The PTSD-Associated Neuropeptide PACAP Modulates Fear Learning Through Sex-Specific Action in the Prefrontal Cortex

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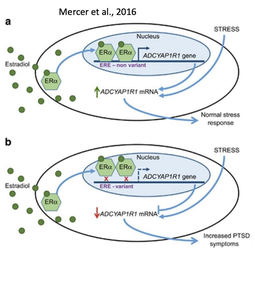
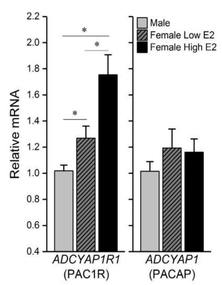
Introduction

Post traumatic stress disorder (PTSD) affects millions of people, but has few effective treatments. The reason for lack of treatments is in part because PTSD's underlying neurobiology and mechanisms for sex differences are poorly understood. PTSD, like other stress-related disorders has a **sex bias**. The lifetime prevalence of PTSD in women (10-12%) is **double** of that in men (5-6%) (Olff, 2017). Studying sex differences in PTSD can provide **novel insi**ght into how a traumatic experience can alter the brain's fear circuitry to lead to long-lasting maladaptive fear states.

Civilian vs. Veterans	Gender	One-Year Prevalence	Lifetime Prevalence
	Female	6%	8%
	Male	2.6%	4.1%
	Female	11.7%	13.4%
	Male	6.7%	7.7%

Estradiol Promotes the Expression of the PACAP Receptor PAC1R

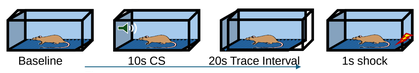




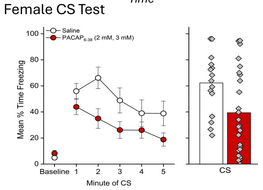
During the proestrus phase of a rats estrous cycle, estradiol (E2) peaks. When E2 levels are high, E2 binds to its receptor creating a dimer thus increasing signaling for the stress-related neuropeptide PACAP. As a result, PACAP has a more robust effect in high E states.

Blocking Prefrontal PACAP Impairs Fear Memory in Females but not Males

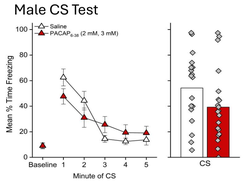
Trace Fear Conditioning



Female CS Test



Male CS Test



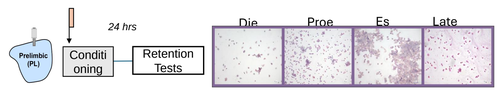
Administration of a PACAP antagonist to **females** decreases freezing levels to the conditioned stimulus compared to saline controls

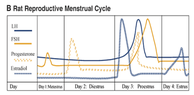
Administration of a PACAP antagonist to **males** does not change freezing levels to the conditioned stimulus compared to saline controls

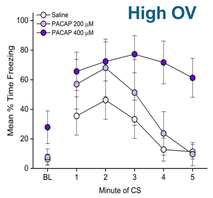
Research Questions: Here we investigate the complex relationship between PACAP, ovarian hormone states and fear learning behavior. We probe the spatial dynamics of PAC1R across the estrous cycle using **RNAscope:** a multiplexing RNA visualization technique. We investigate potential **colocalization of PAC1R mRNA with vGLUT, VGAT, ERα and ERβ.** Additionally, we inject a fluorescent retrograde tracer virus combined with using an immunohistochemistry approach to identify potential regions of PACAP projection to the prelimbic cortex (PL).

PACAP Administration Alters Fear Memory in an Estrous State Dependent Manner

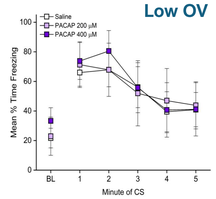
Administering PACAP to the PFC Enhances Cued Fear Memory in High Estradiol Females



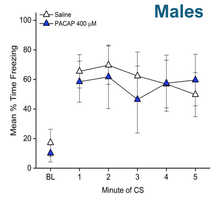




Administration of a **PACAP agonist increases** freezing levels to the conditioned stimulus compared to saline control when females are *trained* in a **high estradiol state**

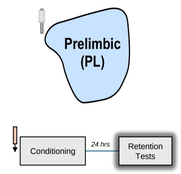


Administration of **PACAP agonist does not significantly increase** freezing to the conditioned stimulus to females *trained* in a low estradiol state



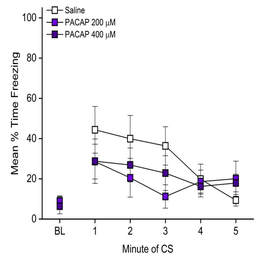
Administration of **PACAP agonist does not significantly increase** freezing to the conditioned stimulus in **males**

PACAP does not Enhance Fear in Low OV state, even in weaker training conditions

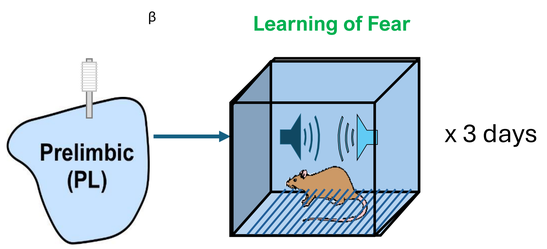


Low-OV rats were trained with only 2 CS-trace-UCS pairings, using a weaker shock (0.5 mA). This lowered learned fear levels to those typical of High-OV females, but PACAP did not enhance fear learning, even at a higher dose.

