

# Examining High Salt Diet, Puberty, and Interactions of Kisspeptin, Neurokinin B, and the Vasopressin Receptor

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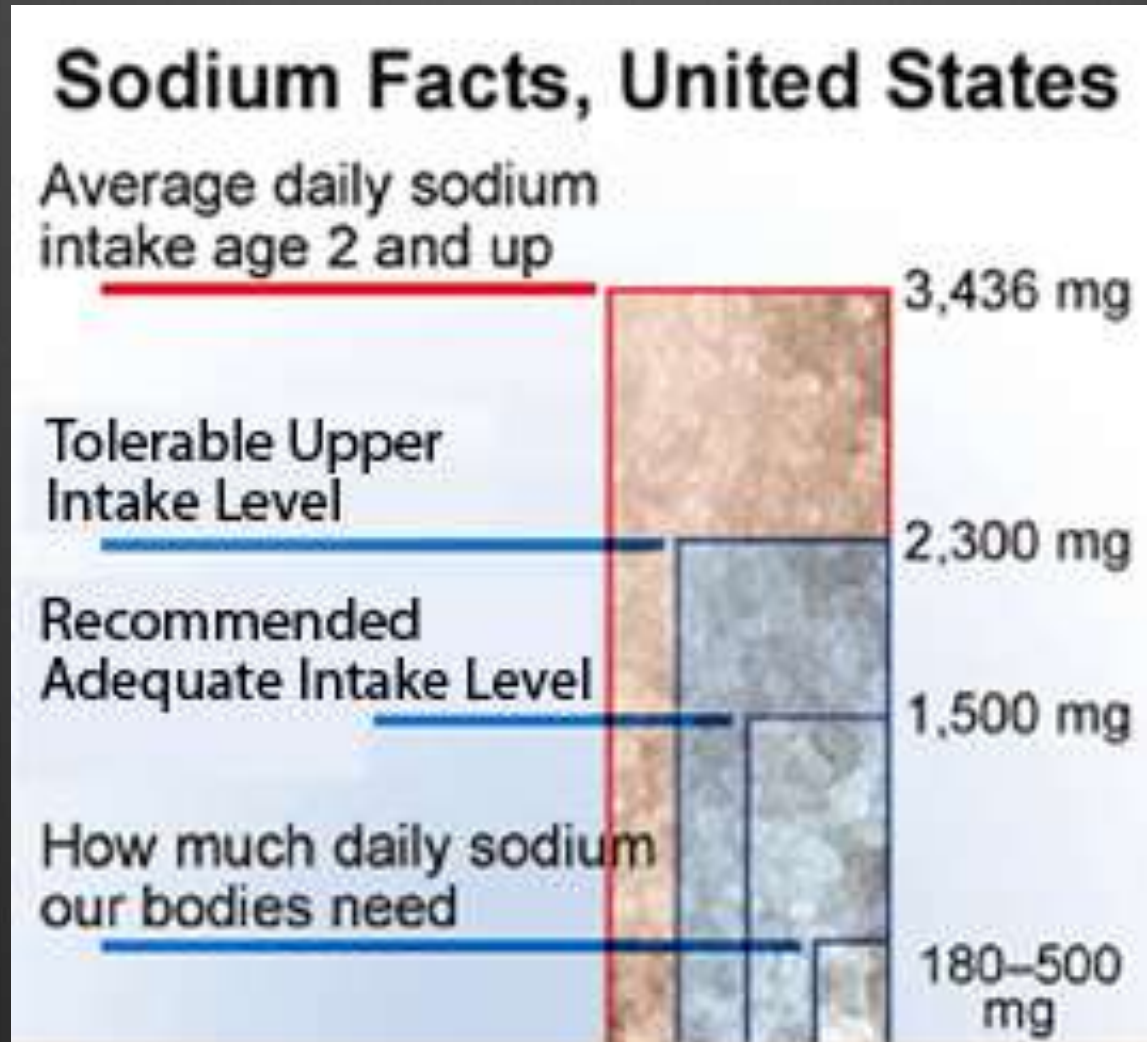
Dori Pitynski

Brooke Fallon

# Background

- Early puberty in females
- Copenhagen Puberty Study- 2,095 girls
  - In 1991, mean age: 10.88 years
  - In 2006, mean age: 9.86 years
- Adverse effects

