Original Article

# Relationship Between Histone Modification and Seizures in Rat Brain

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## ABSTRACT

**Objective:** To see the Effect of prigesterone on GABA transporter-1 & Glutamate transporter-2 expression in the cerebral cortex of developing rats after recurrent seizures.

**Study Design:** Quasi Experimental study.

**Place and Duration of Study:** This study was carried out in Experimental laboratory at Pediatrics Department, Xiangya 2nd Hospital, Central South University Changsha Hunan P.R China, from September 2011 to April 2011.

**Materials and Methods:** This experiment included 90 PN-7d the SD rats, Flurothyl to cause convulsions in group and progesterone in the intervention group, one died, died of status epilepticus, a mortality rate of 2.2% in each group were randomly selected eight test SD rats (72) for the analysis of experimental results. Flurothyl induced seizures stopped after the group of SD rats had no spontaneous seizures is the emergence of limb movement disorder and abnormal reactions.

**Results:** The cerebral cortex GAT-1 immunohistochemistry AOD changes in the control group, PN-13d (ARS-1d), the PN-15d (ARS-3d) and PN-19d Service (ARS-7d), the convulsions rats cerebral cortex GAT-1 immunohistochemistry AOD expression than the control group was significantly higher ($P<0.01$); progesterone in the intervention group, the GAT-1 immunohistochemistry AOD than the convulsions were significantly lower ($P<0.01$) difference between the groups is not significant. The cerebral cortex GAT-1 protein expression was significantly higher and progesterone in the intervention group ($P<0.01$); progesterone at each time point after the intervention of the GAT-1 expression levels than the control group was significantly increased statistically significant ($P <0.01$). Changes of expression of the GLT-1 protein in the rat cerebral cortex in the control group, the PN-13d, PN-15d and PN 19d cerebral cortex of the GLT-1 protein expression was no significant difference ($F = 1.852$, $P= 0.182$).

**Conclusion:** In conclusion , Progesterone through its participation in regulation of neonatal recurrent seizures caused by an imbalance in the brain cerebral cortex, GAT-1, GLT-1 expresion, the maintance of the central nervous system excitation-inhibition of the balance of the system, which play an anticonvulsant effect.

**Key Words:** Histone modification**,** Seizures, Traumatic Brain injury, Sodium Valporate

## INTRODUCTION

Seizure is an uncontrolled electrical activity in the brain, which may produce a physical convulsion, minor physical signs, thought disturbances, or a combination of symptoms. The type of symptoms and seizures depend on where the abnormal electrical activity takes place in the brain, what its cause is, and such factors as the patient's age and general state of health.

The annual incidence for all types of seizures is 1.2 per 1,000 and, for recurrent seizures, is 0.54 per 1,000. Isolated seizures may occur in up to 10% of the general population. Approximately 10–20% of all patients have intractable epilepsy, a disorder characterized by recurrent seizures, is the second most common neurological disorder, affecting more than 50 million people worldwide.[1] Population studies show that seizure incidence is highest in the first month of life,[2] The incidence is highest among young children and the elderly. High-risk groups include persons with a previous history of brain injury or lesions.

Seizure disorders and their classification date back to the earliest medical literature accounts in history. In 1964, the Commission on Classification and Terminology of the International League Against Epilepsy (ILAE) devised the first official classification of seizures, which was revised again in 1981. This classification is accepted worldwide and is based on electroencephalographic (EEG) studies. Based on this system, seizures can be classified as either focal or generalized. Each of these categories can also be further subdivided. A focal (partial) seizure and a generalized seizure. Simple partial seizures can be caused by congenital abnormalities (abnormalities present at birth), tumor growths, head trauma, stroke, and

infections in the brain or nearby structures. Generalized tonic-clonic seizures are associated with drug and alcohol abuse, and low levels of blood glucose (blood sugar) and sodium [3].

