Characterizing Differences in the Superior Colliculus of Genetic Mouse Models for Autism

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Abstract

Genetic factors have been found to contribute to Autism Spectrum Disorder (ASD), a developmental disability characterized by sensory and communication deficits, but current treatment only involves therapy and antipsychotic medications. Finding a model of gene mutations associated with ASD can assist in the development of novel forms of treatment for patients diagnosed with these genetic mutations. Down Syndrome Cell Adhesion Molecule Like-1 (DSCAML1) has been previously studied in mice to have significant effects on developing mouse retinas, but these studies have not extended into other regions of the brain where DSCAML1 is expressed. Initial analyses using expression staining on loss of function DSCAML1 mice has suggested that the superior colliculus, which is involved with sensory processing of visual information, shows variation in expression compared to the wild type. Subsequent analyses evaluated the structure and cell density of the superior colliculus to determine if organization has been altered. Comparison of the superior colliculus and more regions of the brain that are affected between these mutant mice and people with *Dscaml1* mutations may allow for better understanding of DSCAML1's impact on brain function and development to determine if mice will make a good model to test clinical interventions for ASD treatment.

Background

Autism Spectrum Disorder and *Dscam*

Symptoms of ASD develop during infancy and early childhood, when numerous synapses are forming in the brain (Ye et al, 2011). *Dscam* has been found to be mutated in genetic screens of people with ASD. DSCAM functions in synaptic development (Figure 1). Retinal expression of DSCAM has been found to be positively correlated to developmental cell death, and mutations in mice lead to defects in cell spacing and synapse formation (Fuerst et al, 2008).

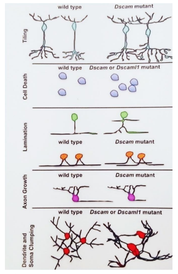


Figure 1. *Dscam/Dscaml1* mutational effects on cellular morphology and synapse formation.

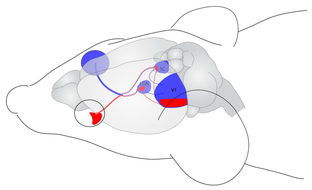
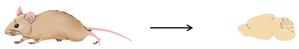


Figure 2. Diagram of the output circuitry from the eyes of an adult mouse. Image provided from Wilks et al, 2013.

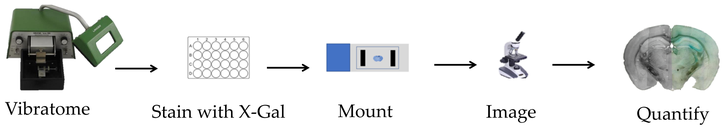
Superior Colliculus

The superior colliculus is important in integrating visual information from the retina and cortex (Wilks et al, 2013). The structure receives information to help formulate a map of the visual field (White et al, 2017). Control of eye movements has long been understood as a mechanism of the superior colliculus and helps for the behavioral localization and focus of the eyes on a particular position.

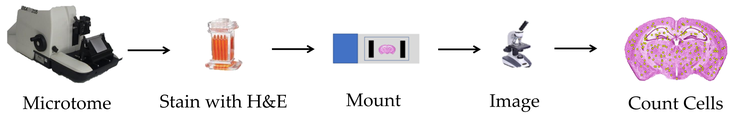
Materials and Methods



X-Gal Staining



Hematoxylin & Eosin Staining



Results

X-Gal

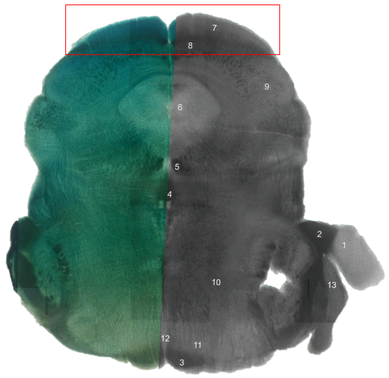


Figure 3. Composite image a coronal section of a loss of function mutant. Intensity in each individual tissue slice was measured at each numbered point.

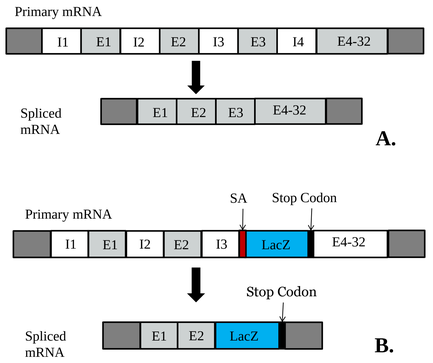


Figure 4. *Dscaml1* LOF mutation involves insertion of LacZ. A. A wild type *Dscaml1* gene does not involve insertion of LacZ. **B.** LacZ interrupts the third intron of the *Dscaml1* gene when a mutation occurs.