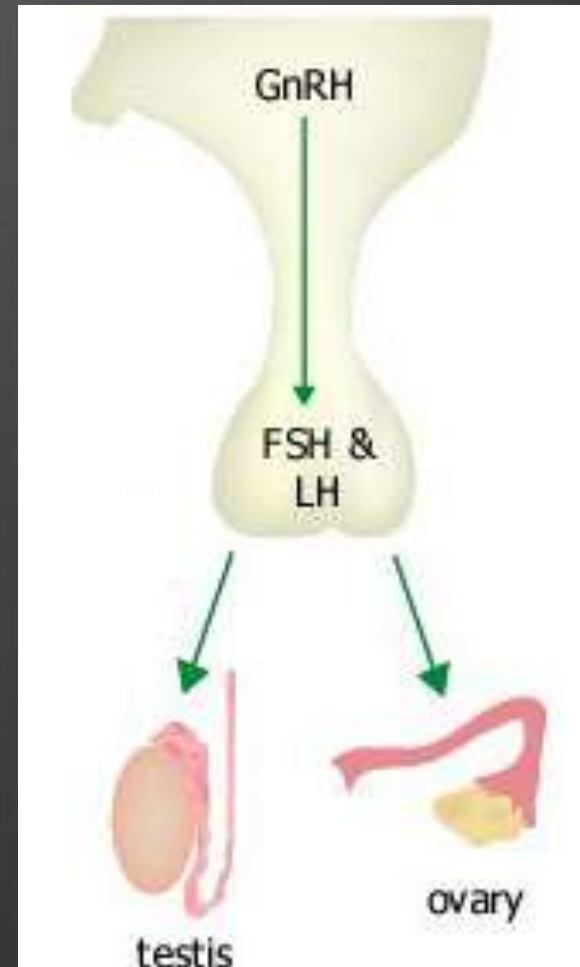
# Innovation



(Centers for Disease Control and Prevention, 2009)

# KNDy cells, GnRH, and the reproductive axis

- Kisspeptin, Neurokinin B, Dynorphin
- GnRH: Gonadotropin releasing hormone (preoptic area)



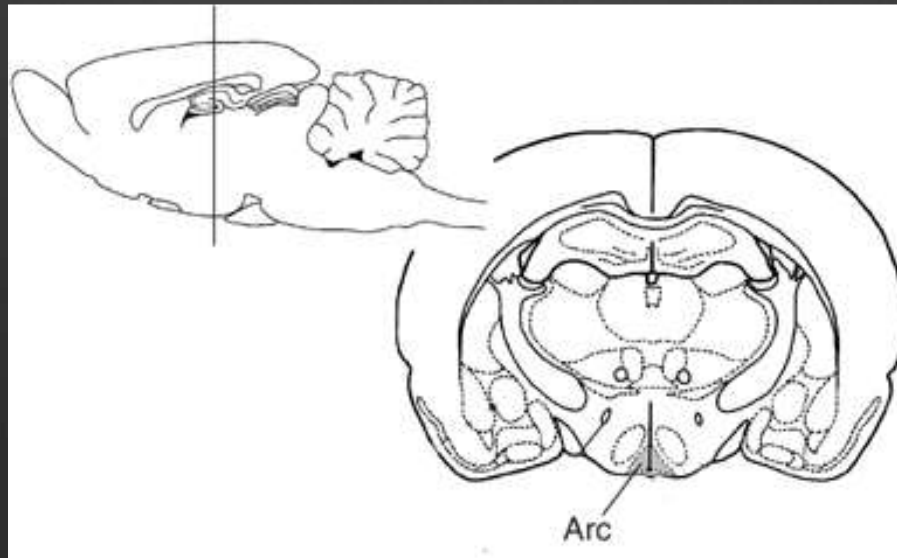
# OVERVIEW

## Specific Aim of Research:

1. Do NKB/Kiss neurons have vasopressin receptors in rat brains?
2. Does salt increase the expression of NKB in rat brains around the time of puberty?

# Vasopressin

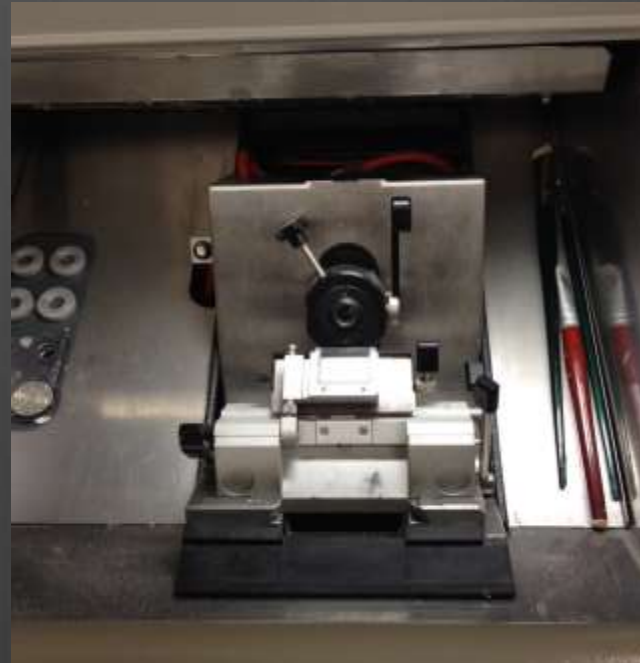
- Arcuate nucleus- site of initiation of puberty
- Kisspeptin neurons have V1aR- AVPV
- Could the same be occurring in arcuate?
- Salt = increased release of vasopressin
- Link between salt and puberty via kiss/NKB



