

Histological study on the effect of single prolonged stress model on the hippocampus of adult male albino rat

Thesis

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بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

قالوا

سبحانك لا علم لنا
إلا ما علمتنا إنك أنت
العليم العظيم

صدق الله العظيم

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List of Abbreviations

ACTH	Adrenocorticotropin
AVP	Arginine vasopressin
Bax	BCL2 associated X protein
Bcl 2	B-cell lymphoma 2
BDNF	Brain derived neurotrophic factor
CA	Cornu Ammonis
CaM	Calmodulin
CaMKIIa	Calmodulin-dependent Kinase IIa
CREB	cAMP response element-binding protein
CRH	Corticotropin releasing hormone
CRP	C-reactive protein
COX	Cytochrome oxidase
DA	Dopamine
DG	Dendate gyrus
DOPAC	3,4-Dihydroxyphenylacetic acid
GABA	Gamma-Aminobutyric acid
GCs	Glucocorticoids
GDNF	Glial derived neurotrophic factor
GFAP	Glial fibrillary acidic protein
GRs	Glucocorticoid receptors
GRP78	Glucose regulated protein 78 kDa
HPA	Hypothalamic-pituitary-adrenal axis

IL-6.....Interleukin 6
MRs.....Mineralocorticoid receptors
NE.....Norepinephrine
NGF.....Nerve growth factor
NMDA.....N-methyl-D-aspartate
PSPhosphatidylserine
PTSDPost-traumatic stress disorder
SPSSingle prolonged stress
sTNF-RIISoluble receptor II for tumor necrosis factors
TNF- αTumor necrosis factor-alpha
UPR.....Unfolded protein response
5-HT5-hydroxytryptamine

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ABSTRACT

Introduction: Post- traumatic stress disorder (PTSD) is a psychiatric disorder which occurs after the experience of life-threatening events as natural disasters, serious accidents, or violent sexual assaults. It has a severe impact on quality of life. PTSD directly affects hippocampal structure and function.

Aim of the work: to study the effect of single prolonged stress (SPS) on the histological structure of hippocampus in adult male albino rats.

Materials and methods: 40 adult male albino rats were randomly divided into two groups control group (group I) and stress group (group II) examined at day 1 (subgroup IIa), day 4 (subgroup IIb), day 7 (subgroup IIc) and day 14 (subgroup IId). Rats were restrained by placing them in plastic restrainers, immediately followed by 20 minutes of forced swimming in 20–24°C water. They were generally anesthetized by ether then they were placed in their home cages. At the end of the experiment blood samples were drawn to measure blood cortisol level. The hippocampi were taken and processed for proper histological, immunohistochemical and electron microscopic studies. Statistical analysis was also done.

Results: light microscopic examination of pyramidal cells of the CA3 region of the hippocampus showed progressive degeneration and a significant decrease in the thickness with passage of time from day 1 to day 14. Sections stained with toluidine blue revealed unapparent Nissl granules in the degenerated pyramidal cells. Transient increase in glial fibrillary acidic protein (GFAP) expression in the cytoplasm of astrocytes in the CA3 region of the hippocampus was detected at day 1 and day 4 followed by reduction in GFAP expression started at day seven and continued to day 14. Electron microscopic examination of the CA3 region in SPS rats showed the pyramidal cells with irregular nuclei, condensed chromatin, dilated rER cisternae, dilated Golgi saccules and vacuolated mitochondria. Many lysosomes were also observed. Changes were more aggravated in subgroups IIc and IId. Blood cortisol level showed significant increase at day 1, 4, and 7 followed by a significant reduction at day 14.

Conclusion: PTSD caused significant structural hippocampal neuronal damage affecting the CA3 hippocampal subfield.

Keywords: Hippocampus. Post- traumatic stress disorder (PTSD). Glial fibrillarly acidic protein (GFAP). Histology. Immuno-histochemical study.

