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Histopathological changes in autism using various histological staining techniques.

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ABSTRACT

Introduction: Autism spectrum disorders (ASDs) are a cluster of related neurodevelopmental disorders characterized by varying degrees of impaired socialization, reduced communication, and limited, repetitive, or stereotyped interests and activities. The lack of a full understanding of the relationship between the brain and the behavior of autistic patients has hindered the development of effective methods for the diagnosis and management of autism. Various animal models have been employed to investigate the core symptoms, possible causes, and potential treatments of autism. Among the several animal models so far assayed, the rat model appears to be an excellent standard experimental system, predominantly because of the ample data already available on the genetics and behavioral phenotyping of various rat strains. Materials and Methods: 6 Wister rats are taken, 4 subjects are induced with autism 2 subjects are control Sacrificed Fixed in 10% formalin processed and staining done. Result and Discussion: In similar studies, several of the genetic models display some or all of the core behavioral features of autism. The animal work has not yet shed light on the mysterious strong male bias found in autism prevalence. Another frontier is represented by intriguing non-human primate models based on immune manipulation and maternal infection. Conclusion: We have observed hair sloughing, black discoloration of ruptured skin, and also coagulation necrosis by observing the epithelial tissues.

Key Words: Autism spectrum disorders (ASDs), neurodevelopmental disorders, autistic patients

INTRODUCTION

Autism spectrum disorders (ASD) are neurodevelopmental disabilities with unknown etiology, which are characterized by two core symptoms: alterations of social communication and restrictive or repetitive behavior. (Baker, Russell Lindsey and Wesibroth, 2013)Despite decades of research the etiology and pathophysiology of disease is still unknown, however, there is a strong interaction between genetic and environmental factors.(Simeon, 2019) One of the intriguing points in autism research is identifying the vulnerable time periods of development deviations without compensatory homeostatic corrections. (Mistry, Rajdev and Mullan, 2016; Simeon, 2019)Notably, brain regions as prefrontal cortex and amygdala continue plastic changes even at postnatal period of development. At this time period brain maturation continues, particularly pruning of synapses and refinement of neuronal connections. (El-Ansary and Al-Ayadhi, 2014)In this context it is important to pay attention to brain development differences in humans and rats. It is estimated that postnatal days 1–10 of rat development corresponds to the 3rd trimester in humans gestation according to the level of brain development (R et al., 2016)The other critical change during the brain maturation is a switch of GABA excitatory effect to inhibitory

A variety of features of autism can be simulated in rodents, including the core behavioral hallmarks of stereotyped and repetitive behaviors, and deficits in social successful interaction and communication. (R et al., 2016)Other behaviors frequently found in autism spectrum disorders (ASD) such as neophobia, enhanced anxiety, abnormal pain sensitivity and eye blink conditioning, disturbed sleep patterns, seizures, and deficits in sensorimotor gating are also present in some of the animal models. (Shibuya and Yamamoto, 1998)Neuropathology and some characteristic neurochemical changes that are frequently seen in autism, as well as alterations in the immune status in the brain and periphery are also found in some of the models. Several known environmental risk factors for autism have been established in rodents, including maternal infection and maternal valproate administration.(Bergroth et al., 1980) Also under investigation are a number of mouse models based on genetic variants associated with autism or on syndromic disorders with autistic features.

With this background this study is aimed to evaluate the various staining techniques and features of autism induced in rat samples

MATERIALS AND METHODS

A total of 4 formalin fixed paraffin embedded tissue blocks of wistar rat samples that were exposed to high fat diet and 2 control rat samples . Four lysine-coated slides were obtained from each block and used for further processing.

Preparation of buffers:

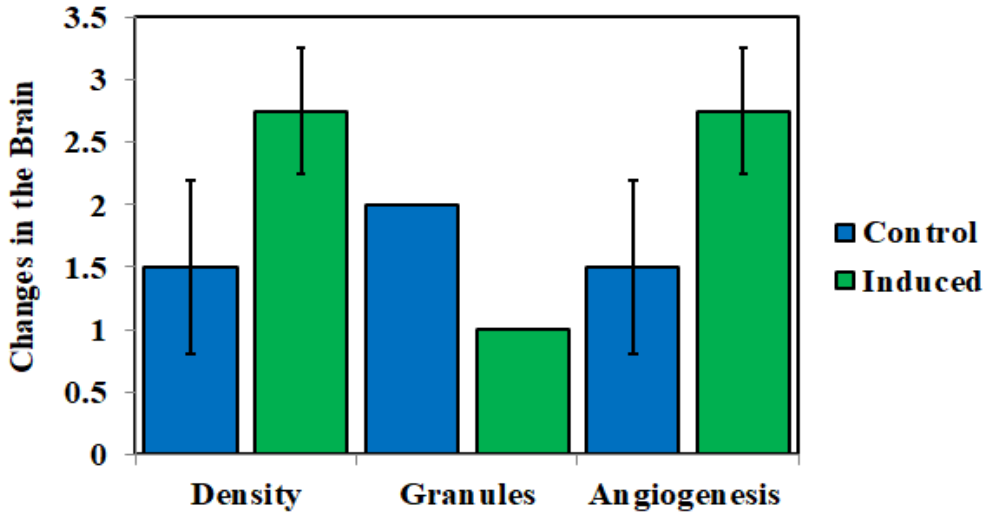
This buffer was prepared by dissolving 8 grams of sodium chloride and 0.6 grams of Tris buffer in 1000 ml of distilled water and the pH was adjusted to 7 by adding sodium chloride. Citrate buffer was prepared by dissolving 1.05 grams of citric acid monohydrate in 500 ml of distilled water and the pH was adjusted to 6.5 by adding diluted hydrochloric acid. The pH of these solutions was adjusted using the pH meter.

