Effect of repeated early maternal separation on the hippocampus and some glands of albino rat mothers and their newly born pups Histological and Immunohistochemical study

Thesis
Submitted for Partial Fulfillment of M.D. Degree of Histology

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2012

بسم الله الرحمن الرحيم

﴿وَالْوَالِدَاتُ يُرْضِعْنَ أَوْلادَهُنَّ حَوْلَيْنِ كَامِلَيْنِ لِمَنْ أَرَادَ أَن يُتِمَّ الرَّضَاعَةَ وَعَلَى الْمَوْلُودِ لَهُ رِزْقُهُنَّ وَكِسْوَتُهُنَّ بِالْمَعْرُوفِ لاَ تُكَلَّفُ نَفْسٌ إِلاَّ وُسْعَهَا لاَ تُضَارَّ وَالِدَةٌ بِوَلَدِهَا وَلاَ مَوْلُودٌ لَّهُ بِوَلَدِهِ وَعَلَى الْوَارِثِ مِثْلُ ذَلِكَ فَإِنْ أَرَادَا فِصَالاً عَن تَرَاضٍ مِّنْهُمَا وَتَشَاوُرٍ فَلاَ جُنَاحَ عَلَيْهِمَا وَإِنْ أَرَدتُّمْ أَن تَسْتَرْضِعُواْ أَوْلادَكُمْ فَلاَ جُنَاحَ عَلَيْكُمْ إِذَا سَلَّمْتُم مَّا آتَيْتُم بِالْمَعْرُوفِ وَاتَّقُواْ اللَّهَ وَاعْلَمُواْ أَنَّ اللَّهَ بِمَا تَعْمَلُونَ بَصِيرٌ﴾

صدق الله العظيم

(البقرة: ٢٣٣)

Acknowledgement

*First and foremost thanks to **Allah** the most kind and most merciful, to whom I relate any success in achieving any work in my life.*

*I would like to express my deepest gratitude and profound respect to **Prof. Dr. Adel Salah El-din Zohdy**, Professor of Histology, Faculty of Medicine-Ain Shams University, for his everlasting encouragement, meticulous help, wide experience, precious instructions and kind supervision. It was such a great honor to work under his guidance.*

*I am deeply grateful to **Prof. Dr. Nagah Mourad Yahia Shalby,** Professor of Histology, Faculty of Medicine-Ain Shams University, for her precious opinions, contributive comments and sincere effort that served much in the construction of this work.*

*I am deeply indebted and extremely grateful to **Prof. Dr. Amel Ali Soliman**, Professor of Histology, Faculty of Medicine-Ain Shams University, for her valuable advices and supervision during various stages of the development of this work. Without her help, patience, continuous encouragement, and skillful scientific guidance, this work could not have been completed.*

*I am also grateful to **Prof. Dr. Mostafa Ismail,** Professor of Histology, Faculty of Medicine-Al-Azhar University, for his great effort, patience and valuable opinions regarding the Morphometric study in this work.*

I would like to extend my thanks to ***Prof. Dr. Faten Sobhy,*** *Professor and Head of Histology department, Faculty of Medicine-Ain Shams University, for her support and encouragement.*

Special thanks to all my professors and my colleagues in Histology department, Faculty of Medicine-Ain Shams University (especially ***Dr. Ghada Galal****, Lecturer of Histology) for their continuous support and valuable advices.*

Dedication

*Words can not express my thanks, gratefulness, respect and love to all members of my **family**, especially my **husband** and my **mother**. Without their help, support, patience and encouragement I would have never achieved any success.*

*To all of them, to **Yahya** & **Seif**, and to little **Nour** I dedicate my work.*

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ACTH……...adrenocorticotrophic hormone
Cort ……..corticosterone
CRH ……corticotrophin releasing hormone
DNA ……..…deoxyribonucleic acid
GR ……glucocorticoid receptor
HMS …….handling-maternal separation
HPA……..hypothalamic-pituitary-adrenal
HPT……..hypothalamic-pituitary-thyroid
hr ……...hours
MR ……...mineralocorticoid receptor
mRNA …….messenger RNA
MS ……maternal separation
PND ..……postnatal day
PVN……...paraventricular nucleus
RNA …….ribonucleic acid
SHRP……stress hyporesponsive period
T_3 ……...triiodothyronine
T_4……tetraiodothyronine
TEM …….transmition electron microscopy

Introduction

The mother-infant relationship is an instinctive phenomenon, and loss of mother care in early life influences neonatal development, behavior and physiologic responses ***[Hofer (1996)*** and ***Francis and Meaney (1999)]***. Furthermore, the early loss may affect the vulnerability of the infant to neuropsychiatric disorders, such as childhood anxiety disorders, personality disorders and depression over his life span ***[Andersen et al. (1999)*** and ***Kagan and Zenther (1999)].*** According to ***Heim et al., 2000 a&c*** and ***Ehlert et al., 2001,*** parental separation is one of the forms of traumatic early life stress in humans, as well as childhood sexual or physical abuse, or preterm birth which has been associated with mood and anxiety disorders.

