Establishment of a Coyote Brain Atlas: Counterstaining Techniques in a Canid Brain

Auria Staheli, Nate Johnson, and Dr. Sara Freeman

UtahStateUniversity
DEPARTMENT OF BIOLOGY

Research Introduction

- Oxytocin is commonly known as "the love hormone," as it is the neurotransmitter responsible for pair bonding in many mammalian species, including humans[2].
- The Freeman neurobiology lab is working to map the location of oxytocin and vasopressin receptors within brains of the common coyote (*Canis latrans*). This work will permit comparisons with the brains of other pair-bond-forming mammals and will further our understanding of how these transmitters and their receptors affect behavior.
- However, inferring the locations of these receptors can be difficult, so counterstaining is a vital step in obtaining accurate regional boundaries in any neuroanatomical research—especially when working in an as-yet undescribed species' brain like the coyote. We used two common counterstains:
  - Acetylcholinesterase (AChE) staining allows for visualization of cholinergic neurons in the brain.
  - Nissl staining provides additional reference points for delineating boundaries between regions, by marking the RNA of dense neural cell bodies.
- These methods allow the same sections that have previously been processed using autoradiography to be counterstained and consequently provides more accurate results since the exact same tissue samples are being examined instead of adjacent sections.

The goal of the current study is to use complementary counterstaining methods to establish a coyote brain atlas. This tool will be used to provide a vital roadmap for neuroanatomical research in this species, particularly with regards to determining the locations of oxytocin and vasopressin receptors.

Methods

Animal specimens. This research utilized four brains opportunistically collected from captive-housed coyotes at the USDA Millville Predator Research Center. These animals were euthanized for 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.

Staining Techniques.

Acetylcholinesterase (AChE) stain: Using previously established procedures, an enzymatic reaction solution was formulated in which the slides soaked for five hours. The slides were then added to a developer solution. Finally, a silver intensification soak allowed for staining of the cholinergic neurons (brown). Slides were coverslipped.

Nissl Stain: Adjacent sections corresponding to those from the AChE stain were stained with purple thionin dye to mark the location of neuronal cell bodies. The slides were hydrated, soaked in thionin for 20 seconds, then dehydrated in ethanol before coverslipping. Areas of the brain that have a higher concentration of neuron cell bodies are stained more darkly.

Imaging. The counterstained slides were placed on a lightbox and photographed with a dSLR camera. All editing, cropping, and light adjustment of the images were done consistently across all specimens to avoid introducing variation.

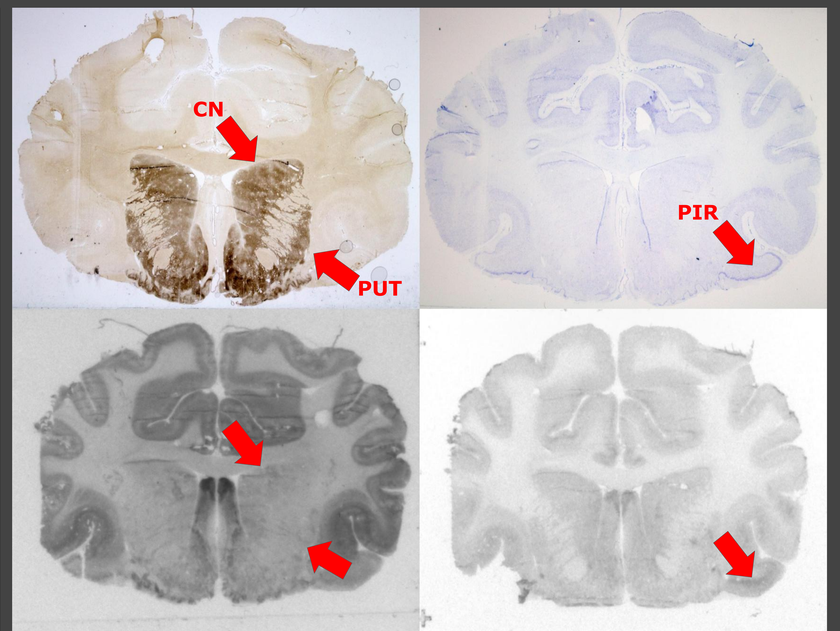


Figure 2. AChE (upper left), Nissl (upper right), vasopressin receptor binding (lower left), oxytocin receptor binding (lower right). Comparing these complementary slides reflects the importance of counterstaining by differentiating brain regions which are unclear via receptor autoradiography (or through use of a single staining technique) alone.

Results

Figure 1 (right).
Sample of coyote brain atlas, showing AChE staining on the top row, and Nissl staining on the bottom row.

