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A New Approach to Staining Oxytocin Receptors in Prairie Vole Brain Tissue

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Introduction

G-protein coupled receptors (GPCR) present a challenge to scientists trying to visualize neural pathways in the brain. Currently there are very limited methods that allow scientists to visualize these receptors in tissues.

Our research aims to create a new staining method that will selectively visualize the Oxytocin Receptors (OXTR), a GPCR. We will be performing this research on Prairie Vole brain tissue.

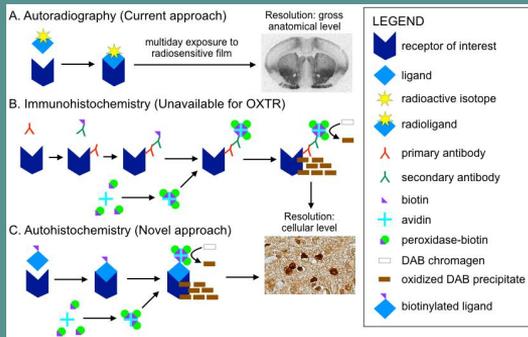


Figure 1. Models of the novel methods used to visualize oxytocin receptors in tissue sections.

Methods

There are two main methods to look for receptors in the brain. Autoradiography and Immunohistochemistry. Our research goals are to combine them.

- Autoradiography- Technique that uses radioactive ligands that selectively bind to the target receptor. Radiosensitive film is used to pick up the energies released from the radioactive ligand. The film turns dark in those areas. See Figure 1A
- Immunohistochemistry- An antibody is designed that binds to a specific sequences in a receptor. The primary antibody will bind to a sequence in the receptor and the secondary antibody will bind onto the primary antibody. The secondary antibody has a biotin attached to avidin bonded to peroxidase-biotin. The Avidin complex attracts a brown stain to deposits brown stain in tissue. See Figure 1B

We will be using a molecule called deamino-lysine vasotocin (dLVT-Biotin) as our ligand as seen in Figure 2. The dLVT-Biotin has an amino acid change at the 8th amino acid that allows the molecule to be bound to Biotin. Figure 1C demonstrates the way the dLVT-Biotin Ligand binds to the OXTR and Avidin (Freeman).

References: NIMH R21MH115680 to Freeman, Sara. "Visualization of oxytocin receptors for translational social neuroscience. July 2018

Current Progress

We have the current dLVT-Biotin but we have reached some difficulty in getting the Avidin to bind to the biotin in th dLVT-Biotin.

Our next step is to obtain another ligand with a different structure to reduce any steric hindrance.

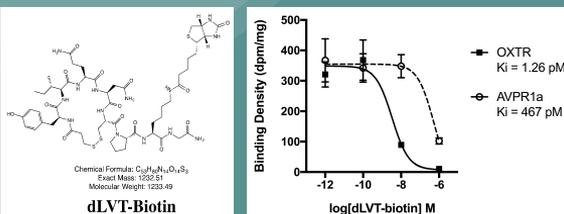


Figure 2 Structure of the dLVT-Biotin.

Figure 3. Competition curve showing dLVT-b binds to OXTR.

Figure 4. Staining with dLVT-biotin (L) and without (R).

