

# Characterization of a new pharmacological tool for targeting the canid oxytocin receptor.

Hillary Ihrig, McKenna Rich, and Sara M Freeman, PhD

## Introduction

- Oxytocin is a hormone that mediates multiple social behaviors such as social recognition, maternal behaviors, and pair-bonding.
- Coyotes display monogamy. In other mammalian models, this behavior has been shown to be influenced by oxytocin.
- Oxytocin and Vasopressin's structural similarities result in binding promiscuity -- both molecules bind to both receptors.
- We will determine the binding affinity of the oxytocin receptor antagonist, L-368,899 using competitive binding autoradiography.
- If this antagonist effectively blocks the coyote oxytocin receptor and not the vasopressin 1a receptor, then it can be used in live coyotes to study oxytocin-dependent social behaviors.

## Methods

- Frozen coyote brains were blocked into slabs and stored at -80C until sectioning.
- Brain sections were sliced using a cryostat at 20 micron thickness, and mounted on microscope slides.
- Competitive binding autoradiography will be done with increasing concentrations of the oxytocin receptor antagonist L-368,899 in competition with the commercially available radioligands: 1) OXTR radioligand <sup>125</sup>I-ornithine vasotocin analog (<sup>125</sup>I-OVTA) and 2) AVPR1a radioligand <sup>125</sup>I-linear vasopressin antagonist (<sup>125</sup>I-LVA).
- Using the data from the autoradiography we will generate a competition curve.

## PROPOSAL

# Does the human-selective oxytocin receptor antagonist L-368,899 bind selectively to the coyote (*Canis latrans*) oxytocin receptor?

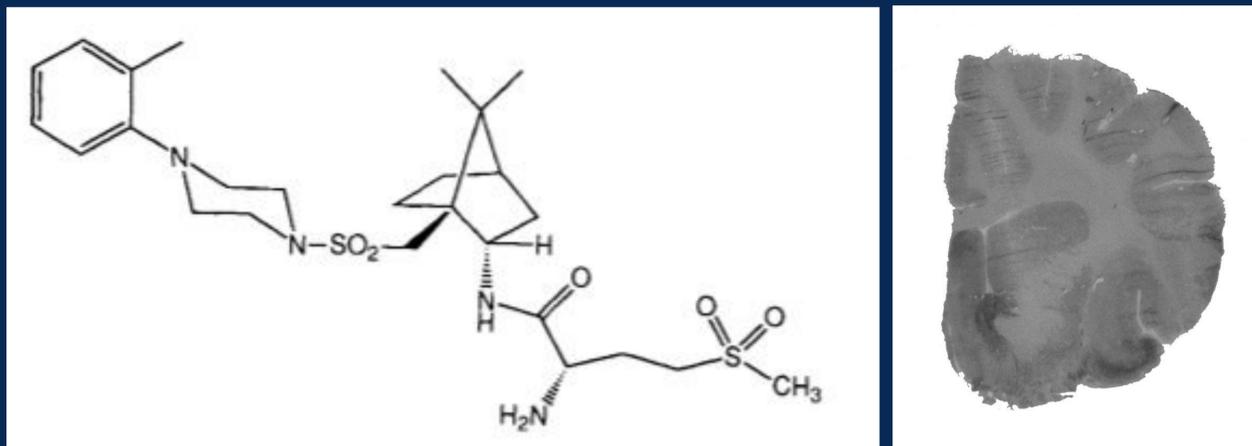
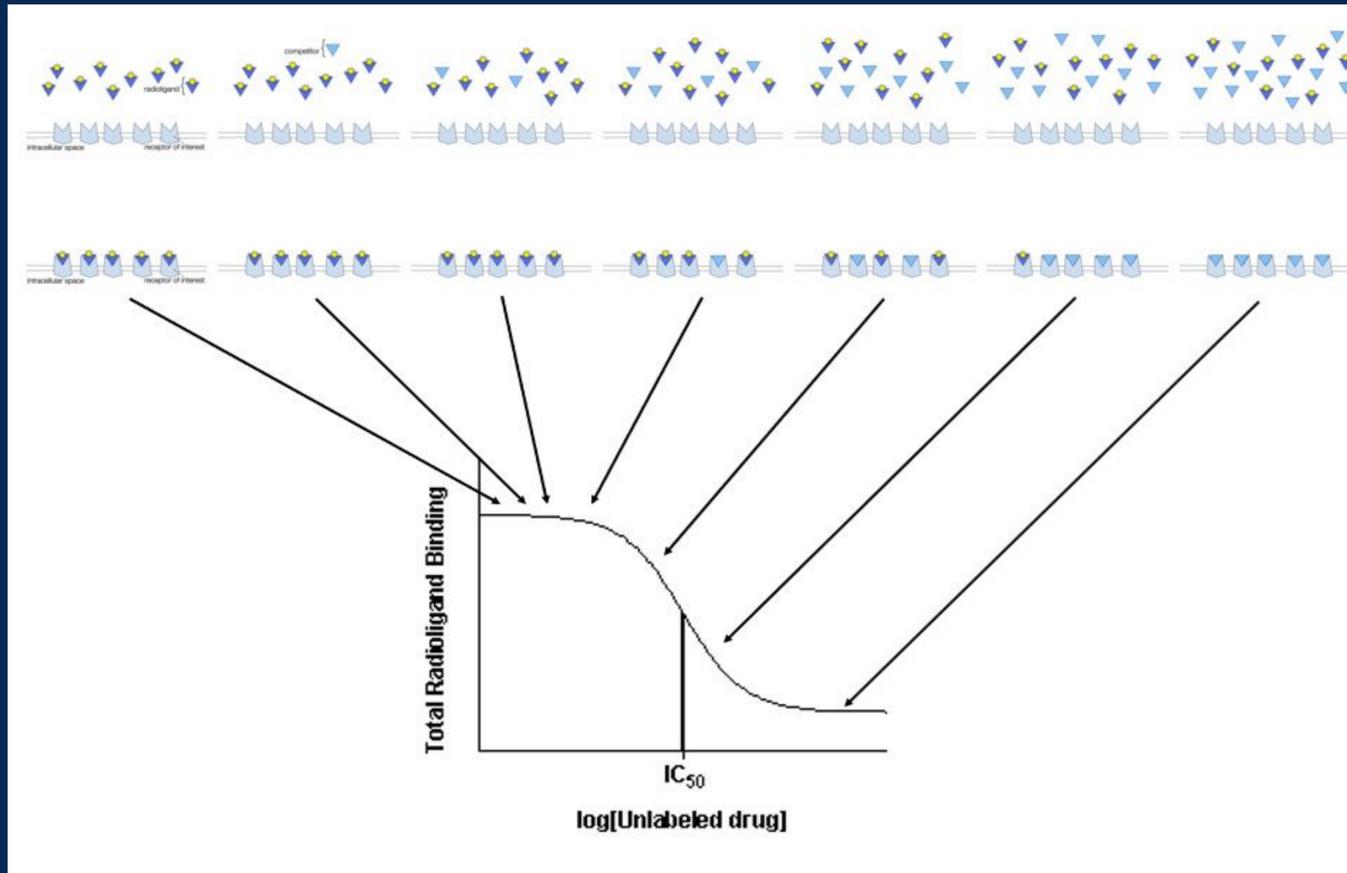


