

CHANGES OF HYPOTHALAMIC-PITUITARY-  
TESTICULAR AXIS IN MALE RATS  
DURING COGNITIVE IMPAIRMENT



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จุฬาลงกรณ์มหาวิทยาลัย  
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A Thesis Submitted in Partial Fulfillment of the Requirements  
for the Degree of Master of Science in Zoology  
Department of Biology  
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การเปลี่ยนแปลงของแกนไฮโปทาลามัส-ต่อมใต้สมอง-อวัยวะในหนูเพศผู้  
ที่อยู่ในภาวะความจำเสื่อม



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พัทธ์ธีรา วิทิตสุวรรณกุล : การเปลี่ยนแปลงของแกนไฮโปทาลามัส-ต่อมใต้สมอง-อัณฑะในหนูเพศผู้  
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จากที่มีรายงานว่าสมองส่วนไฮโปทาลามัส ที่ทำหน้าที่ควบคุมการทำงานของระบบสืบพันธุ์ผ่านแกนไฮโปทาลามัส-ต่อมใต้  
 สมอง-อัณฑะ (HTP) และสมองส่วนฮิปโปแคมปัส ที่ทำหน้าที่เกี่ยวกับการเรียนรู้และการจดจำ สามารถสร้างฮอร์โมนที่เกี่ยวข้องกับระบบ  
 สืบพันธุ์ได้ และมีบทบาทความจำเสื่อมควบคู่ไปกับความเสื่อมของระบบสืบพันธุ์ จึงนำไปสู่คำถามการวิจัยที่ว่าระหว่างระบบสืบพันธุ์และ  
 ระบบความจำ ระบบใดมีความเสื่อมเกิดขึ้นก่อนและมีผลเปลี่ยนแปลงที่สัมพันธ์กันอย่างไร ทำการทดลองในหนูเพศผู้อายุ 4, 6, 8, 10  
 และ 12 เดือน เก็บเลือดเพื่อนำไปตรวจวัดระดับของเทสโทสเตอโรน (T) และลูทีนิงฮอร์โมน (LH) ในซีรัมด้วยวิธี Enzyme-  
 linked immunosorbent assay (ELISA) เก็บอัณฑะ ต่อมสร้างน้ำเลี้ยงอสุจิ ต่อมลูกหมาก และหลอดเก็บอสุจิมาซึ่งน้ำหนัก  
 เก็บสมองส่วนไฮโปทาลามัส (บริเวณแอนเตอร์เวนทรัล เพอริเวนทริคิวลาร์นิวเคลียส (AVPV), อาร์คูเอท นิวเคลียส (ARC),  
 พร็ออปติก (PoA) และ มีเดียน เอมีเนนส์ (ME)) และสมองส่วนฮิปโปแคมปัส มาวัดการแสดงออกของยีนที่เกี่ยวข้องกับฮอร์โมนใน  
 ระบบสืบพันธุ์ในระดับเอ็มอาร์เอ็นเอด้วยวิธี Quantitative real-time reverse transcription polymerase chain  
 reaction (qRT-PCR) และวัดการเรียนรู้และการจดจำด้วยวิธี Morris water maze ผลการศึกษาพบว่าระดับซีรัม T เริ่ม  
 ลดลงเมื่อหนูอายุได้ 8 เดือน และลดลงอย่างมีนัยสำคัญทางสถิติที่อายุ 12 เดือน ระดับ LH ลดลงอย่างมีนัยสำคัญทางสถิติที่อายุ 8 เดือน  
 คำนวณน้ำหนักสัมพัทธ์ของอัณฑะ ต่อมสร้างน้ำเลี้ยงอสุจิ และต่อมลูกหมากลดลงตั้งแต่อายุ 6 เดือน ในขณะที่น้ำหนักสัมพัทธ์ของหลอดเก็บอสุจิ  
 ลดลงตั้งแต่อายุ 10 เดือน การแสดงออกของยีนในสมองส่วนไฮโปทาลามัสเมื่อไล่ตามลำดับการควบคุมทำงาน เป็นดังนี้ AVPV-*Kiss1*  
 ลดลงที่อายุ 12 เดือน PoA-*Kiss1r* และ *Gnrh1* ลดลงตั้งแต่อายุ 12 และ 8 เดือน *Ar*, *Esr1* และ *Esr2* ที่ AVPV,  
 ARC และ ME ลดลงในช่วงอายุ 6-12 เดือน แต่ไม่พบการเปลี่ยนแปลงของ *ARC-Esr1* และของ *ME-Esr1* และ *Esr2*  
 สำหรับสมองส่วนฮิปโปแคมปัสมีการเพิ่มการแสดงออกของยีน *Kiss1*, *Gnrhr*, *Lhb*, *Ar*, *Esr1* และ *Esr2* ที่อายุ 8 เดือนแต่ไม่  
 พบการเปลี่ยนแปลงของยีนที่เกี่ยวข้องกับการสร้างฮอร์โมนเพศ และหนูมีสถานะความจำเสื่อมเกิดขึ้นเมื่อเข้าสู่ช่วงกลางระยะเริ่มต้นที่อายุ 8  
 เดือน จากการศึกษาที่สรุปได้ว่าความเสื่อมในระบบสืบพันธุ์เริ่มเกิดขึ้นที่ระดับบนของแกน HPT (สมองส่วนไฮโปทาลามัสและต่อมใต้  
 สมอง) เมื่อหนูอายุได้ 6-12 เดือน ที่นำไปสู่ความเสื่อมของอวัยวะสืบพันธุ์ (อัณฑะ) เมื่อหนูเข้าสู่ช่วงกลางที่อายุ 12 เดือน ซึ่งการ  
 เปลี่ยนแปลงของแกน HPT ทำให้เกิดการปรับตัวของสมองฮิปโปแคมปัสในหนูอายุ 8 เดือน โดยเพิ่มการแสดงออกของยีนที่เกี่ยวข้อง  
 กับตัวรับฮอร์โมนเพศและการสร้างฮอร์โมนในระบบสืบพันธุ์ (เฉพาะ *Kiss1*, *Gnrhr* และ *Lhb*) เพื่อที่จะรักษาการทำงานด้านการ  
 เรียนรู้และการจดจำ นอกจากนี้ผลการศึกษาในครั้งนี้ก็นำไปสู่การประยุกต์ใช้ที่ว่าในการป้องกันสถานะความจำเสื่อมในผู้ชายควรเริ่มดำเนินการ  
 ตั้งแต่เมื่อเข้าสู่ช่วงกลางระยะเริ่มต้น ก่อนที่ระดับ T ในซีรัมจะลดลงและเกิดความเสื่อมของระบบสืบพันธุ์

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## 5872002223 : MASTER OF SCIENCE

COGNITIVE IMPAIRMENT, HIPPOCAMPUS, HYPOTHALAMUS, REPRODUCTIVE  
SENESCENCE

Patteera Wititsuwankul : CHANGES OF HYPOTHALAMIC-PITUITARY-  
TESTICULAR AXIS IN MALE RATS

DURING COGNITIVE IMPAIRMENT. ADVISOR: Prof. Suchinda  
Malaivijitnond, Ph.D., Asst. Prof. Sukanya Jaroenporn, Ph.D.

From the previous reports denoting that hypothalamus regulating reproductive function via hypothalamic-pituitary-testicular (HPT) axis, and hippocampus controlling learning and memory capacity can synthesize reproductive hormones, and that cognitive impairment particularly occurs during reproductive senescence, it leads to the following research questions; i) which system first enters the aging stage and ii) how the changes are related. Male rats at the ages of 4, 6, 8, 10 and 12 months old were subjected for this study. Blood samples were collected for serum testosterone (T) and luteinizing hormone (LH) assays by enzyme-linked immunosorbent assay (ELISA) techniques. Testes, seminal vesicle, prostate gland and epididymis were collected and weighed. Hypothalamus (including anteroventral periventricular nucleus (AVPV), arcuate nucleus (ARC), preoptic area (PoA) and median eminence (ME)) and hippocampus were collected, and mRNA expression levels of reproductive hormones related genes were determined by quantitative real-time reverse transcription polymerase chain reaction (qRT-PCR) techniques. Learning and memory capacity were tested using Morris water maze test. Serum T level was marginally decreased at 8 months old, but significantly decreased at 12 months old. Serum LH levels were significantly declined from 8 months old. Relative weights of testes, seminal vesicle and prostate gland were significantly lowered at 6 months old, but it occurred at 10 months old for epididymis. Decreases in transcript expression at hypothalamus counted by the controlling pathway were as follows; AVPV-*Kiss1* at 12 months old, PoA-*Kiss1r* and *Gnrh1* from 12 and 8 months old, and *Ar*, *Esr1* and *Esr2* at AVPV, ARC and ME at 6-12 months old, but no changes in ARC-*Esr1*, and ME-*Esr1* and *Esr2* were detected. In hippocampus, *Kiss1*, *Gnrhr*, *Lhb*, *Ar*, *Esr1* and *Esr2* mRNA levels were significantly elevated at 8 months old, but no changes in those of sex steroid synthesis genes. Cognitive impairment was detected when the rats are at the early middle-aged, 8 months old. This study indicates that the reproductive senescence is initiated at the higher (hypothalamus and pituitary) levels of the HPT axis of 6-12 months old male rats which leads to the aging of reproductive (testis) organ when they are middle-aged, 12 months old. In an attempt to retain the cognitive function in response to changes of HPT axis, hippocampus up-regulated sex steroid receptors and locally synthesized reproductive hormone encoding genes (only *Kiss1*, *Gnrhr* and *Lhb*). In light on the results of this study, it implies that the prevention of cognitive impairment in men should be conducted when they are at the early middle age before a significant reduction of serum T level and a reproductive senescence are detected.

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Student's Signature .....  
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# TABLE OF CONTENTS

	<b>Page</b>
ABSTRACT (THAI) .....	D
ABSTRACT (ENGLISH).....	E
ACKNOWLEDGEMENTS.....	F
TABLE OF CONTENTS.....	G
LIST OF TABLES .....	I
LIST OF FIGURES .....	J
LIST OF ABBREVIATIONS.....	L
CHAPTER I GENERAL INTRODUCTION .....	1
Objectives .....	4
CHAPTER II LITERATURE REVIEW .....	5
1. Reproductive system.....	5
1.1 The role of HPT axis .....	6
1.2 Testis .....	7
1.2.1 Structure of the testis .....	7
1.2.2 Testicular function.....	8
1.2.2.1 Spermatogenesis .....	9
1.2.2.2 Steroidogenesis .....	9
1.3 Pituitary gland .....	12
1.3.1 Structure of pituitary gland.....	12
1.3.2 Anterior pituitary gland and LH/FSH production .....	13
1.4 Hypothalamus.....	13
1.4.1 Structure of hypothalamus.....	13
1.4.2 Function of hypothalamus and GnRH production .....	15
2. Cognition .....	19
2.1 Cognitive impairment.....	19



2.2 Structure of hippocampus.....	22
2.3 Local sex steroid synthesis in the hippocampus.....	23
CHAPTER III MATERIALS AND METHODS .....	29
Animals.....	29
Experimental design .....	29
Learning behavior and spatial memory test.....	30
Hormone assays .....	32
Hypothalamus and hippocampus collection and isolation.....	33
Two stage quantitative real time reverse transcription PCR (qrtRT-PCR) .....	33
Statistical analysis.....	37
CHAPTER IV RESULTS.....	38
Detection of the status of androgen deficiency through a significant reduction of serum testosterone levels .....	38
Changes of serum LH levels.....	39
Changes of testes and accessory sex organ weights .....	40
Detection of cognitive impairment using Morris water maze test.....	41
Changes of mRNA expression levels of genes associated with reproductive hormones and their receptors in hypothalamus .....	43
Changes of mRNA expression levels of genes associated with reproductive hormones and their receptors in hippocampus .....	48
CHAPTER V GENERAL DISCUSSION AND CONCLUSION.....	51
Recommendations.....	61
REFERENCES .....	62
APPENDICES .....	73
APPENDIX A; The submitted abstract for the 95 <sup>th</sup> Annual Meeting of the Physiological Society of Japan (PSJ2018). .....	73
APPENDIX B; The submitted manuscript in the Proceedings of the 13 <sup>th</sup> Rangsit University National Graduate Research Conference.....	73
VITA.....	74

## LIST OF TABLES

Table 3.1 Specific primer sequences for qrtRT-PCR .....	35
Table 4.1 Relative weights of testes and accessory sex organs, including seminal vesicles, prostate gland and epididymis of male rats at 4, 6, 8, 10 and 12 months old. ....	40



## LIST OF FIGURES

Figure 2.1 Schematic representation of the male hypothalamic–pituitary–testicular (HPT) axis. ....	7
Figure 2.2 Anatomical structure of a testis (A), histological cross-section of adult mouse testis (B), and a process of spermatogenesis (C). ....	8
Figure 2.3 Biosynthesis of steroid hormones originated from cholesterol. ....	10
Figure 2.4 Schematic of hypothalamic-pituitary-testicular axis. The area of hypothalamus is enlarged and described each of hypothalamic nuclei. ...	15
Figure 2.5 Model of kisspeptin signaling in the brain of male (a) and female (b) rodent. ....	18
Figure 2.6 The anatomy of rat hippocampus and circuit neuronal pathway in the hippocampus. Granule cells of dentate gyrus receives input from entorhinal cortex (layer II). Granule cells project their axons (the mossy fibers) to the pyramidal cells of CA3. Pyramidal cells of CA3 project to pyramidal cells of CA1 through Schaffer collaterals and pyramidal cells of CA1 sent output to entorhinal cortex through the subiculum. ....	23
Figure 2.7 Steroid synthesis pathway in rat hippocampus. ....	26
Figure 2.8 The model of feedback pathways for regulating neurosexsteroid production in the extrahypothalamic brain. ....	27
Figure 3.1 The measurement of learning behavior using Morris water maze test. ....	31
Figure 3.2 The measurement of spatial memory capacity by Morris water maze test. ....	32
Figure 4.1 Serum testosterone levels of male rats at 4, 6, 8, 10 and 12 months old. The data are presented as mean±SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. An asterisk (*) represents $p < 0.05$ compared with the 4 months old rats. ....	38
Figure 4.2 Serum LH levels of male rats at 4, 6, 8, 10 and 12 months old. The data are presented as mean±SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. Asterisks (* and **) represent $p < 0.05$ and $0.01$ compared with the 4 months old rats. ....	39
Figure 4.3 Learning behavior and memory capacity test by Morris water maze test. ....	42
Figure 4.4 The expression of mRNA levels of Kiss1 (A), Ar (B), Esr1 (C) and Esr2 (D) genes in the anteroventral periventricular nucleus (AVPV). The data are presented as mean±SEM. Number in the parenthesis in each bar	

indicates the number of rats used in each month. Asterisks (\*, \*\* and \*\*\*) represent  $p < 0.05$ ,  $0.01$  and  $0.001$  compared with the 4 months old rats.44

- Figure 4.5 The expression of mRNA levels of Kiss1 (A), Ar (B), Esr1 (C) and Esr2 (D) genes in the arcuate nucleus (ARC). The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. Asterisks (\* and \*\*) represent  $p < 0.05$  and  $0.01$  compared with the 4 months old rats. ....45
- Figure 4.6 The expression of mRNA levels of Kiss1r (A) and Gnrh1 (B) genes in the preoptic area (PoA). The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. An asterisk (\*) represents  $p < 0.05$  compared with the 4 months old rats. ....46
- Figure 4.7 The expression of mRNA levels of Ar (A), Esr1 (B) and Esr2 (C) genes in the median eminence (ME). The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. An asterisk (\*) represents  $p < 0.05$  compared with the 4 months old rats. ....47
- Figure 4.8 The expression of mRNA levels of Kiss1 (A), Gnrh1 (B), Lh $\beta$  (C), Kiss1r (D), Gnhr (E) and Lhr (F) genes in hippocampus. The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. Asterisks (\* and \*\*) represent  $p < 0.05$  and  $0.01$  compared with the 4 months old rats. ....49
- Figure 4.9 The expression of mRNA levels of P450scc (A), 3 $\beta$ -hsd (B), P450arom (C), Ar (D), Esr1 (E) and Esr2 (F) genes in hippocampus. The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. An asterisk (\*) represents  $p < 0.05$  compared with the 4 months old rats. ....50
- Figure 5.1 Changes of hypothalamic-pituitary-testicular (HPT) axis and hippocampus in male rats during reproductive senescence and cognitive impairment. .54
- Figure 5.2 The scheme of changes of serum testosterone and LH levels and transcript expression levels of genes associated with reproductive hormones and their receptors in hypothalamus and hippocampus of male rats. ....59

## LIST OF ABBREVIATIONS

3 $\beta$ -HSD	3 $\beta$ -hydroxysteroid dehydrogenase
3 $\beta$ - <i>hsd</i>	3 $\beta$ -hydroxysteroid dehydrogenase gene
17 $\beta$ -HSD	17 $\beta$ -hydroxysteroid dehydrogenase
A $\beta$	Amyloid- $\beta$
AHN	Anterior hypothalamic nucleus
AR	Androgen receptor
<i>Ar</i>	Androgen receptor gene
ARC	Arcuate nucleus
AVPV	Anteroventral periventricular nucleus
CA	Cornu ammonis
cDNA	Complementary deoxyribonucleic acid
DHEA	Dehydroepiandrosterone
DHT	Dihydrotestosterone
DMN	Dorsomedial nucleus
ELISA	Enzyme-linked immunosorbent assay
ER $\alpha$	Estrogen receptor $\alpha$
ER $\beta$	Estrogen receptor $\beta$
<i>Esr1</i>	Estrogen receptor $\alpha$ gene
<i>Esr2</i>	Estrogen receptor $\beta$ gene
FSH	Follicle-stimulating hormone
<i>Fsh<math>\beta</math></i>	Follicle-stimulating hormone $\beta$ -subunit gene

FSHR	Follicle-stimulating hormone receptor
<i>FSHR</i>	Follicle-stimulating hormone receptor gene
Gn	Gonadotropins
GnRH	Gonadotropin-releasing hormone
<i>Gnrh1</i>	Gonadotropin-releasing hormone gene
<i>Gnrhr</i>	Gonadotropin-releasing hormone receptor gene
HPT	Hypothalamic-pituitary-testicular
IVC	Individually ventilated cage
<i>Kiss1</i>	Kisspeptin gene
<i>Kiss1r</i>	Kisspeptin receptor gene
LH	Luteinizing hormone
<i>Lh<math>\beta</math></i>	Luteinizing hormone $\beta$ -subunit gene
LHA	Lateral hypothalamic area
<i>Lhr</i>	Luteinizing hormone receptor gene
ME	Median eminence
mRNA	Messenger ribonucleic acid
<i>P450arom</i>	Aromatase enzyme gene
<i>P450scc</i>	Cytochrome P450 gene
PoA	Preoptic area
PVN	Paraventricular nucleus
qrtRT-PCR	Quantitative real-time reverse transcription polymerase chain reaction
RNA	Ribonucleic acid
SCN	Suprachiasmatic nucleus

SON	Supraoptic nucleus
StAR	Steroidogenic acute regulatory protein
VMN	Ventromedial nucleus



# CHAPTER I

## GENERAL INTRODUCTION

Advancing age is associated with the loss of gonadal function and sex steroid hormone production, so-called reproductive senescence, in both females and males (Golan, Scovell and Ramasamy, 2015; Gunes et al., 2016; Maas, Jochen and Lalande, 1997). In females, ovarian function and serum estrogen levels are abruptly declined at menopause (Hall, 2015; Kermath and Gore, 2012). Although the testicular function and serum androgen levels are gradually and progressively decreased with advancing age, the reproductive senescence is not particularly detectable in males (Harman et al., 2001; Morley and Perry, 2000; Wang, Leung and Sinha-Hikim, 1993), and even some claims that andropause does not exist in men (Vermeulen and Kaufman, 1995). Androgen deficiency could be a causative factor in many symptoms and diseases, including neurodegeneration (Hanninen et al., 1996; Lv et al., 2016; Moffat et al., 2002; Murman, 2015), and an association between androgen deficiency and cognitive decline is substantially reported (Jimenez-Rubio et al., 2017; Zilbermint et al., 2013).

Androgens are mainly synthesized in the testis which is regulated via the hypothalamic-pituitary-testicular (HPT) axis (Vermeulen and Kaufman, 1995). The production of androgens is started with *Kiss1*-expressing neurons in the anteroventral periventricular nucleus (AVPV) and arcuate nucleus (ARC) regions of the hypothalamus producing and secreting kisspeptin. After binding with Kiss1 receptor (encoded by *Kiss1r* gene) on the GnRH neurons at the hypothalamic preoptic area (PoA), kisspeptin implicates in the neuroendocrine regulation of gonadotropin-releasing hormone (GnRH) production (Oakley, Clifton and Steiner, 2009; Smith,



Clifton and Steiner, 2006). GnRH neuron produces and secretes GnRH into the hypothalamic median eminence (ME) before transporting to anterior pituitary gland via hypophyseal portal vessel. At anterior pituitary gland, GnRH binds with GnRH receptor (encoded by *Gnrhr* gene) which, in turn, stimulates the synthesis and secretion of gonadotropins (Gn) which consist of follicle-stimulating hormone (FSH) and luteinizing hormone (LH). FSH binds with FSH receptors (encoded by *FSHR* gene) at Sertoli cells and promotes differentiation of spermatocytes into spermatids (Ji, Kubokawa and Abe, 1995), while LH binds with LH receptors (encoded by *Lhr* gene) onto Leydig cells in the testis and stimulates androgens production (Jin and Yang, 2014; Singh et al., 2017). To this end, androgens elicit negative-feedback effect on hypothalamus and pituitary gland to inhibit kisspeptin (Tng, 2015; Wahab et al., 2016), GnRH, and Gn production via its androgen receptor (AR) (Tilbrook and Clarke, 2001; Veldhuis, Urban and Dufau, 1992). Besides, testosterone (a primary androgen hormone in males) can be aromatized to estrogen and then binds with estrogen receptors ( $ER\alpha$  and  $ER\beta$ ) at hypothalamus and pituitary gland. Recent studies denoted that *Kiss1*- and *Gnrh1*-expressing neurons were a direct target of sex steroid hormones because they expressed both androgen receptor (*Ar*) and estrogen receptors (*Esr*) genes (Poletti et al., 2001; Smith et al., 2005).