# Methods

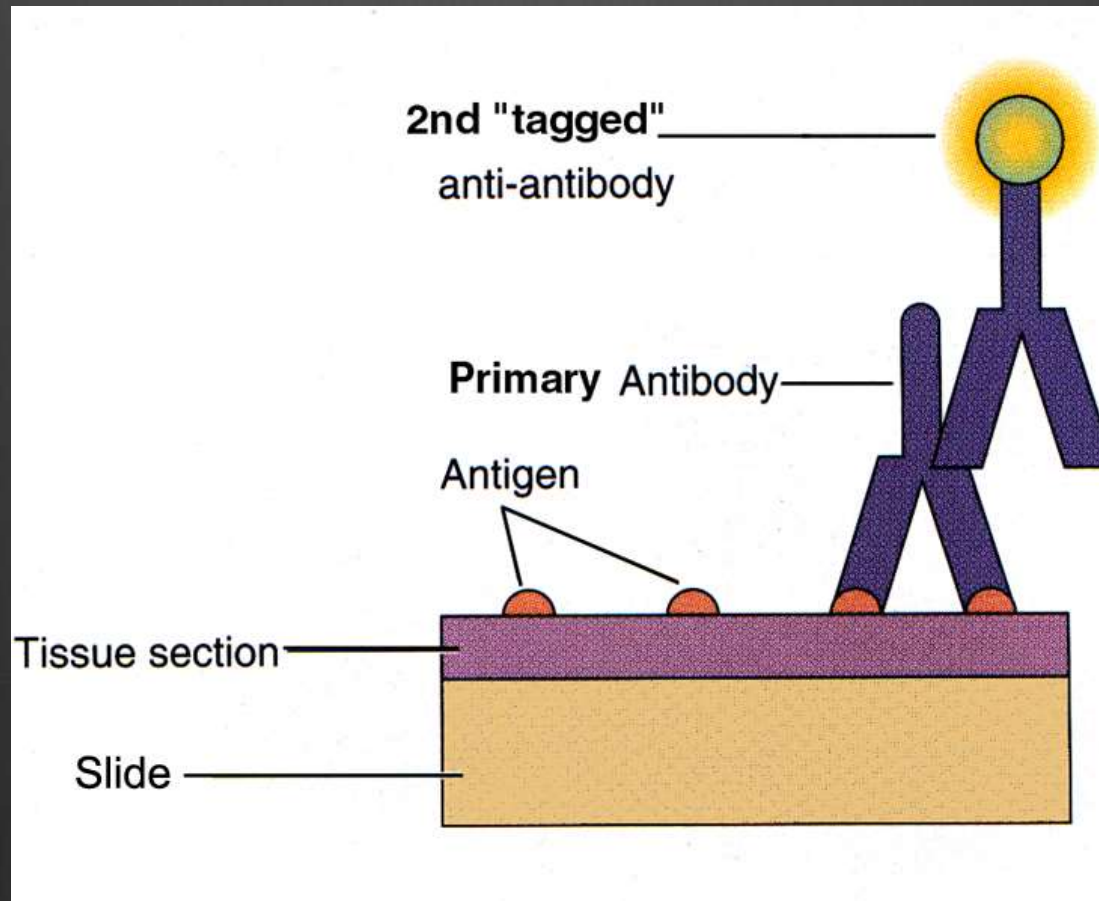
## Brain tissue

- Slicing on the cryostat
- 20 micron slices
- Fixed to slides and labeled for neurotransmitters via immunohistochemistry