INTRODUCTION

Posttraumatic stress disorder (PTSD) is a debilitating psychiatric disorder which develops after exposure to a traumatic highly stressful event **Török et al., (2019)** such as those occurring during natural disasters, sexual abuse and wars **Perlick et al., (2017)**. Posttraumatic stress disorder (PTSD) is complicated by other related disorders such as substance abuse, depression and memory impairments **LoSavio et al., (2017), Shalev et al., (2017)**. It is associated with deterioration of the ability to function in social and family life, leading to marital problems, occupational instability and other social problems **Alzoubi et al., (2018)**. The lifetime prevalence of PTSD is 8% in the general population and 24% in persons exposed to trauma **Wang et al., (2018)**. Many individuals with PTSD recover during two years following traumatic exposure, up to 30–40% remain chronically symptomatic **Yamamoto et al., (2009)**.

Stress is the most potent negative modulator of hippocampal neurogenesis **Dias et al., (2012)**. The hippocampus is an important region in the limbic system of the brain and is known to regulate cognition and memory. It has been reported that the hippocampus not only saves emotion-related information but also formats long-term memory **Han et al., (2015)**.

It is known that chronic stress is one of the most potent negative modulators of hippocampal function **Joëls et al., (2004)**. Many studies were performed on the effect of chronic stress, but the effects of single prolonged stress (SPS) on the hippocampus weren't clearly studied, particularly on the histological light and electron microscopic levels.

AIM OF THE WORK

The aim of this work was to study the effect of single prolonged stress (SPS) on the histological structure of hippocampus in adult male albino rats.

ANATOMY OF THE HIPPOCAMPUS

The hippocampus is a part of the limbic system. It is a C- shaped structure which concaves medially around the mesencephalon. It is formed of a head, a body and a tail. It is located at the medial aspect of the temporal lobe beneath the temporal horn of the lateral ventricle. It has two aspects a ventricular one facing the lateral ventricle and a cisternal one facing the mesencephalon from which it is separated by a transverse fissure. In a coronal section the hippocampus is made of two cortical laminae named cornu Ammonis and dentate gyrus **Destrieux et al., (2013)**.

The hippocampus includes the subiculum, the hippocampus proper, and the dentate gyrus. Each of them is composed of temporal lobe allocortex that is organized into S-shaped scroll along the floor of the lateral ventricle. A band-like structure originating from the fornix of the subiculum is called the fimbria. The hippocampus is also known as Ammon's horn (an Egyptian deity having a ram's head). The main cells of the hippocampus and the subiculum are pyramidal cells; those of the dentate gyrus are granule cells. Dendrites of both granule and pyramidal cells are studded with many dendritic spines. The hippocampus is also rich with inhibitory gamma-Aminobutyric acid (GABA) neurons **Mtui et al., (2015)**

The white fibres covering the ventricular surface of hippocampus is called the alveus. The alveus originates in the hippocampal cortex and course towards the medial border of hippocampus. They converge to form a narrow strip of white matter, the fimbria of hippocampus **Singh and Vishram, (2014)**

The cornu Ammonis (CA) is subdivided into four different regions according to the cytoarchitecture of its pyramidal layer, CA1 (Cornu Ammonis one) which lies close to the subiculum to CA4 which is located within the concavity of the dentate gyrus. The cornu Ammonis is covered by a thin white layer called the alveus. The dentate gyrus is a concave groove containing the CA4 region **Destrieux et al., (2013).**

Microscopical structure of the hippocampus:

The hippocampus is formed of four parts:

- 1- The hippocampus proprius.
- 2- The dentate gyrus.
- 3-The subiculum.
- 4-The area entorhinalis **Keuker et al., (2003).**

- 1- The hippocampus proprius is further subdivided into four regions according to density, size and arborizations of axons and dendrites of the pyramidal cells (CA1– CA4):

- CA1 region is formed of medium sized densely packed pyramidal cells.
- CA2 is the small transitional zone between CA3 and CA1.
- CA3 region contains large less densely packed pyramidal cells.
- CA4 region is the continuation of the CA3 area situated into the concavity of the dentate gyrus **El Falougy et al., (2008)**

Snell and Richard (2010) described the three layers of the hippocampus as:

- The superficial molecular layer consists of nerve fibers and scattered small neurons.
- The pyramidal cell layer is formed of large pyramidal neurons. It is the most characteristic layer of the hippocampus.
- The inner polymorphic layer is similar to the polymorphic layer of the cortex seen elsewhere, and it is formed of fusiform neurons.

2- The dentate gyrus is a coiled structure with opened concave part enclosing the CA4 region of the hippocampus proprius. The dentate gyrus is formed of the