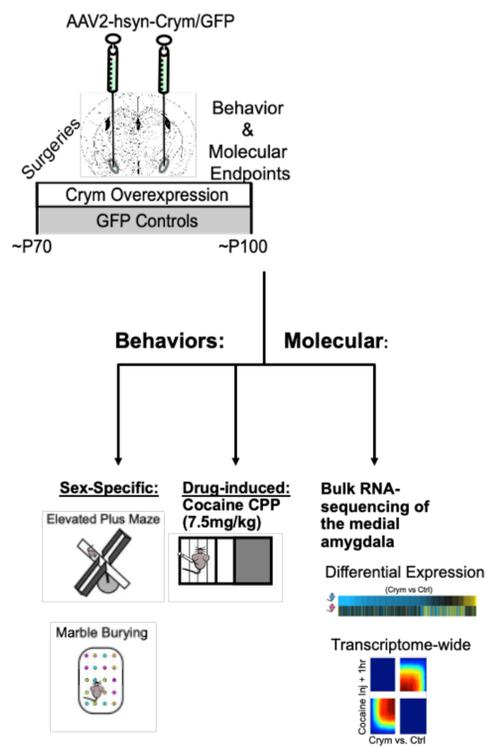


BACKGROUND:

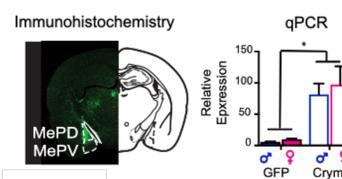
- Hormones play an important role in regulating sex differences in substance use disorder (SUD)
- Hormones also play an important role in natural reward and motivation-related behaviors (e.g. copulation, aggression and parental behaviors)
- Hormones mediate behavior through their actions at their receptors.
 - Nuclear hormone receptors act as epigenetic and transcriptional regulators
- Most studies focused on hormonal regulation of SUD have focused on gonadal hormones
- Thyroid hormone signaling is a likely candidate for regulating sex differences in SUD because of its role in energy and metabolism as many sex-specific motivated behaviors are energetically costly.
- We tested the hypothesis that thyroid hormone signaling plays an important role in the sex-specific behavioral response to cocaine**
- To test this we overexpressed an intracellular thyroid hormone binding protein, Crystallin mu (Crym), in the medial amygdala to disrupt thyroid hormone signaling specifically in a sexually dimorphic reward-associated brain region.**

METHODS :

Experimental Design:



Validation of Targeting and Expression:



RESULTS:

Figure 1. Blocking Thyroid Hormone Signaling through Crym Overexpression in the Medial Amygdala Disrupts Sex-Specific Behaviors

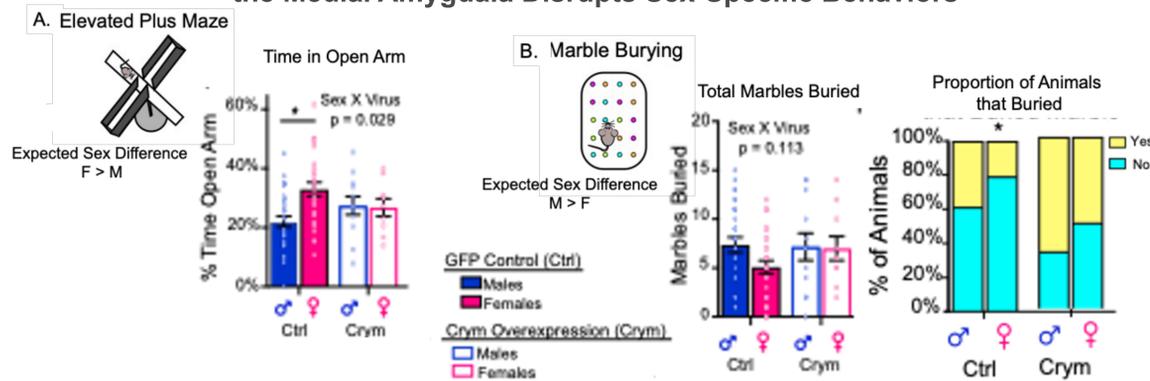


Figure 1: Crym overexpression in the medial amygdala results in a loss of sex-specific behaviors in elevated plus maze (A) and marble burying (B). (A) The predicted sex difference in elevated plus maze behavior was observed in GFP controls (Ctrl; closed bars) with females spending more time in the open arm than males. This sex difference was lost when Crym was overexpressed (open bars). (B) The predicted sex difference in marble burying was also observed in GFP controls with males burying more marbles than females (left). This sex difference was lost when Crym was overexpressed along with a sex differences in the proportion of animals that buried marbles ($p < 0.05$; right).