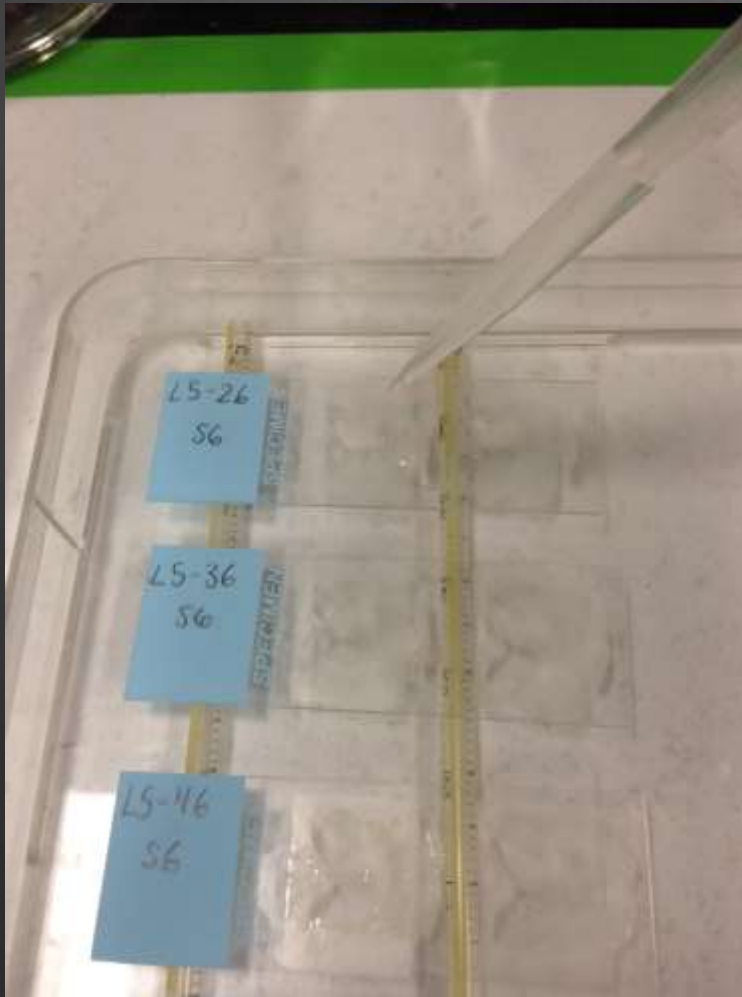
# Immunohistochemistry

- Fluorescence, double label





# Primary Antibody Stain



1% Serum

**Primary  
Antibody**



PBS +  $\text{NaN}_3$

.4% Triton

- 48 hours

Rinse: 1% PBS +  $\text{NaN}_3$

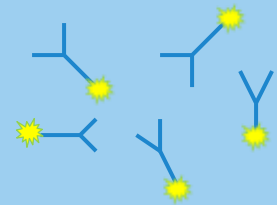
# Secondary Antibody Stain

- Cover-slipped with Vectashield with DAPI



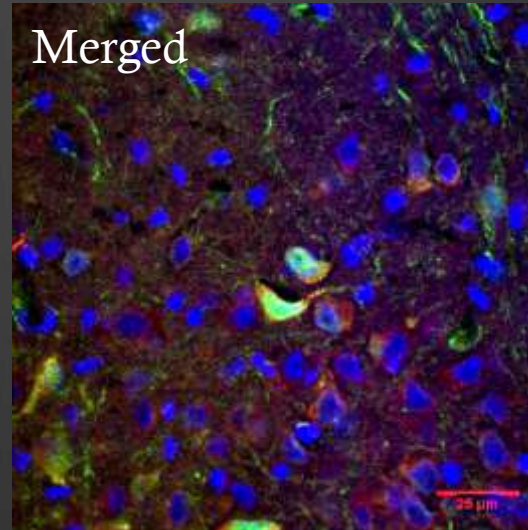
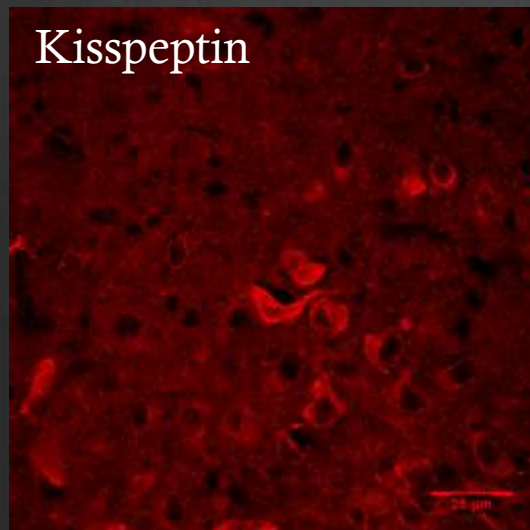
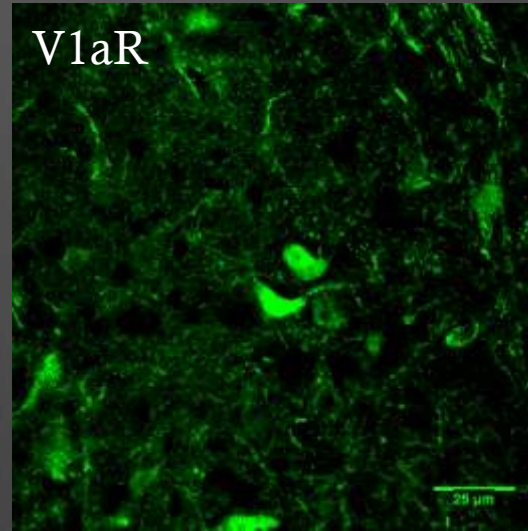
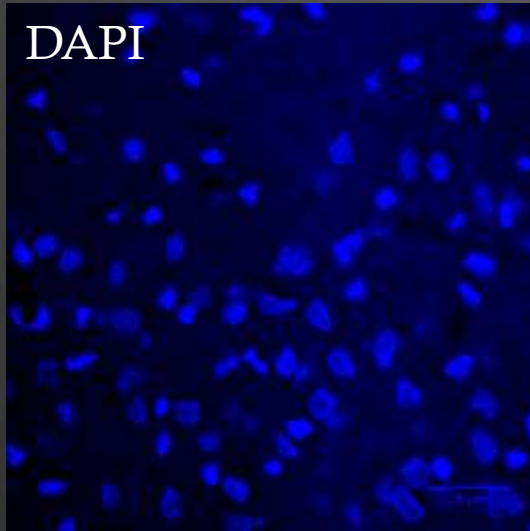
**1% Serum**