Stress was defined as "a mentally or emotionally disruptive or upsetting condition, occurring in response to adverse external influences", as well as "a stimulus or circumstance causing such a condition", ***Nunberg, 1997***.

Stressors detected via the primary sensory organs generated signals which passed through mediating systems located in the amygdale, limbic system and prefrontal cortex. These regions served to process and evaluate stress-related

information and in turn generated responses through regulation of the Hypothalamic-pituitary-adrenal (HPA) and the Locuscoeruleus-norepinephrine (LC-NE) activity and other effector systems. During stress, the HPA axis became critically engaged through its role in activating the release of glucocorticoids with consequent increase in heart rate, blood pressure and metabolism. ***(de Mello et al., 2003)***

So long as maternal separation was a stressful condition that affects new-born babies, it might exert a parallel stressful effect on the mother as well. This is in accord with the opinion of ***Hock and Schirtziger (1992)*** who mentioned that maternal separation anxiety was a construct that described a mother's experience of worry, sadness or guilt during short-term separation from her child.

Accordingly, this work aimed at studying the immediate and late effects of maternal separation on some organs related to psychic, metabolic and lactation functions in both the mother and their new-born babies, mainly from the microscopical point of view.

Review of Literature

An undisturbed development of the brain is essential for normal functioning of the organism during adulthood. Maternal separation is considered as one of the most stressful experiences that can affect brain development. It also has long-term effects, both on mothers and infants.

Thliveris and Connell Jr (1972) studied the ultrastructure of the fetal rat adrenal gland at full-term and during artificially prolonged gestation. They found that cortical and medullary cells contained well developed organelles at all stages. Nonetheless, zonation of the gland was not yet distinct. They added that the zona glomerulosa and zona fasciculata were fairly well delineated. However, the zona reticularis and medulla were as yet poorly defined. The latter regions were intermingled, and thus collectively referred to as the "zona reticulo-medullaris." During prolonged gestation, many cells of the zona fasciculata and zona reticulo-medullaris contained dilated smooth endoplasmic reticulum and numerous enlarged mitochondria. They also observed enlarged mitochondria in medullary cells concurrent with a paucity of catecholamine storage granules. Moreover, meconium staining, which was indicative of fetal stress, was

also observed. The authors suggested that the ultrastructural changes observed were a response by the fetal adrenal gland to fetal stress.

Merry (1975) observed in both normal and stressed adrenal cortices, protrusions of the outer membrane of mitochondria. He added that these protrusions were often seen penetrating lipid droplets. He suggested that these protrusions might have a role in the transport of cholesterol from the lipid droplet to the inner mitochondrial membrane. That could facilitate side-chain cleavage of cholesterol to pregnenolone.

Stone et al., (1976) found that rat pups, 10-12 days old, survived maternal deprivation if kept warm at 35 degrees. They added that normal body temperature facilitated feeding. However, even without food, warm pups survived starvation longer than cool ones. After 72 hours (hr) of separation without food, they found that cool pups failed to increase brain weight, protein, DNA, RNA, and catecholamine contents. On the other hand, warm pups showed developmental growth comparable to that of normally mothered pups. Moreover, they significantly exceeded controls in the rate of accumulation of brain norepinephrine and dopamine**.**

Crutchfield and Dratman (1980) found that nutritional deficiencies in the maternal diet did not alter growth rate. That occurred only if the pups were minimally disturbed in the course of rearing. They also found that chronic handling stress alone did not affect weight gain of pups of normally fed mothers. However, both factors together led to growth retardation without evidence of hypothyroidism. They also noticed that males were more susceptible than females to early dietary deficiencies, radiation and handling stresses.

Khokhlov and Nevorotin (1980) noticed that lactating mammary gland showed increased number of secretory granules. However, the primary lysosome count was less, as compared to that of mammary gland after weaning. They also found abundant secondary lysosomes, autophagosomes, and heterophagosomes in the epithelial cells of the involuting mammary glands. In addition, some Golgi lamellae and smooth endoplasmic reticulum filled with acid phosphatase were often observed. The possible role of the relationship between the secretory and catabolic activities of the epithelial cells during mammary gland tumorogenesis was considered.

Martins and Stokes (1987) found that the activities of the hexose monophosphate dehydrogenases decreased in mammary gland of rat. That change occurred following weaning at mid-lactation (day 14). When dietary intake was restricted at mid-lactation, the activities of the hexose monophosphate dehydrogenases were unaltered in mammary gland.

Lau (1988) found that plasma adrenocorticosteroid levels were more increased in early stages of lactation. However, milk yield was significantly less at the earlier than at the later stage of lactation. Adrenalectomy in conjunction with corticosterone replacement pellets did not alter milk supply or milk release. The subsequent treatment with pentolinium did not affect milk ejection. The author suggested that the isolation/suckling condition might be more stressful earlier in lactation.

DeSantiago et al., (1991) found that the wet weight and total organ protein were higher in mammary glands of lactating female rats. Moreover, the DNA and RNA were also higher during lactation. On the other hand, these values were lower after weaning. They concluded that these changes in protein content were related to an adaptive