Weak training paradigm



PACAP impairs the ability of rats to discriminate between safety and threat

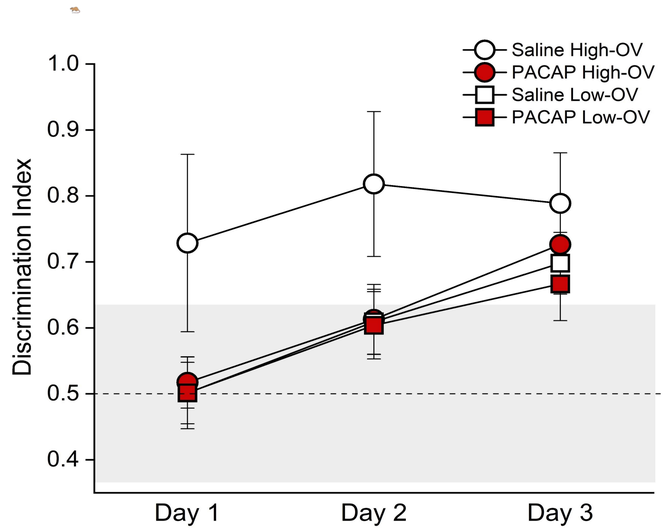


Pseudorandom presentation of CS+ and CS-
CS+ 10s WN
CS- 10s Tone
UCS 1s 0.5 mA shock

Estrous Cycle Tracking began 6 days before start of experiment

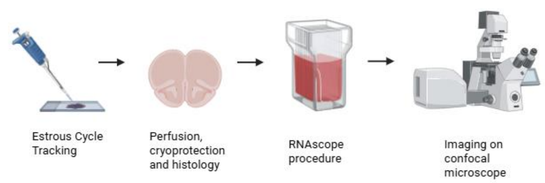
CS + : Indicates shock is coming

CS -: Indicates no shock is coming



Expression patterns of PAC1R colocalization across the estrous cycle

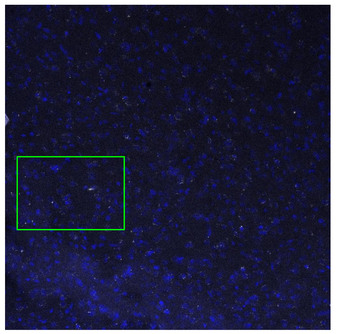
Estradiol has been found to regulate the transcription of PAC1R. We observe fear-modulating effects of PACAP only in females in states of elevated circulating ovarian hormones. We currently investigate PAC1R expression in specific cell types within the PL and how expression changes across the estrous cycle. We aim to reveal how cortical circuits become sensitive to PACAP signaling and influence memory circuits. Here we use RNAscope in the prelimbic region to determine if PAC1R is co-expressed with beta estrogen receptors (ERβs) and VGAT, a marker of GABAergic cells. We hypothesize that we will see high colocalization of PAC1R with Erβs because they are the predominant type of estrogen receptor in the PL, and it has been shown that high E states are associated with higher PAC1R expression. We will continue to investigate the expression of PAC1R in different neuron types to elucidate signaling dynamics in PACAPS role in fear learning.



RNAscope methods: Animals were handled and given daily vaginal lavages for estrous cycle tracking 10-12 days before transcardial perfusion. Brains were rapidly dissected, fixed, cryoprotected and frozen then sliced and mounted according to ACD Bio recommendations. RNAscope staining procedure was completed within a week of histological preparation and followed by imaging on a Nikon A1R + laser scanning confocal microscope. Images were analyzed using QuPath software

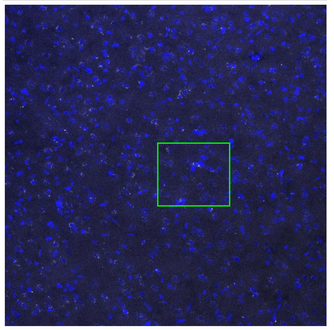
Expression patterns of PAC1R across the estrous cycle

High E



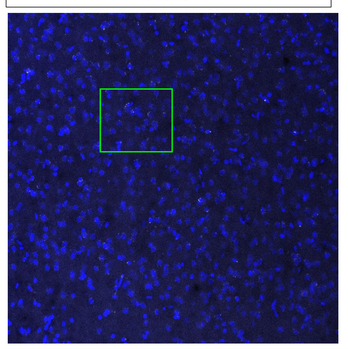
Tissue from rat in high E state.
Mean number of single spots for PAC1R per cell **0.542**
Mean number of clusters for PAC1R per cell **1.222**

Low E



Tissue from rat in low E state.
Mean number of single spots for PAC1R per cell **0.31**
Mean number of clusters for PAC1R per cell **1.11**

Male



Tissue from a male rat.
Mean number of single spots for PAC1R per cell **0.133**
Mean number of clusters for PAC1R per cell **1.047**

Conclusions and Future Directions

- Patterns we observe here are similar to those described in Ressler et al, 2011. Disruptions in PACAP signaling enhance fear to threat-predictive cues and impair cue discrimination.
- This suggests the PL is a key site of action of PACAP in mediating learning and attention-based symptoms.
- We have found that females in elevated OV hormone states are most susceptible to disruptions in PACAP and females in low OV hormone states and males are less suspectable
- Next we will optimize our labs use of RNAscope procedures to determine the expression of PAC1R across the estrous cycle with cell type specific markers and estrogen receptors
- We will carry out our immunohistochemistry study to determine the source of PACAP to the PL

Acknowledgements

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