An electron microscopic investigation of the central oxytocin system the CNTNAP2 mouse model of autism

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Oxytocin (OXT) is a peptide hormone synthesized by neurons in the supraoptic and paraventricular (PVN) nuclei of the hypothalamus with projections to several brain areas. Several studies highlight the pivotal role of OXT in the regulation of social behavior. There is a growing body of evidence for OXT's involvement in neuropsychiatric disorders that result in social deficits such as in autism spectrum disorders (ASD). The loss of the brain-wide distributed *contactin associated protein-like 2* (CNTNAP2) is implicated in a syndromic form of ASD. Since facilitating neuron-glia interaction is a major function of the transmembrane protein CNTNAP2, we reasoned that a potential alteration in the structural relationship between OXT neurons and their neighboring astrocytes could serve as a neuroanatomical correlate of the underlying deficits in social behavior previously reported in the Cntnap2 knockout (KO) mouse. To test this hypothesis, we performed a quantitative ultrastructural analysis focusing on the OXT neurons and astrocytes of the hypothalamic PVN. We used the parameter of astrocyte end-foot coverage of OXT neuron cell body to quantify neuron-glial interaction, finding significant differences in glial endfoot coverage of specifically immunolabeled OXT neurons in Cntnap2 KO mice as compared to WT. The marked structural difference in astrocyte endfeet – OXT neuron interactions in the KO PVN implicates a potential dysregulation in the synaptic and glial input to these neurons that may underlie the observed social deficits present in these mice.

Introduction

Social behavioral deficit is the most characteristic and therapeutically significant component of ASD. Recessive truncating mutations in the CNTNAP2 gene cause a syndromic form of ASD in humans. Cntnap2 KO mice recapitulate core ASD deficits. However, the anatomical effects of this mutation at the cellular and ultrastructural level remain elusive. In light of recent advances in OXT therapy for ASD, we used quantitative electron microscopy to measure glial coverage of oxytocinergic cells of the paraventricular hypothalamic nucleus (PVN) of Cntnap2 KO mice.

Materials and Methods

C57BL/6J mice from WT and CNTNAP2 KO groups (n=2, respectively) were processed. Animals were deeply anesthetized with isoflurane, then perfused transcardially with a mixture of 2% paraformaldehyde (PFA) and 2% glutaraldehyde in 0.1 M phosphate buffer (PB, pH 7.4). After, the brains were removed and processed to ultrathin sections according to Marcello et al. (2018) [1]. Free-floating sections where immersed in 15-30% sucrose solution for at least 12 hours after which the sections were cooled in liquid nitrogen to carefully loosen the cell membranes of the brain tissue for better penetration of the OXT antibody. Monoclonal OXT antibody (PS series 38, highly specific

against mammalian neurophysin) was used at a 1/100 concentration. Pre-embedding immunohistochemistry was performed according to Racz and Weinberg (2006) [2]. Image acquisition was performed by the help of a transmission electron microscope (JEOL, Tokyo, Japan) equipped with a Mega-View-III digital camera and a Soft Imaging System (SIS, Münster, Germany). Glial coverage ratios were calculated from electron micrographs for n=16 WT and n=14 KO OXT neurons. Student t-test and Tukey's test was performed, $p < 0.05$ was considered statistically significant for both.

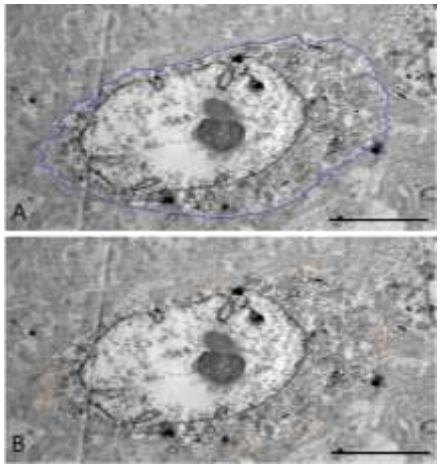

Figure 1. Electron Micrographs showing measurement of glial coverage ratio. A. OXT neuron of PVH with soma perimeter traced in blue, Scale bar: 5 μm. B. Same neuron as in A with glial end-feet traced in orange covering neuronal somatic membrane, Scale bar: 5 μm.

Results

Glial coverage ratio of WT OXT neurons as compared to CNTNAP2 KO in the PVN are WT = 0.5338 +/-0.037, KO = 0.3862 +/-0.04, $p < 0.05$ according to Students-t test and Tukey's test. CNTNAP2 KO OXT neurons have a significantly lower perikaryal glial coverage as compared to WT.

Conclusion

Mice lacking CNTNAP2 exhibit a significant difference in the glial regulation of their central OXT system. A decrease in glial end-feet coverage of OXT neurons in the PVN: raise mechanistic questions of how more surface for possible excitatory or inhibitory input, more intermittent exposure to the surrounding electrochemical milieu, and less glial-neuron interaction may influence the resultant social behavioral phenotype of these mice.

References

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