

# CONTENTS

|  | PAGE |
|--|------|
| ABSTRACT (THAI).....   | iv   |
| ABSTRACT (ENGLISH).....  | v    |
| ACKNOWLEDGEMENTS.....  | vi   |
| CONTENTS.....  | vii  |
| LIST OF TABLES.....  | ix   |
| LIST OF FIGURES.....   | x    |
| LIST OF ABBREVIATIONS.....   | xiv  |
| CHAPTER  |      |
| I INTRODUCTION.....  | 1    |
| II LITERATURE REVIEWS.....   | 4    |
| A Anxiety.....   | 4    |
| B Neural Circuitry of Anxiety.....   | 5    |
| C GABAergic system.....  | 7    |
| D Estrogen.....  | 11   |
| E Experimental models of anxiety.....  | 15   |
| III MATERIALS AND METHODS.....   | 19   |
| A Animals.....   | 20   |
| B Chemicals.....   | 20   |
| C Experimental protocols.....  | 20   |
| D Methods.....   | 22   |
| IV RESULTS.....  | 33   |
| 1 To investigate the effects of estrogen deprivation on anxiety-like behaviors, GABA <sub>A</sub> receptor subunits gene expression and serotonergic activity in brain associated with anxiety in ovariectomized rats..... | 34   |

| CHAPTER  | PAGE |
|--|------|
| 2 To investigate whether lacking of estrogen causes alteration of GABA <sub>A</sub> receptor function and whether these alterations affect serotonergic activity in brain associated with anxiety.....   | 53   |
| 3 To investigate whether estrogen can alleviate anxiety-like behavior in ovariectomized rat and whether the GABA <sub>A</sub> receptor function is modulated by estrogen, which in turn modulates the serotonergic activity in brain areas related to anxiety..... | 65   |
| V DISCUSSION.....  | 78   |
| VI CONCLUSION.....   | 91   |
| REFERENCES.....  | 93   |
| BIOGRAPHY.....   | 114  |

## LIST OF TABLES

|           |   | PAGE |
|-----------|---|------|
| Table 2-1 | Distribution and pharmacological characteristics of the GABA <sub>A</sub> receptor subtype in the rat brain.....  | 10   |
| Table 3-1 | Gene-specific real-time PCR primers for rat GABA <sub>A</sub> receptor subunits and 18s rRNA.....   | 31   |
| Table 4-1 | The body weights, the percent change of body weight and the daily food intake in Ovx and E2 rats at day 7-, 14-, 21- and 28-post-ovariectomy.....                       | 35   |
| Table 4-2 | The uterine weight, percentage of uterine weight to body weight ratio in 7-, 14-, 21- and 28-days Ovx rats.....   | 36   |
| Table 4-3 | The body weight, percentage change of body weight, daily food intake uterine weight and percentage of uterine weight to body weight in Ovx and E <sub>2</sub> rats..... | 66   |

## LIST OF FIGURES

|            | PAGE  |
|------------|---|
| Figure 3-1 | The elevated T-maze composed of two open- and one closed-arms of equal dimension, connected by the center platform..... 24                                  |
| Figure 3-2 | The open field was a rectangular box of dimension, in which the floor was divided into 6 x 8 squares..... 25  |
| Figure 3-3 | Diagrammatic representation of brain cutting block illustrating orientation of brain and placement of razor blades to obtain coronal brain sections..... 27 |
| Figure 3-4 | The chromatogram represents peaks of standard DHBA, 5-HIAA and 5-HT measured by HPLC-EC..... 29   |
| Figure 4-1 | The effects of time of estrogen deprivation on anxiety-like behavior in (A) Ovx groups and (B) E <sub>2</sub> groups..... 37                                |
| Figure 4-2 | The effects of time of estrogen deprivation on (A) escape latency in ETM and (B) locomotor activity in the open field.... 38                                |
| Figure 4-3 | The chromatograms represent 5-HT and 5-HIAA levels in midbrain of (A) Ovx7, (B) Ovx14, (C) Ovx21 and (D) Ovx28 groups measured by HPLC-EC..... 39           |
| Figure 4-4 | The chromatograms represent 5-HT and 5-HIAA levels in midbrain of (A) E7, (B) E14, (C) E21 and (D) E28 groups measured by HPLC-EC..... 40                   |
| Figure 4-5 | The effect of time of estrogen deprivation on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in midbrain..... 41                                    |
| Figure 4-6 | The effect of time of estrogen deprivation on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in amygdala..... 42                                    |