**Secondary  
Antibody**



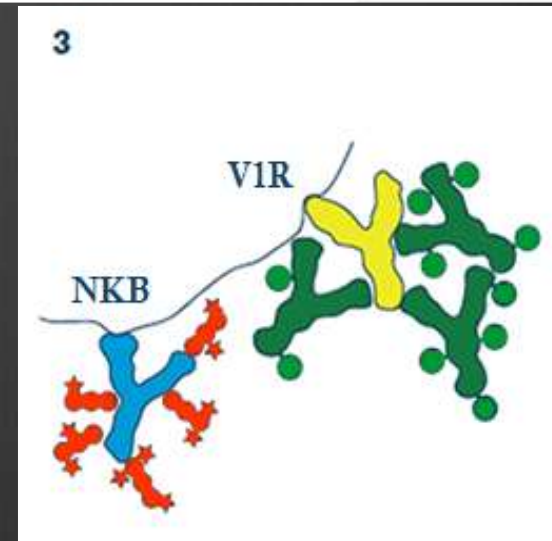
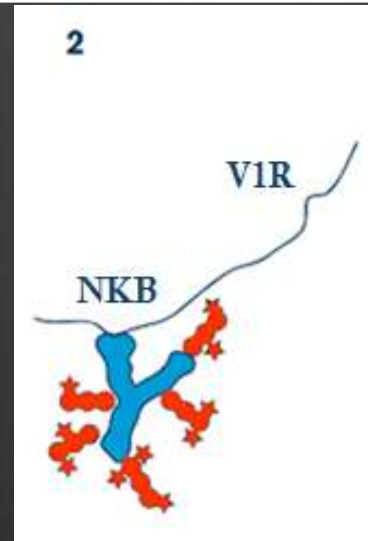
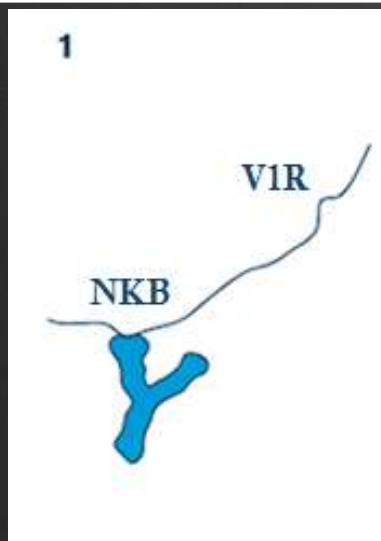
**PBS + NaN<sub>3</sub>  
.4% Triton**

