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# بسم اللّه الرحمن الرحيم

## مركز الشبكات وتكنولوجيا المعلومات

## قسم التوثيق الإلكتروني

Salwa Akl

# جامعة عين شمس

## التوثيق الإلكتروني والميكروفيلم

## قسم

نقسم باللّه العظيم أن المادة التي تم توثيقها وتسجيلها

علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

# Postnatal development of the amygdala of rat offspring after prenatal exposure to valproic acid: A Histological and Morphometric study

Thesis

Submitted for Partial Fulfillment of Master's Degree
in **Anatomy and Embryology**

By

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***Nahla Mohammed Nagiub***

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# List of Abbreviations

| Abb. | Full term |
|---|---|

*AOB* ..... *Accessory Olfactory Bulbs*

*ABR* ..... *Avidin Biotin reagent*

*AC* ..... *Amygdaloid complex*

*ASD* ..... *Autism Spectrum Disorder*

*ASU* ..... *Ain Shams University*

*ANOVA* ..... *Analysis Of Variance*

*BLC* ..... *Basolateral Complex*

*BNST* ..... *Bed Nucleus of Stria Terminalis*

*CARE* ..... *Committee of Animal Research Ethics*

*CNS* ..... *Central Nervous System*

*DAB* ..... *Diaminobenzidine*

*FMR1* ..... *Fragile X Mental Retardation 1*

*GABA* ..... *Gammma-AminoButryic Acid*

*GABAT* ..... *Gamma-Aminobutyrate AminoTransferase*

*GFAP* ..... *Glial Fibrillary Acidic Protein*

*H & E* ..... *Hematoxylin and Eosin*

*MBP* ..... *Myelin Basic Protein*

*MASRI* ..... *Medical Ain Shams Research institute*

*NGS*.....*Normal Goat Serum*

*NLGN*.....*Neuroligin*

*NuAc*.....*Nucleus Accumbens*

*PAG*.....*Periaqueductal Gray*

*PBS*.....*Phosphate Buffered Saline*

*VPA*.....*Valproic acid*

*VTA*.....*Ventral Tegmental Area*

# INTRODUCTION

The limbic system is a good starting point for studying the relationship between brain and mind. It was first described in the first half of the previous century by Paul Broca **(Ter Horst, 2010)**. The limbic system is composed of a group of tightly interconnected brain areas that include the cingulate gyrus, the anterior thalamus, the hypothalamus (mammillary bodies), the hippocampus, and the amygdala **(Catani et al., 2013).**

The term amygdala is derived from both Greek and Latin languages which means almond **(Scatliff & Clark, 1992)**. The amygdala is composed of a group of nuclei located beneath the uncus of the temporal lobe at the anterior end of the hippocampus and the inferior horn of the lateral ventricle **(Baron-Cohen et al., 2000)**.

The amygdala has always been described to have a major role in emotion regulation, social behavior development, and reward learning **(Adolphs, 2010).** The relation between the amygdala and the development of emotional intelligence suggests that structural abnormalities of the amygdala may play an important role in the development of many autistic behaviors **(Gibbard et al., 2018)**.

Postnatal development of the rat's amygdala is described in two stages. The first stage from one to three weeks of age and is characterized by large increase in the volumes of the amygdala nuclei. The second stage from three weeks of age to young adulthood is characterized by a slow and continuous increase in size of most amygdala nuclei **(Chareyron et al., 2012)**.

Precocious development of amygdala is commonly reported in autism spectrum disorder (ASD) youth, and it has a positive correlation with the degree of severity of ASD symptoms **(Barrett et al., 2017)**.

Prenatal exposure to valproic acid (VPA) leads to an ASD phenotype in both humans and rats and has become a common method to model the complexity of ASD symptoms in the laboratory **(Barrett et al., 2017; Sailer et al., 2019)**.