|   | PAGE |
|---|------|
| Figure 4-7 The effect of time of estrogen deprivation on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in frontal cortex.....  | 43   |
| Figure 4-8 The effect of time of estrogen deprivation on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in hippocampus.....   | 44   |
| Figure 4-9 The effect of time of estrogen deprivation on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in nucleus accumbens.....   | 45   |
| Figure 4-10 The effect of time of estrogen deprivation on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in septum.....   | 46   |
| Figure 4-11 The effect of time of estrogen deprivation on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in anterior hypothalamus.....                                      | 47   |
| Figure 4-12 Agarose gel electrophoresis of PCR amplification products of GABA <sub>A</sub> receptor $\alpha$ 2, $\alpha$ 3, $\alpha$ 4 subunits and 18s rRNA.....                   | 48   |
| Figure 4-13 Dissociation curves of PCR amplification products of GABA <sub>A</sub> receptor (A) $\alpha$ 2, (B) $\alpha$ 3, (C) $\alpha$ 4 subunits and (D) 18s rRNA.....           | 49   |
| Figure 4-14 The effect of time of estrogen deprivation on GABA <sub>A</sub> receptor (A) $\alpha$ 2-, (B) $\alpha$ 3- and (C) $\alpha$ 4- subunit gene expressions in midbrain..... | 51   |
| Figure 4-15 The effect of time of estrogen deprivation on GABA <sub>A</sub> receptor (A) $\alpha$ 2-, (B) $\alpha$ 3- and (C) $\alpha$ 4- subunit gene expressions in amygdala..... | 52   |
| Figure 4-16 The effect of benzodiazepine agonist on anxiety-like behavior in the ETM of the (A) Ovx and (B) E2 groups.....  | 54   |
| Figure 4-17 The effect of benzodiazepine agonist on locomotor activity in (A) Ovx groups and (B) E2 groups.....   | 55   |

|             |   |    |
|-------------|---|----|
| Figure 4-18 | The chromatograms represent 5-HT and 5-HIAA levels in midbrain of (A) vehicle-, (B) 0.25 mg/kg diazepam-, (C) 0.5 mg/kg diazepam- and (D) 1.0 mg/kg diazepam- treated Ovx rats as measured by HPLC-EC.....            | 56 |
| Figure 4-19 | The chromatograms represent 5-HT and 5-HIAA levels in midbrain of (A) vehicle-, (B) 0.25 mg/kg diazepam-, (C) 0.5 mg/kg diazepam- and (D) 1.0 mg/kg diazepam- treated E <sub>2</sub> rats as measured by HPLC-EC..... | 57 |
| Figure 4-20 | The effects of benzodiazepine agonist on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in the midbrain.....  | 58 |
| Figure 4-21 | The effects of benzodiazepine agonist on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in the amygdala.....  | 59 |
| Figure 4-22 | The effects of benzodiazepine agonist on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in the frontal cortex   | 60 |
| Figure 4-23 | The effects of benzodiazepine agonist on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in the hippocampus  | 61 |
| Figure 4-24 | The effects of benzodiazepine agonist on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in the nucleus accumbens.....   | 62 |
| Figure 4-25 | The effects of benzodiazepine agonist on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in the septum.....  | 63 |
| Figure 4-26 | The effects of benzodiazepine agonist on (A) 5-HT, (B) 5-HIAA levels and (C) 5-HIAA/5-HT ratios in the anterior hypothalamus.....   | 64 |
| Figure 4-27 | The effects of benzodiazepine agonist on anxiety-like behavior after long-term ovariectomy in Ovx and E <sub>2</sub> rats.....  | 68 |
| Figure 4-28 | The effects of benzodiazepine agonist on escape behavior and locomotor activity in the Ovx and E <sub>2</sub> rats.....   | 69 |

|  | PAGE |
|--|------|
| Figure 4-29 The chromatograms represent 5-HT and 5-HIAA levels in midbrain of (A) Ovx rats treated with vehicle, (B) Ovx rats treated with diazepam, (C) E2 rats treated with vehicle and (D) E2 rats treated with diazepam as measured by HPLC-EC.. | 70   |
| Figure 4-30 The effects of benzodiazepine agonist on the levels of (A) 5-HT, (B) HIAA and 5-HIAA/5-HT ratio in midbrain.....   | 71   |
| Figure 4-31 The effects of benzodiazepine agonist on the levels of (A) 5-HT, (B) HIAA and 5-HIAA/5-HT ratio in amygdala.....   | 72   |
| Figure 4-32 The effects of benzodiazepine agonist on the levels of (A) 5-HT, (B) HIAA and 5-HIAA/5-HT ratio in frontal cortex.....   | 73   |
| Figure 4-33 The effects of benzodiazepine agonist on the levels of (A) 5-HT, (B) HIAA and 5-HIAA/5-HT ratio in hippocampus.....  | 74   |
| Figure 4-34 The effects of benzodiazepine agonist on the levels of (A) 5-HT, (B) HIAA and 5-HIAA/5-HT ratio in nucleus accumbens.  | 75   |
| Figure 4-35 The effects of benzodiazepine agonist on the levels of (A) 5-HT, (B) HIAA and 5-HIAA/5-HT ratio in septum.....   | 76   |
| Figure 4-36 The effects of benzodiazepine agonist on the levels of (A) 5-HT, (B) HIAA and 5-HIAA/5-HT ratio in anterior hypothalamus.....  | 77   |

