทำให้ทราบถึงอิทธิพลของดนตรีที่อาจมีหรือไม่มีผลต่อการเรียนรู้ และหากดนตรีก่อให้เกิดประโยชน์แง่บวก ก็อาจนำมาประยุกต์ใช้ร่วมกับการการเรียนการสอนเพื่อเพิ่มประสิทธิภาพการเรียนรู้ให้ดียิ่งขึ้น
3. ทำให้นักเรียนทราบสภาพจิตใจของตนเองในช่วงเวลาต่างๆ ทั้งก่อนสอบ ระหว่างสอบและหลังสอบซึ่งจะนำไปสู่การแก้ไขปัญหาได้อย่างตรงจุดและรวดเร็วยิ่งขึ้น
4. ทำให้ทราบข้อมูลเพิ่มเติมเกี่ยวกับอิทธิพลของดนตรีที่มีผลต่อการเรียนรู้และความเครียด ซึ่งสามารถนำความรู้ที่ได้รับมาไปช่วยพัฒนาการด้านการเรียนรู้และความจำในเยาวชนทั่วไปได้

6. ความเสี่ยงหรือความไม่สบายที่คาดว่าจะเกิดขึ้นกับผู้ยินยอมตนให้ทำการวิจัยในการเข้าร่วมการศึกษาวิจัย

เนื่องจากมีการเก็บตัวอย่างน้ำลายในช่วงเวลาก่อนสอบ ผู้ยินยอมตนอาจรู้สึกตื่นเต้น ร้อนใจ หรือกังวลเกี่ยวกับการสอบ อันตรายอาจจะเกิดขึ้นในขณะที่เคี้ยวก้อนสำลีโดยผู้ยินยอมตนมีอาการวิตกกังวลทำให้การเคี้ยวก้อนสำลีนั้นอาจจะมีชิ้นส่วนของสำลีหลุดลงไปในลำคอและทำให้เกิดการอุดตันที่หลอดลมได้ ดังนั้นกลุ่มผู้วิจัยจะแนะนำให้ผู้ยินยอมตนรู้สึกผ่อนคลาย และระมัดระวังในการเก็บตัวอย่างน้ำลาย

7. การเตรียมผลิตภัณฑ์ หรือกระบวนการรักษาที่พิสูจน์จากการทำวิจัยแล้วว่าปลอดภัย

ในการทดสอบทางด้านสุขภาพจิตจะกระทำโดยแพทย์ผู้ชำนาญด้านจิตวิทยาเด็กและวัยรุ่นโดยเฉพาะ ดังนั้นผู้ยินยอมตนจะได้รับการปกป้องจากความกังวลหรือความเครียดใดๆ ที่อาจเกิดขึ้นขณะที่ทดสอบด้วยแบบประเมินและวิเคราะห์ความเครียดด้วยตนเองของกรมสุขภาพจิต กระทรวงสาธารณสุข นอกจากนี้การดำเนินการวิจัยจะได้รับการควบคุมดูแลจากแพทย์ร่วมคณะวิจัย อีกทั้งเครื่องมือที่ใช้ในการวิจัยก็เป็นอุปกรณ์ทางการแพทย์ที่ได้มาตรฐาน สะอาดและปลอดภัย ได้มาตรฐานเดียวกันกับอุปกรณ์ที่ใช้ในโรงพยาบาล คลินิก

และห้องวิจัยทางการแพทย์ทั้งในและต่างประเทศ โดยการทดลองนี้ไม่มีการใช้ยา หรือสารเคมีใดๆ ในอุปกรณ์ที่ใช้เก็บตัวอย่างน้ำลาย ทั้งนี้หากผู้ยินยอมตนหรือผู้ปกครองยังมีข้อสงสัยหรือยังไม่มั่นใจในความปลอดภัย ก็สามารถสอบถามจากกลุ่มผู้วิจัยจนกว่าจะได้รับคำตอบจนเป็นที่พอใจ

8. ทางเลือกในการรักษา หรือวิธีการตรวจวินิจฉัยอื่น ที่อาจเป็นประโยชน์แก่ผู้ยินยอมตนให้ทำการวิจัย

วิธีการวินิจฉัยหลักที่มักใช้กันทั่วไปในการตรวจประเมินความเครียด คือการให้ทำแบบสอบถามและสัมภาษณ์เป็นรายบุคคล แต่ความถูกต้องและแม่นยำในการวินิจฉัยก็ขึ้นอยู่กับความรู้และประสบการณ์ของแพทย์เป็นสำคัญ ปัจจุบันยังไม่มีวิธีการใดๆที่สามารถวินิจฉัยโรคเครียดในเด็กนักเรียนได้อย่างชัดเจน ดังนั้นจึงควรต้องมีผลทางห้องปฏิบัติการควบคู่ไปด้วย ส่วนวิธีการรักษาที่เป็นที่ยอมรับคือ การให้ยา แต่แพทย์บางคนอาจใช้การบำบัดแพทย์ทางเลือกอื่นเข้ามาร่วมด้วยเพื่อเพิ่มประสิทธิภาพของการรักษาและลดการใช้ยา เช่น ดนตรีบำบัด การออกกำลังกาย กิจกรรมบำบัด การรับประทานอาหารที่มีประโยชน์ การใช้น้ำมันหอมระเหยบำบัด และอื่นๆ