Hippocampus is the brain area that functions on learning and memory. In the past, it was known that androgen and estrogen (after binding with AR and ERs) are key factors functioning on maintaining the cognitive capability (Mohamad, Im Nirwana and Chin, 2018; Zárate, Stevnsner and Gredilla, 2017). Thus, if the androgen deficient status happens in males, it should lead to cognitive impairment (Hsu et al., 2015; Moffat et al., 2002). However, it was recently proposed that kisspeptin, GnRH

and LH could interact directly with hippocampal neurons and affect on cognition, regarding the discovery of transcript expression of *Kiss1r*, *Gnrhr* and *Lhr* genes at hippocampus (Arai, 2009; Beyenburg et al., 2000; Jennes et al., 1995; Leblanc et al., 1988; Lei et al., 1993; Tsai et al., 2015). Apart from those receptors expression, hippocampus also locally synthesizes the reproductive hormones such as kisspeptin, GnRH, LH, androgens and estrogens (Fester et al., 2011; Prange-Kiel et al., 2013) as seen in the HPT axis (Meethal et al., 2009; Rosati et al., 2011).

Thus, it is interesting to know when a reproductive senescence and a cognitive impairment can be detected in male Sprague-Dawley rats aged 4-12 months old. For a reproductive senescence, a significant reduction of serum testosterone level is used as an indicator in this study. For a cognitive impairment, an impairment in spatial learning and memory determined by Morris water maze test is used as an indicator. As mentioned above that the HPT and the hippocampus could likewise produce reproductive hormones, thus transcript expression of genes associated with reproductive hormones and their receptors at the two brain regions and the association between them were assessed. Hopefully, the results of this study can shade some light on the association between the reproductive senescence and cognitive impairment and lead to the exploration of the suitable treatments in the future.

## Objectives

1. To determine changes of reproductive hormone levels secreted by pituitary gland and testis and accessory sex organ weights in male rats during cognitive impairment
2. To determine changes of reproductive hormone-related genes in hypothalamus and hippocampus in cognitive impaired male rats
3. To investigate an association of the age of rats and the expression of reproductive hormone-related genes in hypothalamus and hippocampus in male rats during reproductive senescence and cognitive impairment



## **CHAPTER II**

### **LITERATURE REVIEW**

An average lifespan of humans is currently progressive and longer than those in the past which brings, as a result, an increase in aging population and aging diseases. In men, aging is generally accompanied by the decline in serum androgen levels, namely androgen deficient status (Swerdloff and Wang, 1993), which is an indicator of reproductive senescence. Many age-related diseases including neurodegeneration and cognitive impairment are attributed to the androgen deficiency (Lv et al., 2016; Swerdloff and Wang, 1993). Low levels of androgen in aging men appear to effect on spatial abilities and cognitive function (Hier and Crowley, 1982; Muller et al., 2005). Thus, androgen deficiency is the key factor of reproductive senescence and cognitive impairment.

Thus, this chapter summarizes the basic knowledge about reproductive senescence and cognitive impairment which were separated into two parts; i)reproductive system which described about HPT axis and their structures and hormonal synthesis in each component of the axis, and ii)cognition which described about cognitive function, cognitive impairment, structure and local steroid synthesis in the hippocampus.

#### **1. Reproductive system**

The male reproductive system includes testes and a number of accessory sex organs. Testis which plays a major role on male gamete production (or

spermatogenesis) and sex steroid hormone synthesis (or androgen production) is subsequently regulated by hypothalamus and pituitary gland, so-called the HPT axis. Each level produces unique hormones that stimulate or inhibit the subsequent level of the axis.

### 1.1 The role of HPT axis

Considering the top-down on the HPT axis, the hypothalamus produces and secretes GnRH, which is encoded by *Gnrh1* gene in the GnRH neurons in the PoA. GnRH is transported through the hypophyseal portal vessel to the anterior pituitary gland, where it binds to GnRH receptors on the gonadotrope cells, to modulate the production and secretion of the Gn, which consist of FSH and LH. Both FSH and LH are secreted into the blood circulation and bind to their receptors (FSHR and LHR) in the testis. At the testis, FSH stimulates Sertoli cells and plays a role in spermatogenesis (Ji, Kubokawa and Abe, 1995; Ramaswamy and Weinbauer, 2014). LH stimulates Leydig (or interstitial) cells to produce androgens (Jin and Yang, 2014). Testosterone is a primary androgen which is secreted to the blood circulation and binds to AR on target cells in several organs such as accessory sex organs (including epididymis, prostate gland, and seminal vesicle), bone, muscle and brain. Moreover, it can be converted to estradiol by aromatase enzyme (encoded by *P450arom*) and then binds to ERs. To keep the homeostasis, testosterone in turn has a negative feedback effect on hypothalamus and pituitary gland to inhibit the pulsatile release of GnRH and LH/FSH, respectively (Tilbrook and Clarke, 2001; Veldhuis, Urban and Dufau, 1992) (Figure 2.1).

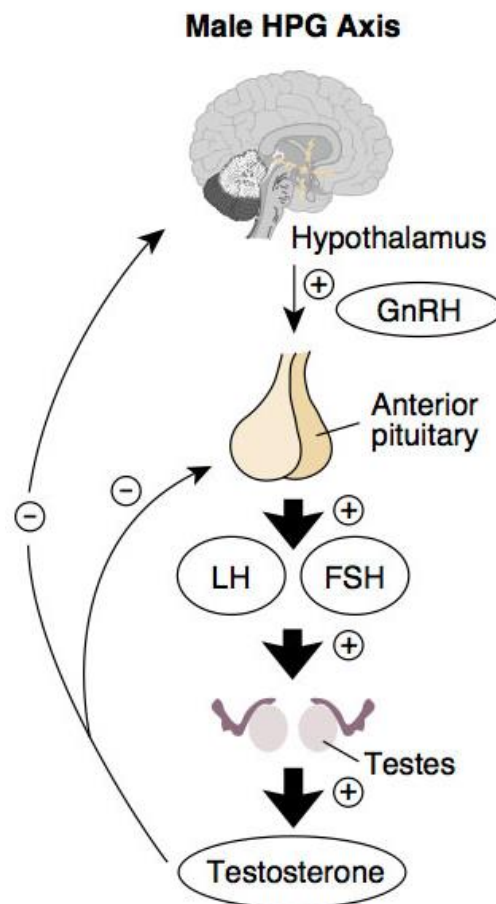


Figure 2.1 Schematic representation of the male hypothalamic–pituitary–testicular (HPT) axis.

Source: Kong et al. (2014)

## 1.2 Testis

### 1.2.1 Structure of the testis

Testes are a pair oval-shaped organ that locate within the scrotum. Each testis is covered by a fibrous capsule, the tunica albuginea, which invaginates to form septa that divide into lobules inside the testis. Within each lobule, it contains the convoluted folded tubules or the seminiferous tubules. Within the seminiferous tubule, it contains Sertoli cells which extend from the basement membrane to the lumen of the tubule and support the development and differentiation of germ cells into

spermatozoa. Sertoli cells are connected together by a tight junction and formed a blood-testis barrier; a physical barrier between blood vessels and seminiferous tubules, and isolating the developmental stages of germ cells. In each lobule, seminiferous tubule ends to the rete testes located within the mediastinum and connected to epididymis. Spaces between seminiferous tubule can be seen the Leydig cells (Tortora and Derrickson, 2014) (Figure 2.2A, B).

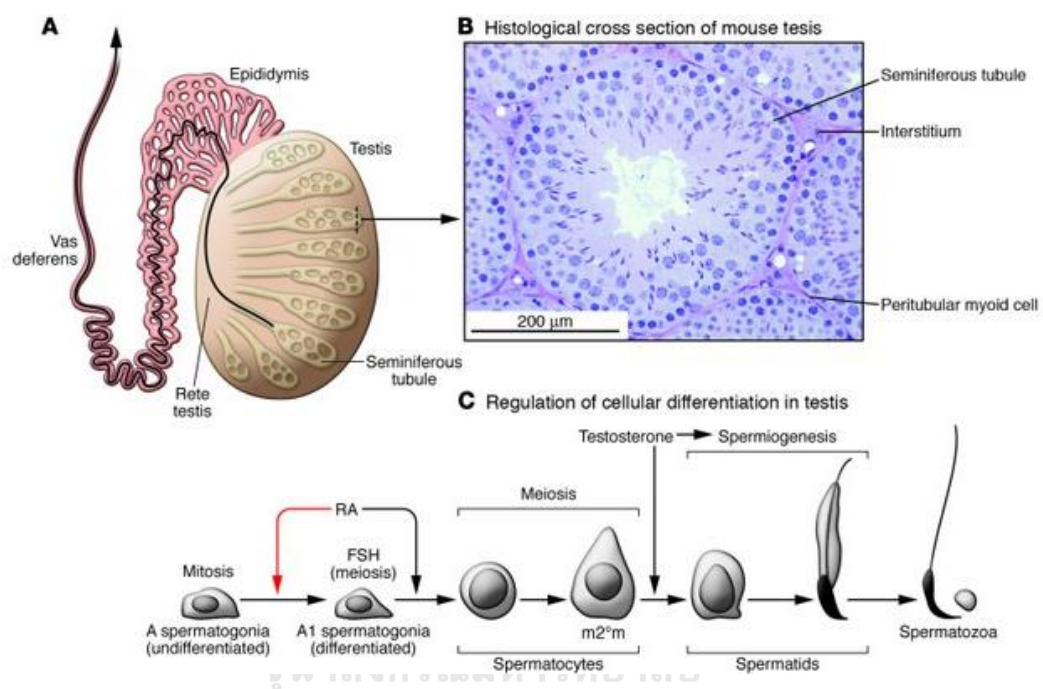


Figure 2.2 Anatomical structure of a testis (A), histological cross-section of adult mouse testis (B), and a process of spermatogenesis (C).

Source: Hogarth and Griswold (2010)

### 1.2.2 Testicular function

The main function of testis is to produce sperm (spermatogenesis) and synthesize testosterone (steroidogenesis).

### 1.2.2.1 Spermatogenesis

Spermatogenesis is a process of sperm production which is developed from primordial germ cells in the seminiferous tubule. Spermatogenesis can be divided into three phases; proliferative, meiotic and differentiation (Hess, 1998) (Figure 2.2C).

- Proliferative phase; spermatogonia, diploid germ cells, are proliferated by mitotic division and developed to primary spermatocytes.
- Meiotic phase; primary spermatocytes begin the meiosis division to produce haploid spermatid germ cell.
- Differentiation phase or spermiogenesis; haploid spermatids undergo dramatic structural changes into the mature spermatozoa (Hess, 1998).

### 1.2.2.2 Steroidogenesis

Steroidogenesis is the process of steroid hormone synthesis. In male, testis is a main organ that produces and secretes sex steroid hormones. Testosterone is a major male sex hormone required for development of the male reproductive organs and secondary sexual characteristics. Testosterone which is synthesized by the Leydig cells locating inside the testis is metabolized from cholesterol that is transferred into the inner mitochondrial membrane by steroidogenic acute regulatory protein (StAR) and converted to pregnenolone by cytochrome P450 (encoded by *P450scc* gene). LH initiates the production of pregnenolone in Leydig cells by regulating StAR and cytochrome P450 (Payne and Youngblood, 1995). Pregnenolone is converted to testosterone through two pathways; delta-4 ( $\Delta^4$ ) and delta-5 ( $\Delta^5$ ).



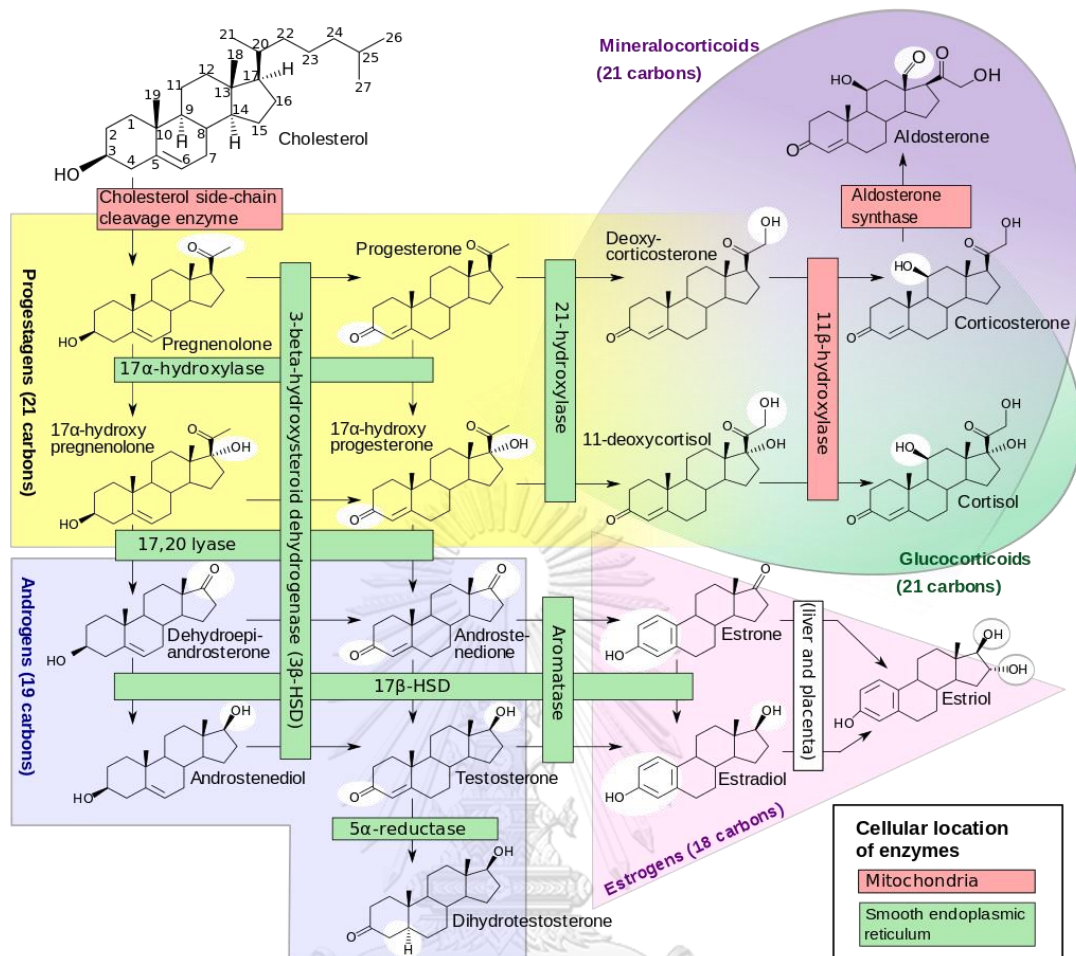


Figure 2.3 Biosynthesis of steroid hormones originated from cholesterol.

The mitochondrial cytochrome P450 converts cholesterol to pregnenolone. The delta-4 pathway proceeds to progesterone and then converted to 17 $\alpha$ -hydroxyprogesterone, and androstenedione, respectively. The delta-5 pathway, pregnenolone converted to 17 $\alpha$ -hydroxypregnenolone, dehydroepiandrosterone (DHEA) and terminated at androstenediol. Both androstenedione and androstenediol can be converted to testosterone.

Source: Haggstrom (2014).

For the delta-4 pathway, the position of an unsaturated bond occurs between C-4 and C-5 of ring A, while for a delta-5 pathway, an unsaturated bond occurs between C-5 and C-6 of ring B during the conversion of pregnenolone to testosterone (Preslock, 1980), and the detail of conversion is described below.

- The delta-4 pathway; pregnenolone is converted to progesterone by  $3\beta$ -hydroxysteroid dehydrogenase ( $3\beta$ -HSD) and  $\Delta^{5,4}$ -isomerase enzymes, and then converted to  $17\alpha$ -hydroxyprogesterone, androstenedione and testosterone by  $17\alpha$ -hydroxylase, C<sub>17-20</sub>-lyase, and  $17\beta$ -hydroxysteroid dehydrogenase ( $17\beta$ -HSD) enzymes, respectively.
- The delta-5 pathway; pregnenolone is converted to  $17\alpha$ -hydroxypregnenolone by  $17\alpha$ -hydroxylase enzyme, and to dehydroepiandrosterone (DHEA), 5-androstene- $3\beta$ ,  $17\beta$ -diol (androstenediol) and testosterone by C<sub>17-20</sub>-lyase,  $17\beta$ -HSD and  $3\beta$ -HSD enzymes, respectively.

In addition to pregnenolone, all other compounds in the delta-5 pathway can also be converted to the corresponding delta-4 compounds (Preslock, 1980; Stanczyk, 2009) (Figure 2.3). At the end of the steroidogenesis in the testis, testosterone is secreted into the blood circulation, which is transported to target organs that express its receptors. Moreover, testosterone can be converted to dihydrotestosterone (DHT), its more biologically active form, by  $5\alpha$ -reductase, and can be converted to estradiol by aromatase. Both testosterone and DHT act via the AR (Chang et al., 1995), and estradiol acts via ERs (both ER $\alpha$  and ER $\beta$ ) (Dickson and Clark, 1981).

## 1.3 Pituitary gland

### 1.3.1 Structure of pituitary gland

Pituitary gland is the brain area that locates at the base of the brain (below the hypothalamus). The pituitary gland is subdivided into two lobes; posterior lobe (or neurohypophysis) that contains axons and nerve ending of neurons and anterior lobe (or adenohypophysis) that contains endocrine cells.

Posterior pituitary gland is a neural tissue which contains axons and nerve ending of neurons from supraoptic and paraventricular nucleus of the hypothalamus. Neurohypophyseal hormones, including oxytocin and antidiuretic hormone, are synthesized in the neurons of hypothalamus and released through the neuronal axon into posterior pituitary gland before passing to the blood vessels (Osamura, 1983).

Anterior pituitary gland consists of endocrine cells that produce and secrete peptide hormones which are controlled by releasing hormones from the hypothalamus. Osamura (1983) divided pituitary secretory cells into two groups, based on the orange G and PAS staining, including acidophilic (orange G-positive) and basophilic (PAS-positive) cells.

- Acidophilic (orange G-positive) cells subdivide into growth hormone and luteotropic hormone cells.
- Basophilic (PAS-positive) cells subdivide into corticotropes (secreting adrenocorticotrophic hormone), thyrotropes (secreting thyrotrophic hormone), and gonadotropes (secreting LH and FSH).

### 1.3.2 Anterior pituitary gland and LH/FSH production

Gonadotrope cells in anterior pituitary gland secrete Gn which includes LH and FSH. LH and FSH are a family of glycoprotein hormones which are composed of two ( $\alpha$  and  $\beta$ ) subunits. The  $\alpha$ - subunit is common to both LH and FSH, but the  $\beta$  subunit is specific for their structure and function which is separately encoded by *Lh $\beta$*  and *Fsh $\beta$*  genes (Mullen, Cooke and Crow, 2013). The production and secretion of Gn are regulated by the hypothalamic GnRH pulsatile. A pulsatile of GnRH (both amplitude and frequency) is essential to maintain Gn secretion (Marshall et al., 1993) . High pulse frequency of GnRH is more effective in releasing LH, while low pulse frequency is effective in releasing FSH (Jayes, Britt and Esbenshade, 1997). In the rat, both  $\alpha$ - and  $\beta$ -subunit transcriptions of Gn were suppressed by the GnRH antagonist but in different actions; low dose of GnRH antagonist suppressed *Lh $\beta$*  mRNA more than *Fsh $\beta$*  mRNA levels, while all three Gn subunits;  $\alpha$ -subunit, *Lh $\beta$*  and *Fsh $\beta$*  were suppressed by high dose of GnRH antagonist (Wierman, Rivier and Wang, 1989).



## 1.4 Hypothalamus

### 1.4.1 Structure of hypothalamus

Hypothalamus is a key regulator of homeostasis in animals. It is a small region of the brain in the ventral part of the diencephalon and locates in the basal forebrain below the thalamus. It connects the brain to the pituitary gland via infundibular stalk (Tortora and Derrickson, 2014).

Hypothalamus is composed of many small nuclei and can be subdivided into three zones; periventricular, medial and lateral hypothalamic area (Elizondo-Vega et al., 2015).

- The periventricular zone includes PoA, suprachiasmatic nucleus (SCN), paraventricular nucleus (PVN), ARC and posterior nucleus.
- The medial zone includes medial preoptic nucleus, anterior hypothalamic nucleus (AHN), ventromedial nucleus (VMN), dorsomedial nucleus (DMN) and premammillary nucleus.
- The lateral hypothalamic area (LHA) includes lateral preoptic nucleus, lateral hypothalamic nucleus, tuberomammillary nucleus and supraoptic nucleus (SON).

Each nucleus contains several neuronal types and produces many neuropeptides or neurotransmitters to communicate with other brain areas.

## Hypothalamic–Pituitary–Testicular (HPT) Axis

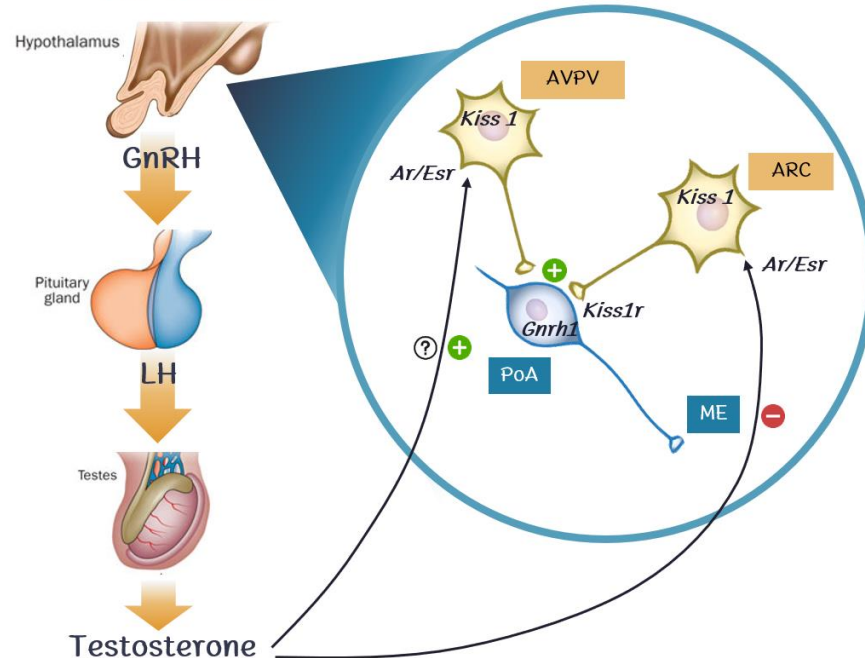


Figure 2.4 Schematic of hypothalamic-pituitary-testicular axis. The area of hypothalamus is enlarged and described each of hypothalamic nuclei. AVPV: anteroventral periventricular nucleus; ARC: arcuate nucleus; PoA: preoptic area and ME: median eminence (the basal area of hypothalamus that contains nerve ending of neurosecretory cells).