Figure 2. Blocking Thyroid Hormone Signaling through Crym Overexpression Increases Preference for Cocaine in Males but not Females

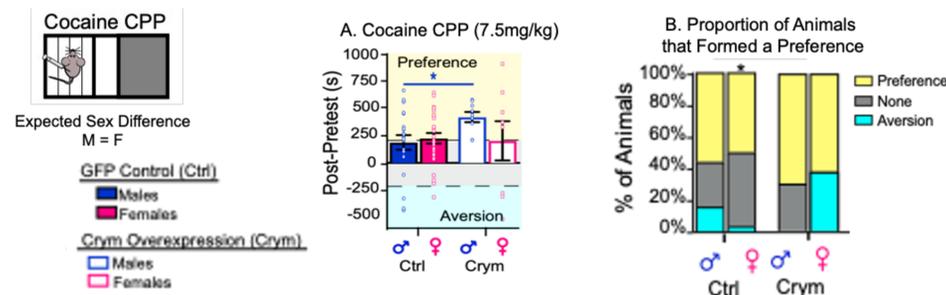


Figure 2. Crym overexpression in the medial amygdala results a sex-specific effect on cocaine induced behavior (A-B). (A) Cocaine sensitivity was assessed via cocaine conditioned place preference (CPP) at a dose of 7.5 mg/kg. Crym overexpression (open bars) increased the preference for cocaine in males when compared to their GFP control (closed bars) counterparts ($p < 0.05$). This was not observed in females (pink). Rather, a bimodal distribution in preference scores was observed after Crym overexpression females - animals either formed a preference (yellow) or an aversion (blue) for cocaine. These effects were observed at the population level as well (right).

Figure 3: Blocking Thyroid Hormone Signaling through Crym Overexpression in the Medial Amygdala Disrupts Sex-Specific Transcription