# Results from Kisspeptin/ V1aR double label



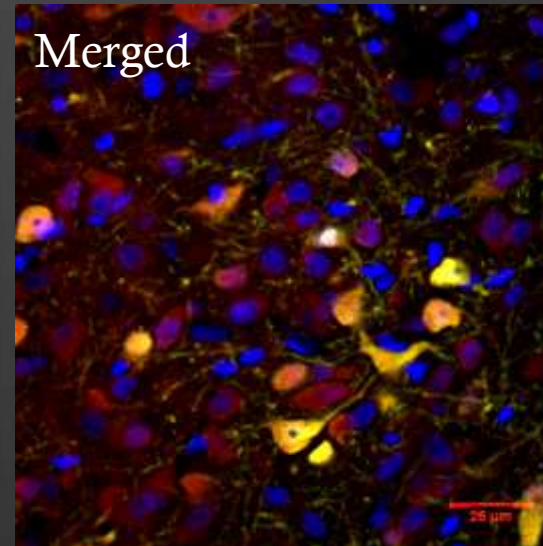
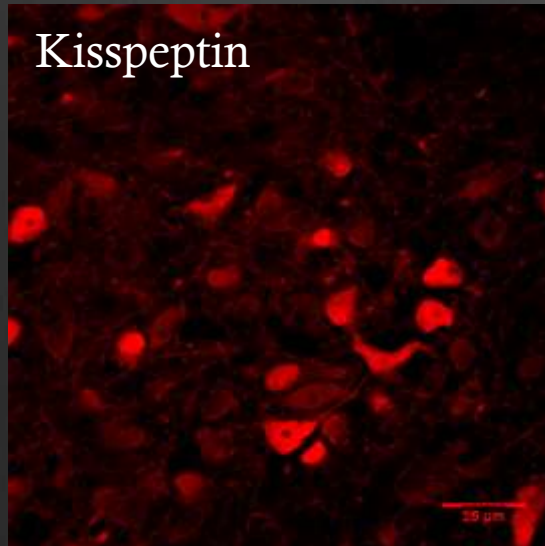
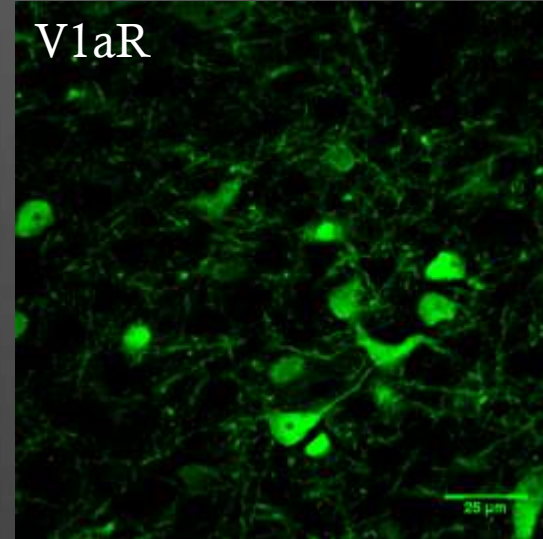
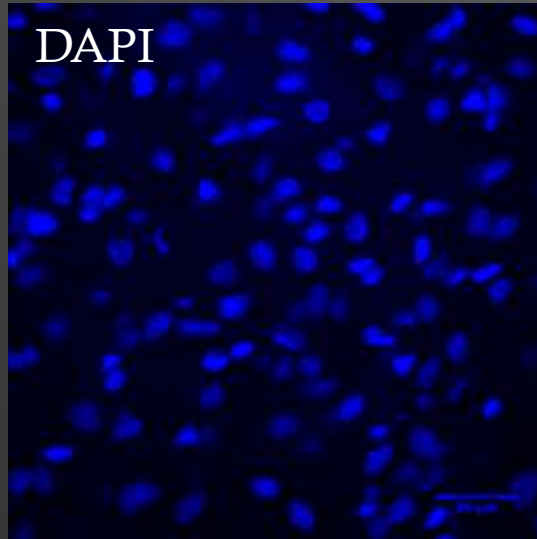
# Neurokinin B/V1aR double label

- Antibodies raised in the same host species
- FAB fragment





# Results of Neurokinin B/V1aR double label



# Results of Neurokinin B/V1aR double label

- Control:
  - Non-specific binding and high background
  - Not all sites covered by FAB