### 1.4.2 Function of hypothalamus and GnRH production

Hypothalamus is a part of the nervous system that has diverse functions in regulating the homeostasis of our bodies by mediating autonomic, behavioral and endocrine function such as temperature regulation, sleep, hunger, mood, blood pressure, and reproduction (Pearson and Placzek, 2013; Tortora and Derrickson, 2014). It also connects between nervous system and endocrine system. Thus, it controls the activity of endocrine glands especially pituitary gland through the releasing (neuropeptides) hormones (Halász, 2000). Their hormones are released to

pituitary gland at the ME, a basal area of hypothalamus that contains the nerve ending of neurosecretory cells, and are transported via nervous or blood vessel pathways to posterior and anterior pituitary gland, respectively (Card, 2001; Javorsky et al., 2011).

Concerning the reproductive system, GnRH is a key hormone synthesized by hypothalamus and released to anterior pituitary gland. GnRH stimulates the production and secretion of Gn in the anterior pituitary gland (Clarke and Cummins, 1982; Kaiser et al., 1997). GnRH is a decapeptide hormone (pGlu-His-Trp-Ser-Tyr-Gly-Leu-Arg-Pro-Gly-NH<sub>2</sub>) (Marques et al., 2015), which encoded by *Gnrh1* gene in GnRH neuron. GnRH cell bodies locate in the PoA of the hypothalamus, and project axons to the ME. Hypothalamus releases GnRH in a pulsatile pattern and transports it through the hypophyseal portal vessel to the anterior pituitary gland (Clarke and Cummins, 1982; Kaiser et al., 1997).

Previous studies proposed that the production and secretion of GnRH are stimulated or inhibited by various neurotransmitter/neuromodulators; e.g. catecholamines, epinephrine, and norepinephrine stimulated, while opioids ( $\beta$ -endorphin) inhibited (Sagrillo et al., 1996). In 2003, kisspeptin was discovered and found its role on reproduction by regulating GnRH/LH secretion (Tena-Sempere, 2006) and controlling sexual maturation and puberty (de Roux et al., 2003; Seminara et al., 2003). Kisspeptin is a neuropeptide that is encoded by *Kiss1* gene in kisspeptin neuron. *Kiss1* gene encodes the 145-amino acid precursor and is cleaved to 54, 14, 13 or 10 amino acids (Oakley, Clifton and Steiner, 2009). Kisspeptin neurons are distributed in the hypothalamus at AVPV, ARC and PVN, its fibers are abundant within the preoptic, periventricular, dorsomedial and arcuate nuclei of rodent hypothalamus (Clarkson et al., 2009; Gottsch et al., 2004). In rodent, the expression

of kisspeptin in AVPV and ARC region are increased during pubertal development (Semaan, Tolson and Kauffman, 2013). Kisspeptin in the AVPV is sexually differentiated; a greater number in females than in males, but in ARC no difference in density and distribution between sexes is detected (Kauffman, Clifton and Steiner, 2007). Kisspeptin regulates the GnRH production after binding with GPR54 receptor (or Kiss1 receptor), which encoded by *Kiss1r* gene, on the GnRH neurons at PoA (Irwig et al., 2004; Oakley, Clifton and Steiner, 2009; Smith, Clifton and Steiner, 2006). GnRH neurons are direct targets for the action of kisspeptin that stimulates Gn secretion through a GnRH-dependent mechanism (Irwig et al., 2004). Gottsch et al. (2004) found that LH and FSH secretion was increased after injecting kisspeptin to adult male C57BL/6 mice, but suppressed when treating with acyline (a GnRH antagonist) before kisspeptin injection. The loss of GPR54 receptor function affects development of reproductive organs by reducing the size of testes and ovaries (Seminara et al., 2004).

Kisspeptin neuron is a direct target of gonadal steroid hormones because it contains both AR and ERs. Smith et al. (2005) found that the expression of *Kiss1* mRNA was regulated by both testosterone and estradiol in the brain of male rodent. The expression of *Kiss1* mRNA and cell number in ARC were increased in castrated male rodent and reduced by testosterone or DHT treatment (Smith et al., 2005). Conversely, the expression in AVPV was decreased in castrated rodent, and partially restored by testosterone and fully restored by estradiol treatment, however, treatment with DHT had no effect on Kiss1 cell number (Irwig et al., 2004; Kauffman et al., 2007; Smith et al., 2005). The increase in *Kiss1* mRNA levels at ARC and decrease at AVPV after castration correlated with increased and decreased LH secretions,



respectively (Kauffman et al., 2007). It shows that kisspeptin in ARC is the site that mediates the negative feedback effect of testosterone on GnRH/LH secretion whereas kisspeptin in AVPV is sensitive to estrogen and plays a role in GnRH/LH surge in females (Kauffman, Clifton and Steiner, 2007). Although in males testosterone also showed a positive effect on kisspeptin neuron but it had no effect on GnRH/LH surge (Figure 2.5) (Kauffman, Clifton and Steiner, 2007).

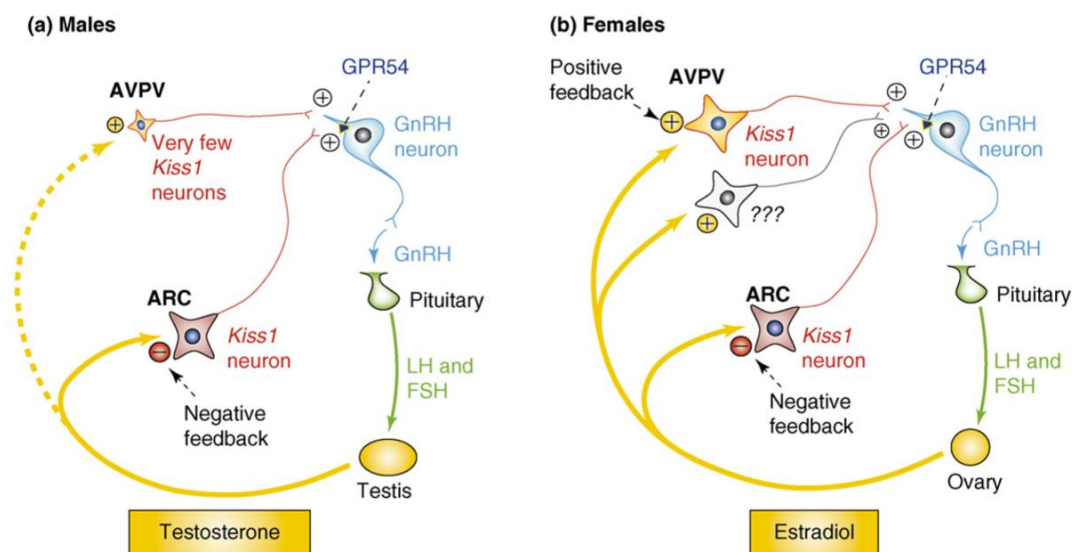


Figure 2.5 Model of kisspeptin signaling in the brain of male (a) and female (b) rodent.

Source: Kauffman, Clifton and Steiner (2007)

## 2. Cognition

Cognitive function is the intellectual activity that includes mental processes such as attention, processing speed, learning and memory, executive function, verbal fluency, and working memory (González-Ortega, Martínez-Cengotitabengoa and González-Pinto, 2017). Hippocampus is the brain region that critically involves in cognition, learning and memory formation. Hippocampus is a major component of the brain in memory consolidation (Gräff et al., 2012; Vertes, 2005) and plays an important role in spatial learning and memory (Good, 2002; Sweatt, 2004). In most rodent studies, spatial memory has been assessed using the Morris water maze that was designed by Richard Morris (Morris, 1981). It is a device to assess the rat's ability to learn to navigate to a specific location. The rats with hippocampal lesion are impaired in spatial learning behavior in the Morris water maze test, that is, they show an impairment in searching the hidden platform (Morris et al., 1982; Pearce, Roberts and Good, 1998).

### 2.1 Cognitive impairment

Cognitive impairment is when a person has trouble remembering, learning new things, concentrating, or making decisions that affect their everyday life (Centers for Disease Control and Prevention, 2011). It ranges from mild cognitive impairment to dementia such as Alzheimer's disease that can be caused by neurodegeneration. Three hypotheses are proposed as neuropathological hallmarks of neurodegenerative diseases including extracellular amyloid- $\beta$  ( $A\beta$ ) plaques, intracellular neurofibrillary tangles and neuronal synaptic loss.

- Extracellular accumulation of insoluble 40- or 42-amino acid amyloid- $\beta$  peptide (A $\beta$ 40 and A $\beta$ 42) is one of the major pathological lesions of neurodegenerative diseases. A $\beta$ 40 and A $\beta$ 42 are generated from amyloid precursor protein and are major components of senile plaques (Hardy, 2009; Serrano-Pozo et al., 2011).
- Intracellular neurofibrillary tangles are aggregates of hyperphosphorylated tau proteins within the neurons and glia of people with neurodegenerative disease. Normally, tau protein is to promote assembly of the microtubule protein subunit-tubulin into microtubules and to stabilize their structures. Hyperphosphorylation of tau leads to destabilization of the microtubule structure (Iqbal, Liu and Gong, 2016).
- Synaptic change, especially the loss of synapses and/or synaptic proteins, is effect to synaptic plasticity and neuronal communication, which play a key role in Alzheimer's disease, probably contributing directly to the profound cognitive impairment (Lu and Chow, 1999; Scheff and Price, 2006).

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Although the cause of cognitive impairment is unclear, one of the major risk factors for cognitive impairment is age. Cognitive function declines with advancing age (Bettio, Rajendran and Gil-Mohapel, 2017). The physiological process of aging involving progressive decline in the cognitive functions is structural changes both grossly and cellular levels of hippocampus such as reduced neuronal count (Issa et al., 1990), number of synaptic connections (Geinisman et al., 1995) and hippocampal volume (Golomb et al., 1993; Jernigan et al., 1991), which lead to the deterioration in learning and memory ability.

Moreover, age-related loss of sex steroid hormones in both females and males also associated with neurodegeneration and cognitive decline (Hier and Crowley, 1982; Leifke et al., 2000; Lv et al., 2016; Moffat et al., 2002; Muller et al., 2005). Sex steroid hormones bind with AR and ERs and affect learning and memory at hippocampus (Beyenburg et al., 2000; Tsai et al., 2015). Sex steroid hormones and its receptors regulate morphology, plasticity, neurogenesis and physiology of hippocampus in both male and female rodents (Fester and Rune, 2015). In adult males, androgens have profound effects on hippocampal structure and function such as inducing formation of excitatory spine synapses on the dendrites of pyramidal neurons (MacLusky et al., 2006) and enhancing the survival of new dentate gyrus neurons (Hamson et al., 2013), via an AR-dependent mechanism. Moffat et al. (2002) found that hypogonadal men had significantly lower scores on memory measures and visuospatial performance, and a faster rate of decline in visual memory. However, it can be improved by testosterone supplementation (Cherrier, Craft and Matsumoto, 2003). Patients with mild cognitive impairment or Alzheimer's disease and supplemented with testosterone for 6 weeks showed significantly better scores regarding spatial memory, constructional abilities, and verbal memory (Cherrier et al., 2005). Recently, it was found that estrogen and androgen can be locally synthesized in hippocampus (Kimoto et al., 2001; Mukai et al., 2006) and affected on its structure and function such as modulating the synaptic plasticity (Mukai et al., 2006; Prange-Kiel et al., 2006).

## 2.2 Structure of hippocampus

Hippocampus is one of the brain regions making up the limbic system. It locates in the medial temporal lobe, under the cerebral cortex. Mammal has two hippocampi, one in each cerebral hemisphere (Taupin, 2007). The hippocampal system consists of two interlocked cell layers; the dentate gyrus and Ammon's horn or cornu ammonis (CA). The dentate gyrus is an input region which receives input from entorhinal cortex and consists of granule cells (Hayman et al., 1998). The CA fields of the hippocampus are subdivided into four (CA1, CA2, CA3 and CA4) regions, which consist of pyramidal cells (Hayman et al., 1998). The CA1 and CA3 are the largest regions. CA1 region is adjacent to the subiculum, and CA3 region is adjacent to the fimbria/fornix region which receives input from the dentate gyrus. The CA2 region is a small region between CA1 and CA3. The CA4 is the small region that locates in the hilus of the dentate gyrus (Figure 2.6) (Brotons-Mas, O'Mara and Sanchez-Vives, 2006; Hayman et al., 1998; Taupin, 2007; Wible, 2013).

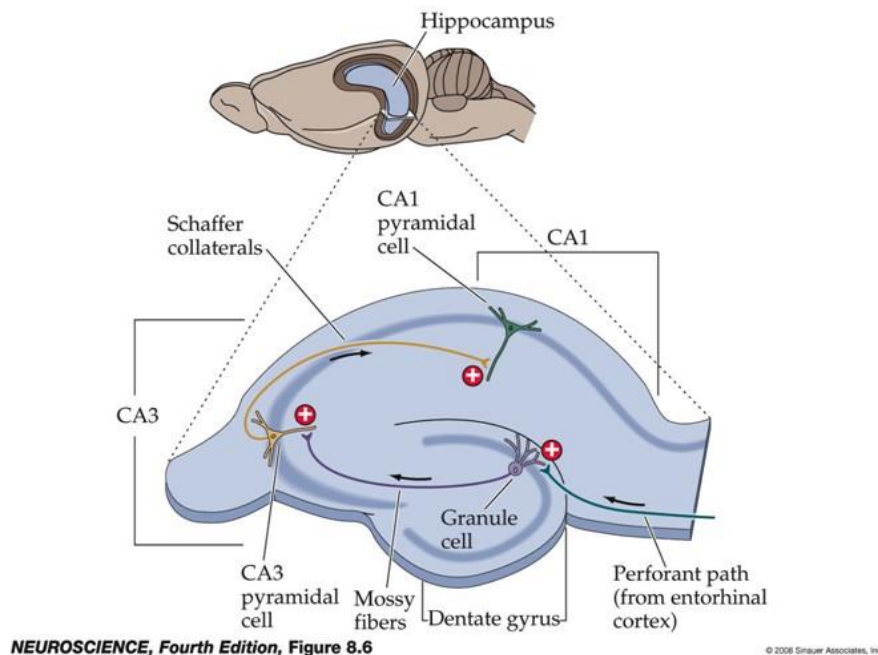


Figure 2.6 The anatomy of rat hippocampus and circuit neuronal pathway in the hippocampus. Granule cells of dentate gyrus receives input from entorhinal cortex (layer II). Granule cells project their axons (the mossy fibers) to the pyramidal cells of CA3. Pyramidal cells of CA3 project to pyramidal cells of CA1 through Schaffer collaterals and pyramidal cells of CA1 sent output to entorhinal cortex through the subiculum.

Source: Purves et al. (2008)

### 2.3 Local sex steroid synthesis in the hippocampus

Although testosterone is produced mainly in Leydig cells of the testes, it has been recently reported to be synthesized in hippocampus. Other than testosterone, the hippocampus also locally synthesizes other reproductive hormones, i.e., kisspeptin, GnRH, LH, androgens and estrogens, and expresses reproductive hormone receptors such as *Gnrhr*, *Lhr*, *Ar* and *Ers* as seen in the HPT axis (Meethal et al., 2009).

In the HPT axis, GnRH axons extend to the ME of the hypothalamus and secrete GnRH to stimulate the secretion of LH and FSH at pituitary gland via GnRH

receptor (Perrett and McArdle, 2013). However, GnRH can also effect outside the reproductive axis because the previous study found that some GnRH axons extend to other regions of the central nervous system including the limbic system (Silverman, Jhamandas and Renaud, 1987) and *Gnrhr* is also expressed in extra-pituitary tissues, including adrenal, kidney, heart, bladder and brain regions (cerebral cortex, cerebellum and limbic system especially in the hippocampus) (Albertson et al., 2008; Badr and Pelletier, 1987; Jennes et al., 1997; Skinner et al., 2009). In hippocampus, *Gnrhr* expresses in the pyramidal cells from CA1 to CA4 of cornu ammonis region and granule cells of dentate gyrus (Chu, Gao and Huang, 2008; Jennes et al., 1995; Leblanc et al., 1988; Wilson et al., 2006). GnRH is also detected in the cerebrospinal fluid (Caraty and Skinner, 2008). Thus, it was proposed that GnRH secreted from the pituitary gland can cross the blood-brain barrier into the cerebrospinal fluid of the third ventricle, but extremely high doses are required and affect other brain regions (Caraty and Skinner, 2008).

Recent studies reported that GnRH induced biosynthesis of reproductive hormones in the hippocampus after binding with its receptors (Fester et al., 2011; Prange-Kiel et al., 2008). GnRH induced an up-regulation of LH $\beta$  subunit in M17 human neuroblastoma cells (Wilson et al., 2006), and induced expression of *Lh $\beta$*  mRNA in SH-SY5Y human neuroblastoma cells (Rosati et al., 2011). Moreover, it was found that FSH and FSH receptor immunoreactivity co-expressed with *Gnrhr* in pyramidal neurons of CA1 to CA4 region and granular neurons of dentate gyrus (Chu, Gao and Huang, 2008). However, the function of FSH and the effect of GnRH on FSH expression in hippocampal neurons are unclear (Chu, Gao and Huang, 2008). *Lh $\beta$*  mRNA is also co-expressed with *Gnrhr* in the hippocampal neurons (Wilson et

al., 2006). LH and FSH are distributed widely throughout the central nervous system, i.e., hypothalamus, amygdala, cerebral cortex and hippocampus (Bowen et al., 2002; Chu, Gao and Huang, 2008; Emanuele et al., 1983).

Aside from GnRH and Gn, the active biosynthesis of sex steroid hormones also occurs in the brain (Hamson et al., 2013). This synthesis can be either *de novo* from the endogenous precursor (cholesterol) or derived from classical steroids which enter through bloodstream into the nervous system. Hippocampus expresses the steroidogenic enzyme encoding genes such as *StAR*, *P450scc*, *17 $\beta$ -HSD*, *3 $\beta$ -HSD*, *5 $\alpha$ -reductase* and *P450arom* (Do Rego et al., 2009; Hojo et al., 2008; Pelletier, 2010). StAR is the rate-limiting step of steroidogenesis that mediates the transfer of cholesterol from the outer to the inner mitochondrial membrane. Its immunoreactivity is expressed in neurons of CA1–CA3 and in the dentate gyrus of rat brain (King et al., 2002) and co-localized with cytochrome P450, aromatase and another enzymes in the steroidogenesis pathway (Hojo et al., 2004; Kimoto et al., 2001; Wehrenberg, Prange-Kiel and Rune, 2001). Many past studies found that GnRH can regulate steroidogenesis in the hippocampal neurons (Fester et al., 2011; Prange-Kiel et al., 2013) via regulating steroidogenic enzyme activity (Kretz et al., 2004; Prange-Kiel et al., 2008; Prange-Kiel et al., 2003). For example, incubating the SH-SY5Y cells with 1.0 nM GnRH significantly up-regulated StAR and P450scc expression and consequently estradiol production (Rosati et al., 2011). LH can also induce steroid synthesis via LH receptor by modulating the expression of LH receptor and increasing StAR and P450scc expression in rat primary hippocampal neurons (Liu et al., 2007). Webber et al. (2006) found that the expression of LH receptors co-localized with



StAR in rat hippocampal neurons. Thus, this indicates that GnRH and LH can directly induce local steroid synthesis in hippocampus.

Both male and female hippocampus can produce estradiol, testosterone and DHT (Hojo et al., 2014; Kretz et al., 2004) by converting cholesterol to pregnenolone, dehydroepi-androsterone, androstenediol, testosterone, DHT and estradiol, respectively (Hojo et al., 2004; Hojo et al., 2011), as those seen in the steroidogenesis pathway in reproductive organs (Figure 2.7).

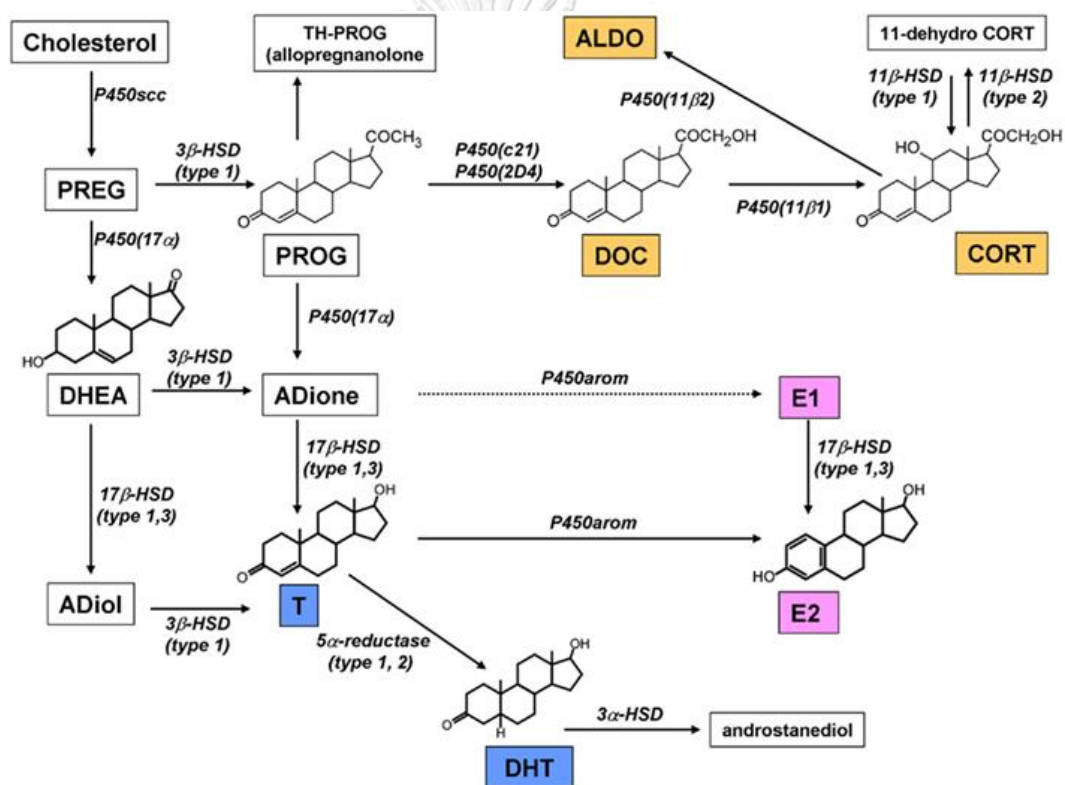


Figure 2.7 Steroid synthesis pathway in rat hippocampus.

