



Piloting the use of oxytocin receptor and vasopressin receptor mRNA dog probes in the coyote (*Canis latrans*)

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Introduction

- Oxytocin and vasopressin are two structurally similar neuropeptides that modulate social behavior such as: pair bonding, aggression, and attachment in humans and animals.
- Oxytocin receptors (OXTR) and vasopressin receptors (AVPR1a) are G-protein coupled receptors. We have started to map out the densities of these two receptors in coyote brains.
- Coyotes have been shown to exhibit social behavior such as pair bonding, making them a great species to study the effects of oxytocin and vasopressin in monogamous relationships.
- Commercially available probes are available for *in situ* hybridization to visualize the mRNA of OXTR and AVPR1a in different species like dogs and humans.

Methods

- We used opportunistically acquired coyote brain tissue that has been frozen at -80 degrees celsius and sectioned at 20 μ m.

DNA Extraction and Polymerase Chain Reaction

- Protocol was followed from: "DNeasy Blood & Tissue Handbook" to extract the DNA from frozen brain tissue.
- Mytaq was mixed with distilled water, primers, and template DNA for the polymerase chain reaction mixture.
- A thermocycler was set to the appropriate annealing temperatures for the primers, then we set it to run 30 cycles.
- PCR product was then sent to Etonbio for sanger sequencing.

RNAscope *In Situ* Hybridization

- The ACDbio procedure for *in situ* hybridization was followed. mRNA probes designed for dog genes along with coyote brain tissue that was sliced on a cryostat set at -15°C and sliced in 20 μ m was used.

Oxytocin and vasopressin receptor mRNA probes designed from the dog genome are effective for *in situ* hybridization in coyote brain tissue.

Figure 1: Oxytocin receptor levels in coyote brain tissue using receptor autoradiography (A) and *in situ* hybridization (B,C).

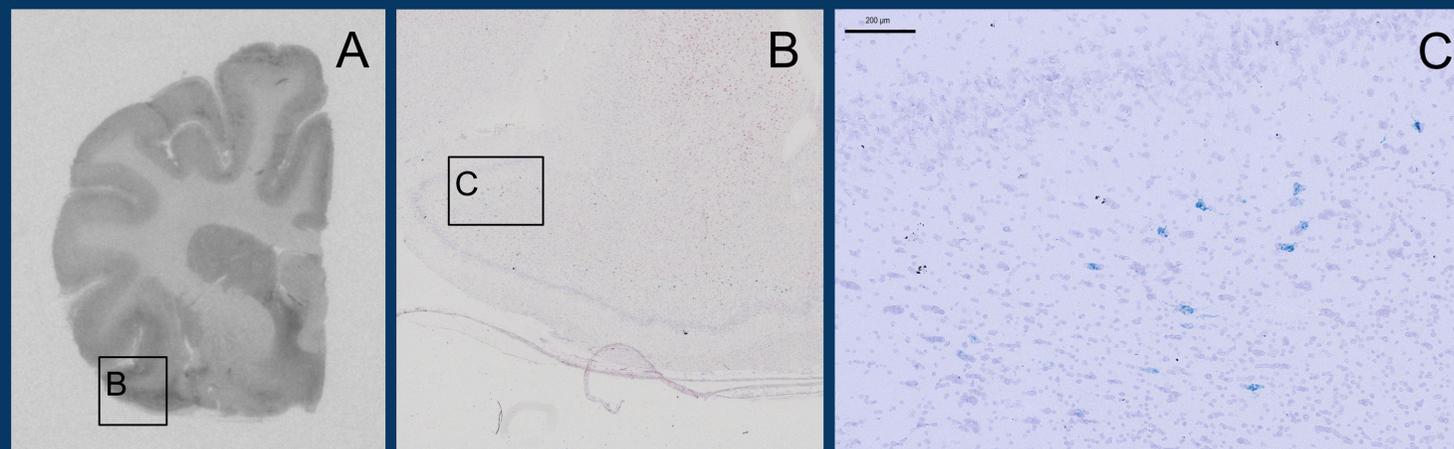
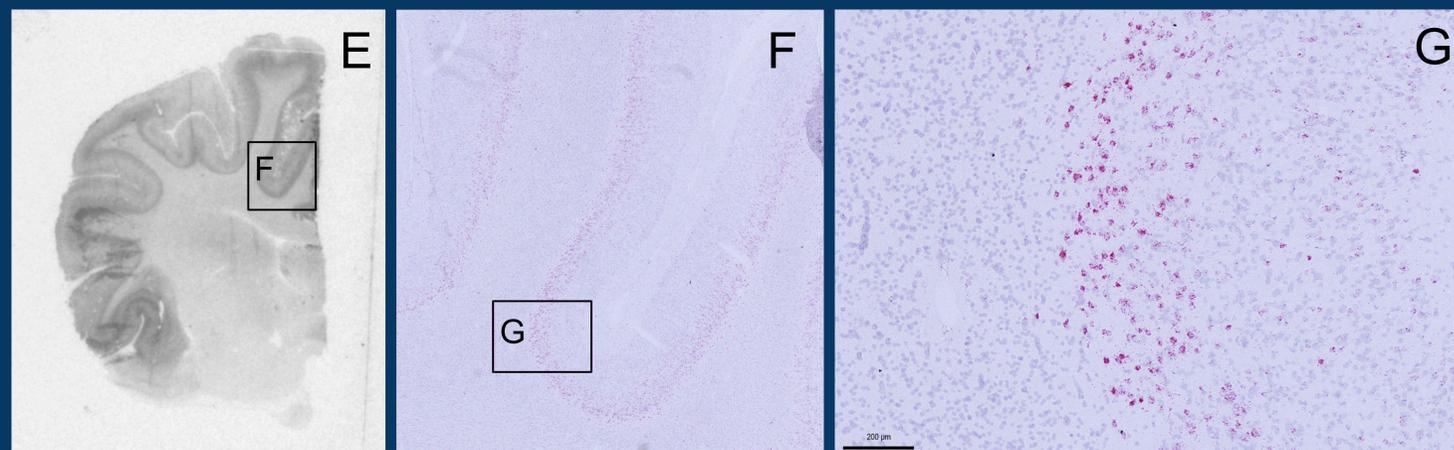