## MATERIALS AND METHODS

This experiment included 90 PN-7d the SD rats, Flurothyl to cause convulsions in group and progesterone in the intervention group, one died, died of status epilepticus, a mortality rate of 2.2% in each group were randomly selected eight test SD rats (72) for the analysis of experimental results. Flurothyl induced seizures stopped after the group of SD rats had no spontaneous seizures is the emergence of limb movement disorder and abnormal reactions.

## RESULTS

The cerebral cortex GAT-1 immunohistochemistry AOD changes in the control group, mainly distributed in the cell membranes of rat cerebral cortical neurons in the PN-13d, the PN-15d and PN 19d, the control group between the GAT-1 immunohistochemistry AOD values were significant differences (F= 0.218, *P*=0.806). PN-13d (ARS-1d), the PN-15d (ARS-3d) and PN-19d Service (ARS-7d), the convulsions rats cerebral cortex GAT-1 immunohistochemistry AOD expression than the control group was significantly higher (*P*<0.01); progesterone in the intervention group, the GAT-1 immunohistochemistry AOD than the convulsions were significantly lower (*P*<0.01) difference between the groups is not significant (Table 1,Figure 1)

Table No.1: The Cerebral Cortex GAT-1 immunohistochemistry AOD changes ( $\bar{x} \pm s$ ) (n = 8)

| | ARS-1d | ARS-3d | ARS-7d |
|---|---|---|---|
| Control group | 1733±85 | 1763±99 | 1751±86 |
| Seizure group | 2038±76[a] | 2055±92[a] | 2067±105[a] |
| Progesterone intervention group | 1854±84[a,b] | 1869±103[a,b] | 1853±94[a,b] |
| F-value | 28.260 | 18.278 | 22.953 |
| *P*-value | <0.001 | <0.001 | <0.001 |

a *P* <0.05 vs. the control group,; b *P* <0.05 vs. the seizure group

The cerebral cortex GAT-1 protein expression was observed in control group, the PN-13d, PN-15d and PN-19d rat cerebral cortex GAT-1 expression was significant difference; seizure group rats of ARS-1d (PN-13d), ARS-3d (PN-15d ) and ARS-7d (PN-19d), the GAT-1 protein expression was significantly higher and progesterone in the intervention group (*P*<0.01); progesterone at each time point after the intervention of the GAT-1 expression levels than the control group was significantly increased statistically significant (*P* <0.01). (Table 2, Figure 2).

Table No.2: The Cerebral Cortex GAT-1 protein expression ( $\bar{x} \pm s$ ) (n = 8)

| | ARS-1d | ARS-3d | ARS-7d |
|---|---|---|---|
| Control group | 0.344±0.006 | 0.340±0.010 | 0.346±0.010 |
| Seizure group | 0.553±0.014[a] | 0.569±0.010[a] | 0.567±0.010[a] |
| Progesterone intervention group | 0.466±0.012[a,b] | 0.439±0.01[a,b] | 0.464±0.016[a,b] |
| F-value | 679.102 | 1038.766 | 492.971 |
| *P*-value | <0.001 | <0.001 | <0.001 |

a *P* <0.05 vs. the control group,; b *P* <0.05 vs. the seizure group

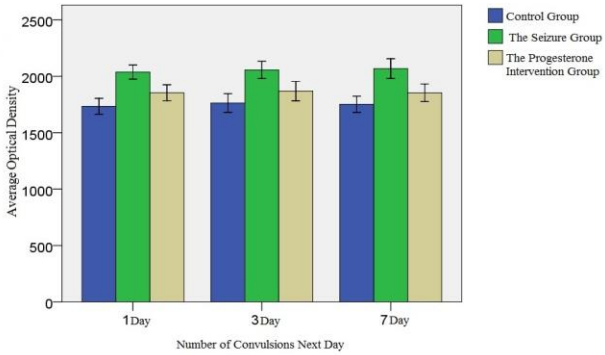


Figure No.1: the cerebral cortex GLT-1 immunohistochemistry AOD changes (n = 8)