The dynamic relationship between genetics, environmental factors, and epigenetic mechanisms render vulnerability to potentially numerous neurodevelopmental disorders, such as autism spectrum disorders (ASD) (**Sailer et al., 2019**). Animal models of ASD are many and each represents a suggested risk factor and pathology of ASD. There are models related to genetic factors that are concerned with abnormal genetic

conditions associated with ASD like fragile X mental retardation gene (Fmr1)and neuroligin 3 and 4 genes (NLGN). Environmental factor models in which different environmental risk factors that may contribute to ASD have also been studied in animals. Thalidomide and valproic acid models, in which the mothers who took these drugs during early pregnancy have shown increased incidence of autism in their offspring **(Gadad et al., 2013).**

Moreover, animal studies have shown that prenatal exposure to VPA resulted in disturbed social behavior, fear expression, anxiety-like behavior, with many changes corresponding to ASD-like symptomatology **(Lin et al., 2013; Banerjee et al., 2014; Barrett et al., 2017).** However, the histological characteristics of the amygdala received little attention in these studies, although proper understanding of the structural aberrations is of great utility in the search for pathogenesis and new treatment modalities for ASD.

# AIM OF THE WORK

The aim of the current work was to study the structural and morphometric changes of the amygdala of rat's offspring prenatally exposed to valproic acid and to compare it with the control.

Objectives of the current study were to:

1- Describe the histological changes of the amygdala of rat's offspring aged one, two and three weeks in both normal and after maternal exposure to valproic acid during pregnancy.

2- Evaluate the morphometric parameters of the amygdala of the control & VPA treated rats such as:

   a: Surface area of the amygdala and number of neurons and oligodendrocytes.

   b: Number of astrocytes in GFAP-immune stained sections.

# REVIEW OF LITERATURE

## Limbic system

### Anatomy :

The limbic system is an aggregation of brain structures that lies below the cerebral cortex, above the brainstem and lateral to the thalamus **(Rajmohan & Mohandas, 2007).** The term limbic is a Latin word meaning border and refers to the cortical structures that form a border around the diencephalon and midbrain on the medial surface of the cerebral hemispheres **(white et al., 2007).**

The structures that constitute the limbic system have no universal agreement. They include limbic cortex (cingulate and parahippocampal gyri), hippocampal formation (the dentate gyrus, hippocampus, subicular complex), hypothalamus, amygdala and the septal area **(Rajmohan & Mohandas, 2007).**

Other structures have been added to the description of the limbic system, including; nucleus accumbens, major areas of the prefrontal cortex, habenula, anterior thalamic nuclei, parts of the basal ganglia, ventral tegmental area, and limbic midbrain areas, including the periaqueductal grey **(Standring, 2016)**.