Figure 2: Vasopressin 1a receptor levels in coyote brain tissue using receptor autoradiography (E) and *in situ* hybridization (F,G).



Additional Results

- mRNA oxytocin and vasopressin receptor probes designed for dogs work on coyote tissue.
- mRNA sequences for dog and coyote oxytocin and vasopressin receptors have a homology of 97% and 93%, respectively.

% identity (mRNA sequence)			OXTR			AVPR1a		
			Rodent	Primate	Canid	Rodent	Primate	Canid
			MOUSE	HUMAN	DOG	MOUSE	HUMAN	DOG
OXTR	Rodent	MOUSE	100	87.09	88.06	65.93	65.78	67.19
		RAT	87.21	87.77	86.98	65.28	64.52	66.07
	Primate	HUMAN	87.09	100	88.06	66.27	67.63	68.82
		RHESUS	97.26	96.49	88.98	66.59	67.52	68.9
	Canid	DOG	88.06	88.06	100	65.92	67.35	70.18
COYOTE		87.5	89.81	97.82	66.49	68.64	71.27	
MOUSE		65.93	66.27	65.92	100	83.19	80.5	
AVPR1a	Rodent	RAT	65.5	65.68	65.76	84.42	82.45	81.51
		HUMAN	65.78	67.93	67.35	87.16	100	86.19
	Primate	RHESUS	66.53	68.41	67.97	87.57	94.6	86.16
		DOG	67.19	68.82	70.18	89.12	86.19	100
	Canid	COYOTE	72.69	73.64	75.19	83.75	86.02	93.4

Figure 3: A table that shows the % homology in oxytocin and vasopressin receptor mRNA sequences between different mammalian species.

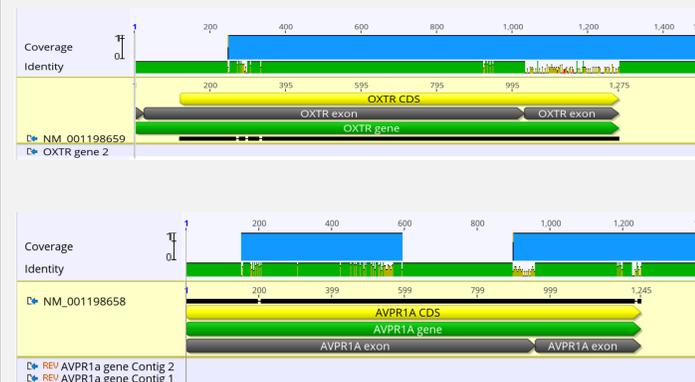
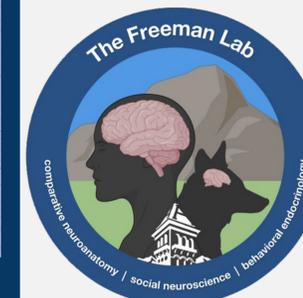


Figure 4: Images depicting the similarities between the oxytocin receptor (top) and vasopressin 1a receptor (bottom) mRNA sequences in dogs and coyotes.

Future Directions

- RT-PCR will be used to acquire the full mRNA sequence of these two genes.
- Further experiments using these probes will be performed to better understand the concentration of these two mRNA sequences in coyote brain tissue.
- Custom probes could be designed to validate the results of this experiment.



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