Source: Hojo et al. (2011)

The local *de novo* sex steroid synthesis pathway in hippocampus is not well understood. However, Rosati et al. (2011) proposed that the local *de novo* sex steroid synthesis in hippocampus had a level of regulation as seen in the HPT axis. Because they found that SH-SY5Y cells up-regulated an expression of *Lhβ* mRNA after

incubation with GnRH for 90 min, increased an expression of *StAR* mRNA and protein after 90 min to 3 h of incubation, increased *P450<sub>scc</sub>* after 3 to 6 h of incubation, and increased estradiol synthesis after 24, 48, and 72 h of incubation, respectively. Their study was consistent with the model of the endocrine and paracrine/autocrine regulatory feedback loops for the regulation of neurosteroid synthesis in the brain of Meethal et al. (2009). Meethal et al. (2009) proposed that GnRH from hypothalamus (or extrahypothalamic brain) transported via efferent pathways into the hippocampus bound with GnRH receptor (GnRHR) and induced Gn production which in turn signaled via Gn receptors on neurons around the dentate gyrus to stimulate the neurosexsteroid synthesis (Figure 2.8).

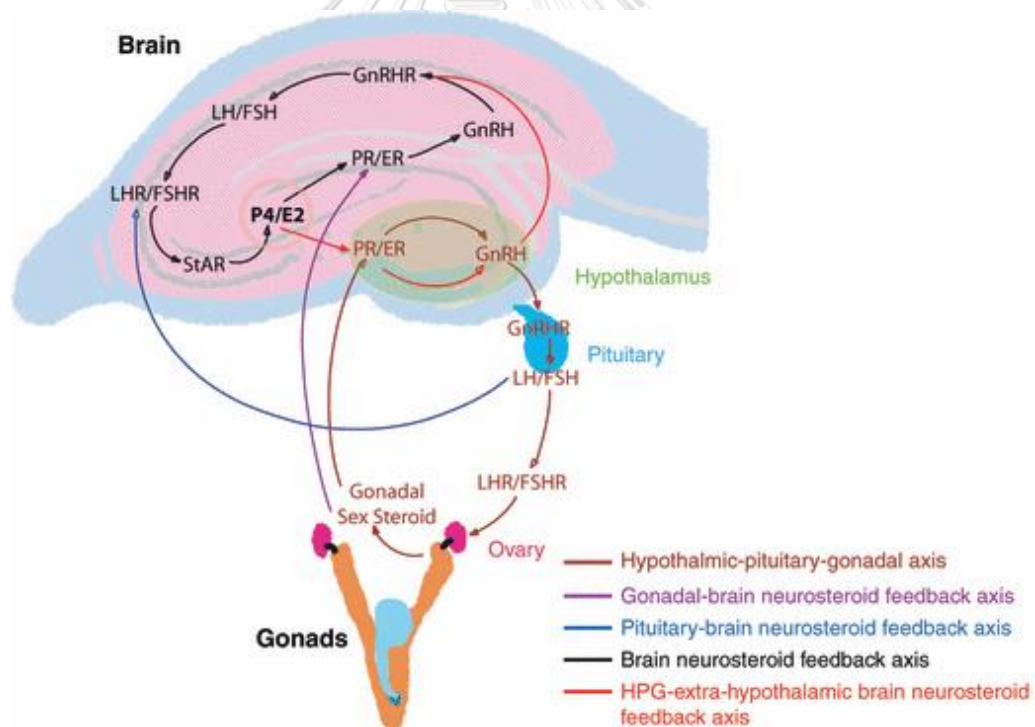


Figure 2.8 The model of feedback pathways for regulating neurosexsteroid production in the extrahypothalamic brain.

Source: Meethal et al. (2009)

Moreover, the hippocampal neurons express both AR and ERs in the CA1 to CA3 of cornu ammonis region and granule cells of dentate gyrus. For ERs, hippocampal neuron contains both ER $\alpha$  and  $\beta$  (Österlund et al., 1998; Prange-Kiel et al., 2003; Shughrue, Lane and Merchenthaler, 1997). Expression of these receptors was increased with age (Beyenburg et al., 2000; Tsai et al., 2015) and regulated by sex steroid hormones (Weiland et al., 1997). Albertson et al. (2008) found that ER $\beta$  is co-localized with GnRH receptor immunoreactivity neurons in the hippocampus. The amount of GnRH receptor was increased in gonadectomized rats compared with intact rats of both sexes (Jennes et al., 1995). Gonadal steroids, in turn, regulate the action of GnRH by regulating the expression of GnRH receptors in the hippocampus.

Taken together, this chapter gives the basic knowledge to the readers that hypothalamus (via HPT axis) and hippocampus are the two brain regions which are associated with sex steroid hormone production and action via AR and ERs. Moreover, the hippocampus can also locally synthesize the reproductive hormones such as kisspeptin, GnRH, LH, androgens and estrogens as those seen in the HPT axis. Therefore, the androgen deficiency might affect the expression of reproductive hormone-related genes in both hypothalamus and hippocampus and subsequently leads to cognitive impairment.

## **CHAPTER III**

### **MATERIALS AND METHODS**

#### **Animals**

Adult male Sprague-Dawley rats, 2 months old, were purchased from the National Laboratory Animal Center, Mahidol University, Thailand. Two animals were reared in each individually ventilated cage (IVC) in a room with controlled lighting (lights on 0600-1800 h) and temperature ( $22\pm 1^{\circ}\text{C}$ ) at Chulalongkorn University Laboratory Animal Center (CULAC), Thailand. The animals were fed with a standard rat chow diet (Teklad Global Diets<sup>®</sup>: ENVIGO Harlans laboratories, Indianapolis, USA) and water *ad libitum*. The rats were reared until they became 4, 6, 8, 10 and 12 months old and used in this study. The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the CULAC, Chulalongkorn University (Protocol review number: 1573004).

#### **Experimental design**

The rats at the age of 4, 6, 8, 10 and 12 months old were tested a learning behavior and memory capacity using a Morris water maze test for 6 consecutive days before euthanized by carbon dioxide. After euthanized, the whole brains of rats were immediately collected and kept frozen at  $-80^{\circ}\text{C}$  until the hypothalamus and hippocampus were isolated and the mRNA expression levels of genes associated with reproductive hormones were determined. Blood samples were collected for serum LH and testosterone level assays. Testes and accessory sex organs, including prostate

gland, seminal vesicles and epididymis, were dissected, trimmed free of fat remnants and weighed. Animals were also weighed for their body mass. Thus, the relative organ weight was calculated by dividing organ weight by body weight and multiply by 100.

### **Learning behavior and spatial memory test**

The Morris water maze test was performed in a circular pool with a 180 cm diameter and 50 cm depth following Anukulthanakorn et al. (2013). For learning behavior test, each rat was given four trials per day for five consecutive days to find the hidden platform. For each trial, the rat was allowed to swim for a maximum of 90 sec to find the platform. The first trial was started by placing the rat in the water facing the pool wall in one of the four quadrants and then rotating the position clockwise to cover all four quadrants in the subsequent trials. From the next day onwards, the test was started at a quadrant different from the previous day. When the rat successfully found the platform, it was allowed to rest for 30 sec on the platform. If the rat could not find the platform within 90 sec, it was guided to the platform manually and was then given a 30 sec rest on the platform, and a score of 90 sec was recorded. The latency to reach the platform and the swimming distance to find the hidden platform were measured using a video tracking system (Smart Junior, Panlab-Harvard Apparatus, Barcelona, Spain). The movement patterns of the rats to find the platform were categorized into four strategies; line, taxis, random and circular, following Anukulthanakorn et al. (2013). The frequency of each strategy accounted for each rat, only on the day-5 of the learning behavior test, was used for analysis (Figure 3.1).

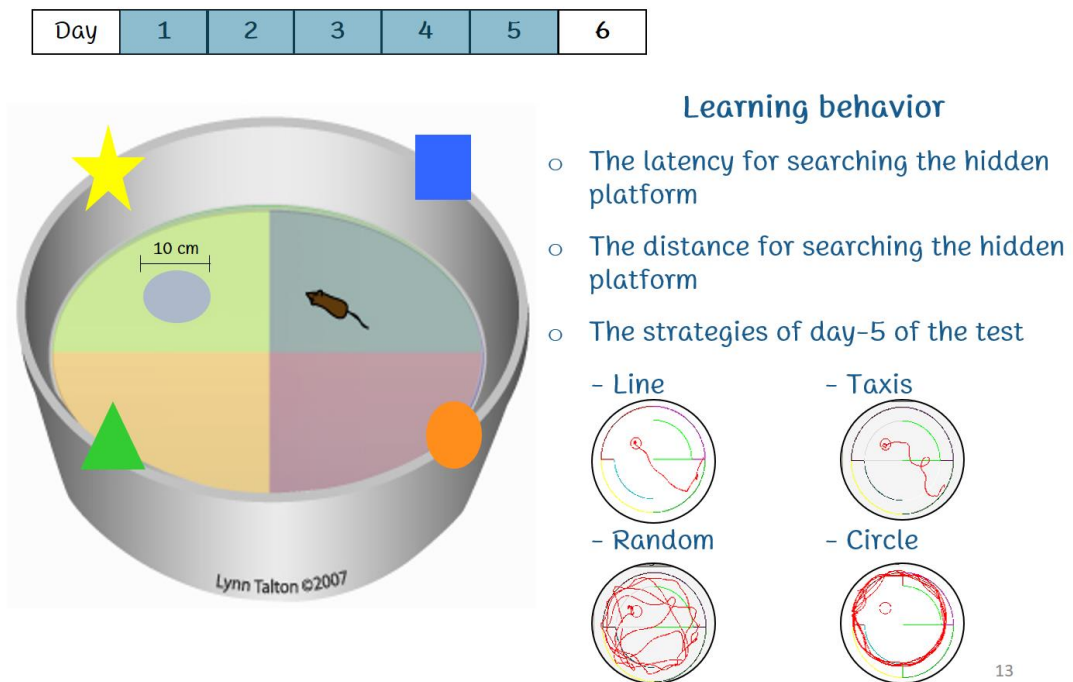


Figure 3.1 The measurement of learning behavior using Morris water maze test.

Three parameters measured are the latency for searching the hidden platform, the distance for searching the hidden platform, and the strategies (or the movement patterns) for searching the hidden platform of day-5 of the test. The strategies were categorized into line, taxis, random, and circular.

After the rats completed the 5-day learning behavior test, they were assessed the memory capacity by the spatial probe test on day-6. The platform was removed from the pool to check the memory of the animal for platform location. The rat was released to the pool at the opposite quadrant of platform location and allowed to swim for 30 sec. Number of platform crossing, time and distance spent in the target quadrant were recorded by the video tracking system (Smart Junior, Panlab-Harvard Apparatus, Barcelona, Spain) (Figure 3.2).

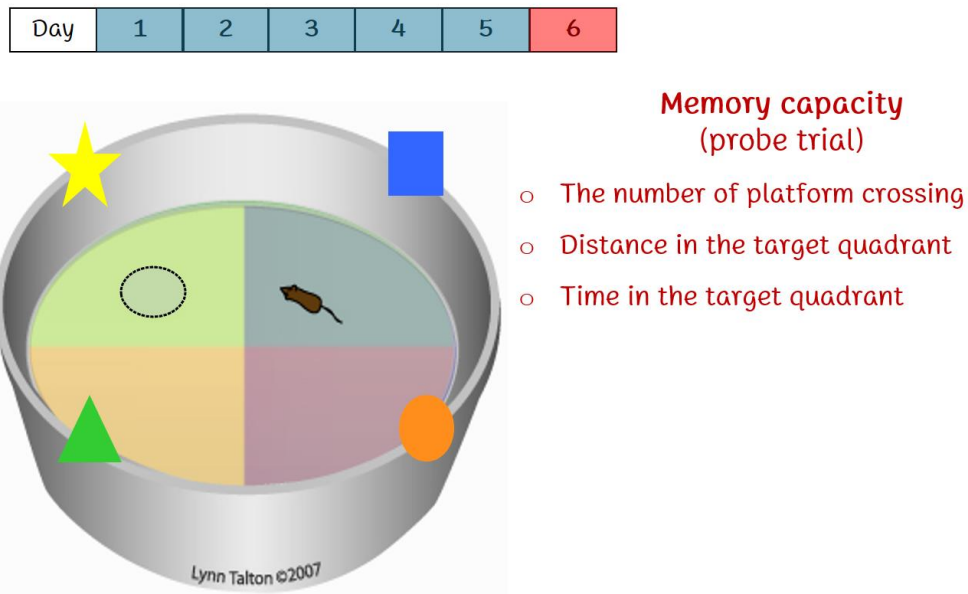


Figure 3.2 The measurement of spatial memory capacity by Morris water maze test.

Three parameters measured are the number of platform crossing, distance and time spent in the target quadrant. Noting that the platform is withdrawn when the spatial memory test is performed.

### Hormone assays

After the blood samples were collected, the blood sera were separated by centrifugation at 1600 xg for 20 min at 4 °C and kept frozen at -20 °C until serum LH and testosterone levels were assayed. Serum LH and testosterone levels were measured by Rat LH ELISA Kit no. CSB-E12654r (Cusabio, Hubei, China) and Testosterone ELISA Kit no. ab108666 (Abcam, MA, USA), respectively. Intra-assay coefficients of variation were 8.05% for LH and 6.52% for testosterone. The limits of detection (LOD) of the assay were 0.3 mIU/ml for LH and 0.2 ng/ml for testosterone.

### **Hypothalamus and hippocampus collection and isolation**

Fresh brains were carefully removed from the skulls, frozen immediately on a dry-ice chip and stored at  $-80\text{ }^{\circ}\text{C}$  for subsequent RNA extraction. The dissection of the brains was performed by transverse section using stainless steel brain matrices with  $200\text{ }\mu\text{m}$  of the thickness. The transverse section was started from bregma  $+5.64\text{ mm}$  to bregma  $-6.60\text{ mm}$  according to the rat brain Paxinos Atlas (Paxinos and Watson, 2005). Each section was placed on an ice-cold stainless-steel plate, the regions of hypothalamus including PoA (bregma  $+0.48\text{ mm}$  to  $-0.84\text{ mm}$ ), AVPV (bregma  $+0.12\text{ mm}$  to  $-0.12\text{ mm}$ ), ARC (bregma  $-1.72\text{ mm}$  to  $-3.36\text{ mm}$ ) and ME (bregma  $-1.80\text{ mm}$  to  $-3.36\text{ mm}$ ) and hippocampus (bregma  $-1.72\text{ mm}$  to  $-6.60\text{ mm}$ ) were dissected and transferred to a frozen Eppendorf tube.

Each brain region was extracted the total RNA using  $1,000\text{ }\mu\text{l}$  TRIzol® Reagent (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's protocols. The quantity and the purity of the RNA samples were checked by spectrometer based on absorbance measurements at  $260$  and  $280\text{ nm}$  wavelength. The  $260/280$  ratio of  $2.0\pm 0.1$  was accepted for RNA purity.

### **Two stage quantitative real time reverse transcription PCR (qrtRT-PCR)**

Transcript expression levels were examined using two-stage qrtRT-PCR. In the first stage,  $5\text{ }\mu\text{g}$  of the hippocampal total RNA sample and  $400\text{ ng}$  of each region of hypothalamus total RNA samples were reverse transcribed to cDNA in a  $20\text{ }\mu\text{l}$  reaction mixture containing  $4\text{ }\mu\text{l}$  of 5xRT Buffer,  $1\text{ }\mu\text{l}$  of dNTP mix,  $1\text{ }\mu\text{l}$  of Random Hexamer Primer Mix,  $1\text{ }\mu\text{l}$  of Oligo (dT) 18 Primer Mix,  $1\text{ }\mu\text{l}$  of reverse transcriptase ( $200\text{ U}/\mu\text{l}$ ) and  $1\text{ }\mu\text{l}$  of RNase inhibitor ( $10\text{ U}/\mu\text{l}$ ), using the Tetro cDNA Synthesis kit



(Bioline Reagent Ltd., London, UK). The samples were incubated for 10 min at 25 °C, 30 min at 45 °C, and finally 5 min at 85 °C. The obtained cDNA was diluted in five volumes of DEPC-treated water prior to use.

The second stage was performed using a StepOne™ Plus Real-Time PCR System (Applied Biosystems, CA, USA) in a 20 µl reaction mixture containing 10 µl of SensiFAST SYBR® Hi-ROX mix, 0.8 µl each of the forward and reverse primer (10 µM), 5 µl of the cDNA sample and 3.4 µl of DEPC-treated water. The reaction was performed at 95 °C for 2 min, then 40 cycles of 95 °C for 5 sec, annealing temperature of each gene (see Table 3.1) for 10 sec and 72 °C for 10 sec, followed by a dissociation curve step. The relative expression levels of the target genes were calculated by the  $2^{-\Delta\Delta C_t}$  method. In addition, the S28RNA gene was amplified by the same approach to act as a standardization reference gene. Relative mRNA levels were normalized to the S28RNA housekeeping gene and fold changes were expressed in relation to 4 months old control levels.

Primer sequence of each gene for qRT-PCR was shown in Table 3.1. In hypothalamus, the expression levels of *Kiss1r* and *Gnrh1* in PoA, *Kiss1* in AVPV and ARC, and *Ar*, *Esr1* and *Esr2* in AVPV, ARC and ME were examined. In hippocampus, the expression levels of *Kiss1*, *Kiss1r*, *Gnrh1*, *Gnrhr*, *Lhβ*, *Lhr*, steroidogenic enzymes including *P450scc*, *3β-hsd* and *P450arom*, and sex steroid hormone receptors including *Ar*, *Esr1* and *Esr2* were examined.

Table 3.1 Specific primer sequences for qrtRT-PCR

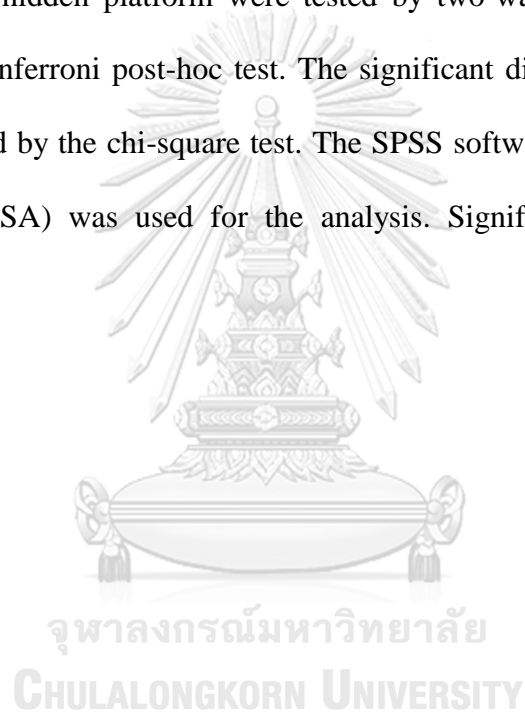
Target gene	Primer sequence (5' -3')	Product size (bp)	Accession no.	Annealing temperature
<i>Kiss1</i>	forward	TGGCACCTGTGGTGAACCCCTGAAC	NM_181692.1	60
	reverse	ATCAGGGGACTGCGGGTGGCACAC		
<i>Kiss1r</i>	forward	TGTGCAAAATTCGTCAACTACATCC	NM_001301151.1	60
	reverse	AGCACCGGGCGGAAACAGCTGC		
<i>Gnrh1</i>	forward	GCCGCTGTTGTTCTGTGACT	NM_012767.2	56
	reverse	TTCCTCTTCAATCAGACGTTCC		
<i>Gnrhr</i>	forward	AATCATCTTCGCCCTCACAC	NM_031038.3	56
	reverse	AGCACGGGTTTAGAAAAAGCA		
<i>Lhb</i>	forward	CTCCCGTGCCTCAGCCAGTGTGC	NM_012858.2	58
	reverse	GAAGAGGAGAAGGCCGGGGAGGTG		
<i>Lhr</i>	forward	AATCACGAGCCTCCTGGTC	NM_012978.1	58
	reverse	GCATCTGGTTCTGGAGCACA		
<i>Ar</i>	forward	GGGCAATTCGACCATATCTG	NM_012502.1	58
	reverse	CCCTTTGGCGTAACCTCCCTT		
<i>Esr1</i>	forward	ACCAATGCACCATCGATAAGAAC	NM_012689.1	58
	reverse	TCCTTTTCGTATCCCCGCTTTC		
<i>Esr2</i>	forward	GCGTTTGGTCATGTGAAGGA	NM_012754.1	58
	reverse	GCCGGTTCCTTGCTATGGTACAC		

Table 3.1 Specific primer sequences for qRT-PCR (con't)

Target gene	Primer sequence (5'-3')	Product size (bp)	Accession no.	Annealing temperature
<i>P450scc</i>	forward TAATTACAAGATTCCAGCCAA	245	NM_017286.3	58
	reverse CTTCAATTCTGAAGTTTCCCA			
<i>3β-hsd</i>	forward AGCCTGTGTCCAAAGCTAGTGT	161	XM_017591325.1	58
	reverse CTCGGCCATCTTTTGTCTGTAT			
<i>P450arom</i>	forward TGGCAGATTCTTGTGGATGG	118	NM_017085.2	58
	reverse CGAGGACTTGCTGATGATGAGT			
<i>S28RNA</i>	forward GCCCGAAACGATCTCAAACCT	217	V01270	
	reverse GCCACCGTCCTGCTGTCTAT			

### Statistical analysis

All data are presented as mean  $\pm$  standard error of the mean (SEM), and displayed normality of distribution and homogeneity of variance. One-way ANOVA with LSD post-hoc test was used to determine the differences of means of hormone and mRNA levels, relative testis and accessory sex organ weights, number of platform crossing, and time and distance in the target quadrant. The latency and the distance of searching for the hidden platform were tested by two-way ANOVA with repeated measures with Bonferroni post-hoc test. The significant difference of the movement patterns was tested by the chi-square test. The SPSS software program (version 22.0, SPSS Inc., IL, USA) was used for the analysis. Significance levels were set at  $p < 0.05$ .



