

Oxytocin in Pair Bonding: Staining and Mapping of OXTR in the Coyote brain

Logan Hale, Ryan Williams, Dr. Sara Freeman

THE FREEMAN LAB



College of Science
Utah State University

Introduction/ Methods

Introduction

Oxytocin is a neuropeptide that binds to a specific receptor in the brain called oxytocin receptors (OXTR) (Figures 1 and 2). It plays a significant role in social bonding and behavior in mammals. It is involved in a wide range of physiological processes, such as lactation, uterine contractions, maternal behavior, emotions, and social bonding (Walum et al., 2012).

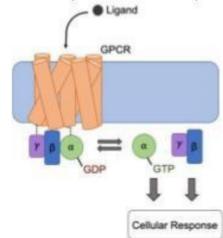


Figure 1: Simple depiction of the receptor oxytocin binds to, this receptor is called a G-protein coupled receptor (GPCR).

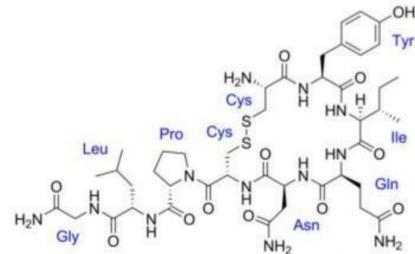


Figure 2: Chemical structure of Oxytocin a complex but important hormone in many different systems and endocrinological pathways.

Understanding the distribution of OXTR in the brain is crucial for identifying the neural pathways that regulate social behavior and bonding in monogamous species like coyotes.

Methods

Our sample size consisted 3 Female and 2 Male coyote brains extracted with help from the Utah Veterinary Diagnostic Lab. The following steps were taken to prepare specimens and map OXTR.

1. Brain Preparation and Slicing: A cryostat was used to slice sections of the brain at 20 μ m thickness at around -15°C .
2. Slide Preparation: Slices were thaw-mounted on glass microscope slides and stored at -80°C .
3. Autoradiography: Brain sections were sorted and processed for autoradiography by washing the slides to prepare them for radioligand uptake.
4. Radioligand Uptake: The radioligand ^{125}I -Ornithine Vasotocin Analog (^{125}I -OVTA) was used at a concentration of 50 nM to label OXTRs present in the tissue sections (Figure 3).
5. Film Exposure: After final washes and drying, the slides were then exposed to film for 7 days.
6. Image Processing: The film images were digitally processed using MCID Core to measure receptor densities (Freeman et al., 2014).

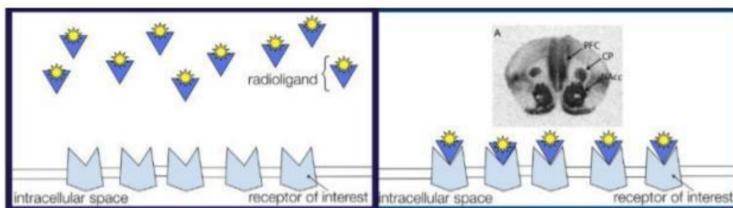


Figure 3: Graphic representation of the utilization of radioligands for autoradiography and mapping.

Results

- **Hypothesis:** We hypothesized that we would find OXTR in the same locations that have been identified to have high OXTR density in other monogamous mammals: the hippocampus, central and lateral amygdala, BNST, nucleus accumbens, and ventromedial hypothalamus (Lee et al., 2009).

