

The Grieving Brain

Effect of Pairmate Loss on Coyote Oxytocin Receptor Densities

Kaleb Keller, Utah State University | Sara Freeman PhD, Utah State University

Introduction

- Complicated grief can be a severe mental health disorder and in humans, losing a spouse is associated with a 48% increased risk of death.⁴
- Understanding the biological underpinnings of grief and loss could help find treatments and therapies.
- The neurohormone oxytocin is a polypeptide important in modulating social behavior such as parenting and pair bonding within mammals.
- Coyotes exhibit social, sexual, and genetic monogamy which is rare within mammalian species.²
- Previous research has shown that socially monogamous prairie voles display a decrease in OXTR binding in the nucleus accumbens after separation from a pairmate.¹
- In addition, previous research by undergraduates in the Freeman Lab has shown restricted variance in OXTR binding in the lateral septum and nucleus accumbens, suggesting neuroplasticity after partner loss.³
- **We hypothesized that more posterior brain structures that have shown high affinity for OXTR in monogamous prairie voles and non-human primates including the hippocampus, periaqueductal gray, and superior colliculus would show a decrease in OXTR binding after pairmate loss.**

Methods

- Six coyote brain specimens, 3 widowed females and 3 paired females, were procured opportunistically from the USDA's predator research facility in Millville, Utah.
- Specimens were frozen in hemispheres 2-3 hours postmortem and blocked and stored at -80°C until they were sliced.
- The specimens were sectioned at -15°C to 20 µm thickness on the cryostat and mounted to glass slides.
- The slides then underwent receptor autoradiography and were washed and then bathed in a solution containing ¹²⁵I-ornithine vasotocin analog.
- The slides were then exposed to radiosensitive film for seven days.
- Digital densitometry was used to quantify OXTR binding densities in the brain regions of interest.

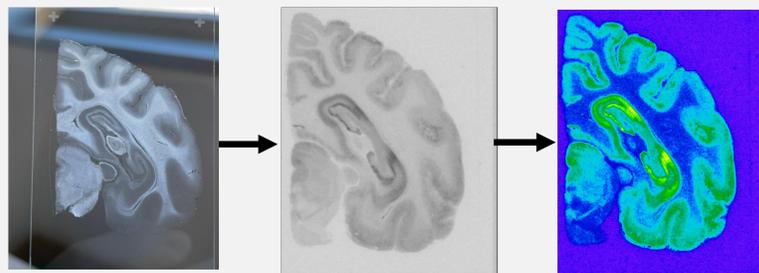


Figure 1: Tissue mounted to glass slide. Figure 2: Radiosensitive film post exposure to radioactive tissue. Figure 3: Digital densitometry

Effect of partner loss on OXTR in the Hippocampus

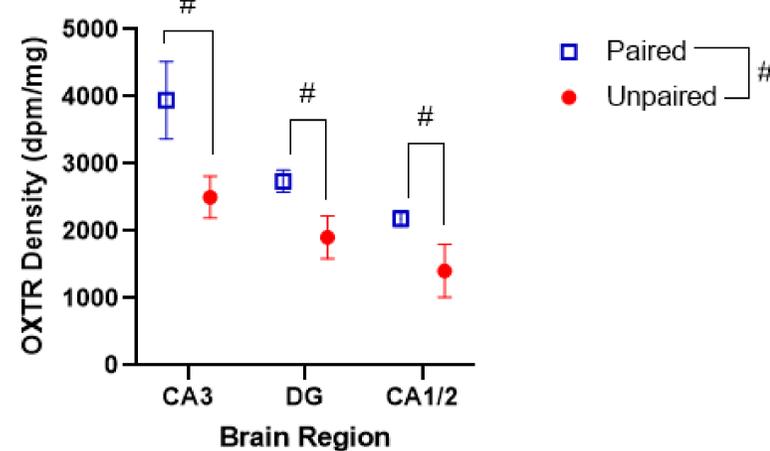


Figure 4: Comparison of OXTR binding between paired and unpaired coyotes in the hippocampus

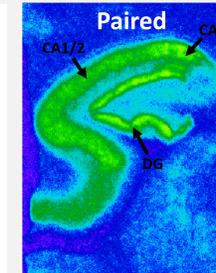


Figure 5: OXTR distribution in the hippocampus of a paired coyote.

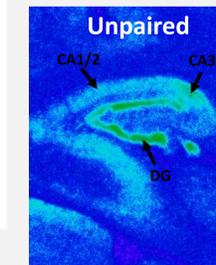


Figure 6: OXTR distribution in the hippocampus of an unpaired coyote.