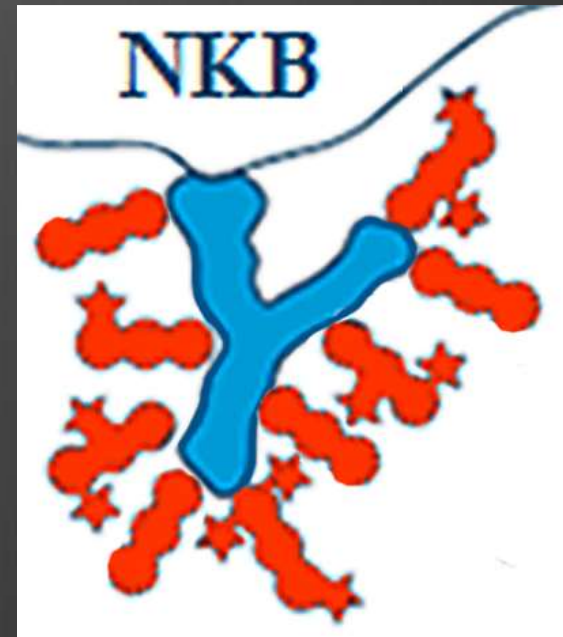
# Unconjugated FAB



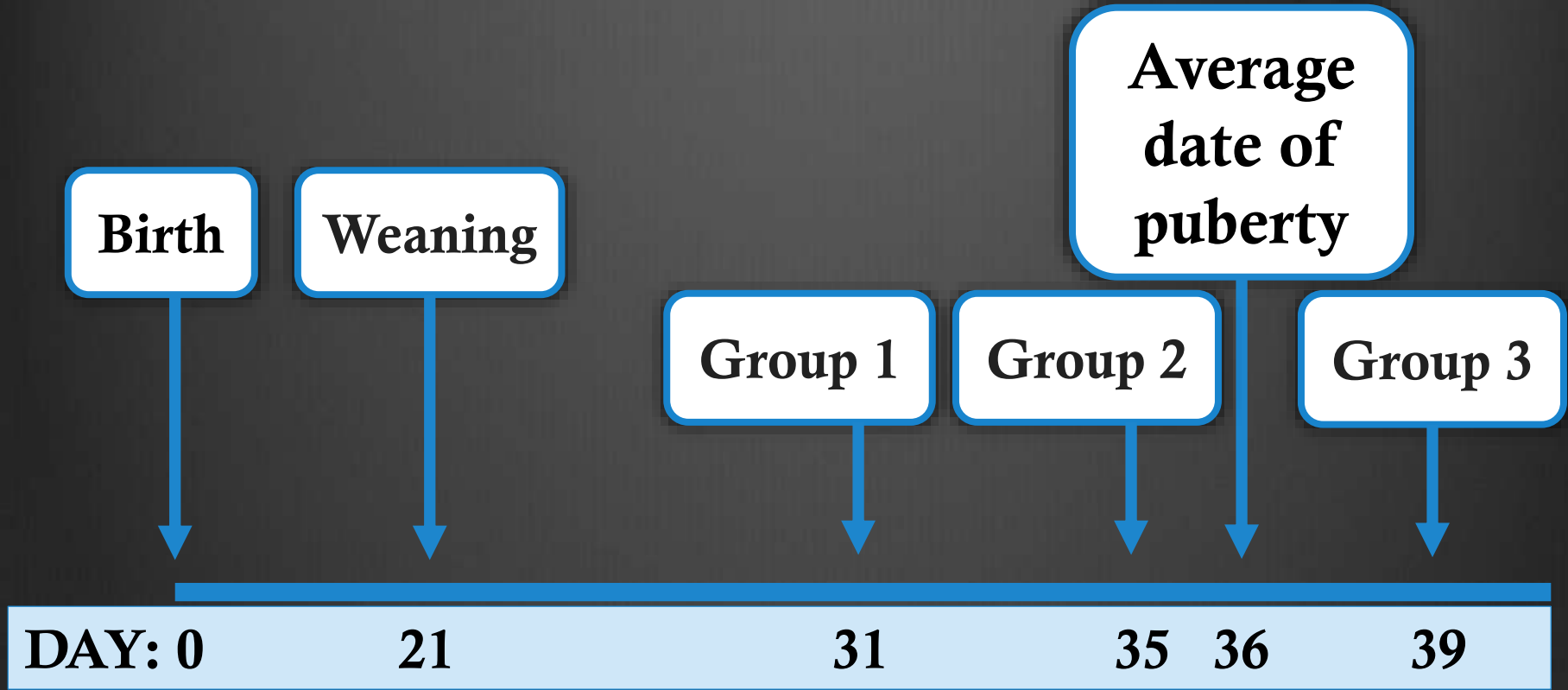
**Unconjugated  
FAB- no  
fluorescence**



