Exploring Canid Monogamy: Characterization of the Distribution of Oxytocin Receptors in the Brain of the Coyote (*Canis latrans*)

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Introduction

- Oxytocin is a neuropeptide that has been shown to be a factor in species that display social monogamy[1]. The neural actions of oxytocin at the oxytocin receptor in the brain are necessary for social memory of familiar individuals of the same species.
- Much research has been done on oxytocin receptors (OXTR) in the brains of socially monogamous rodents and non-human primates, and these studies have demonstrated a critical role of oxytocin in the neurobiology of social attachment.
- Coyotes are a unique species in the context of social research because they are socially monogamous and have been shown to also exhibit sexual and genetic monogamy.

The goal of this study is to quantify the distribution of OXTR throughout the coyote forebrain in order to compare their receptor map to other known monogamous species and to lay the neuroanatomical foundation for future studies of the oxytocin system of coyotes.

Methods

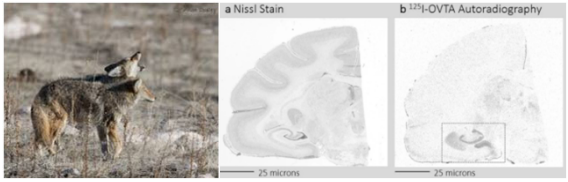
Animal specimens. In order to map coyote OXTR, we utilized six brains (3 males; 3 females) that were opportunistically collected from captive-housed coyotes at the USDA Millville Predator Research Center. These animals were euthanized for other reasons unrelated to this study.

Specimen preparation. The brain samples were fresh frozen on dry ice within hours of death and cut into blocks. The blocks were then sectioned at 20 microns using a cryostat, and the slices mounted on glass slides.

Receptor autoradiography. These slides were processed according to established methods to visualize OXTR using receptor autoradiography[2]. This process uses radioactively labeled ligands that bind to OXTR and emit radiation, which is detected by radiosensitive film (Figure 1). We then took these films and quantified them by using digital densitometry. Binding densities were normalized by subtracting each specimen's average background from each region. We analyzed the data using a repeated measures, two-way ANOVA to look for effects of sex and region.

Future Research

Previous research has established that monogamous titi monkeys have high levels of OXTRs in their hippocampus[5]. These high levels have been associated with parenting behaviors in further studies[4]. As we have found a similar pattern in coyotes, further research should see if OXTR density in the hippocampus is also associated with parenting behavior in coyotes as well as titi monkeys.



In this study we found high densities of OXTRs in many areas that are involved in the olfactory pathway, including the olfactory bulbs, olfactory tubercles, and the piriform cortex. Coyotes, like all canids, are scent marking species and use odor for social communication. These findings have implications for the neural processing of social information in the coyote brain and the possible involvement of oxytocin.

Results

We found there to be several areas of interest to have a high density of OXTRs. These areas include, but not limited to, the CeAmyg, Olf Tub, Hippocampus, DHypo, and the PVN. We tested if there was any differences between males (n=3) and females (n=3) and did not find any significant differences except in the CeAmyg. It should be noted that we only had one male and two female samples for the CeAmyg.

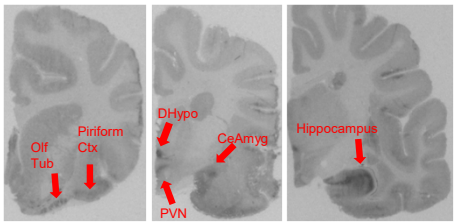


Figure 1. Density of OXTRs in different areas of interest throughout the coyote forebrain.

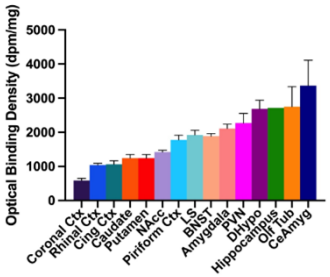
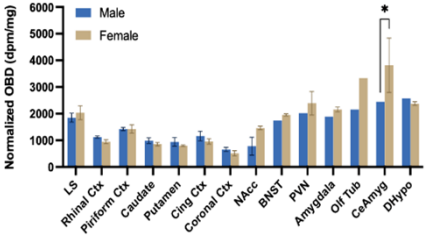


Figure 2. Sex Differences in OXTR binding. The only area shown to have a sex difference in our samples was the Central Amygdala.



NAcc - Nucleus Accumbens; LS - Lateral Septum; BNST - bed nucleus of the stria terminalis; PVN - Paraventricular Nucleus; DHypo - Dorsal Hypothalamus; Olf Tub - Olfactory tubercles; CeAmyg - Central Amygdala

Citations

1. Young, L. J. & Wang, Z. The neurobiology of pair bonding. *Nature Neuroscience* **7,** 1048–1054 (2004).
2. Freeman SM, Walum H, Inoue K, Smith AL, Goodman MM, Bales KL, Young LJ. Neuroanatomical distribution of oxytocin and vasopressin 1a receptors in the socially monogamous coppery titi monkey (*Callicebus cupreus*). Neuroscience 2014; 273: 12–23.
3. Palazzi, Xavier; The Beagle Brain in Stereotaxic Coordinates; Springer Science & Business Media; 2011.
4. Baxter, A., Anderson, M., Seelke, A.M. *et al.* Oxytocin receptor binding in the titi monkey hippocampal formation is associated with parental status and partner affiliation. *Sci Rep*10, 17301 (2020). https://doi.org/10.1038/s41598-020-74243-1
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