## CHAPTER IV

### RESULTS

#### Detection of the status of androgen deficiency through a significant reduction of serum testosterone levels

Serum testosterone levels tended to decrease at 6 months old ( $2.050 \pm 0.497$  ng/ml;  $p=0.627$ ) and were marginally decreased at 8 months old ( $1.282 \pm 0.311$  ng/ml;  $p=0.105$ ) comparing to the 4 months old ( $2.375 \pm 0.576$  ng/ml), and significantly decreased at 12 months old ( $0.889 \pm 0.210$  ng/ml) (Figure 4.1). This indicates that the reproductive senescence in male Sprague-Dawley rats occurs during their middle age of 12 months old.

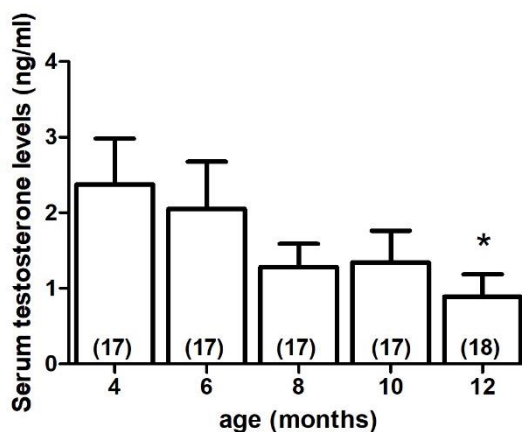


Figure 4.1 Serum testosterone levels of male rats at 4, 6, 8, 10 and 12 months old. The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. An asterisk (\*) represents  $p < 0.05$  compared with the 4 months old rats.

### Changes of serum LH levels

Serum LH levels were significantly decreased earlier than that of the testosterone levels, it was detected from 8 months old rats ( $27.521 \pm 4.152$  mIU/ml) to 12 months old ( $18.274 \pm 2.238$  mIU/ml) comparing to the 4 months old rats ( $42.046 \pm 7.066$  mIU/ml) (Figure 4.2).

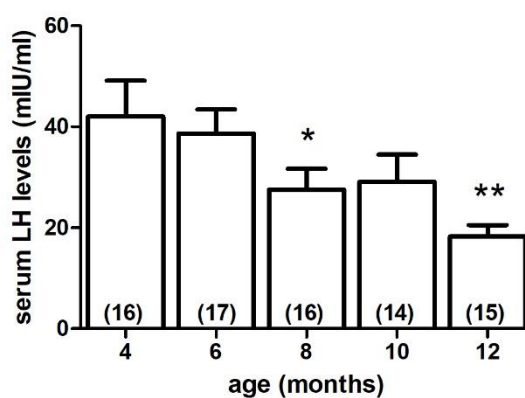


Figure 4.2 Serum LH levels of male rats at 4, 6, 8, 10 and 12 months old. The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. Asterisks (\* and \*\*) represent  $p < 0.05$  and  $0.01$  compared with the 4 months old rats.

### Changes of testes and accessory sex organ weights

The reduction of relative weights of testes and accessory sex organs including seminal vesicle, prostate gland and epididymis could be detected earlier than the decline of serum testosterone and LH levels. The relative weights of testes, seminal vesicle and prostate gland were significantly lowered in 6, 8, 10 and 12 months old rats comparing to the 4 months old rats, except the testes weight of the 8 months old rats. A significant decrease of epididymis was detected slower at 10 and 12 months old of rats (Table 4.1).

Table 4.1 Relative weights of testes and accessory sex organs, including seminal vesicles, prostate gland and epididymis of male rats at 4, 6, 8, 10 and 12 months old.

Age (months)	Number of rats used	Relative testes weight ( $\times 10^2$ )	Relative accessory sex organ weights ( $\times 10^2$ )		
			Seminal vesicles	Prostate gland	Epididymis
4	9	0.900 $\pm$ 0.017	0.411 $\pm$ 0.015	0.169 $\pm$ 0.006	0.349 $\pm$ 0.004
6	9	0.803 $\pm$ 0.034 *	0.333 $\pm$ 0.012 **	0.147 $\pm$ 0.005 *	0.318 $\pm$ 0.021
8	9	0.835 $\pm$ 0.023	0.337 $\pm$ 0.020 **	0.142 $\pm$ 0.009 **	0.322 $\pm$ 0.016
10	9	0.791 $\pm$ 0.016 *	0.310 $\pm$ 0.015 ***	0.149 $\pm$ 0.004 *	0.294 $\pm$ 0.009 **
12	9	0.717 $\pm$ 0.057 ***	0.283 $\pm$ 0.015 ***	0.133 $\pm$ 0.008 ***	0.266 $\pm$ 0.015 ***

The data are presented as mean $\pm$ SEM. Asterisks (\*, \*\* and \*\*\*) represent  $p < 0.05$ , 0.01 and 0.001 compared with the 4 months old rats.

### **Detection of cognitive impairment using Morris water maze test**

For learning behavior, the latencies of searching for the hidden platform were reduced during five consecutive days of test in all five groups of rats (Figure 4.3A). Comparing between groups, there were no significant changes in the profiles of the latency of 6 months old rats compared to the 4 months old. However, the latencies were significantly longer at the 8, 10 and 12 months old rats.

The patterns of changes in the distance to reach the platform were similar to those of the latency; the distance declined following the five consecutive days of test in all five groups of rats (Figure 4.3B). A significantly longer distance to reach the platform, comparing to the 4-month old rats, was detected in the 8, 10 and 12 months old rats.

The strategies of searching for the hidden platform were mainly line and taxis in 4 and 6 months old rats (Figure 4.3C). However, a random pattern was used more often by 8, 10 and 12 months old rats (17%, 30% and 37%, respectively;  $P < 0.05$ ) compared to the 4-month old rats (0%). The use of circle pattern was observed only in the 10 months old rats (3%).

Along with those of worsen learning behaviors, the memory capacity test of rats also indicated a worsen memory when the rats became older. The number of platform crossing (Figure 4.3D) and the distant spent (Figure 4.3E) in the target quadrant were significantly lowered in 8, 10 and 12 months old rats compared to the 4 months old, while a significant decrease in a time spent (Figure 4.3F) in target quadrant was detected earlier at 6 months old.



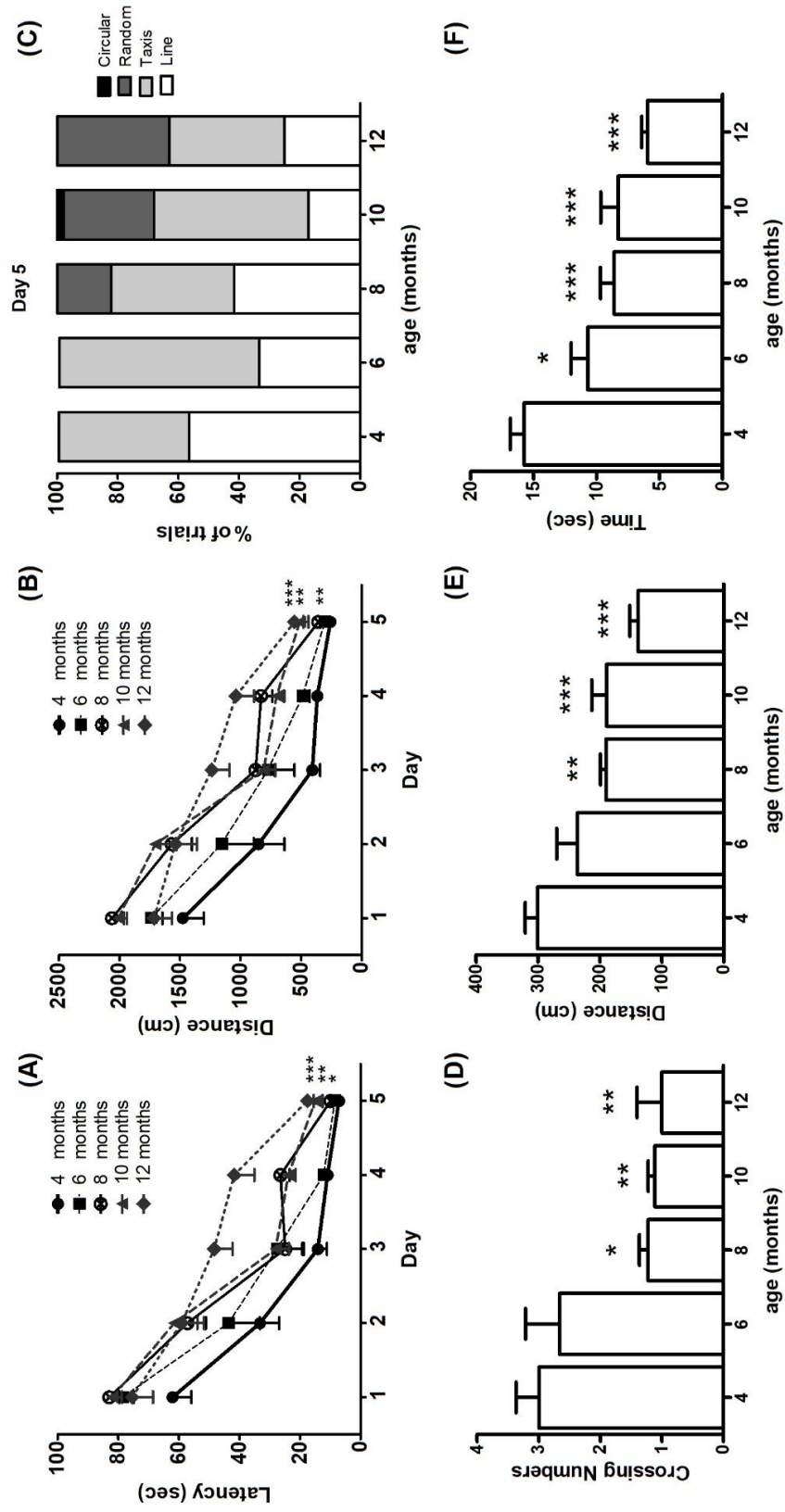


Figure 4.3 Learning behavior and memory capacity test by Morris water maze test. (see explanation of this figure in page 43)

Figure 4.3 Learning behavior and memory capacity test of male rats (9 rats/group) at 4, 6, 8, 10 and 12 months old by Morris water maze test. The data are presented as mean±SEM. For learning behavior test, the latency (A) and distance (B) for searching the hidden platform of five consecutive days and the strategies of day-5 of the test (C) are shown. For memory capacity test, the number of platform crossing (D), distance (E) and time (F) in the target quadrant of probe trial on day-6 are shown. Asterisks (\*, \*\* and \*\*\*) represent  $p < 0.05$ ,  $0.01$  and  $0.001$  compared with the 4 months old rats.

### **Changes of mRNA expression levels of genes associated with reproductive hormones and their receptors in hypothalamus**

The expression of mRNA levels of gene associated with reproductive hormones and their receptors was determined in four areas of hypothalamus, including AVPV, ARC, PoA, and ME.

At AVPV, the transcript expression levels of *Kiss1*, *Ar*, *Ers1* and *Ers2* genes were determined (Figure 4.4). Like the changes of serum testosterone and LH levels, male rats showed a marginal decrease in *Kiss1* mRNA level at 8 months old ( $p=0.344$ ), comparing to that of the 4 months old, and a significant decrease at 12 months old (Figure 4.4A). A decrease in expression of three sex-steroid hormone receptor genes (*Ar*, *Ers1* and *Ers2*) was observed earlier than that of the *Kiss1* gene. The decreased *Esr1* and *Esr2* mRNA levels were starting at 6 months old (Figure 4.4 C and D), while the *Ar* mRNA level was first detected at 8 months old (Figure 4.4B).

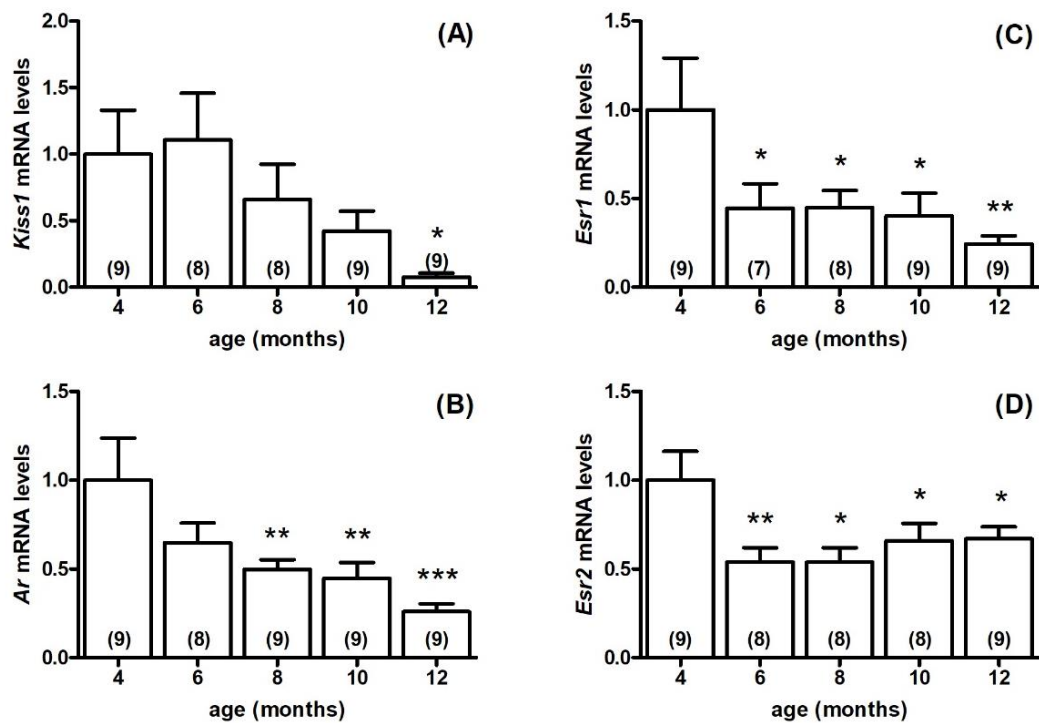


Figure 4.4 The expression of mRNA levels of Kiss1 (A), Ar (B), Esr1 (C) and Esr2 (D) genes in the anteroventral periventricular nucleus (AVPV). The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. Asterisks (\*, \*\* and \*\*\*) represent  $p < 0.05$ ,  $0.01$  and  $0.001$  compared with the 4 months old rats.

At ARC, similar to the AVPV the transcript expression levels of *Kiss1*, *Ar*, *Esr1* and *Esr2* genes were determined (Figure 4.5). The expression of *Kiss1* mRNA levels tended to decrease with advancing age, however, it did not reach a statistical significance throughout 12 months old of rats (Figure 4.5A). A similar significant trend as seen in those of AVPV was detected in *Ar* and *Esr2* mRNA levels. The *Esr2* mRNA level was significantly decreased from 6 months old (Figure 4.5D), and the *Ar* mRNA level was significantly decreased from 10 months old (Figure 4.5B). No significant difference of *Esr1* mRNA levels was detected throughout the 12 months of age in male rats (Figure 4.5C).

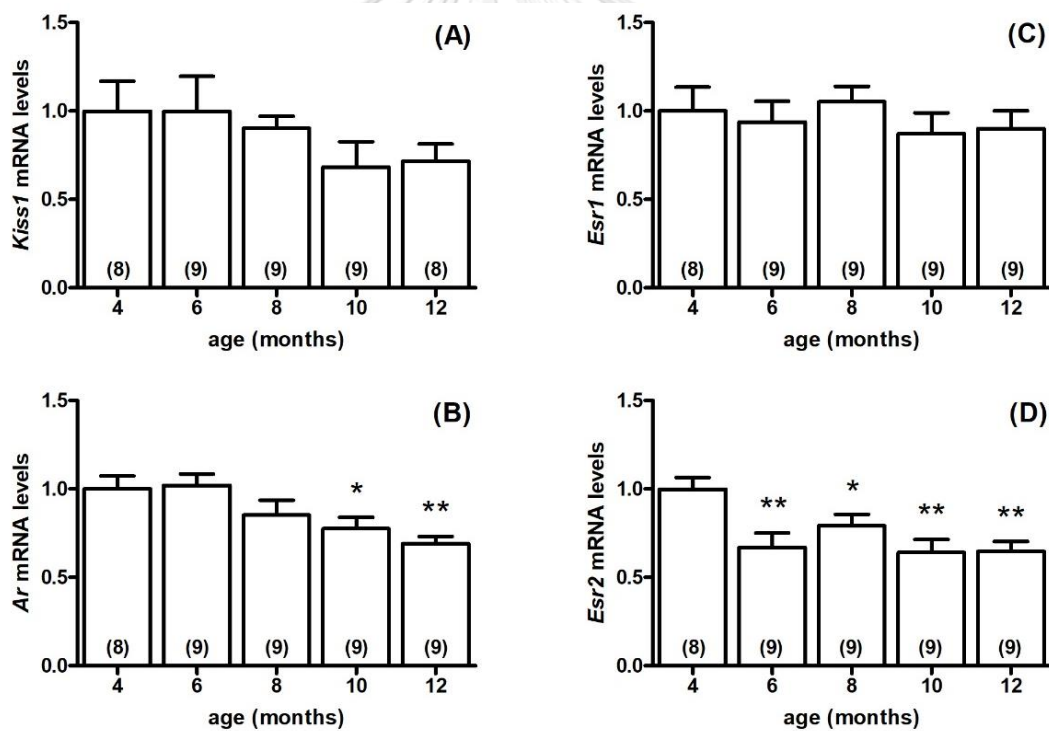


Figure 4.5 The expression of mRNA levels of *Kiss1* (A), *Ar* (B), *Esr1* (C) and *Esr2* (D) genes in the arcuate nucleus (ARC). The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. Asterisks (\*) and (\*\*\*) represent  $p < 0.05$  and  $0.01$  compared with the 4 months old rats.

At PoA where the GnRH neurons are mainly found and in relation to its function, only two genes, *Kiss1r* and *Gnrh1*, were determined in this brain region (Figure 4.6). In the same line with that of the AVPV, the mRNA expression level of *Kiss1r* in PoA showed a marginal decrease in the 8 months old rats ( $p=0.112$ ) and was significantly decreased in the 12 months old rats compared to the 4 months old rats (Figure 4.6A). Rats showed a significant reduction of *Gnrh1* mRNA levels starting at 8 months old although a marginal decrease was at 6 months old ( $p=0.065$ ) (Figure 4.6B).

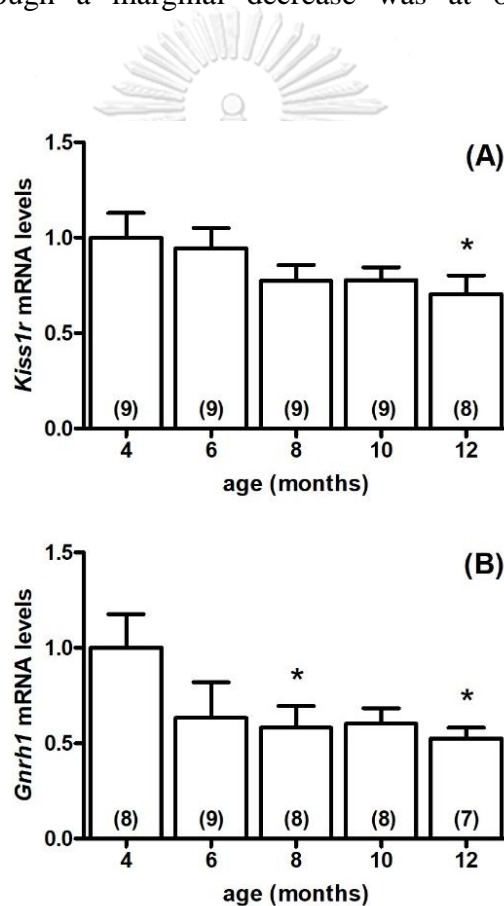


Figure 4.6 The expression of mRNA levels of *Kiss1r* (A) and *Gnrh1* (B) genes in the preoptic area (PoA). The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. An asterisk (\*) represents  $p < 0.05$  compared with the 4 months old rats.

At ME, as this area of hypothalamus does not produce Kiss and GnRH but the GnRH was released from PoA to here before flowing into the hypophyseal portal vessel and transporting to the anterior pituitary gland, thus only *Ar*, *Esr1* and *Esr2* mRNA levels were measured. Only the *Ar* mRNA level was decreased with advancing age, when a significant difference was detected at 12 months old (Figure 4.7).

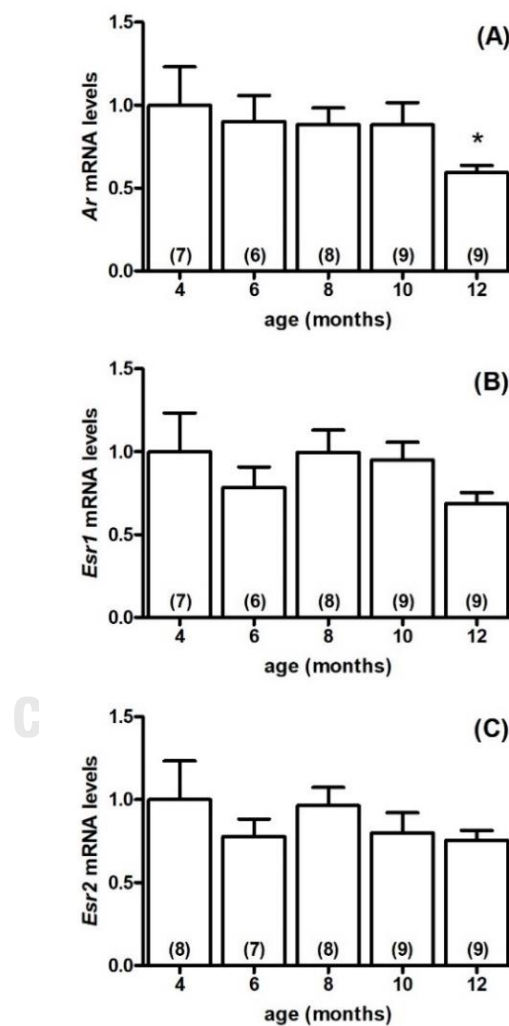


Figure 4.7 The expression of mRNA levels of *Ar* (A), *Esr1* (B) and *Esr2* (C) genes in the median eminence (ME). The data are presented as mean $\pm$ SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. An asterisk (\*) represents  $p < 0.05$  compared with the 4 months old rats.