Effect of Partner Loss on OXTR in the Midbrain

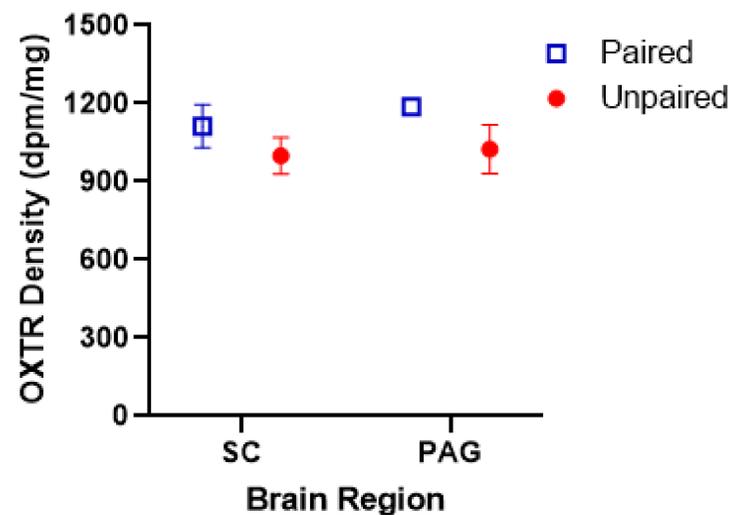


Figure 7: Comparison of OXTR binding between paired and unpaired coyotes in the midbrain

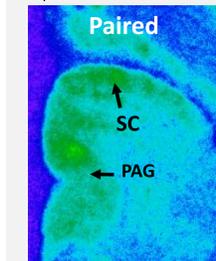


Figure 8: OXTR distribution in the midbrain of a paired coyote.

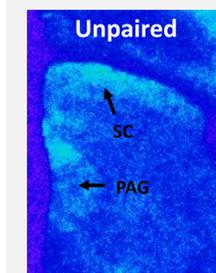


Figure 9: OXTR distribution in the midbrain of an unpaired coyote

Results

- A two-way ANOVA showed a trending for a main effect of paired status in the mean binding density across all regions (midbrain and hippocampus, $P=0.06$).
- Follow-up analyses using an unpaired t-test on each brain region showed that the mean binding density in the hippocampus was trending toward significance ($P=0.075$).
- Additional follow-up analyses using an unpaired t-test on each brain region showed the mean binding density in each subregion of the CA3, DG, and CA1/2 was trending toward significance ($0.05 < p < 0.10$).
- There were no statistically significant differences in the midbrain regions.

Discussion

- While the data was not quite statistically significant, there is certainly a trend in the data that suggests there is a reduction of OXTR after pairmate loss.
- The hippocampus specifically shows data trending towards significance, indicating that this region may be particularly sensitive to the effects of social loss.
- There was also a large variance between each animal which may reflect the natural variation that occurs between individuals but could also be a result of a limited sample size.
- The large variance and small sample size could have contributed to the lack of statistical significance.
- Nearly a quarter of the percent of total variation was due to pair status, suggesting pair status does influence OXTR densities.

Conclusion

- The data suggests that partner loss reduces the signaling of oxytocin, specifically in the hippocampus, which is done via a reduction of OXTR.
- The loss of OXTR after pairmate loss could contribute to the negative psychological and physiological consequences of social loss.
- Follow-up studies should use a larger sample size and investigate other structures that were not part of this study such as various cortical regions.

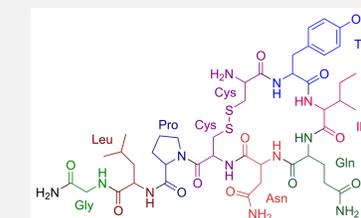


Figure 10: Oxytocin Structure



Figure 11: Pair of Coyotes
Photograph by Sean Crane, Minden Pictures

References

1. Bosch, O. J., & Young, L. J. (2018). *Oxytocin and social relationships: From attachment to Bond Disruption*. Current topics in behavioral neurosciences. Retrieved April 6, 2023, from <https://pubmed.ncbi.nlm.nih.gov/28812266/>
2. Hennessy, C. A., Dubach, J., & Gehrt, S. D. (2012, June 28). *Long-term pair bonding and genetic evidence for monogamy among urban coyotes (Canis latrans)*. Academic.oup.com. Retrieved April 6, 2023, from <https://academic.oup.com/jmammal/article/93/3/732/837894>
3. Neilson & Freeman, USU SRS'22
4. Sullivan, A. R., & Fenelon, A. (2014, January). *Patterns of widowhood mortality*. The journals of gerontology. Series B, Psychological sciences and social sciences. Retrieved March 31, 2023, from <https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3968855/>

Acknowledgments

Members of the Freeman Lab for help sectioning and help performing the autoradiography. We gratefully acknowledge the Millville Predator Research Center for providing the specimens.