UNIVERSITI PUTRA MALAYSIA

COMPARISON OF SEX HORMONE MANIPULATION EFFECTS DURING PERINATAL PERIOD ON MRNA EXPRESSION OF SLC9A4, NR3C2, HTR5B AND MAS1, IN HIPPOCAMPUS AND FRONTAL CORTEX OF MALE AND FEMALE RATS

BENYAMIN KARIMI

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By

BENYAMIN KARIMI

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfillment of Requirements for the Degree of Master of Science

November 2013

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DEDICATION

This thesis is dedicated to my beloved parents and my lovely family, to my love, "Pardis", and to all unknown soldiers of science, all around the world, sacrificing their lives to seek the truth.

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirement for the Degree of Master of Science

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November 2013

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Spatial memory and the ability to navigate through space are known as sexually dimorphic traits among mammals, and numerous studies have shown that these traits can be altered by means of sex hormone manipulation. Hippocampus, the main organ involved in this kind of memory, has specific signature genes, with high expression level compared to other regions of the brain. Based on their expression levels and the role that products of these genes can play in processes like signal transduction, mediation of hormone effects and long term potentiation, these genes can be considered as genes necessary for routine tasks of hippocampus. Male and female rat pups were injected with estradiol and testosterone, respectively, at early stage of their lives to examine the effect of sex hormone manipulation on mRNA expression of Slc9a4, Nr3c2, Htr5b and Mas1, using comparative quantitative real-time polymerase chain reaction. Another parallel study was also conducted, using another set of animals, to test the spatial ability after administration of sex hormones under identical conditions. The results showed that expressions of these genes are strongly influenced by sex hormones in both the frontal cortex and hippocampus, especially in male hippocampus, in which expression of all genes were up-regulated. Htr5b was the only gene that was affected only in the males. Expression of Mas1, contrary to expectations, showed stronger changes in its expression in cortex than in hippocampus. Nr3c2 was down regulated in all samples but up regulated in male hippocampus; Slc9a4 also showed a huge up-regulation in male hippocampus compared to other samples. It was also evident from the Morris Water Maze test results that sex hormone manipulation significantly alters the ability in spatial memory and navigation.

Abstrak tesis yang dikemukakan kepada senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PERBANDINGAN KESAN MANIPULASI HORMON JANTINA SEMASA TEMPOH PERINATAL KE ATAS EXPRESI mRNA SLC9A4, NR3C2, HTR5B DAN MAS1 DALAM HIPOCAMPUS DAN KORTEKS FRONTAL TIKUS JANTAN DAN BETINA

Oleh

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Pengerusi: Prof. Madya Hafidzi Mhmd Noor, PhD

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Memori spatial dan keupayaan untuk mengemudi melalui ruang dikenali sebagai sifat dimorfik seksual di kalangan mamalia, dan banyak kajian telah menunjukkan bahawa sifat-sifat ini boleh diubah melalui manipulasi hormone jantina. Hippocampus, organ utama yang terlibat dalam jenis memori ini, memiliki gen signature tertentu, dengan tahap ekspresi yang tinggi berbanding dengan kawasan lain di otak.

Berdasarkan tahap ekspresi mereka dan peranan yang produk gen-gen ini boleh bermain dalam proses seperti transduksi isyarat, pengantaraan kesan hormon dan *potentiation* jangka panjang, gen-gen ini boleh dianggap sebagai gen yang diperlukan untuk tugas-tugas rutin hippocampus.

Anak tikus jantan dan betina telah disuntik dengan estradiol dan testosterone, masing-masing, pada peringkat awal kehidupan mereka untuk mengkaji kesan manipulasi hormon jantina ke atas ekspresi mRNA Slc9a4, Nr3c2, Htr5b dan Mas1, dengan menggunakan *comparative quantitative real-time polymerase chain reaction*. Satu lagi kajian juga telah dijalankan, frmgan menggunakan set haiwan yang lain, untuk menguji keupayaan spatial selepas diberi hormon jantina di bawah keadaan yang sama.

Hasil kajian menunjukkan bahawa ekspresi gen-gen ini dipengaruhi kuat oleh hormon jantina dalam kedua-dua korteks frontal dan hippocampus, terutama dalam hippocampus jantan, di mana ekspresi semua gen dipertingkatan. Htr5b adalah satu-satunya gen yang dipengaruhi hanya pada jantan. Ekspresi Mas1m, bertentangan dengan dijangka, menunjukkan perubahan besar dalam ekspresinya dalam korteks daripada hippocampus. Nr3c2 turun dikawal selia dalam semua sampel tetapi tambah dikawal selia dalam hippocampus jantan; Slc9a4 juga menunjukkan tambahan dikawal selia yang besar dalam hippocampus jantan berbanding dengan sampel lainnya. Ia juga jelas daripada keputusan ujian Morris Water Maze bahawa manipulasi hormon jantina mengubah

secara berkesan keupayaan dalam memori spatial dan pelayaran.

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I certify that a Thesis Examination Committee has met on 14 November 2013 to conduct the final examination of Benyamin Karimi on his thesis entitled "Comparison of Sex Hormone Manipulation Effects during Perinatal Period on MRNA Expression of SLC9A4, NR3C2, HTR5B And MAS1 in Hippocampus and Frontal Cortex of Male and Female Rats" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Master of Science.