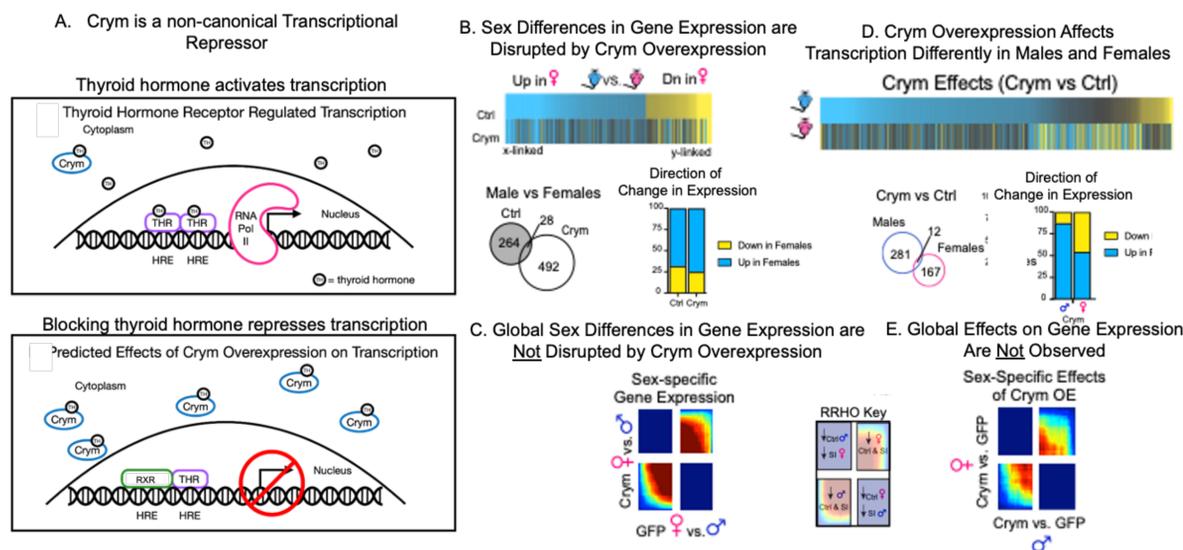


Figure 3. (A) Thyroid hormone mediated transcription occurs when thyroid hormone binds its receptor in the nucleus. Crym acts as a transcriptional repression by sequestering thyroid hormone in the cytoplasm and preventing thyroid hormone mediated transcription. (B) Crym overexpression disrupts sex-specific expression. Heatmap and Venn diagram of sex differences differentially expressed genes (DEGs) in GFP controls (Ctrl) indicate little overlap of DEGs in males vs females after Crym overexpression. (C) Rank rank hypergeometric overlap (RRHO) indicates almost complete overlap of global patterns of sex differences in expression between Ctrl and Crym. (D) Sex-specific analysis of Crym's impact on transcription reveals that males regulate more genes than females and those genes are more likely to be repressed. (E) RRHO reveals subtle effects on transcriptome-wide expression patterns between males and females after Crym overexpression.

RESULTS (cont'd):

Figure 4. Crym Overexpression Induces a Similar Transcriptional Profile to the First Dose of Cocaine in Males but not Females.

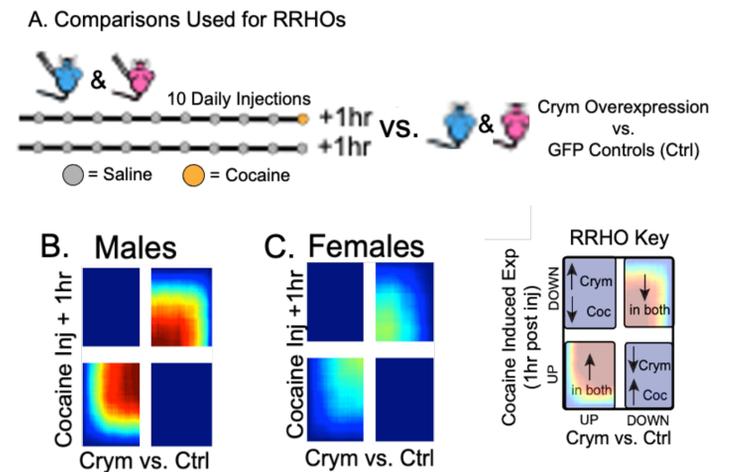


Figure 4. (A) Threshold free comparisons of cocaine induced gene expression patterns compared to those induced by Crym overexpression using rank rank hypergeometric overlap (RRHO). (B) RRHO of Crym induced genes and cocaine-induced genes reveals that transcriptional patterns after Crym overexpression closely reflect those induced by the first dose of cocaine in males but not females.

SUMMARY & CONCLUSIONS:

Crym Effects on Behavior:

- Crym overexpression in the medial amygdala results in a loss of sex differences in known sex-specific behaviors
- Crym overexpression in the medial amygdala results in an increase in cocaine preference in males but not females
- Females display a bimodal distribution of cocaine induced behavior - either a preference or aversion.

Crym Effects on Transcription:

- Crym overexpression disrupts baseline sex differences in DEGs but does not disrupt global sex-specific transcriptional patterns.
- Crym overexpression results in sex-specific expression changes in males and females and results in more transcriptional repression in males.
- Crym overexpression results in a transcriptional pattern similar to that induced by the first dose of cocaine in males but not females.
- The transcriptional changes induced by Crym overexpression reflect the behavioral impacts of Crym overexpression

Conclusions:

- Blocking thyroid hormone through Crym overexpression yields sex-specific effects on behavior and transcription.
- Thyroid hormone signaling in the medial amygdala may be important for sex-specific behavior
- Thyroid hormone mediated transcription may be regulated in a sex-specific manner.

Future Directions:

- Our lab is currently investigating the epigenetic mechanisms underlying sex differences in thyroid hormone regulation of behavior and transcription.

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