Figure 1 (top)- Molecular binding used to generate a competition growth curve  
 Figure 2 (bottom left)- L-368,889 chemical structure  
 Figure 3 (bottom right)- autoradiogram of OXTR binding in coyote forebrain

## Projected Results

### Oxytocin receptors vs Vasopressin receptors

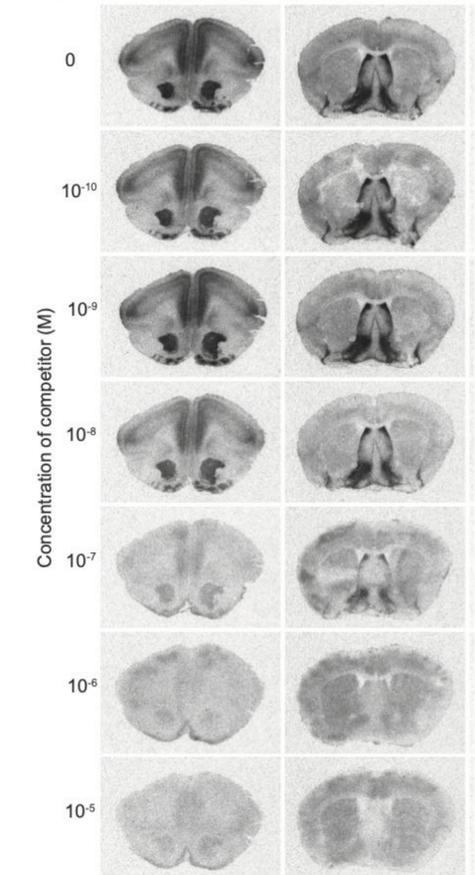


Figure 4 (above)- Radioligand receptor expression with increasing concentrations of oxytocin and vasopressin respectively.

## Competition Curves

Binding of vasopressin and oxytocin radioligands with increasing concentrations of oxytocin receptor antagonist

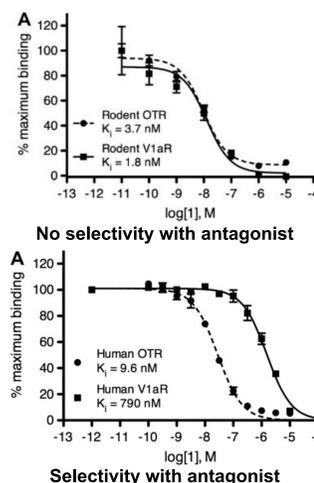


Figure 5 (top)- vole brain sample  
 Figure 6 (bottom)- human brain sample

## REFERENCES

- Boccia, M. L., Goursaud, A. - S., Bachevalier, J., Anderson, K. D., & Pedersen, C. A. (2007). Peripherally administered non-peptide oxytocin antagonist, L368,899®, accumulates in limbic brain areas: A new pharmacological tool for the study of social motivation in non-human primates. *Hormones and Behavior*, 52(3), 344-351.
- Freeman, S. M., Walum, H., Inoue, K., Smith, A. L., Goodman, M. M., Bales, K. L., & Young, L. J. (2014). Neuroanatomical distribution of oxytocin and vasopressin 1a receptors in the socially monogamous coppery titi monkey (*Callicebus cupreus*). *Neuroscience*, 273, 12-23.
- Smith, A. L., Freeman, S. M., Stehouwer, J. S., Inoue, K., Voll, R. J., Young, L. J., & Goodman, M. M. (2012). Synthesis and evaluation of C-11, F-18 and I-125 small molecule radioligands for detecting oxytocin receptors. *Bioorganic and Medicinal Chemistry*, 20(8), 2721-2738.
- Smith, A. S., Ågmo, A., Birnie, A. K., & French, J. A. (2010). Manipulation of the oxytocin system alters social behavior and attraction in pair-bonding primates, callithrix penicillata. *Hormones and Behavior*, 57(2), 255-262.