Astrocyte Connexin43 Localization is Altered by Potential Therapeutic Compound in a Mouse Model of Autism

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Background: Connexin 43 (Cx43), an astrocyte gap junction protein, regulates neuronal activity by facilitating the reuptake of potassium (Figure 1).[1] Cx43 has been implicated in autism spectrum disorder (ASD) and conditions of neuroinflammation, such as epilepsy and stroke, where reduced expression of Cx43 worsens outcome.[2,3] To better understand the pharmacological profile of a novel drug for ASD, we analyzed the expression of multiple glial proteins including Cx43 and GFAP in CNTNAP2 KO mice.

Materials and Methods: Our sample consisted of 9 CNTNAP2 KO mice that were 6 months old. Osmotic pumps delivered Drug X to 4 mice and saline to 5 mice over the course of 4 weeks**.** The mice were sacrificed and all brains underwent a standard formaldehyde fixation and immunostaining procedure for GFAP and Cx43 markers. A Nikon confocal microscope captured the following layers of the hippocampus seen below (Figures 2 and 3): (A) dentate gyrus, (B) molecular layer, (C) stratum lacunosum-moleculare, and (D) stratum radiatum. A freehand selection of each layer established the area of pixel luminescence analysis in ImageJ software. Both the image acquisition and data analysis were done in a manner blind to the treatment. Unpaired t-tests were used to analyze the data.

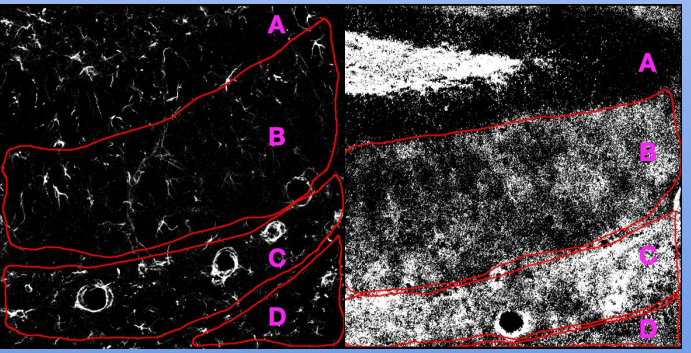


Figure 2: GFAP staining

Figure 3: Cx43 staining

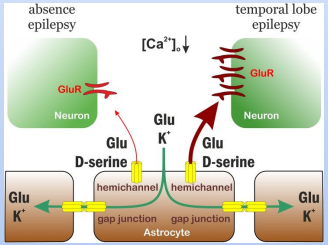


Figure 1[1]: Astrocyte Cx43 gap junctions promote an intracellular flow and spatial distribution of potassium and glutamate.

Vincze, Peter, Szabo et al. Connexin 43 Differentially Regulates Epileptiform Activity in Models of Convulsive and Non-convulsive Epilepsies. *Frontiers in Cellular Neuroscience*. 2019;13:173

Results: No significant difference in GFAP quantity was observed between the drug and control groups (p>0.1). In contrast, Cx43 is significantly increased in the drug-treated mice when compared to the control (p<0.05) (Figures 4 and 5).

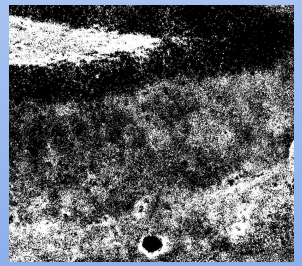


Figure 4: Cx43 expression in control group Mouse 11

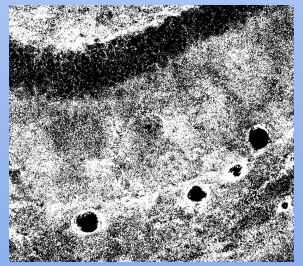
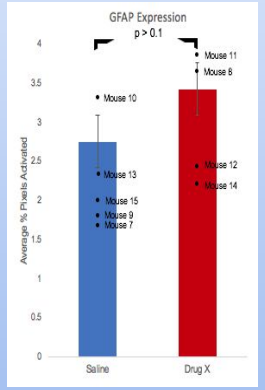
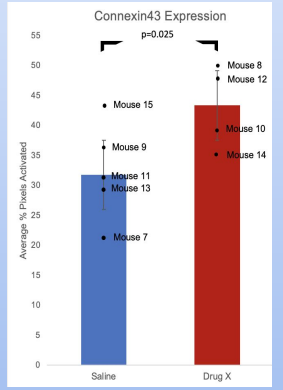


Figure 5: Cx43 expression in treatment group Mouse 12

Discussion: Drug X increases Cx43 gap junction localization in astrocytes which may improve the clearance of synaptic glutamate. This effect could reduce circuit hyperexcitability ultimately providing a neuroprotective effect in conditions such as ASD and epilepsy, where insufficient inhibitory input causes pathological activation and excitotoxicity.[3]





Conclusion: Mice treated with Drug X demonstrated a statistically significant increase in Cx43, potentially leading to improved synaptic clearance of potassium and glutamate. This mechanism represents potential for treatment of epilepsy in ASD by reducing widespread neuronal circuit hyperactivity. Further studies with larger sample sizes, and mice treated at younger ages will help to further elucidate the therapeutic potential of Drug X.

References:

1.Vincze, Peter, Szabo et al. Connexin 43 Differentially Regulates Epileptiform Activity in Models of Convulsive and Non-convulsive Epilepsies. *Frontiers in Cellular Neuroscience*. 2019;13:173

2. Nakase, Fushiki, Naus. Astrocytic Gap Junctions Composed of Connexin 43 Reduce Apoptotic Neuronal Damage in Cerebral Ischemia. *Stroke*. 2003;34:1987-1993

3. Ozog, Siushansian, Naus. Blocked Gap Junctional Coupling Increases Glutamate-Induced Neurotoxicity in Neuron-Astrocyte Co-Cultures. 2002;61(2):132-141