## DISCUSSION

Traumatic brain injury is a serious and complex injury that occurs in approximately 1.4 million people each year in the United States. [4] TBI is associated with a broad spectrum of symptoms and disabilities, including a risk factor for developing neurodegenerative disorders, such as Alzheimer's disease. [5-7]. The inflammatory cascade is characterized by proinflammatory cytokines [9, 10] which can exacerbate other pathologies. Although the role of inflammation in experimental TBI is well established, no truly efficacious and approved anti-nflammatory therapies are currently available for the treatment of traumatic brain injury. Under normal conditions microglia are in a resting state, characterized by a small cell body with fine, ramified processes and low expression of surface antigens. CNS injury triggers rapid changes in the morphology and function of microglia. Hypertrophic microglia with thickened and slightly shorter processes are in a reactive state which suggests a largely passive response to injury. In contrast, activated microglia and phagocytic microglia (or macrophages) have a more aggressive role which,

depending upon the circumstances; can produce deleterious effects to the CNS through secretion of various inflammatory molecules. [14, 15] Residential microglia activation and infiltration of macrophages from the peripheral blood is well known to contribute to post-injury inflammation after TBI. [16,17] Such secondary injuries often occur following the initial TBI insult and contribute to further brain pathology and neurological impairment. [18,19] Changes in epigenetic gene expression influence normal neuroplasticity, learning, and memory [20], the signaling events mediating such changes are unknown. Epigenetics has been defined as "the study of the processes that mediate metastable and somatically heritable states of gene expression without altering the DNA sequence". [24]

After post injury survival, rats were perfused with 4% paraformaldehyde and sections of the mid-dorsal hippocampus were stained with hematoxylin and eosin (H&E) for neuronal density or reacted with antibodies for immunohistochemistry. Immunohistochemical staining was performed by an avidin–biotin–peroxidase method (ABC) using an acetyl–histone H3 (lys 9) antibody (40 µg/ml, New England Biolabs), a histone H3 antibody (100 µg/ml, Upstate Biotechnology), and a dimethyl (lys 9 and lys 4) histone H3 antibody (20 µg/ml, Upstate Biotechnology). Regional relative optical density was used for statistical analysis (one-way ANOVA analysis followed by a Bonferroni post hoc analysis). Antibody specificity was examined in naive rats using Western blot analyses (1 µg/ml of each antibody). The ipsilateral hippocampal CA3 and CA1 sectors are selectively vulnerable to TBI [25], in their study, they have confirmed that acute seizure, in this case ECS, increases H4 acetylation selectively at the BDNF P2 promoter. Interestingly, such chromatin remodeling appears to shift toward the P3 and P4 promoters under chronic ECS conditions. It is noteworthy that whereas only BDNF P4 mRNA levels were significantly increased 24 hr after chronic ECS, H3 acetylation was induced at both the P4 and P3 promoters. This increase at P3, in the absence of increased BDNF P3 mRNA levels, could be explained by the fact that the P3 and P4 promoters are only 0.8 kb apart in the primary BDNF transcript [30], thus permitting some of the histone enrichments at P4 to also be detected at the P3 promoter. H3 acetylation at Lys9 and Lys14, similar to H4 acetylation, is found in transcriptionally active promoters [31]. Here, they similarly showed that acute ECS treatment increases H3 phosphoacetylation; however, this change was delayed compared with the induction of H4 acetylation and c-fos mRNA levels. Chromatin remodeling is normally described as a dynamic process induced by transient histone modifications. However, they observed several chromatin modifications that were changed in chronic ECS conditions and persisted 24 hr after the last seizure. Thus, it is likely that adaptations in chromatin structure exert not only short-term, transient effects, but also longer-term effects on gene activity. Specifically, the down regulation of H4 acetylation at the c-fos and CREB promoters, and the up regulation of H3 acetylation at the BDNF P3 and P4 promoters, provide proximal mechanisms by which chronic ECS might alter the expression of these three genes in the hippocampus. Such changes may well play an important role in modulating neuroplasticity in the adult brain. Indeed, the sustained activation of BDNF expression after chronic ECS, which we hypothesize may be mediated in part via H3 acetylation at the P3 and P4 promoters, could contribute to the antidepressant effects of ECS [21]. May be mediated in part via reduced H4 acetylation at the promoter of the gene. Histone acetyltransferases (HATs; enzymes that increase histone acetylation), or proteins that regulate these enzymes. It is generally believed that HDACs and HATs are controlled mainly at the level of their recruitment to target promoters, but some evidence suggests that at least CREB-binding protein, a type of HAT, may be regulated directly through Ca2+ signaling. [22]

