Mapping the Connectivity of Oxytocin Receptor Neurons in the Dorsal Tenia Tecta of Mice

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The Dorsal Tenia Tecta (DTT)

- The dorsal tenia tecta (DTT) is an understudied region of the primary olfactory cortex.
- Prior work has demonstrated a link between DTT signaling and stress in rats[4].
- Preliminary analyses from our laboratory have indicated that this enigmatic brain region is interconnected with neural regions involved in stress, memory, and olfactory processing.
- Behavioral studies from our lab paired with microendoscope imaging have shown that the DTT responds to monoamine odor detection in a bidirectional fashion, eliciting both inhibitory and excitatory neural responses.

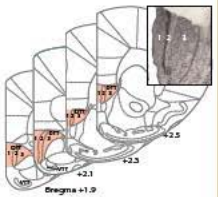

Figure 1: Coronal sections showing the location of the DTT and ventral tenia tecta (VTT). Figure from Paxinos and Franklin (2019) and LaPlante (2013).

Oxytocin Receptor (OXTR) in the DTT

- Oxytocin (OXT) is a neuropeptide involved in social behavior including social bonding, maternal behavior, aggression, and anxiety[3].
- OXT receptors (OXTRs) are present across a variety of brain regions involved in stress and social behavior. However, the highest binding density of an OXTR-specific ligand has been found in olfactory brain regions across developmental ages[5].
- The role of OXTR in the processing of odor stimuli remains to be fully understood. However, previous work in our lab has found that it is not necessary for baseline non-social odor processing[6], implying a more nuanced social-behavioral role for OXTR signaling in olfactory processing.
- To further uncover the role of the oxytocin system in olfactory processing, we have decided to investigate the neuronal connectivity and activity of the DTT in OXTR-expressing neurons.

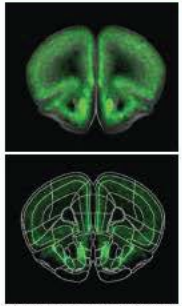

Figure 2: Averaged OXTR density at P56 (Green) overlaid in an age matched reference brain. Figure from Newmaster et al., 2020[9].

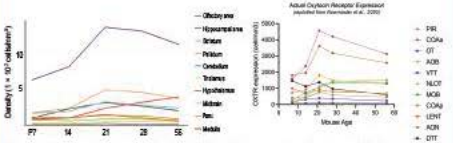

Figure 3: Average OXT neuron density within 10 different major subregions of the brain[9].

Research Goal

Identify the connectivity of the OXTR-expressing neurons in the dorsal tenia tecta (DTT) to further uncover the role of OXTR in olfactory processing, and develop a greater understaning of the function of the DTT.

Injection of AAV Viral Tracer into the DTT of mice

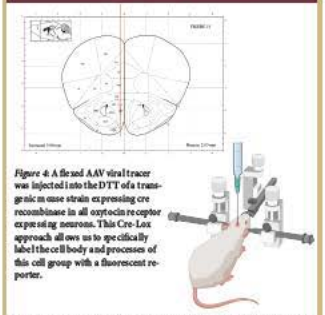

Figure 4: A flexed AAV viral tracer was injected into the DTT of a transgenic mouse strain expressing cre recombinase in all oxytocin receptor expressing neurons. This Cre-Lox approach allows us to specifically label the cell body and processes of this cell group with a fluorescent reporter.

Figure 5: Using a stereotaxic approach, the AAV viral tracer injection was injected into the DTT causing fluorescence in OXTR expressing neurons.

Transcardial Perfusion and Neural Tissue Collection

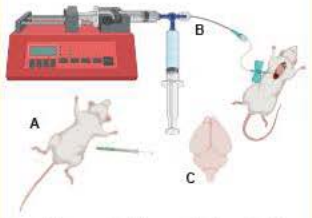

Figure 6: After surgery, mice (N=1) recovered for three weeks to allow for optimal expression of the tracer. (A) Mice were then injected with a Somnasol Euthanasia solution, (B) then were transcardially perfused via a syringe pump. (C) The brain was removed and post-fixed overnight. Brains were consequently rinsed in PBS and cryoprotected in preparation for embedding. The tissue was embedded in Tissue Tek optimal cutting temperature (OCT) compound then stored in an -80° C freezer prior to cryostat microsectioning.

Cryostat Sectioning

Figure 7: Brains were serially sectioned on a cryostat in 20 µm coronal sections. The resulting slides were stored in the -80° C freezer prior to microscope imaging.

Imaging

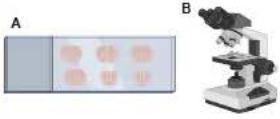

Figure 8: (A) Slides were removed from the -80° C freezer, thawed, counterstained with a fluorescent Nissl Stain (Neurotrace). (B) Slides were imaged using an epifluorescent Zeiss microscope. Using the 10x and 20x objective lenses, images of each section were taken using three different color channels.

ABBA and QuPath Image Processing

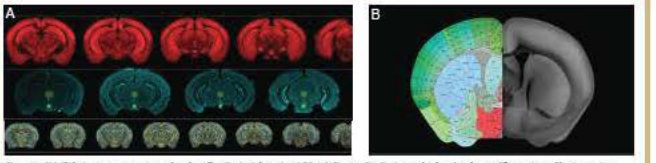

Figure 9: (A) Slide images were registered to the Allen Brain Atlas using ABBA (Aligning Big Brains and Atlases) software. The registered brain sections were imported into QuPath, an imaging software used to analyze the presence and density of fibers in each brain region. (B) Once slide images were aligned in ABBA and processed in QuPath, the exact brain region containing expressing specific fluorescence from the AAV viral tracer was able to be identified (Image from Yongsoo Kim lab, Penn State University).

Preliminary Results

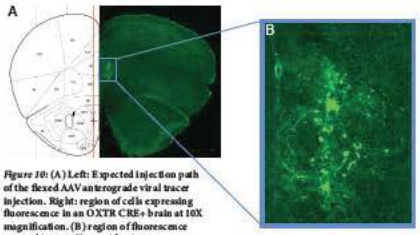

Figure 10: (A) Left: Expected injection path of the flexed AAV anterograde viral tracer injection. Right: region of cells expressing fluorescence in an OXTR CRE+ brain at 10X magnification. (B) region of fluorescence zoomed in to 20X magnification.

- Fluorescence was expressed in a CRE-dependent manner in the neural tissue of the OXTR CRE+ brain, but not within the wildtype tissue as expected.
- The region of fluorescence was not localized to the dorsal tenia tecta (DTT) as intended. The targeting was too rostral and dorsal.

Conclusion and Future Directions

- Within this preliminary experiment, the flexed AAV viral injection did not reach the target region of the DTT. Adjustments to the injection procedure will be implemented to ensure the DTT is properly targeted in future injections.
- The connectivity of OXTR neurons of the DTT will hopefully help elucidate the function of the DTT and the role of the OXT system in higher order olfactory processing.

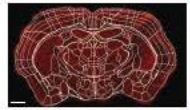

Figure 11: A brain section processed with NeuroTrace Nissl Stain was aligned and registered to the Allen Brain Atlas using ABBA.

Acknowledgements

We gratefully acknowledge the support of the Laboratory Animal Resources (LAR) personnel for veterinary care and animal husbandry. Thank you to the members of the Dewan Lab: Sam Caton, Austin Pauley, Christopher May, and Ellie Williams. We would also like to acknowledge the members of the Hammock Lab for providing and genotyping the mice utilized in this project. Thank you to the Women in Math Science and Engineering (WIMSE) organization for funding this research through the Research Experience Program (REP).

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