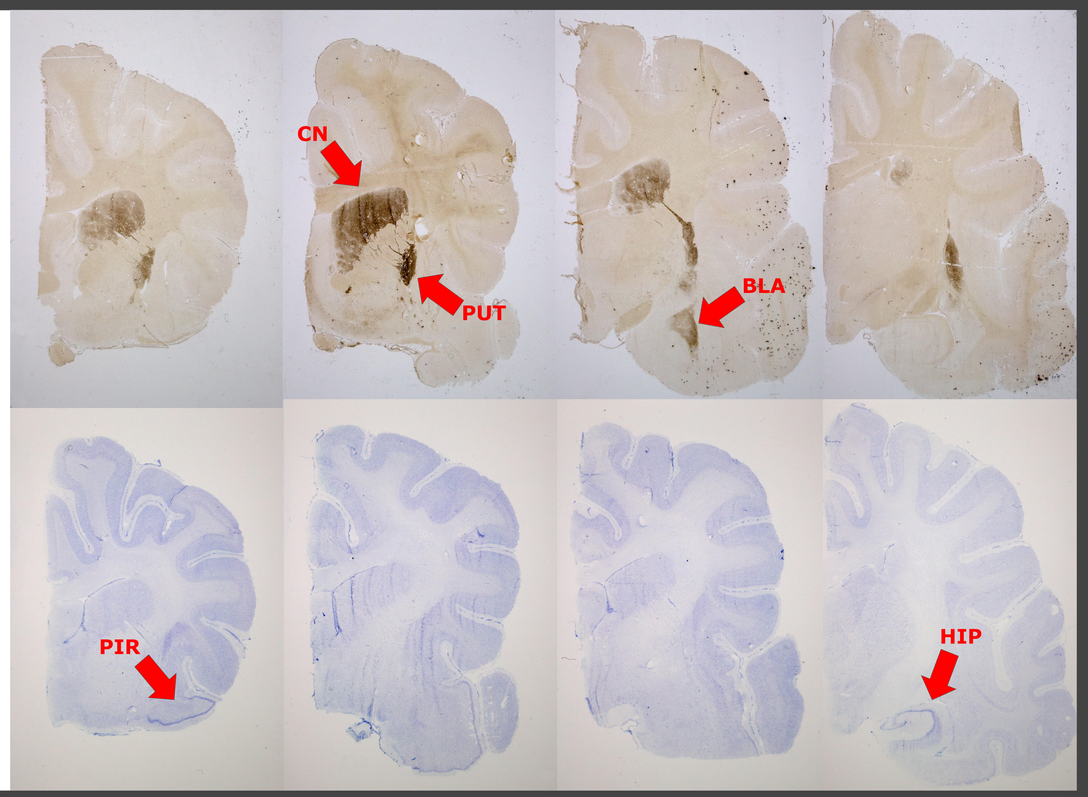
Knowing that the caudate nucleus, putamen, and basolateral amygdala are rich in cholinergic neurons allows for determination of these locations when receptor autoradiography is used.

Similarly, other regions such the hippocampus and piriform cortex contain a greater density of neuron cell bodies and are therefore better illustrated via the thionin Nissl stain technique.

Due to their ability to stain different regions of the brain and determine independent neuroanatomical landmarks, these stains are used in a complementary manner.

Brain Region Abbreviation Legend:
(Figure 1 & Figure 2)

BLA: basolateral nucleus
CN: caudate nucleus
HIP: hippocampus
PIR: piriform cortex
PUT: putamen



Conclusions & Future Directions

- From these results, we concur that differential counterstaining is an effective method for determining neuroanatomical landmarks. With these counterstained images, an atlas can be developed. Figure 1 illustrates only a small portion of the scope, which the eventual full reference will encompass.
- Brain atlases provide the ability to identify and localize structures for future labelling and quantification[1]. These complementary counterstained sets of brain sections will be used to interpret the results of ongoing research to describe the oxytocin and vasopressin receptor distributions in the brain (see Figure 2, above) via receptor autoradiography. These staining methods are used in brain atlases for mice, rats, dogs, monkeys, and humans, which allows for direct comparison of the resulting coyote brain sections to these well defined brain atlas images in order to identify and label the regions of the coyote brain.

References

1. Nowinski, W. L. (2016). Usefulness of brain atlases in neuroradiology: Current status and future potential. *The neuroradiology journal, 29*(4), 260-268.
2. Young, L. J., & Wang, Z. (2004). The neurobiology of pair bonding. *Nature neuroscience, 7*(10), 1048-1054.

Acknowledgements

Members of the Freeman Lab assisted with brain sectioning and autoradiography. We gratefully acknowledge the USDA Millville Predator Research Center for providing specimens.

USDA