## LIST OF ABBREVIATIONS

|           |   |
|-----------|---|
| °C        | degree Celsius                          |
| µg        | microgram                               |
| µl        | microliter                              |
| µm        | micrometer                              |
| µM        | micromolar                              |
| 5-HIAA    | 5-hydroxyindoleacetic acid              |
| 5-HT      | serotonin                               |
| 5-HTP     | 5-hydroxytryptophan                     |
| 8-OH-DPAT | 8-hydroxy-2-(di-N-propylamino) tetralin |
| A         | amygdala;                               |
| AADC      | aromatic L-amino acid decarboxylase     |
| ACh       | acetylcholine                           |
| Ag        | chemical symbol; Silver                 |
| AgCl      | silver chloride                         |
| aH        | anterior hypothalamus                   |
| am        | ante meridiem                           |
| BDZs      | benzodiazepines                         |
| bp        | base pair                               |
| BW        | body weight                             |
| cDNA      | complementary deoxyribonucleic acid     |
| cm        | centimeter                              |
| CP        | caudate putamen                         |
| CT        | threshold cycle                         |
| d         | day                                     |
| DA        | dopamine                                |
| DFI       | daily food intake                       |
| DHBA      | 3,4-dihydroxy-benzyl-amine hydrobromide |
| DNase I   | deoxyribonuclease I                     |
| DSM       | diagnostic and statistical manual       |
| DWG       | daily weight gain                       |



|                  |   |
|------------------|---|
| E2               | 17 $\beta$ -estradiol   |
| EC <sub>50</sub> | effective concentration   |
| EPM              | elevated plus-maze  |
| ER               | estrogen receptor   |
| ERT              | estrogen replacement therapy  |
| ETM              | elevated T-maze   |
| FC               | frontal cortex  |
| GABA             | gamma-aminobutyric acid   |
| GAD              | glutamic acid decarboxylase   |
| gm               | gram  |
| GP               | globus pallidus   |
| H                | hippocampus   |
| HPA              | hypothalamic-pituitary-axis   |
| HPLC-EC          | high-performance liquid chromatography -<br>electrochemical detection |
| hr               | hour  |
| i.p.             | intraperitoneal injection   |
| kg               | kilogram  |
| lx               | lux   |
| M                | molarity  |
| MAOI             | monoamine oxidase inhibitors  |
| MDMA             | 3,4-methylenedioxymethamphetamine                                     |
| mg               | milligram   |
| min              | minute  |
| ml               | milliliter  |
| mM               | millimolar  |
| mRNA             | messenger RNA   |
| n                | number  |
| nA               | nano ampere   |
| NA               | nucleus accumbens   |
| NE               | norepinephrine  |
| ng               | nanogram  |

|      |  |
|------|--|
| nm   | nanometer                              |
| No.  | number                                 |
| NPY  | neuropeptide Y                         |
| OCD  | obsessive-compulsive disorder          |
| OT   | olfactory tubercle                     |
| Ovx  | ovariectomized                         |
| PAG  | periaqueductal gray                    |
| PCR  | polymerase chain reaction              |
| PD   | panic disorder                         |
| pH   | posterior hypothalamus                 |
| pm   | post Meridiem                          |
| PTSD | post-traumatic stress disorder         |
| RC   | remaining cortex                       |
| RNA  | ribonucleic acid                       |
| rRNA | ribosomal RNA                          |
| s    | seconds                                |
| S    | septum                                 |
| SEM  | standard errors of mean                |
| SN   | substantia nigra                       |
| SSRI | selective serotonin reuptake inhibitor |
| T    | thalamus                               |
| TCAs | tricyclic antidepressants              |
| UW   | uterine weight                         |
| V    | volt                                   |
| veh  | vehicle                                |
| VT   | ventral tegmentum                      |