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This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

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Declaration by graduate student

I hereby declare that:

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

~	-	approximately
AGD	-	Ano-genital Distance
bp	-	base pair
cDNA	-	complementary DNA
dH_2O	-	distilled water
DNA	-	deoxyribonucleic acid
dNTPs	-	deoxyribonucleic acid Tri-phosphates
dsDNA	-	double stranded DNA
g	-	Gravity force
gr	-	gram
hr	-	hour
IU	-	International Units
Kb	-	kilo base pair
M	-	Molarity
$MgCl_2$	-	magnesium chloride
Min	-	minute
ml	-	milliliter
mM	-	milli molar
MWM	-	Morris Water Maze
mRNA	-	messenger RNA
N	-	Normality
NaCl	-	sodium chloride
ng	-	Nano gram
No.	-	Number
N-terminal		the amino (NH_2)–terminal of a polypeptide
^oC	-	degree centigrade
OD	-	optical density
PCR	-	polymerase chain reaction
pH	-	isoelectric point
RNA	-	ribonucleic acid
RNase	-	Ribonuclease
rpm	-	revolutions per minute
s	-	Second
SP	-	signal peptide
sp.	-	species
subsp.	-	subspecies
Taq	-	*Thermus aquaticus*
v/v	-	volume per volume
w/v	-	weight per volume
%	-	percent

µl	-	micro liter
µg	-	micro gram

CHAPTER 1

INTRODUCTION

Sexually dimorphic traits are among the most important traits of those living creatures that reproduce sexually, for they are directly involved with the survival of the species and reproduction of the individuals. Examples of such dimorphic traits include the differences in size, morphology, behavior and ornamentation (Lande, 1980; Rice, 1984; Shine, 1989; Fairbairn *et al.*, 2008).

The underlying reasons for the profound differences in these traits have been the subject of many philosophical and scientific controversies and disputes. Every so often a gene or a group of them is found related to a special trait. Like anything else about mind and body of a living being, these differences are also related to the genes and the function of environment on them (Dunn *et al.*, 2001; Plavcan, 2001).

Spatial navigation which is one of the most important sexual dimorphic traits in mammals is the ability of the animal to locate a specific place by means of spatial cues (Coyne, 2009). Spatial memories are formed after an animal gathers and processes sensory information about its surroundings, especially through vision and proprioception. A cognitive map is required for proper spatial navigation and it is a mental model of object's spatial configuration that permits navigation along optimal path between arbitrary pairs of points (Newman *et al.*, 2007).

Historically, the male has been the food providers for the family by homing in on the food source; therefore, they have to be good at locating objects from a distance using spatial memory and navigation cues as tools in securing food sources while avoiding predators at the same time (Maguire *et al.*, 1999a; Bird and Burgess, 2008; Epstein, 2008). On the other hand the female typically tends to the offspring, gathers food in the vicinity of the home base (nest, den, etc.) and defends her charge in the males' absence. Such task requires a broader and panoramic vision. They have to execute multiple tasks simultaneously and they do not need to match the male in spatial abilities.

These different tasks have been believed to be main underlying reasons for divergent evolution of spatial navigation in males and females; however, this theory has its flaws which need to be addressed accordingly. The first reason that this theory might fail to explain these differences is that in those species of mammals in which males and females have different tasks, and those in which the duties of males and females seem to be interchanged, males are still better in spatial navigation than females. Another reason is that if males have become better in spatial navigation because of their hunting skills, which has also resulted in superiority of those successful males who were able to pass on their genes to the next generation, then there is equal chance that female offspring would also inherit those genes and they would have also evolved with better spatial navigation abilities than they seem to have now (Sherry *et al.*, 1992; Clint *et al.*, 2012).

The alternative theory might just be that these differences are like many other secondary sex characteristics which have resulted as side effects of sex hormones, similar to male baldness, puberty acnes, and facial hair. It must be noted that living beings evolve as a function of natural selection on their genes and the way they are expressed and, therefore, for the alternative theory to be correct sex hormones must somehow affect expression of genes in different brain regions. As hippocampus is believed to be the main organ involved in spatial navigation and spatial memory, in this study it has been tried to explore expression patterns of highly expressive genes in hippocampus and the cortex. (Macphail and Bolhuis, 2001; Thomas, 2010; Clint *et al.*, 2012; Jack *et al.*, 2013).

Hippocampus is a coupled structure similar to the cerebral cortex, with which it is closely related. Regardless of these structural similarity and close connection, according to investigations, hippocampus and cortex have the most diverse gene expression profiles compared to other mammalian brain parts (Andersen *et al.*, 2007; Stansberg *et al.*, 2007; Thompson *et al.*, 2008). Among 10 genes that have been shown to have higher expression in hippocampus, *Mas1, Nr3c2, Slc9a4 and Htr5b*, play important roles in different regions of brain and it is hypothesized that expression of all four of these genes or at least some of them will significantly change in the hippocampus, under administration of the opposite sex hormone at early stage of an animal's life.

This study, in general, was an attempt to investigate the genetic basis of spatial navigation and to determine whether sex differences observed in an animal's phenotype occur during embryonic period or at puberty. In particular, it was aimed at answering the following questions:

- Do sexually dimorphic traits in general and spatial navigation in particular are developed as a result of evolutionary pressure or they can be assumed as side effects of testosterone?
- What will be the effects of sex hormone manipulation in neonatal period on spatial navigation ability of male and female rats?
- Does sex hormone manipulation during neonatal period affect expression of highly expressed genes in hippocampus and are the potential effects are different from cortex?
- Will any significant differences be detected in the expression of highly expressed genes in hippocampus and cortex between male and female rats?

The specific objectives of the study were:

- To explore the effects of sex hormone manipulation on spatial navigation performance of male and female rats;

- To assess the influence of sex hormone manipulation during neonatal period on expression of highly expressed genes in the hippocampus and cortex of male and female rats.

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