Valproic acid (VPA) is a widely used preventive treatment for seizures and bipolar disorder [8], Using a rodent model of TBI, we examined if post-injury VPA administration is neuroprotective, and improves motor and cognitive outcomes. Our preclinical findings show that post-injury systemic administration of VPA: 1) reduced cortical contusion volume, 2) improved blood-brain barrier integrity (BBB), 3) reduced hippocampal MAP2 disruptions, and 4) improved motor and cognitive function. Valproate [2-propylpentanoic acid] (VPA) is a simple branched- chain fatty acid with well established efficacy for seizures. [33] It is also commonly prescribed for bipolar disorder, acute mania and migraines. [34] The therapeutic concentration of valproate is 40–100 mg/ml, and it has a serum half-life of 8–17 hours in adults. This therapeutic concentration is achieved by a loading dose followed by maintenance doses. In rodents, an i.p. dose gives rise to a peak serum post injection concentration that rapidly decreases post-infusion. [35]. Consistent with this, Shein et al., have shown that acute treatment of mice following TBI with the HDAC inhibitor ITF2357 reduces contusion volume and improves motor function. [36] Further, Lyeth and colleagues have shown that DMA-PB (a novel HDAC inhibitor) attenuates the TBI-associated decrease in histone acetylation and reduces microglia-mediated inflammation. [32] However, post-injury treatment with SAHA did not improve motor or cognitive function, suggesting that the HDAC-inhibiting activity of VPA is not sufficient to improve behavioral outcome following TBI. we have recently reported that post-injury treatment with sodium butyrate, a non-selective HDAC inhibitor, also failed to improve cognitive function when administered acutely following TBI. [12] We

have previously shown that the activation of ERK following TBI is neuroprotective, and its inhibition exacerbates TBI-associated motor and cognitive deficits. [11] Although our western blots did not reveal any significant increase in ERK phosphorylation in response to VPA injection, its activation at an earlier/later time point than that examined here (45 min post-injection) cannot be ruled out. Lastly, while not specifically examined in the current study, valproate has also been shown to enhance GABAergic neurotransmission. Previous studies have shown that administra- tion of a GABA agonist acutely following injury can be used to reduce hyperactivity and improve neurological outcome. [27, 28] Consistent with these benefits, food restriction has been shown to be associated with a recovery of spatial memory following global ischemia. [23], one potential limitation of valproate therapy to improve cognitive function is that it appears to only be effective post- injury. Further experiments will be required to establish the exact time window, the best routes of administration, and if this improvement can be observed following different injury magnitudes. [26]

While GluR-mediated excitation is enhanced in the immature brain, receptors and the synthetic enzyme (glutamic acid decarboxylase) for the major inhibitory neurotransmitter γ-aminobutyric acid (GABA) do not achieve maximal expression levels until the fourth postnatal week in rats. Furthermore, whereas GABA is inhibitory and hyperpolarizing in the mature brain, this neurotransmitter can be excitatory and depolarizing in the immature brain. GABA release can activate GABAA receptors (GABAARS), which are ligand-gated chloride channels. Studies have demonstrated that the NKCC1 inhibitor bumetanide can block seizures in a rodent model of neonatal seizures, and this agent might have potential in the clinic as an antiepileptogenic therapy. [13]

# CONCLUSION

Our review concludes that the importance of better understanding the role of these enzymes and associated proteins and the signaling pathways that regulate them, not only in activity-dependent transcription but also in models relevant for chronic adaptation in the brain is required. Even though many researches were done regarding histone modification in seizures in experimental animals especially in rats ,the results are encouraging but still research are not enough to implement in humans. Thus still further researches on mammals and human itself is required then only its positive implementation can be done in human beings.

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