9. ขอบเขตการดูแลรักษาความลับของข้อมูลต่างๆ ของผู้ยินยอมตนให้ทำการวิจัย

ข้อมูลทุกอย่างของผู้ยินยอมตนจะถูกเก็บเป็นความลับไม่มีการเผยแพร่สู่สาธารณชน ยกเว้นข้อมูลที่อยู่ในรูปผลสรุปการวิจัยโดยรวม และจะไม่มีการระบุชื่อของผู้ยินยอมตนคนใดโดยเฉพาะ การเปิดเผยข้อมูลที่เกี่ยวกับผู้ยินยอมตนจะกระทำได้ในกรณีที่มีเหตุผลจำเป็นทางวิชาการและมีผลกระทบต่อความปลอดภัยโดยตรงของตัวผู้ยินยอมตนเอง ซึ่งการกระทำดังกล่าวนี้จะกระทำก็ต่อเมื่อได้รับความเห็นชอบจากผู้ปกครองของผู้ยินยอมตน หัวหน้าโครงการวิจัยชีววิทยาระบบประสาทและพฤติกรรม และผู้อำนวยการสถาบันวิจัยและพัฒนาวิทยาศาสตร์และเทคโนโลยี มหาวิทยาลัยมหิดล พร้อมทั้งลงลายมือชื่อรับรองทุกครั้ง ส่วนการเก็บรักษาประวัติและข้อมูลของผู้ยินยอมตนนั้น จะมีการจัดเก็บเป็นเอกสารลับแยกเป็นหมวดหมู่เฉพาะในห้องปฏิบัติการวิจัย

10. การดูแลรักษาที่ผู้วิจัยจะจัดให้

หากเกิดเหตุไม่พึงประสงค์ หรือผู้ยินยอมตนได้รับอันตรายใดๆ ระหว่างการดำเนินการวิจัย แพทย์ที่เข้าร่วมในคณะวิจัยจะเข้ามาตรวจอาการและดูแลรักษาอย่างเต็มที่ และแม้ว่ายังมีอาการไม่พึงประสงค์ ซึ่งพิสูจน์ได้ว่าเป็นเหตุอันเนื่องมาจากโครงการวิจัยภายหลังจากการวิจัยสิ้นสุดแล้ว ผู้ปกครองของผู้ยินยอมตนสามารถติดต่อคณะผู้วิจัยเพื่อทำการรักษาผู้ยินยอมตนได้

11. กรณีเกิดอันตรายหรือผลไม่พึงประสงค์จากการวิจัย ผู้ยินยอมตนให้ทำการวิจัยจะได้รับการดูแลรักษาโดยไม่ต้องเสียค่าใช้จ่ายอย่างไรบ้าง

คณะผู้วิจัยจะเป็นผู้รับผิดชอบในด้านค่าใช้จ่ายที่จะต้องทำการรักษาโดยไม่คิดมูลค่าใดๆ และผู้ปกครองของผู้ยินยอมตนจะได้รับการชดเชยรายได้ที่สูญเสียไประหว่างการดูแลผู้ยินยอมตนขณะรับการรักษาพยาบาลดังกล่าว

12. ในกรณีที่เกิดอันตรายจากการวิจัยถึงขั้นพิการหรือเสียชีวิต ผู้ยินยอมตนให้ทำการวิจัยหรือทายาทจะได้รับการชดเชยอย่างไร

หากเกิดเหตุสุดวิสัยถึงขั้นพิการหรือเสียชีวิตซึ่งพิสูจน์ได้ว่าเป็นผลมาจากการวิจัย ผู้ยินยอมตนจะได้รับเงินทดแทนความพิการที่อาจเกิดขึ้น และหากถึงขั้นเสียชีวิตผู้แทนโดยชอบตามกฎหมายจะเป็นผู้รับผลประโยชน์ตามที่กฎหมายระบุไว้

13. สิทธิที่ผู้ยินยอมตนให้ทำการวิจัยจะถอนตัวออกจากโครงการวิจัยได้ทุกเมื่อ โดยไม่กระทบต่อการ ดูแลรักษาที่พึงได้รับตามปกติ

ผู้ยินยอมตนจะได้รับการยินยอมให้ถอนตัวออกจากโครงการวิจัยได้ทุกเมื่อตามความสมัครใจ เมื่อผู้ยินยอมตนไม่พอใจ ไม่สามารถดำเนินการตามแผนงานวิจัยที่ระบุไว้ได้ ผู้วิจัยพิจารณาเห็นถึงความไม่ปลอดภัยต่อผู้ยินยอมตนหากยังดำเนินการวิจัยต่อไป หรือเมื่อผลการวิจัยได้ข้อยุติได้ผลการวิเคราะห์ที่ชัดเจนแล้ว และหากผู้ยินยอมตนเป็นผู้ป่วยของจิตแพทย์ในคณะวิจัยอยู่ก่อนแล้ว ผู้ยินยอมตนก็จะยังคงได้รับการดูแลรักษาตามปกติ

14. ชื่อ ที่อยู่ และเบอร์โทรศัพท์ของแพทย์ที่ผู้ปกครองของผู้ยินยอมตนสามารถติดต่อได้โดยสะดวก ทั้งในและนอกเวลาราชการกรณีมีเหตุจำเป็นหรือฉุกเฉิน

1. ชื่อและนามสกุล พญ. หัทยา ดำรงค์ผล

ที่อยู่ โครงการวิจัยชีววิทยาระบบประสาทและพฤติกรรม
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