### **Changes of mRNA expression levels of genes associated with reproductive hormones and their receptors in hippocampus**

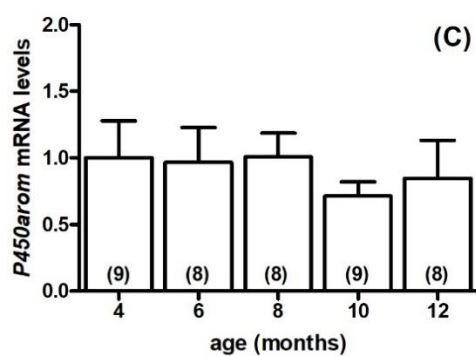
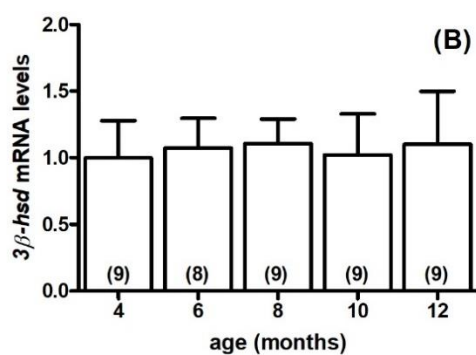
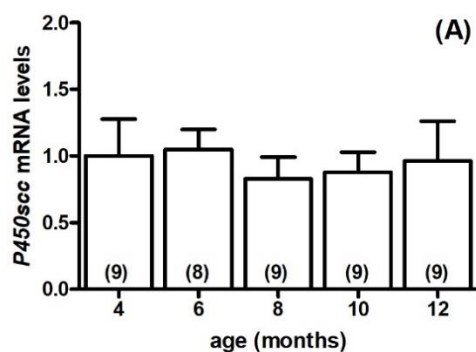
The determination was divided into two sections; *Kiss1/Kiss1r*, *Gnrh1/Gnrhr* and *Lh/Lhr* (both hormones and receptors) genes, and sex steroid hormone (synthesis and receptors) genes (Figure 4.8). Unlike the hypothalamus, the transcript expression levels of *Kiss1/Kiss1r-Gnrh1/Gnrhr-Lh/Lhr* genes were either not changed or spiked up in some ages of rats. Significant elevations were detected at 8 months old for *Kiss1* (Figure 4.8A) and *Gnrhr* (Figure 4.8E) mRNA levels and at 6 and 8 months old for *Lhb* mRNA levels (Figure 4.8C).

Interestingly, no significant alterations of mRNA levels of sex steroid hormone synthesis related genes, including *P450scc*, *3 $\beta$ -hsd* and *P450arom*, were detected throughout 12 months of age in male rats (Figure 4.9A, B and C). However, the mRNA expression levels of *Ar*, *Esr1* and *Esr2* were all spiked up in 8 months old rats (Figure 4.9D, E and F). Notably, the increase in those hippocampal mRNA levels of 8 months old rats was the age that they also showed the cognitive impairment tested by Morris water maze test as mentioned earlier.





## HORMONE SYNTHESIS



## HORMONE RECEPTORS

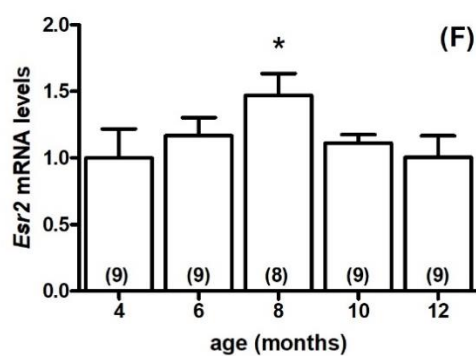
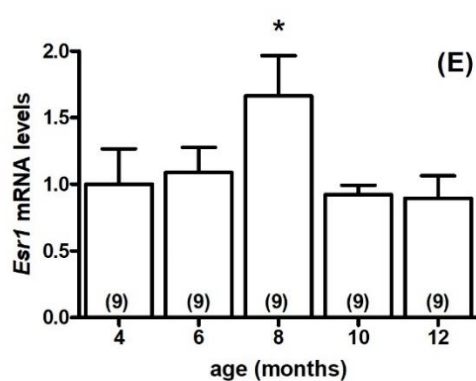
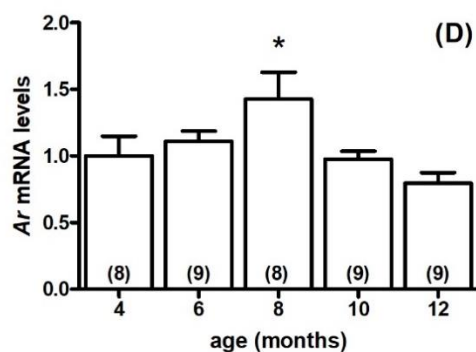


Figure 4.9 The expression of mRNA levels of *P450scc* (A), *3β-hsd* (B), *P450arom* (C), *Ar* (D), *Esr1* (E) and *Esr2* (F) genes in hippocampus. The data are presented as mean ± SEM. Number in the parenthesis in each bar indicates the number of rats used in each month. An asterisk (\*) represents  $p < 0.05$  compared with the 4 months old rats.

## CHAPTER V

### GENERAL DISCUSSION AND CONCLUSION

Age-related reproductive senescence is associated with the loss of sexual function and dysregulation of the HPT axis (Golan, Scovell and Ramasamy, 2015; Gunes et al., 2016). In this study, using a significant reduction of serum testosterone levels as an indicator, the reproductive senescence of male Sprague-Dawley rats occurred at 12 months old (or middle age) which agreed with the previous studies in Wistar rats (Sokanovic et al., 2014) and Harlan Sprague-Dawley rats (Wu and Gore, 2010; Wu, Lin and Gore, 2009). The decline of testosterone affects many organs that contain AR, especially testes and accessory sex organs. In the previous study, it was found that the weights of seminal vesicle and prostate gland were significantly decreased after two days of castration (Yamasaki et al., 2001) and the rats injected with ethane dimethane sulphonate and GnRH antagonist to abolish the testicular testosterone production had significantly reduced testis, prostate and seminal vesicle weights (van Roijen et al., 1997). Unexpectedly, the relative testes and accessory sex organ weights of the rats in this study were significantly decreased at 6 (for relative testis, seminal vesicle and prostate gland weights) or 10 months old (for relative epididymis weight), while the serum testosterone levels were only moderately reduced. This implies that high levels of testosterone are required to maintain testes and accessory sex organ structure and function in males Wistar rat (van Roijen et al., 1997) and rhesus monkeys (Mangat, 1979).

A testosterone decline was corresponded with a significant reduction of mRNA expression levels of *Kiss1/Kiss1r-Gnrh1* genes at hypothalamus (*Kiss1* at AVPV at 12 months old, *Kiss1r* and *Gnrh1* at PoA at 12 and 8 months old, respectively), as well as peripheral LH (primarily pituitary) levels at 8 months old (see Figure 5.1 for the conclusion of changes of HPT axis during the reproductive senescence). Resemble to these results, orchidectomy-induced androgen deficient rats showed a decrease in hypothalamic GnRH synthesis (Park, Dai Park and Kim, 1988; Rudenstein et al., 1979). Moreover, Wang, Leung and Sinha-Hikim (1993) found that the reduction of testosterone levels is associated with a significant decrease in serum LH levels in male Brown-Norway rats. This confirms that the deterioration of HPT axis leads to an androgen deficient status (Gruenewald et al., 2000). From these results, it can be proposed that the moderate decrease in testicular testosterone levels was attributed to the initiation of changes (at 8 months old of rat or before reaching the 12 months old middle-aged stage) at the higher levels of the HPT axis. Straightforwardly, that is, mRNA levels of *Kiss1* at AVPV and *Kiss1r* at PoA tended to be decreased at 8 months old of rats, while a significant reduction of *Gnrh1* mRNA levels in hypothalamic PoA and LH produced by the anterior pituitary gland were detected at 8 months old comparing to the 4 months old rats. In correspond with these initiative declines of *Kiss1/Kiss1r* and *Gnrh1* transcript expression at hypothalamic level at 8 months old, the sex steroid receptors, both *Ar* and *Esr*, at AVPV and ARC were also significantly decreased at around 8 (6 or 10) months old, which implies that the actions of sex steroid hormones upon their receptors in hypothalamus were also diminished at this stage of life. Similarly, Haji et al. (1981) reported that androgen binding with cytosol AR was decreased when the rats are at 300-330 days (or 10-11

months) old compared with 90-100 days (or 3-3.5 months) old in male Sprague-Dawley rats and they also proposed that the reduction of serum testosterone levels was caused by a dysfunction of the negative feedback system of the androgen to AR. By contrast, the AR and ER $\alpha$ -immunoreactive cell numbers and density in the AVPV and PoA were not significantly different between the young (3 months old) and the middle-aged (12 months old) rats (Wu, Lin and Gore, 2009). These results indicate that the reproductive senescence is initiated at the higher center (hypothalamus-pituitary level) of HPT axis before male rats entering the middle age and is subsequently evident at testicular level during the 12 months old middle age. The *Kiss1* gene encodes for kisspeptins, which have been implicated in the neuroendocrine regulation of GnRH production (Oakley, Clifton and Steiner, 2009; Smith, Clifton and Steiner, 2006), and the previous studies denote that the decline in GnRH secretion from the hypothalamus plays a key role in reproductive senescence (Kermath and Gore, 2012; Yin and Gore, 2006).

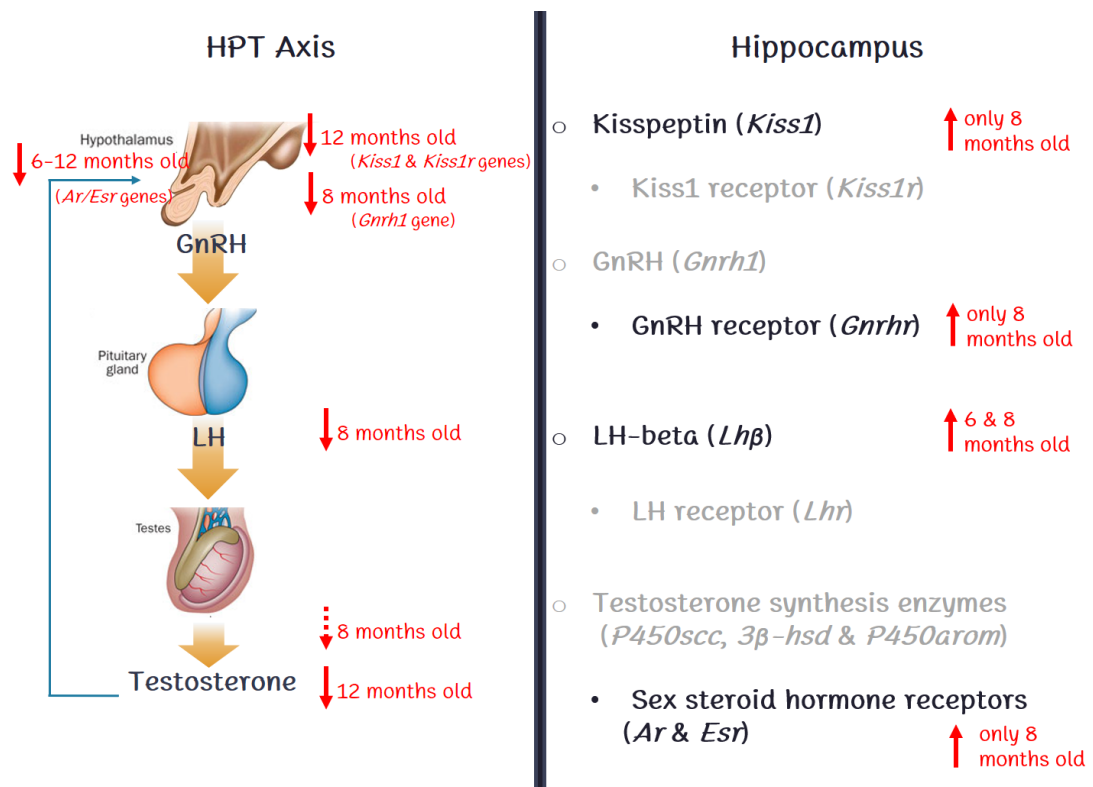


Figure 5.1 Changes of hypothalamic-pituitary-testicular (HPT) axis and hippocampus in male rats during reproductive senescence and cognitive impairment.

At the HPT axis, the reproductive senescence indicating by a significantly lowered serum testosterone level of male rats could be detected at 12 months old, in parallel with a significant reduction of mRNA expression levels of *Kiss1/Kiss1r* genes at hypothalamus. However, the onset of changes was initiated at 8 months at the hypothalamus and pituitary levels which consequently led to a marginal decline of serum testosterone level. At hippocampus, *Kiss1*, *Gnrhr*, *Lhb*, *Ar*, *Esr1* and *Esr2* mRNA levels were significantly elevated at 8 months old, but no changes in the expression levels of *Kiss1r*, *Gnrh1*, *Lhr* and sex steroid synthesis encoding (steroidogenic enzymes) genes were detected. Cognitive impairment was detected when the rats were at 8 months old.

Furthermore, the dysfunction of reproductive system is associated with cognitive function (Hier and Crowley, 1982). Learning and memory gradually decline with advancing age in rodents and humans (Beatty, 1988). Using a repeated acquisition water maze task test, the spatial memory capacity of 18- and 24-month old rats was reported to be lower than the young Fischer 344 rats (Frick et al., 1995) and Sprague-Dawley rats (Wyss et al., 2000). However, if the Morris water maze test was used for determination of spatial learning and memory performance in Fischer 344 rats, some change was noticeable as early as 12–15 months of age (Shukitt-Hale, Mouzakis and Joseph, 1998). Interestingly, male Sprague-Dawley rats in the present study elicited an impaired learning and memory capacity, detected by the Morris water maze test, at 8 months old which were earlier than the previous study (Wyss et al., 2000). In the past, androgen deficiency was profoundly counted as one of the risk factors for cognitive impairment (Boulware, Kent and Frick, 2012; Roberts et al., 1997). Recently, it was reported that an elevated serum LH level induced by ovariectomy could induce the cognitive impairment in female rats (Berry et al., 2008; Bryan et al., 2010; Ziegler and Thornton, 2010). By contrarily, serum LH level was significantly decreased in 8-12 months old intact male rats in this study. Thus, the induction of the cognitive impairment in middle-aged male rats in this study should be mainly due to a decrease in serum testosterone levels (mainly produced by testes via HPT axis). In an attempt to retain the cognitive function in response to the moderately lowered serum testosterone levels occurring at 8 months old, hippocampus up-regulated the transcript expression levels of sex steroid hormone receptors (*Ar*, *Esr $\alpha$*  and *Esr $\beta$* ) in 8-month old rats, while the local sex steroid hormones synthesis was not altered, indicating by no changes of *P450scc*, *3 $\beta$ -hsd* and *P450arom* mRNA levels

(see Figure 5.1 for the conclusion of changes of hippocampus during reproductive senescence and cognitive impairment). Taken together, these results indicate that systemic testicular testosterone levels had a greater effect on cognition than that of the local *de novo* sex steroid synthesis (or neuro-steroidogenesis) at hippocampus.

Other than the upregulation of sex steroid hormone receptor genes at hippocampus, *Kiss1*, *Gnrhr* and *Lhb* mRNA levels were also significantly elevated in 8 months old rats, except the *Lhb* mRNA level that was increased earlier at 6 months old. So far, no reports have been found whether *Kiss1*, GnRH, LH and testosterone hormones which were locally synthesized in the hippocampus have an axis of regulation as seen in that of the hypothalamus (or HPT axis homolog). Although the presence of a local endocrine axis between GnRH/GnRHR on estrogen synthesis was proposed, it is based solely on the *in vitro* neuroblastoma study (Rosati et al., 2011). Besides, whether neuro-testosterone could elicit the negative feedback circuit on *Kiss*, GnRH and/or gonadotroph neurons in hippocampus is unclear. To assess if the HPT axis homolog and negative feedback loop exist in the hippocampus is complicated because of the technical difficulties associated with the determination of locally produced and peripherally circulating reproductive hormones. However, the previous study found that reproductive hormone of HPT axis can cross blood-brain barrier to the brain and bind to its receptor (Caraty and Skinner, 2008; Lukacs et al., 1995). For example, the testicular testosterone hormone could affect *Kiss1* mRNA expression in hippocampus of male rats. It increased up to 0.5 folds after two weeks of orchidectomy (Arai, 2009). Thus, it was proposed that hippocampus and hypothalamus kisspeptins are under the same mechanism of transcriptional control. Some fragmented components of hypothalamic-pituitary-gonadal axis homolog were

also reported in female rats; for example, LH signals via hippocampal LH receptors to stimulate neuro-steroid synthesis (Lei et al., 1993; Liu et al., 2007).

The previous study proposed that *Kiss1* gene was transcribed within the hippocampal dentate gyrus, but the levels was lower than in the hypothalamus (Arai, 2009; Arai and Orwig, 2008). Moreover, its receptor has also been highly expressed in the hippocampus (Arai, 2009). Thus, the other action of kisspeptin, besides that on reproduction, such as cognition was assessed (Telegdy and Adamik, 2013). Kisspeptin increased synaptic transmission in the hippocampus through the activation of MAP-kinase-related signaling pathway, thus it may play a role in the cognition (Arai, 2009). The injection of kisspeptin could improve memory through the activation of kisspeptin receptor and GnRHR in mice (Jiang et al., 2015). It is possible that kisspeptin might be the linkage between hypothalamus and hippocampus to maintain cognitive function. Similarly, GnRH from hypothalamus or extrahypothalamic brain transported via efferent pathways into the hippocampus and signals via neuronal GnRHR to induce gonadotropin production, which in turn, signals via gonadotropin receptors on neurons around the dentate gyrus to synthesize neuro-sex steroid (Meethal et al., 2009; Rosati et al., 2011).

Since the remarkably increased *Lhb* mRNA expression levels were detected in hippocampus of the 6 and 8 months old rats, it could be proposed that a highly locally synthesized LH level at hippocampus is an inductive factor on cognitive impairment in middle-aged intact rats. This hypothesis contradicts with the previous studies denoting that a high peripheral (primarily pituitary) LH level was associated with a cognitive decline (Casadesus et al., 2007; Hyde et al., 2010; Rodrigues et al., 2008). However, a group of researchers observed an increase in cortical neuronal levels of



LH in Alzheimer's brain, and they proposed that elevated serum and neuronal LH levels, coupled with a decreased sex steroid level, induced an Alzheimer's disease pathology (Bowen et al., 2002).

In conclusion, this finding corroborates the previous studies suggesting that cognitive impairment is associated with androgen deficiency, and the deterioration at the higher (hypothalamus and pituitary) levels of the HPT axis is the initiative step. Although the reproductive senescence at testicular level is evident in the middle-aged, 12-month old rats, the cognitive impairment occurs earlier (at 8 months old) before the rats enter the middle age. In light on the results of this study, it indicates that the prevention of cognitive impairment in men should be considered before the significantly lowered serum testosterone levels are detected. The scheme of changes of serum testosterone and LH levels and transcript expression levels of genes associated with reproductive hormones and their receptors in hypothalamus and hippocampus of male rats is presented in Figure 5.2.

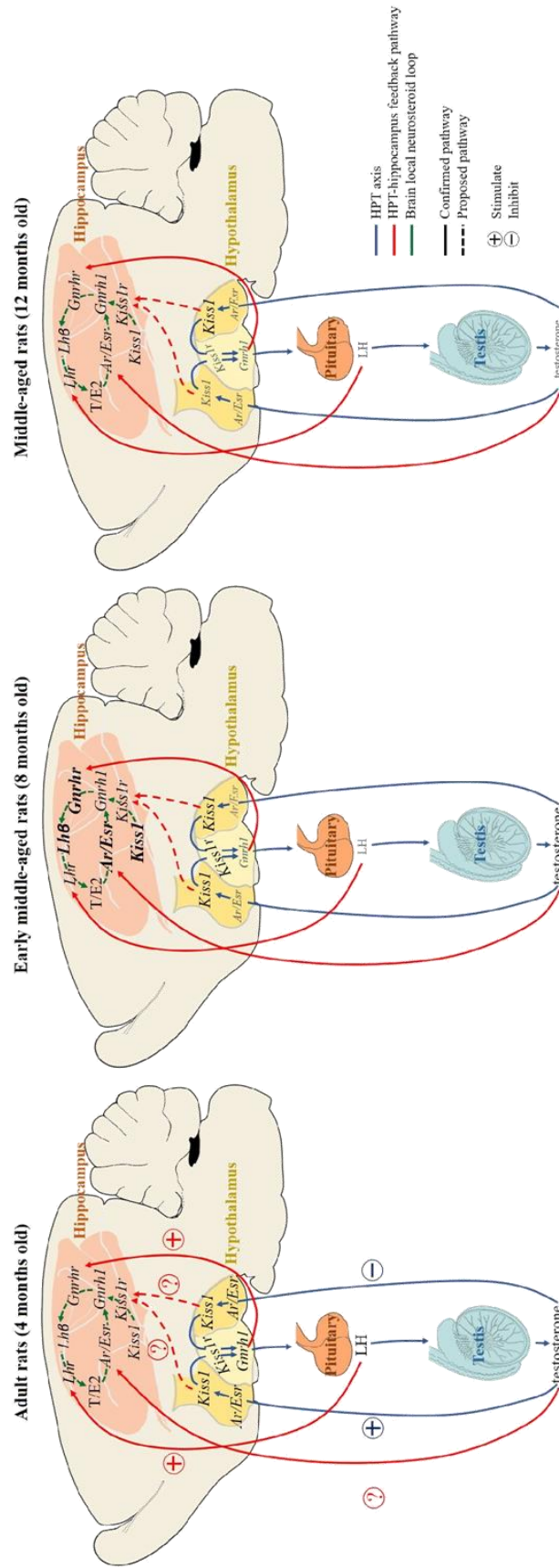


Figure 5.2 The scheme of changes of serum testosterone and LH levels and transcript expression levels of genes associated with reproductive hormones and their receptors in hypothalamus and hippocampus of male rats. (see explanation of this figure in page 60)

Figure 5.2 The scheme of changes of serum testosterone and LH levels and transcript expression levels of genes associated with reproductive hormones and their receptors in hypothalamus and hippocampus of male rats.