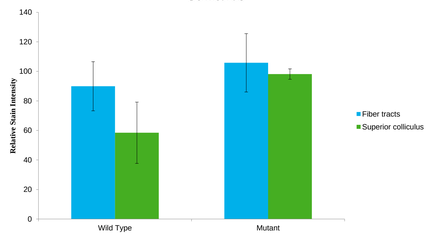


Figure 5. Data analysis of X-gal staining intensity of wild type and mutant fiber tracts and superior colliculus. Two sample t-tests confirmed the superior colliculus was not significantly different than fiber tracts and that the two sets of fiber tracts were significantly different.

Hematoxylin & Eosin

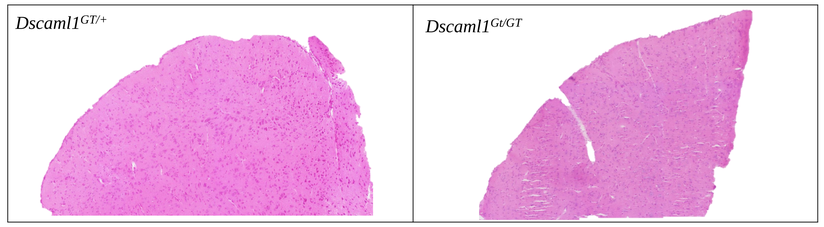


Figure 6. H&E stained wild type and mutant superior colliculus.

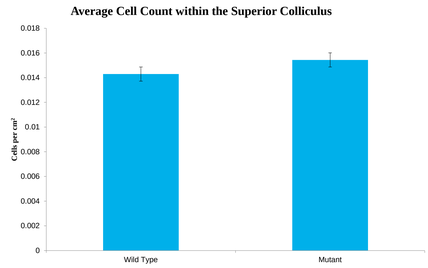


Figure 7. Cell density in the super colliculus of wild type and mutant mice. The p value was found to be 0.3180.

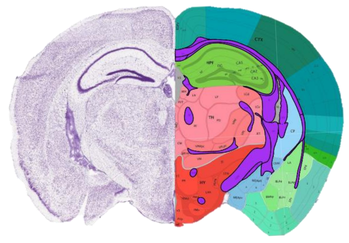
Conclusions

- X-Gal staining is able to target areas of the brain where *Dscaml1* is expressed, and optimal cell body staining can be achieved with altered staining protocols.
- Intensity values show that the fiber tracts consistently have the highest expression levels, and the superior colliculus exhibits *Dscaml1* expression at these levels.
- Mutant staining intensity of the superior colliculus was significantly greater than wild type intensity, suggesting higher expression in mutant brains.
- In previous studies, H&E stained phenotypes demonstrated that loss of function DSCAM-L1 mutants have higher cell densities than the wild type counterparts.
- Observational evidence suggests cells of the superior colliculus to be less organized and more cluttered in loss of function mutants.

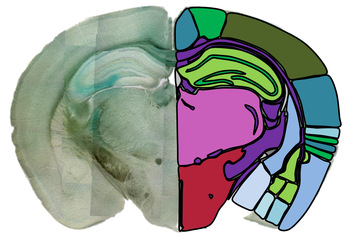
Future Work

- Continue cell counts with both wild type and loss of function mice and perform statistical analysis to quantify structural differences.
- Use spatial analysis to determine if cellular organization is significantly different in mutants.
- Identify other target regions in the brain by further analysis of X-Gal staining, such as the lateral geniculate nucleus.
- Compare differences seen in mutant mouse brains with those from MRIs of human subjects.
- Work with the Computer Science team at LCSC to develop an atlas from the brain sections in both X-Gal and H&E.

Allen Brain Atlas



Annotated X-Gal Section



References

Fuerst, P. G., Koizumi, A., Masland, R. H., & Burgess, R. W. (2008). Neurite arborization and mosaic spacing in the mouse retina require DSCAM. Nature, 451(7177), 470–474. http://doi.org/10.1038/nature06514

Ye H, Liu J, Wu J, Y. (2011) Cell Adhesion Molecules and Their Involvement in Autism Spectrum Disorder. Neurosignals;18(2), 62-71 https://doi.org/10.1159/000322543

Wilks, T. A., Harvey, A. R., Rodger, J. (2013). Seeing with Two Eyes: Integration of Binocular Retinal Projections in the Brain. IntechOpen, DOI: 10.5772/56491.

White, B. J., Kan, J. Y., Levy, R., Itti, L., Munoz, D. P. (2017). Superior colliculus encodes visual saliency before the primary visual cortex. Proceedings of the National Acadamy of Sciences of the United States of America, 114 (35) 9451-9456. https://doi.org/10.1073/pnas.1701003114

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