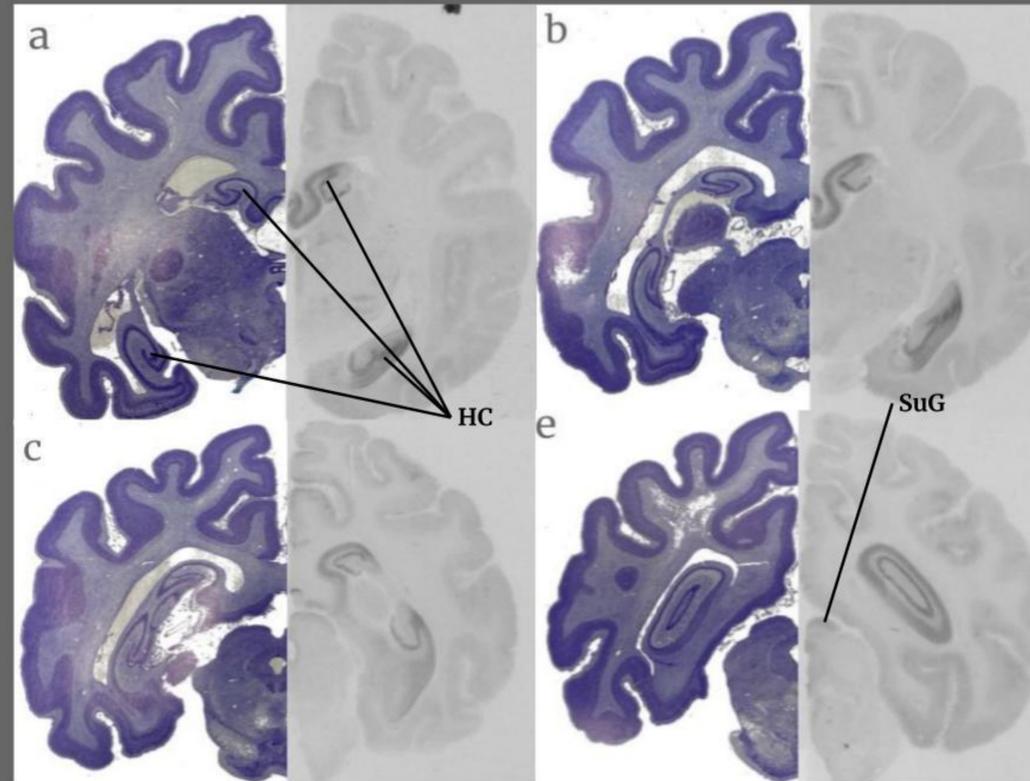


Figure 4: Comparative pictures of nissl stained beagle brain slices from the 'The Beagle Brain in Stereotaxic Coordinates Vol. 1' (purple images on the left) and Coyote brain slices that have undergone OXTR mapping (grayscale images on the right). The dark areas indicates more cell bodies in the nissl stained images from the beagle brain atlas whereas dark areas on the OXTR mapped slices indicates a higher density of OXTR.

Results

As shown in Figure 4 there are 2 areas that contain a high density of OXTR, Both the Hippocampus (HC) and the superficial gray layer of the superior colliculus (SuG). The contrast between the nissl stained Beagle Brain Atlas pictures and the OXTR stained photos is to highlight that the OXTR densities do not simply increase with an increase in cell bodies but are localized to specialized areas of the Coyote's brain.

- **Hippocampus:** The hippocampus is a region of the brain that is involved in learning and memory, as well as spatial navigation. It has also been implicated in the regulation of social behavior and emotions. One possible reason why there is a high density of OXTR in the coyote's hippocampus is because this region may play a crucial role in the processing and integration of social and emotional information, which is known to be modulated by oxytocin.
- **Superficial gray layer of the superior colliculus:** The superior colliculus is responsible for integrating visual, auditory, and somatosensory information to direct eye and head movements towards relevant stimuli. Therefore oxytocin receptors in this brain region of coyotes suggests that the (SuG) may be a key region for regulating social behavior, particularly in directing visual attention towards social cues.

Future Research/ Discussion

Discussion

The high density of oxytocin receptors in the HC and the SuG of the coyote brain provides important insights into the neural basis of social bonding in this species. Previous research has shown that the hippocampus is involved in social recognition and memory, which suggests that oxytocin may play a role in these processes. The main function of the SuG is coordinating shifts in visual attention, with our findings of OXTR in this brain region further research can be performed to test how this brain region is connected with social behavior.

Future Research

- Investigate the role of OXTR in regulating social behavior in coyotes through behavioral studies
 - Study the relationship between OXTR expression and reproductive success in coyotes
 - Examine the impact of environmental factors, such as food availability and social group size, on OXTR expression in coyotes
 - Investigate the quantitative difference between OXTR in males and females, and see whether or not time spent with their pair mate affects the amount of OXTR



Acknowledgements

I want to thank Jacey Hopkin, Olivia Griffin, Kaleb Keller, Rachel Tong, and Maggie Zentner for contribution to brain slicing. We would like to thank USDA Millville Predator Research Facility for providing the specimens and the pathologists at Utah Veterinary Diagnostic Laboratory for performing the brain removal.

References

- 1: 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. <https://doi.org/10.1016/j.neuroscience.2014.04.055>
- 2: Walum, H., Lichtenstein, P., Neiderhiser, J. M., Reiss, D., Ganiban, J. M., Spotts, E. L., Pedersen, N. L., Anckarsäter, H., Larsson, H., & Westberg, L. (2012). Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. *Biological Psychiatry*, 71(5), 419–426. <https://doi.org/10.1016/j.biopsych.2011.09.002>
- 3: Lee, H.-J., Macbeth, A. H., Pagani, J., & Young, W. S. (2009). Oxytocin: The great facilitator of life. *Progress in Neurobiology*. <https://doi.org/10.1016/j.pneurobio.2009.04.001>
- 4: Palazzi, X. (2011). *The Beagle Brain in Stereotaxic Coordinates (Vol. 1)*. Springer New York, NY.