Left: in adult male rats, testosterone production is controlled by the HPT axis (blue line). GnRH, LH and sex steroid hormones cross the blood-brain barrier and bind with GnRHR, LHR, AR and ERs at hippocampal neurons (red line). Hippocampus can also locally synthesize kisspeptin, GnRH, LH, androgens and estrogens as seen in that of the HPT axis (green line).

Middle: the reproductive senescence is initiated by deterioration at the hypothalamus and pituitary levels of the HPT axis. A significant reduction (indicating by reduced font size and lighter shade) of *Gnrh1* (at PoA) and *Ar* and *Esr* (at AVPV and ARC) mRNA expression and pituitary LH production, and a moderate reduction of serum testosterone levels can be detected. Hippocampus responds to a moderately reduced serum testicular testosterone levels by up-regulating (indicating by increased font size and heavier shade) *Kiss1*, *Gnrhr* and *Lhb* and sex steroid hormone receptors (*Ar*, *Esr1* and *Esr2*), but not the local sex steroid hormone synthesis (*P450scc*, *3 $\beta$ -hsd* and *P450arom*) mRNA levels. Rats show the evidence of cognitive impairment at this age.

Right: at 12 months old middle age, rats show a sign of reproductive senescence, indicating by a significant reduction of serum testosterone levels. Hypothalamic *Kiss1* (at AVPV) and *Kiss1r* (at PoA) mRNA expression levels decrease, while hippocampal *Kiss1*, *Gnrhr* and *Lhb* mRNA levels return to the 4 months old levels. In light of these results, it indicates that systemic testicular testosterone level has a greater effect on cognition than that of the neuronal sex steroids locally de novo synthesized at hippocampus.

## Recommendations

In light of the results of this study, it suggests that several studies need to be conducted further as follows;

1. Since this study discovered that the moderate (non-significant) reduction of serum testosterone levels can induce the spatial learning and memory impairment, it is interesting to know if the treatment of synthetic testosterone at this time point can rescue the spatial learning and memory capacity of the males.
2. Since the testosterone production is controlled by the HPT axis, it is interesting to know if the long-term treatment of synthetic testosterone can disturb the homeostasis of the HPT axis and if the treatment can induce other side effects such as prostate cancer.
3. Since the change of estrogen receptors can be detected in both hypothalamus and hippocampus, and the use of synthetic hormones can cause many side effects, it is interesting to know if the treatment of natural products such as phytoestrogens can rescue the cognitive impairment in males without any adverse side effects comparing to the synthetic estrogens.
4. Since the risk factor for cognition is not only an androgen deficiency, other factors such as advancing age, gender, genetic factors, brain trauma and cardiovascular-related factors can induce cognitive impairment. Thus, it is interesting to know if those factors can synergize or potentiate the effect of androgen deficiency on cognitive impairment.

## REFERENCES

- Albertson, A. J., Navratil, A., Mignot, M., Dufourny, L., Cherrington, B. and Skinner, D. C. 2008. Immunoreactive GnRH type I receptors in the mouse and sheep brain. Journal of chemical neuroanatomy 35: 326-333.
- Anukulthanakorn, K., Malaivijitnond, S., Kitahashi, T., Jaroenporn, S. and Parhar, I. 2013. Molecular events during the induction of neurodegeneration and memory loss in estrogen-deficient rats. General and Comparative Endocrinology 181: 316-323.
- Arai, A. C. 2009. The role of kisspeptin and GPR54 in the hippocampus. Peptides 30: 16-25.
- Arai, A. C. and Orwig, N. 2008. Factors that regulate KiSS1 gene expression in the hippocampus. Brain Research 1243: 10-18.
- Badr, M. and Pelletier, G. 1987. Characterization and autoradiographic localization of LHRH receptors in the rat brain. Synapse 1: 567-571.
- Beatty, W. W. 1988. Preservation and loss of spatial memory in aged rats and humans: implications for the analysis of memory dysfunction in dementia. Neurobiology of Aging 9: 557-561.
- Berry, A., Tomidokoro, Y., Ghiso, J. and Thornton, J. 2008. Human chorionic gonadotropin (a luteinizing hormone homologue) decreases spatial memory and increases brain amyloid-beta levels in female rats. Hormones and Behavior 54: 143-152.
- Bettio, L. E. B., Rajendran, L. and Gil-Mohapel, J. 2017. The effects of aging in the hippocampus and cognitive decline. Neuroscience and Biobehavioral Reviews 79: 66-86.
- Beyenburg, S., Watzka, M., Clusmann, H., Blümcke, I., Bidlingmaier, F., Elger, C. E. and Stoffel-Wagner, B. 2000. Androgen receptor mRNA expression in the human hippocampus. Neuroscience letters 294: 25-28.
- Boulware, M. I., Kent, B. A. and Frick, K. M. 2012. The impact of age-related ovarian hormone loss on cognitive and neural function. Current Topics in Behavioral Neurosciences 10: 165-184.
- Bowen, R. L., Smith, M. A., Harris, P. L., Kubat, Z., Martins, R. N., Castellani, R. J., Perry, G. and Atwood, C. S. 2002. Elevated luteinizing hormone expression colocalizes with neurons vulnerable to Alzheimer's disease pathology. Journal of Neuroscience Research 70: 514-518.
- Brotans-Mas, J. R., O'Mara, S. and Sanchez-Vives, M. V. 2006. Neural processing of spatial information: what we know about place cells and what they can tell us about presence. Presence: Teleoperators and Virtual Environments 15: 485-499.
- Bryan, K. J., Mudd, J. C., Richardson, S. L., Chang, J., Lee, H. G., Zhu, X., Smith, M. A. and Casadesus, G. 2010. Down-regulation of serum gonadotropins is as effective as estrogen replacement at improving menopause-associated cognitive deficits. Journal of Neurochemistry 112: 870-881.
- Caraty, A. and Skinner, D. C. 2008. Gonadotropin-releasing hormone in third ventricular cerebrospinal fluid: endogenous distribution and exogenous uptake. Endocrinology 149: 5227-5234.
- Card, J. P., "eLS," in Hypothalamus, eds. (John Wiley & Sons, Ltd, 2001)
- Casadesus, G., Milliken, E. L., Webber, K. M., Bowen, R. L., Lei, Z., Rao, C. V., Perry,

- G., Keri, R. A. and Smith, M. A. 2007. Increases in luteinizing hormone are associated with declines in cognitive performance. Molecular and Cellular Endocrinology 269: 107-111.
- Chang, C., Saltzman, A., Yeh, S., Young, W., Keller, E., Lee, H. J., Wang, C. and Mizokami, A. 1995. Androgen receptor: an overview. Critical Reviews in Eukaryotic Gene Expression 5: 97-125.
- Cherrier, M. M., Craft, S. and Matsumoto, A. H. 2003. Cognitive changes associated with supplementation of testosterone or dihydrotestosterone in mildly hypogonadal men: a preliminary report. Journal of Andrology 24: 568-576.
- Cherrier, M. M., Matsumoto, A. M., Amory, J. K., Asthana, S., Bremner, W., Peskind, E. R., Raskind, M. A. and Craft, S. 2005. Testosterone improves spatial memory in men with Alzheimer disease and mild cognitive impairment. Neurology 64: 2063-2068.
- Chu, C., Gao, G. and Huang, W. 2008. A study on co-localization of FSH and its receptor in rat hippocampus. Journal of Molecular Histology 39: 49-55.
- Clarke, I. J. and Cummins, J. T. 1982. The temporal relationship between gonadotropin releasing hormone (GnRH) and luteinizing hormone (LH) secretion in ovariectomized ewes. Endocrinology 111: 1737-1739.
- Clarkson, J., d'Anglemont de Tassigny, X., Colledge, W. H., Caraty, A. and Herbison, A. E. 2009. Distribution of kisspeptin neurones in the adult female mouse brain. Journal of Neuroendocrinology 21: 673-682.
- Centers for Disease Control Prevention. 2011. Cognitive impairment: A call for action, now [Online]. Available from: [https://www.cdc.gov/aging/pdf/Cognitive\\_impairment/cogimp\\_policy\\_final.pdf](https://www.cdc.gov/aging/pdf/Cognitive_impairment/cogimp_policy_final.pdf)
- de Roux, N., Genin, E., Carel, J. C., Matsuda, F., Chaussain, J. L. and Milgrom, E. 2003. Hypogonadotropic hypogonadism due to loss of function of the KiSS1-derived peptide receptor GPR54. Proceedings of the National Academy of Sciences 100: 10972-10976.
- Dickson, R. and Clark, C. 1981. Estrogen receptors in the male. Archives of andrology 7: 205-217.
- Do Rego, J. L., Seong, J. Y., Burel, D., Leprince, J., Luu-The, V., Tsutsui, K., Tonon, M.-C., Pelletier, G. and Vaudry, H. 2009. Neurosteroid biosynthesis: enzymatic pathways and neuroendocrine regulation by neurotransmitters and neuropeptides. Frontiers in neuroendocrinology 30: 259-301.
- Elizondo-Vega, R., Cortes-Campos, C., Barahona, M. J., Oyarce, K. A., Carril, C. A. and García-Robles, M. A. 2015. The role of tanycytes in hypothalamic glucosensing. Journal of Cellular and Molecular Medicine 19: 1471-1482.
- Emanuele, N. V., Anderson, J., Andersen, E., Connick, E., Baker, G., Kirsteins, L. and Lawrence, A. M. 1983. Extrahypothalamic brain luteinizing hormone: characterization by radioimmunoassay, chromatography, radioligand assay and bioassay. Neuroendocrinology 36: 254-260.
- Fester, L., Prange-Kiel, J., Jarry, H. and Rune, G. M. 2011. Estrogen synthesis in the hippocampus. Cell and Tissue Research 345: 285-294.
- Fester, L. and Rune, G. M. 2015. Sexual neurosteroids and synaptic plasticity in the hippocampus. Brain Research 1621: 162-169.
- Frick, K. M., Baxter, M. G., Markowska, A. L., Olton, D. S. and Price, D. L. 1995. Age-related spatial reference and working memory deficits assessed in the water

- maze. Neurobiology of Aging 16: 149-160.
- Geinisman, Y., Detolledo-Morrell, L., Morrell, F. and Heller, R. E. 1995. Hippocampal markers of age-related memory dysfunction: behavioral, electrophysiological and morphological perspectives. Progress in neurobiology 45: 223-252.
- Golan, R., Scovell, J. M. and Ramasamy, R. 2015. Age-Related Testosterone Decline is due to Waning of Both Testicular and Hypothalamic-Pituitary Function. The aging male : the official journal of the International Society for the Study of the Aging Male 18: 201-204.
- Golomb, J., de Leon, M. J., Kluger, A., George, A. E., Tarshish, C. and Ferris, S. H. 1993. Hippocampal atrophy in normal aging: an association with recent memory impairment. Archives of neurology 50: 967-973.
- González-Ortega, I., Martínez-Cengotitabengoa, M. and González-Pinto, A., "Handbook of Cannabis and Related Pathologies," in Chapter 27 - Cannabis Use and First-Episode Psychosis Patients (FEP), eds. (San Diego: Academic Press, 2017), pp. 257-266. In V. R. Preedy (Ed.).
- Good, M. 2002. Spatial memory and hippocampal function: where are we now? Psicológica 23.
- Gottsch, M. L., Cunningham, M. J., Smith, J. T., Popa, S. M., Acohido, B. V., Crowley, W. F., Seminara, S., Clifton, D. K. and Steiner, R. A. 2004. A role for kisspeptins in the regulation of gonadotropin secretion in the mouse. Endocrinology 145: 4073-4077.
- Gräff, J., Woldemichael, B. T., Berchtold, D., Dewarrat, G. and Mansuy, I. M. 2012. Dynamic histone marks in the hippocampus and cortex facilitate memory consolidation. Nature Communications 3: 991.
- Gruenewald, D. A., Naai, M. A., Marck, B. T. and Matsumoto, A. M. 2000. Age-related decrease in hypothalamic gonadotropin-releasing hormone (GnRH) gene expression, but not pituitary responsiveness to GnRH, in the male Brown Norway rat. Journal of Andrology 21: 72-84.
- Gunes, S., Hekim, G. N. T., Arslan, M. A. and Asci, R. 2016. Effects of aging on the male reproductive system. Journal of Assisted Reproduction and Genetics 33: 441-454.
- Haggstrom, M. 2014. Diagram of the pathways of human steroidogenesis. WikiJournal of Medicine 1: 1.
- Haji, M., Kato, K. I., Nawata, H. and Ibayashi, H. 1981. Age-related changes in the concentrations of cytosol receptors for sex steroid hormones in the hypothalamus and pituitary gland of the rat. Brain Research 204: 373-386.
- Halász, B., "Neuroendocrinology in Physiology and Medicine," in The Hypothalamus as an Endocrine Organ, eds. (Totowa, NJ: Humana Press, 2000), pp. 3-21. In P. M. Conn & M. E. Freeman (Eds.).
- Hall, J. E. 2015. Endocrinology of the Menopause. Endocrinology and Metabolism Clinics of North America 44: 485-496.
- Hamson, D. K., Wainwright, S. R., Taylor, J. R., Jones, B. A., Watson, N. V. and Galea, L. A. 2013. Androgens increase survival of adult-born neurons in the dentate gyrus by an androgen receptor-dependent mechanism in male rats. Endocrinology 154: 3294-3304.
- Hanninen, T., Koivisto, K., Reinikainen, K. J., Helkala, E. L., Soininen, H., Mykkanen, L., Laakso, M. and Riekkinen, P. J. 1996. Prevalence of ageing-associated

- cognitive decline in an elderly population. Age and Ageing 25: 201-205.
- Hardy, J. 2009. The amyloid hypothesis for Alzheimer's disease: a critical reappraisal. Journal of Neurochemistry 110: 1129-1134.
- Harman, S. M., Metter, E. J., Tobin, J. D., Pearson, J. and Blackman, M. R. 2001. Longitudinal effects of aging on serum total and free testosterone levels in healthy men. Baltimore Longitudinal Study of Aging. The Journal of Clinical Endocrinology and Metabolism 86: 724-731.
- Hayman, L. A., Fuller, G. N., Cavazos, J. E., Pflieger, M. J., Meyers, C. A. and Jackson, E. F. 1998. The hippocampus: normal anatomy and pathology. AJR American Journal of Roentgenology 171: 1139-1146.
- Hess, R. 1998. Spermatogenesis, Overview. 4.
- Hier, D. B. and Crowley, W. F., Jr. 1982. Spatial ability in androgen-deficient men. The New England Journal of Medicine 306: 1202-1205.
- Hogarth, C. A. and Griswold, M. D. 2010. The key role of vitamin A in spermatogenesis. The Journal of Clinical Investigation 120: 956-962.
- Hojo, Y., Hattori, T.-a., Enami, T., Furukawa, A., Suzuki, K., Ishii, H.-t., Mukai, H., Morrison, J. H., Janssen, W. G. and Kominami, S. 2004. Adult male rat hippocampus synthesizes estradiol from pregnenolone by cytochromes P45017 $\alpha$  and P450 aromatase localized in neurons. Proceedings of the National Academy of Sciences of the United States of America 101: 865-870.
- Hojo, Y., Higo, S., Kawato, S., Hatanaka, Y., Ooishi, Y., Murakami, G., Ishii, H., Komatsuzaki, Y., Ogiue-Ikeda, M. and Mukai, H. 2011. Hippocampal synthesis of sex steroids and corticosteroids: essential for modulation of synaptic plasticity. Frontiers in endocrinology 2: 43.
- Hojo, Y., Murakami, G., Mukai, H., Higo, S., Hatanaka, Y., Ogiue-Ikeda, M., Ishii, H., Kimoto, T. and Kawato, S. 2008. Estrogen synthesis in the brain—role in synaptic plasticity and memory. Molecular and cellular endocrinology 290: 31-43.
- Hojo, Y., Okamoto, M., Kato, A., Higo, S., Sakai, F., Soya, H., Yamazaki, T. and Kawato, S. 2014. Neurosteroid synthesis in adult female rat hippocampus, including androgens and allopregnanolone. Journal of Steroids and Hormonal Science 4: 002.
- Hsu, B., Cumming, R. G., Waite, L. M., Blyth, F. M., Naganathan, V., Le Couteur, D. G., Seibel, M. J. and Handelsman, D. J. 2015. Longitudinal Relationships between Reproductive Hormones and Cognitive Decline in Older Men: The Concord Health and Ageing in Men Project. The Journal of Clinical Endocrinology and Metabolism 100: 2223-2230.
- Hyde, Z., Flicker, L., Almeida, O. P., McCaul, K. A., Jamrozik, K., Hankey, G. J., Chubb, S. and Yeap, B. B. 2010. Higher luteinizing hormone is associated with poor memory recall: the health in men study. Journal of Alzheimer's Disease 19: 943-951.
- Iqbal, K., Liu, F. and Gong, C.-X. 2016. Tau and neurodegenerative disease: the story so far. Nature Reviews Neurology 12: 15.
- Irwig, M. S., Fraley, G. S., Smith, J. T., Acohido, B. V., Popa, S. M., Cunningham, M. J., Gottsch, M. L., Clifton, D. K. and Steiner, R. A. 2004. Kisspeptin activation of gonadotropin releasing hormone neurons and regulation of KiSS-1 mRNA in the male rat. Neuroendocrinology 80: 264-272.



- Issa, A. M., Rowe, W., Gauthier, S. and Meaney, M. J. 1990. Hypothalamic-pituitary-adrenal activity in aged, cognitively impaired and cognitively unimpaired rats. Journal of Neuroscience 10: 3247-3254.
- Javorsky, B. R., Aron, D. C., Findling, J. W. and Tyrrell, J. B., "Greenspan's Basic & Clinical Endocrinology, 9e," in Chapter 4. Hypothalamus and Pituitary Gland, eds. (New York, NY: The McGraw-Hill Companies, 2011), In D. G. Gardner & D. Shoback (Eds.).
- Jayes, F. C., Britt, J. H. and Esbenshade, K. L. 1997. Role of gonadotropin-releasing hormone pulse frequency in differential regulation of gonadotropins in the gilt. Biology of Reproduction 56: 1012-1019.
- Jennes, L., Brame, B., Centers, A., Janovick, J. A. and Conn, P. M. 1995. Regulation of hippocampal gonadotropin releasing hormone (GnRH) receptor mRNA and GnRH-stimulated inositol phosphate production by gonadal steroid hormones. Molecular brain research 33: 104-110.
- Jennes, L., Eyigor, O., Janovick, J. A. and Conn, P. M. 1997. Brain gonadotropin releasing hormone receptors: localization and regulation. Recent progress in hormone research 52: 475-490; discussion 490-471.
- Jernigan, T. L., Archibald, S. L., Berhow, M. T., Sowell, E. R., Foster, D. S. and Hesselink, J. R. 1991. Cerebral structure on MRI, Part I: Localization of age-related changes. Biological psychiatry 29: 55-60.
- Ji, Z. S., Kubokawa, K. and Abe, S. 1995. Promotion of differentiation of newt primary spermatocytes into spermatids by mammalian FSH via Sertoli cells. Journal of Experimental Zoology 272: 374-383.
- Jiang, J. H., He, Z., Peng, Y. L., Jin, W. D., Wang, Z., Han, R. W., Chang, M. and Wang, R. 2015. Kisspeptin-13 enhances memory and mitigates memory impairment induced by A $\beta$ 1-42 in mice novel object and object location recognition tasks. Neurobiology of learning and memory 123: 187-195.
- Jimenez-Rubio, G., Herrera-Perez, J. J., Hernandez-Hernandez, O. T. and Martinez-Mota, L. 2017. Relationship between androgen deficiency and memory impairment in aging and Alzheimer's disease. Actas espanolas de psiquiatria 45: 227-247.
- Jin, J.-M. and Yang, W.-X. 2014. Molecular regulation of hypothalamus-pituitary-gonads axis in males. Gene 551: 15-25.
- Kaiser, U. B., Jakubowiak, A., Steinberger, A. and Chin, W. W. 1997. Differential effects of gonadotropin-releasing hormone (GnRH) pulse frequency on gonadotropin subunit and GnRH receptor messenger ribonucleic acid levels in vitro. Endocrinology 138: 1224-1231.
- Kauffman, A. S., Clifton, D. K. and Steiner, R. A. 2007. Emerging ideas about kisspeptin- GPR54 signaling in the neuroendocrine regulation of reproduction. Trends in Neurosciences 30: 504-511.
- Kauffman, A. S., Gottsch, M. L., Roa, J., Byquist, A. C., Crown, A., Clifton, D. K., Hoffman, G. E., Steiner, R. A. and Tena-Sempere, M. 2007. Sexual differentiation of Kiss1 gene expression in the brain of the rat. Endocrinology 148: 1774-1783.
- Kermath, B. A. and Gore, A. C. 2012. Neuroendocrine control of the transition to reproductive senescence: Lessons learned from the female rodent model. Neuroendocrinology 96: 1-12.