Normal Protocol:

Sections of 2.5µmin thickness were cut and mounted on a lysine coated slide after removing from the incubator the slides were kept for deparaffinization by placing in two changes of xylene, each change lasting for ten minutes followed by isopropyl alcohol for 5 minutes. The antigen retrieval was done as follows, the slides were placed in a citrate buffer and kept in a pressure cooker for two whistles. The slides were then cooled and agitated in Tris buffer for two changes of five minutes each, after which the slides were marked with a PAP pen which creates a water repellent barrier that keeps staining reagents localized on the tissue sections. The slides were placed in the humidor after applying the peroxide block for 30 minutes. The slides were again washed in two changes of Tris buffer. After which the slides were covered with the primary antibody, Alpha- SMA, CK and s- 100 for sixty minutes after which the slides were washed with Tris buffer. The secondary antibody was applied for thirty minutes and again washed with Tris buffer. The slides were then covered with a DAB buffer (novolink) in the ratio of 1:20 for a minute. The slides were then washed in Tris buffer and the counterstain was achieved with haematoxylin, the slides were dried and mounted.

RESULTS:

The expression of autism is significantly high



mean std deviation	3.000	1.2500	3.000	1.2500	1.5000	3.000
P significance	.010	.006	.010	.006	.008	.205

DISCUSSION

A recent study reported that long-term (9–12 days) exposure to VPA (100 mg/kg) in utero resulted in an increased number of neocortical neurons in rat pups postnatally . (Yasuzumi and Oura, 1964)Various methods have been suggested to generate ASD-like animal models, including administration of PPA or VPA. Both PPA and VPA have similar effects including inhibition of histone deacetylase, altering carnitine activity and mitochondrial metabolism .(Hirabayashi and Nakagawa, 2010)

In late stages of development (P70) behavioral alterations remained only in prenatally treated rats. This also indicates the severity of the prenatal model in comparison with postnatal one, which exhibits reversible manner of damage. (Banaszewska and Andraszek, 2023) Furthermore, electrophysiological studies confirmed the existence of several disconnections between hippocampus, prefrontal cortex and amygdala . Particularly, an increased frequency of peristimulus spikes were recorded (Mbs, Mhfs, Mps) in all mPFC neurons (both hippocampal and cerebellum HFS) in prenatally VPA treated rats and a contrary trend of low frequency in postnatally VPA treated group versus control. (Idris and Khan, 2012)Thus, our results of increased peristimulus frequency in all populations of tested neurons supported the data reported by Ijima et al and Rinaldi et al. which evidence about epigenetic modifications elicited by VPA results in hyperactivity and hyperplasticity in the PFC.(Idris and Khan, 2012; Jain, Jain and Jain, 2020)

We report extensive histopathological characterization of the brain of the BTBR inbred mouse strain, and describe novel changes in specific markers within key forebrain regions. Specifically, a significant increase in the expression of the oligodendrocyte precursor NG2 in the ACC and marked reductions in the number of neural precursors positive for DCX, PSA-NCAM and NeuroD in the hippocampus were seen. Despite the presence of complete callosal agenesis, surprisingly few changes in the majority of markers were found in most brain regions Given the number of neuronal-, glial-, synaptic- and neurotransmitter-related markers we examined, the modest extent of global changes in neurostructural proteins in response to such a marked perturbation of normal brain development is striking. No evidence of structural or antigenic changes was seen in most brain regions using markers such as MAP2 for neuronal dendrites, Timm staining for mossy fibers, and AchE histochemistry for cholinergic pathways, and no specific changes in the expression of excitatory (VGluT1) or inhibitory (GAD67, GAD65, PVA) markers were seen.

CONCLUSION

In conclusion, previously described studies show that a single prenatal exposure to VPA results in lifelong behavioral impairments similar to core symptoms of autism. In comparison to well-known and widely used embryonic models, postnatal administration of VPA also led to the autistic features in adolescent rats which showed a tendency to normalization in adulthood. We found pronounced structural changes in the brain target regions of prenatally VPA-treated groups, and an absence of abnormalities in postnatally VPA-treated groups, which confirmed the different severity of VPA across different stages of brain development.

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