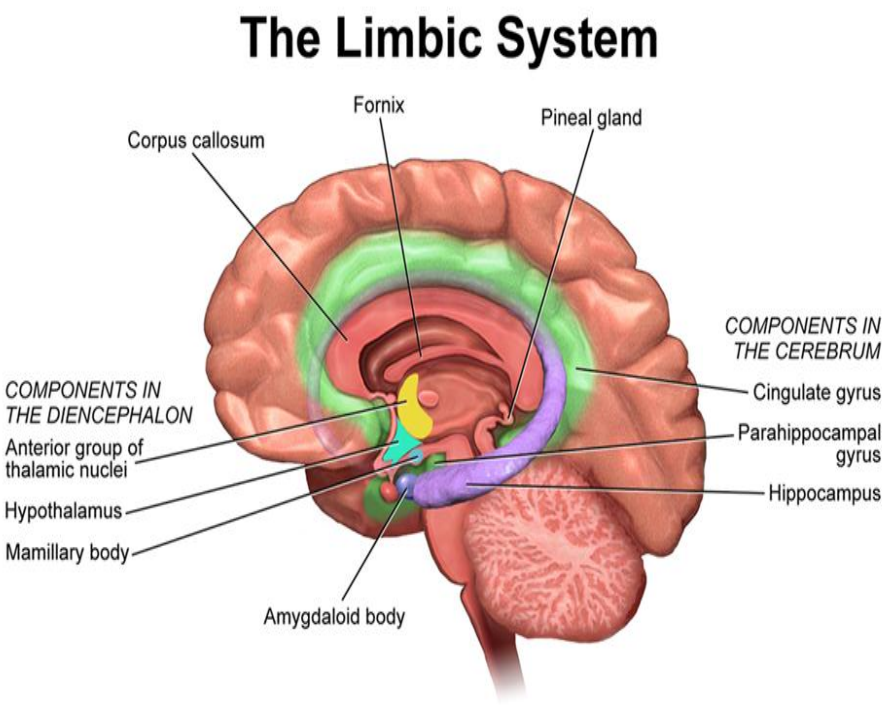


**Figure 1: Components of the limbic system (Suhaimi et al., 2020).**

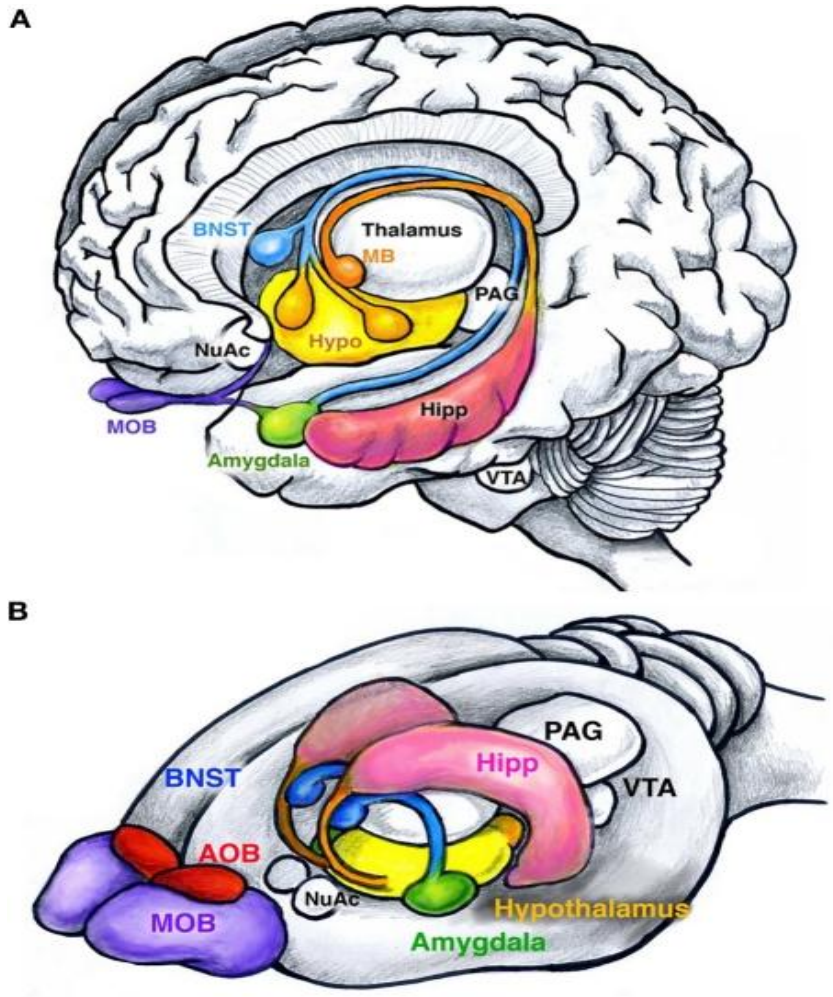


**Figure 2: Main structures of the human and rodent limbic system (A) Human brain showing the amygdala (green), bed nucleus of stria terminalis (BNST, blue), hypothalamus (yellow), and hippocampus (pink). The hippocampus (pink) attaches to the mamillary bodies (orange) through the fimbria-fornix. Olfactory inputs are received by the olfactory bulbs (MOB, purple). Other structures include the nucleus accumbens (NuAc), ventral tegmental area (VTA), and the periaqueductal gray (PAG). (B) Similar structures are found in rodents. Note the enlarged olfactory bulbs compared to humans, and the presence of the accessory olfactory bulbs (AOB, red). Together these structures facilitate the execution and reinforcement of innate behaviors (Sokolowski and Corbin, 2012).**