- Kimoto, T., Tsurugizawa, T., Ohta, Y., Makino, J. y., Tamura, H.-o., Hojo, Y., Takata, N. and Kawato, S. 2001. Neurosteroid synthesis by cytochrome p450-containing systems localized in the rat brain hippocampal neurons: N-methyl-D-aspartate and calcium-dependent synthesis. Endocrinology 142: 3578-3589.
- King, S. R., Manna, P. R., Ishii, T., Syapin, P. J., Ginsberg, S. D., Wilson, K., Walsh, L. P., Parker, K. L., Stocco, D. M. and Smith, R. G. 2002. An essential component in steroid synthesis, the steroidogenic acute regulatory protein, is expressed in discrete regions of the brain. Journal of Neuroscience 22: 10613-10620.
- Kong, L., Tang, M., Zhang, T., Wang, D., Hu, K., Lu, W., Wei, C., Liang, G. and Pu, Y. 2014. Nickel nanoparticles exposure and reproductive toxicity in healthy adult rats. International Journal of Molecular Sciences 15: 21253-21269.
- Kretz, O., Fester, L., Wehrenberg, U., Zhou, L., Brauckmann, S., Zhao, S., Prange-Kiel, J., Naumann, T., Jarry, H. and Frotscher, M. 2004. Hippocampal synapses depend on hippocampal estrogen synthesis. Journal of Neuroscience 24: 5913-5921.
- Leblanc, P., Crumeyrolle, M., Latouche, J., Jordan, D., Fillion, G., L'Heritier, A., Kordon, C., Dussailant, M., Rostène, W. and Haour, F. 1988. Characterization and distribution of receptors for gonadotropin-releasing hormone in the rat hippocampus. Neuroendocrinology 48: 482-488.
- Lei, Z., Rao, C. V., Kornyei, J., Licht, P. and Hiatt, E. 1993. Novel expression of human chorionic gonadotropin/luteinizing hormone receptor gene in brain. Endocrinology 132: 2262-2270.
- Leifke, E., Gorennoi, V., Wichers, C., Von Zur Mühlen, A., Von Büren, E. and Brabant, G. 2000. Age-related changes of serum sex hormones, insulin-like growth factor-1 and sex-hormone binding globulin levels in men: cross-sectional data from a healthy male cohort. Clinical endocrinology 53: 689-695.
- Liu, T., Wimalasena, J., Bowen, R. L. and Atwood, C. S. 2007. Luteinizing hormone receptor mediates neuronal pregnenolone production via up-regulation of steroidogenic acute regulatory protein expression. Journal of Neurochemistry 100: 1329-1339.
- Lu, B. and Chow, A. 1999. Neurotrophins and hippocampal synaptic transmission and plasticity. Journal of Neuroscience Research 58: 76-87.
- Lukacs, H., Hiatt, E. S., Lei, Z. M. and Rao, C. V. 1995. Peripheral and intracerebroventricular administration of human chorionic gonadotropin alters several hippocampus-associated behaviors in cycling female rats. Hormones and Behavior 29: 42-58.
- Lv, W., Du, N., Liu, Y., Fan, X., Wang, Y., Jia, X., Hou, X. and Wang, B. 2016. Low Testosterone Level and Risk of Alzheimer's Disease in the Elderly Men: a Systematic Review and Meta-Analysis. Molecular Neurobiology 53: 2679-2684.
- Maas, D., Jochen, A. and Lalande, B. 1997. Age-Related Changes in Male Gonadal Function. Drugs and Aging 11: 45-60.
- MacLusky, N. J., Hajszan, T., Prange-Kiel, J. and Leranth, C. 2006. Androgen modulation of hippocampal synaptic plasticity. Neuroscience 138: 957-965.
- Mangat, H. K. 1979. Evaluation of various doses of testosterone on accessory reproductive organs and plasma testosterone in intact and gonadectomized rhesus monkeys (*Macaca mulatta*). Andrologia 11: 449-452.

- Marques, P., Skorupskaite, K., Rozario, K. S., Anderson, R. A. and George, J. T. 2015. Physiology of GnRH and gonadotropin secretion.
- Marshall, J., Dalkin, A., Haisenleder, D., Griffin, M. and Kelch, R. 1993. GnRH pulses--the regulators of human reproduction. Transactions of the American Clinical and Climatological Association 104: 31.
- Meethal, S. V., Liu, T., Chan, H. W., Ginsburg, E., Wilson, A. C., Gray, D. N., Bowen, R. L., Vonderhaar, B. K. and Atwood, C. S. 2009. Identification of a regulatory loop for the synthesis of neurosteroids: a steroidogenic acute regulatory protein-dependent mechanism involving hypothalamic-pituitary-gonadal axis receptors. Journal of Neurochemistry 110: 1014-1027.
- Moffat, S. D., Zonderman, A. B., Metter, E. J., Blackman, M. R., Harman, S. M. and Resnick, S. M. 2002. Longitudinal assessment of serum free testosterone concentration predicts memory performance and cognitive status in elderly men. The Journal of Clinical Endocrinology and Metabolism 87: 5001-5007.
- Mohamad, N. V., Ima-Nirwana, S. and Chin, K.-Y. 2018. A Review on the Effects of Testosterone Supplementation in Hypogonadal Men with Cognitive Impairment. Current drug targets 19: 898-906.
- Morley, J. E. and Perry, H. M., 3rd. 2000. Androgen deficiency in aging men: role of testosterone replacement therapy. Journal of Laboratory and Clinical Medicine 135: 370-378.
- Morris, R. G. M. 1981. Spatial localization does not require the presence of local cues. Learning and Motivation 12: 239-260.
- Morris, R. G. M., Garrud, P., Rawlins, J. N. P. and O'Keefe, J. 1982. Place navigation impaired in rats with hippocampal lesions. Nature 297: 681.
- Mukai, H., Takata, N., Tanabe, N., Hojo, Y., Furukawa, A., Kimoto, T. and Kawato, S. 2006. Hippocampal synthesis of estrogens and androgens which are paracrine modulators of synaptic plasticity: synaptocrinology. Neuroscience 138: 757-764.
- Mullen, M. P., Cooke, D. J. and Crow, M. A., "Gonadotropin," in Structural and functional roles of FSH and LH as glycoproteins regulating reproduction in mammalian species, eds. (InTech, 2013).
- Muller, M., Aleman, A., Grobbee, D. E., de Haan, E. H. and van der Schouw, Y. T. 2005. Endogenous sex hormone levels and cognitive function in aging men: is there an optimal level? Neurology 64: 866-871.
- Murman, D. L. 2015. The Impact of Age on Cognition. Seminars in Hearing 36: 111-121.
- Oakley, A. E., Clifton, D. K. and Steiner, R. A. 2009. Kisspeptin signaling in the brain. Endocrine Reviews 30: 713-743.
- Osamura, R. Y., "Endocrine System," in Pituitary Gland, Rat, eds. (Berlin, Heidelberg: Springer Berlin Heidelberg, 1983), pp. 121-129. In T. C. Jones, U. Mohr, R. D. Hunt, & C. C. Capen (Eds.).
- Österlund, M., Kuiper, G. G., Gustafsson, J.-Å. and Hurd, Y. L. 1998. Differential distribution and regulation of estrogen receptor- $\alpha$  and- $\beta$  mRNA within the female rat brain1. Molecular brain research 54: 175-180.
- Park, Y., Dai Park, S. and Kim, K. 1988. Testosterone stimulates LH-RH-like mRNA level in the rat hypothalamus. Brain Research 451: 255-260.
- Paxinos, G. and Watson, C. 2005. The Rat Brain in Stereotaxic Coordinates - The New

Coronal Set 5th Edition.

- Payne, A. H. and Youngblood, G. L. 1995. Regulation of expression of steroidogenic enzymes in Leydig cells. Biology of Reproduction 52: 217-225.
- Pearce, J. M., Roberts, A. D. and Good, M. 1998. Hippocampal lesions disrupt navigation based on cognitive maps but not heading vectors. Nature 396: 75-77.
- Pearson, C. A. and Placzek, M. 2013. Development of the medial hypothalamus: forming a functional hypothalamic-neurohypophyseal interface. Current Topics in Developmental Biology 106: 49-88.
- Pelletier, G. 2010. Steroidogenic enzymes in the brain: morphological aspects. Progress in brain research 181: 193-207.
- Perrett, R. M. and McArdle, C. A. 2013. Molecular Mechanisms of Gonadotropin-Releasing Hormone Signaling: Integrating Cyclic Nucleotides into the Network. Frontiers in endocrinology 4: 180.
- Poletti, A., Rampoldi, A., Piccioni, F., Volpi, S., Simeoni, S., Zanisi, M. and Martini, L. 2001. 5Alpha-reductase type 2 and androgen receptor expression in gonadotropin releasing hormone GT1-1 cells. Journal of Neuroendocrinology 13: 353-357.
- Prange-Kiel, J., Fester, L., Zhou, L., Lauke, H., Carretero, J. and Rune, G. M. 2006. Inhibition of hippocampal estrogen synthesis causes region-specific downregulation of synaptic protein expression in hippocampal neurons. Hippocampus 16: 464-471.
- Prange-Kiel, J., Jarry, H., Schoen, M., Kohlmann, P., Lohse, C., Zhou, L. and Rune, G. M. 2008. Gonadotropin-releasing hormone regulates spine density via its regulatory role in hippocampal estrogen synthesis. The Journal of cell biology 180: 417-426.
- Prange-Kiel, J., Schmutterer, T., Fester, L., Zhou, L., Imholz, P., Brandt, N., Vierk, R., Jarry, H. and Rune, G. M. 2013. Endocrine regulation of estrogen synthesis in the hippocampus? Progress in histochemistry and cytochemistry 48: 49-64.
- Prange-Kiel, J., Wehrenberg, U., Jarry, H. and Rune, G. M. 2003. Para/autocrine regulation of estrogen receptors in hippocampal neurons. Hippocampus 13: 226-234.
- Preslock, J. P. 1980. Steroidogenesis in the mammalian testis. Endocrine Reviews 1: 132-139.
- Purves, D., Augustine, G. J., Fitzpatrick, D., Hall, W. C., LaMantia, A.-S., McNamara, J. O. and White, L. E. 2008. Neuroscience. 4th Edition. united states: Sinauer Associates, Inc.
- Ramaswamy, S. and Weinbauer, G. F. 2014. Endocrine control of spermatogenesis: Role of FSH and LH/ testosterone. Spermatogenesis 4: e996025.
- Roberts, J. A., Gilardi, K. V., Lasley, B. and Rapp, P. R. 1997. Reproductive senescence predicts cognitive decline in aged female monkeys. Neuroreport 8: 2047-2051.
- Rodrigues, M. A., Verdile, G., Foster, J. K., Hogervorst, E., Joesbury, K., Dhaliwal, S., Corder, E. H., Laws, S. M., Hone, E. and Prince, R. 2008. Gonadotropins and cognition in older women. Journal of Alzheimer's Disease 13: 267-274.
- Rosati, F., Sturli, N., Cungi, M. C., Morello, M., Villanelli, F., Bartolucci, G., Finocchi, C., Peri, A., Serio, M. and Danza, G. 2011. Gonadotropin-releasing hormone modulates cholesterol synthesis and steroidogenesis in SH-SY5Y cells. The

- Journal of Steroid Biochemistry and Molecular Biology 124: 77-83.
- Rudenstein, R. S., Bigdeli, H., McDonald, M. H. and Snyder, P. J. 1979. Administration of gonadal steroids to the castrated male rat prevents a decrease in the release of gonadotropin-releasing hormone from the incubated hypothalamus. The Journal of Clinical Investigation 63: 262-267.
- Sagrillo, C. A., Grattan, D. R., McCarthy, M. M. and Selmanoff, M. 1996. Hormonal and neurotransmitter regulation of GnRH gene expression and related reproductive behaviors. Behavior Genetics 26: 241-277.
- Scheff, S. W. and Price, D. A. 2006. Alzheimer's disease-related alterations in synaptic density: neocortex and hippocampus. Journal of Alzheimer's Disease 9: 101-115.
- Semaan, S. J., Tolson, K. P. and Kauffman, A. S. 2013. The Development of Kisspeptin Circuits in the Mammalian Brain. Advances in Experimental Medicine and Biology 784: 221-252.
- Seminara, S. B., Messenger, S., Chatzidaki, E. E., Thresher, R. R., Acierno Jr, J. S., Shagoury, J. K., Bo-Abbas, Y., Kuohung, W., Schwinof, K. M. and Hendrick, A. G. 2004. The GPR54 gene as a regulator of puberty. Obstetrical and Gynecological Survey 59: 351-353.
- Seminara, S. B., Messenger, S., Chatzidaki, E. E., Thresher, R. R., Acierno, J. S., Jr., Shagoury, J. K., Bo-Abbas, Y., Kuohung, W., Schwinof, K. M., Hendrick, A. G., Zahn, D., Dixon, J., Kaiser, U. B., Slaugenhaupt, S. A., Gusella, J. F., O'Rahilly, S., Carlton, M. B., Crowley, W. F., Jr., Aparicio, S. A. and Colledge, W. H. 2003. The GPR54 gene as a regulator of puberty. The New England Journal of Medicine 349: 1614-1627.
- Serrano-Pozo, A., Frosch, M. P., Masliah, E. and Hyman, B. T. 2011. Neuropathological Alterations in Alzheimer Disease. Cold Spring Harbor Perspectives in Medicine: 1: a006189.
- Shughrue, P. J., Lane, M. V. and Merchenthaler, I. 1997. Comparative distribution of estrogen receptor- $\alpha$  and - $\beta$  mRNA in the rat central nervous system. The Journal of Comparative Neurology 388: 507-525.
- Shukitt-Hale, B., Mouzakis, G. and Joseph, J. A. 1998. Psychomotor and spatial memory performance in aging male Fischer 344 rats. Experimental Gerontology 33: 615-624.
- Silverman, A.-J., Jhamandas, J. and Renaud, L. P. 1987. Localization of luteinizing hormone-releasing hormone (LHRH) neurons that project to the median eminence. Journal of Neuroscience 7: 2312-2319.
- Singh, V., Agrawal, N. K., Verma, R. and Singh, K., "Male Infertility: Understanding, Causes and Treatment," in HPG Axis: The Central Regulator of Spermatogenesis and Male Fertility, eds. (Singapore: Springer Singapore, 2017), pp. 25-36. In R. Singh & K. Singh (Eds.).
- Skinner, D. C., Albertson, A. J., Navratil, A., Smith, A., Mignot, M., Talbott, H. and Scanlan-Blake, N. 2009. Effects of gonadotrophin-releasing hormone outside the hypothalamic-pituitary-reproductive axis. Journal of Neuroendocrinology 21: 282-292.
- Smith, J. T., Clifton, D. K. and Steiner, R. A. 2006. Regulation of the neuroendocrine reproductive axis by kisspeptin-GPR54 signaling. Reproduction 131: 623-630.
- Smith, J. T., Dungan, H. M., Stoll, E. A., Gottsch, M. L., Braun, R. E., Eacker, S. M.,

- Clifton, D. K. and Steiner, R. A. 2005. Differential regulation of KiSS-1 mRNA expression by sex steroids in the brain of the male mouse. Endocrinology 146: 2976-2984.
- Sokanovic, S. J., Janjic, M. M., Stojkov, N. J., Baburski, A. Z., Bjelic, M. M., Andric, S. A. and Kostic, T. S. 2014. Age related changes of cAMP and MAPK signaling in Leydig cells of Wistar rats. Experimental Gerontology 58: 19-29.
- Stanczyk, F. 2009. Production, Clearance, and Measurement of Steroid Hormones.
- Sweatt, J. D. 2004. Hippocampal function in cognition. Psychopharmacology (Berl) 174: 99-110.
- Swerdloff, R. S. and Wang, C. 1993. Androgen deficiency and aging in men. Western journal of medicine 159: 579.
- Taupin, P. 2007. The hippocampus: neurotransmission and plasticity in the nervous system. Nova Publishers.
- Telegdy, G. and Adamik, Á. 2013. The action of kisspeptin-13 on passive avoidance learning in mice. Involvement of transmitters. Behavioural Brain Research 243: 300-305.
- Tena-Sempere, M. 2006. GPR54 and kisspeptin in reproduction. Human Reproduction Update 12: 631-639.
- Tilbrook, A. J. and Clarke, I. J. 2001. Negative feedback regulation of the secretion and actions of gonadotropin-releasing hormone in males. Biology of Reproduction 64: 735-742.
- Tng, E. L. 2015. Kisspeptin signalling and its roles in humans. Singapore medical journal 56(12): 649.
- Tortora, G. J. and Derrickson, B. 2014. Principles of anatomy and physiology. 14 edition. New York, United States: John Wiley & Sons Inc.
- Tsai, H.-W., Taniguchi, S., Samoza, J. and Ridder, A. 2015. Age- and sex-dependent changes in androgen receptor expression in the developing mouse cortex and hippocampus. Neuroscience journal 2015.
- van Rooijen, J. H., Ooms, M. P., Weber, R. F., Brinkmann, A. O., Grootegoed, J. A. and Vreeburg, J. T. 1997. Comparison of the response of rat testis and accessory sex organs to treatment with testosterone and the synthetic androgen methyltrienolone (R1881). Journal of Andrology 18: 51-61.
- Veldhuis, J. D., Urban, R. J. and Dufau, M. L. 1992. Evidence that androgen negative feedback regulates hypothalamic gonadotropin-releasing hormone impulse strength and the burst-like secretion of biologically active luteinizing hormone in men. The Journal of Clinical Endocrinology and Metabolism 74: 1227-1235.
- Vermeulen, A. and Kaufman, J. M. 1995. Ageing of the hypothalamo-pituitary-testicular axis in men. Hormone Research 43: 25-28.
- Vertes, R. P. 2005. Hippocampal theta rhythm: a tag for short-term memory. Hippocampus 15: 923-935.
- Wahab, F., Atika, B., Shahab, M. and Behr, R. 2016. Kisspeptin signalling in the physiology and pathophysiology of the urogenital system. Nature Reviews Urology 13: 21.
- Wang, C., Leung, A. and Sinha-Hikim, A. P. 1993. Reproductive aging in the male brown-Norway rat: a model for the human. Endocrinology 133: 2773-2781.
- Webber, K. M., Stocco, D. M., Casadesus, G., Bowen, R. L., Atwood, C. S., Preville, L. A., Harris, P. L., Zhu, X., Perry, G. and Smith, M. A. 2006. Steroidogenic acute

- regulatory protein (StAR): evidence of gonadotropin-induced steroidogenesis in Alzheimer disease. Molecular neurodegeneration 1: 14.
- Wehrenberg, U., Prange-Kiel, J. and Rune, G. 2001. Steroidogenic factor-1 expression in marmoset and rat hippocampus: co-localization with StAR and aromatase. Journal of Neurochemistry 76: 1879-1886.
- Weiland, N., Orikasa, C., Hayashi, S. and McEwen, B. 1997. Distribution and hormone regulation of estrogen receptor immunoreactive cells in the hippocampus of male and female rats. Journal of Comparative Neurology 388: 603-612.
- Wible, C. G. 2013. Hippocampal physiology, structure and function and the neuroscience of schizophrenia: a unified account of declarative memory deficits, working memory deficits and schizophrenic symptoms. Behavioral Sciences (Basel) 3: 298-315.
- Wierman, M. E., Rivier, J. E. and Wang, C. 1989. Gonadotropin-releasing hormone-dependent regulation of gonadotropin subunit messenger ribonucleic acid levels in the rat. Endocrinology 124: 272-278.
- Wilson, A. C., Salamat, M. S., Haasl, R. J., Roche, K. M., Karande, A., Meethal, S. V., Terasawa, E., Bowen, R. L. and Atwood, C. S. 2006. Human neurons express type I GnRH receptor and respond to GnRH I by increasing luteinizing hormone expression. Journal of Endocrinology 191: 651-663.
- Wu, D. and Gore, A. C. 2010. Changes in androgen receptor, estrogen receptor alpha, and sexual behavior with aging and testosterone in male rats. Hormones and Behavior 58: 306-316.
- Wu, D., Lin, G. and Gore, A. C. 2009. Age-related changes in hypothalamic androgen receptor and estrogen receptor alpha in male rats. Journal of Comparative Neurology 512: 688-701.
- Wyss, J. M., Chambless, B. D., Kadish, I. and van Groen, T. 2000. Age-related decline in water maze learning and memory in rats: strain differences. Neurobiology of Aging 21: 671-681.
- Yamasaki, K., Sawaki, M., Muroi, T. and Takatsuki, M. 2001. Changes in the weights of the accessory sex organs in male CD/IGS rats (*Rattus norvegicus*) after castration. Contemporary Topics in Laboratory Animal Science 40: 25-26.
- Yin, W. and Gore, A. C. 2006. Neuroendocrine control of reproductive aging: roles of GnRH neurons. Reproduction 131: 403-414.
- Zárate, S., Stevnsner, T. and Gredilla, R. 2017. Role of estrogen and other sex hormones in brain aging. Neuroprotection and DNA repair. Frontiers in aging neuroscience 9: 430.
- Ziegler, S. G. and Thornton, J. E. 2010. Low luteinizing hormone enhances spatial memory and has protective effects on memory loss in rats. Hormones and Behavior 58: 705-713.
- Zilbermint, M. F., Wisniewski, A. B., Xu, X., Selnes, O. A. and Dobs, A. S. 2013. Relationship between sex hormones and cognitive performance in men with substance use. Drug and alcohol dependence 128: 250-254.

## APPENDICES

### **APPENDIX A; The submitted abstract for the 95<sup>th</sup> Annual Meeting of the Physiological Society of Japan (PSJ2018).**

Wititsuwankul, P., Jaroenporn, S., Fainanta, T. and Malaivijitnond, S. 2018.

Association between reproductive senescence and cognitive impairment in male rats. Proceedings of the 95<sup>th</sup> Annual Meeting of the Physiological Society of Japan: The Journal of Physiological Sciences 68: 85.

### **APPENDIX B; The submitted manuscript in the Proceedings of the 13<sup>th</sup> Rangsit University National Graduate Research Conference.**

Wititsuwankul, P., Malaivijitnond, S., Jaroenporn, S. and Fainanta, T. 2018.

Reproductive aging of hypothalamus-pituitary-testicular axis in middle-aged male rats. Proceedings of the 13<sup>th</sup> RSU National Graduate Research Conference: 3003-3011.



## VITA

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**PUBLICATION** She published a part of her research work as a research article in the Proceedings of the 13th Rangsit University National Graduate Research Conference 2018.

**AWARD RECEIVED** She was awarded the research grant from the 90th Anniversary of Chulalongkorn University Fund (Ratchadaphiseksomphot Endowment Fund). During her Master study in 2016, she had a chance to participate in the 3rd Advanced School of Neuroscience of the International Brain Research Organization (IBRO) at Monash University, Malaysia which she had conducted a research project entitled, "The effect of light on dopamine system in valopa mutant zebrafish". Patteera was also experienced both international and national oral presentations. For the international one, she presented a part of her thesis entitled, "Association between reproductive senescence and cognitive impairment in male rats" in the 95th Annual Meeting of the Physiological Society of Japan (PSJ2018), Kagawa, Japan on March 29th, 2018. For the national meeting, she presented her work entitled, "Reproductive aging of hypothalamus-pituitary-testicular axis in middle-aged male rats" in the 13th Rangsit University National Graduate Research Conference, Rungsit University, Bangkok, Thailand on August 16th, 2018.