**Conjugated  
FAB-  
fluorescence**



# NKB activation in the medial amygdala



**Control**

**2%**

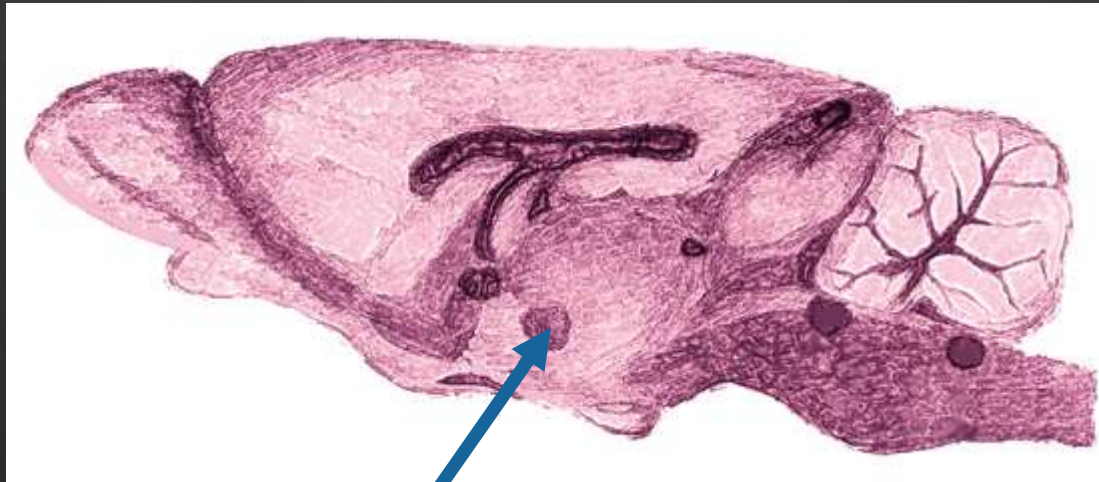
**4%**

**8%**



# Medial Amygdala

- Involved in salt appetite, NK3R
- Sends neuronal projections to GnRH neurons
- Salt can activate NKB system
- Area of integration?



**AMYGDALA**

# Methods

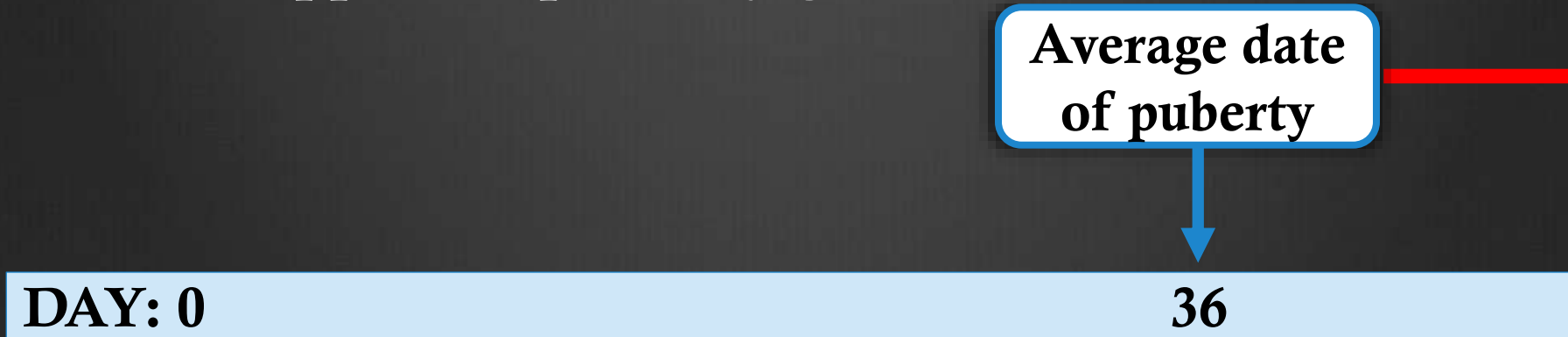
- Weighed and fed every 3<sup>rd</sup> day, food intake and outtake measured
- Check for puberty- vaginal opening
- Brain punch of medial amygdala
- QPCR to analyze NKB/NK3R

# Expected Results

- If salt activates pituitary-gonadal axis



- If salt suppresses pituitary-gonadal axis



- Determine if salt activates or suppresses NKB

# Summary and Future Directions

- Co-localization of kisspeptin and vasopressin in arcuate nucleus
  - Further evaluation needed
- Next- NKB with unconjugated FAB
- Tissue extracted from ~100 animals from time point and salt diet groups.
- QPCR to determine quantity of NKB/NK3R in medial amygdala

# Acknowledgements

- Professor Donal Skinner
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