# Understanding Sex Differences of eCB Modulation Through Fear Conditioning On Adolescent Rats

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## Background

- The endocannabinoid system regulates bodily functions through interactions with receptors CB1 and CB2.
- Fear learning, also known as aversive learning, is dependent on the function of the amygdala and hypothalamus.
  - CB1 receptors are populated here and modulates activity, which modulates fear behavior.
- Purpose of fear conditioning: endocannabinoid signaling in the hippocampus and amygdala modulates fear learning behavior and memory.
- Male rats exhibit higher CB1 receptor binding sites than females, but females have more efficient CB1 receptors
  - There are sex differences in the endocannabinoid system and in fear learning behavior.

## Methodology

Surgical Procedure & Drug Injection

One rat at a time (Female (9 vehicle, 6 JZL)) and male (9 vehicle, 7 JZL)) was handled from the cages and anesthetized under Isoflurane (75 mg/kg BW) anesthesia. The rat was then mounted in a stereotaxic instrument with anesthesia facilitating through the mouthpiece. All surgical procedures abided by aseptic, sterile conditions. Two bilateral holes were drilled in the skull to correspond to the dorsal hippocampus (Stereotaxic coordinates: anterior -3.5; lateral +/– 2.5; ventral -2.5), along with two other holes for the screws. One cannula with two stainless steel needles was implanted, secured with dental cement, and capped. Rodents recovered 5-7 days prior to fear conditioning and given meloxicam. For vehicle and JZL 184 drug delivery, an infusion cannula was connected to a polyethylene tube to a 5µl micro syringe. Using an infusion pump, bilateral injections were injected into the dorsal hippocampus with the cannulas at a rate of 5µl/min. The infusion cannula remained in place for another minute to allow for diffusion and minimize backflow.

Fear Conditioning

On day 1 (Acquisition Day), male rats were injected with the drug JZL 184 or no drug: one rat was then placed in each of the conditioning chambers (3 total) per trial for 10 minutes. A mild electric shock of 0.8 milliamps was given at minutes 7, 8, and 9 for 2 seconds. On day 2 (Recall Day), male rats were placed in their same cages, given a vehicle, and subjected to the same time interval but with no mild shock. For Day 1 the rat associated the shock with the box and in Day 2 the strength of memory recall was measured. For data collection, freezing was recorded and measured as an absence of movement for at least 3 seconds. The percentage of time frozen was quantified for a minute over 10 minutes. This experiment lasted for ~ 10 weeks.

## Research Question

Hypothesis: Sex differences in endocannabinoid signaling contributes to sex differences in fear learning.

## Purpose

To provide insight on the endocannabinoid system and how neurological disorders work pathologically.

## Results of Memory Formation

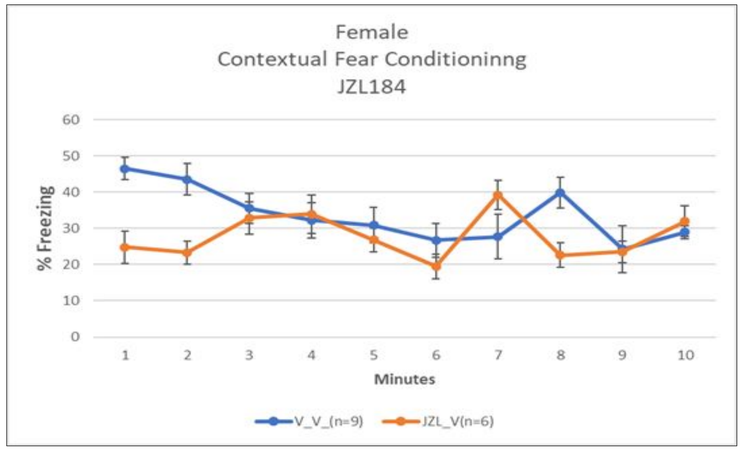


**Figure 1.** Female Contextual Fear Conditioning. Data representing the mean percent time female adolescent rats were freezing during memory recall day (day 2).

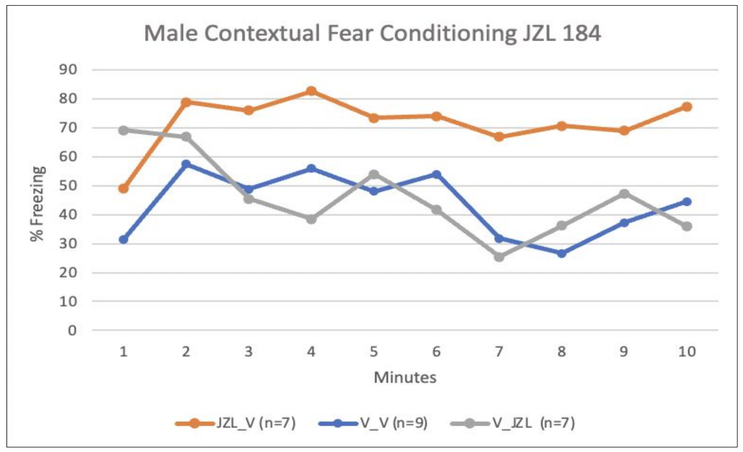


**Figure 2.** Male Contextual Fear Conditioning. Data representing the mean percent time male adolescent rats were freezing during memory recall and acquisition day (day 2).

## Discussion

- Lower threshold: synaptic connections in female rats may be more easily activated or more sensitive to stimuli, which could impact their behavioral responses.
- Looked at memory formation of male and female adolescent rats during fear conditioning, specifically when the JZL 184 drug was injected on day 1 to increase endocannabinoids.
- With the increase of endocannabinoid levels, there is a weaker memory in female rats compared to control female rats **(Figure 1);** there is a stronger memory in male rats compared to the control male rats regardless of drug administration on acquisition or recall day **(Figure 2).**
- The rats with JZL 184 did not extinguish despite having low freezing levels **(Figure 1**); JZ_V males do not show short-term extinction while V_V and V_JZL males do **(Figure 2).**
- Differences in synaptic activity thresholds between male and female rats may contribute to variations in their learning, memory, and emotional responses.

## Future Implications

Will look at the memory retrieval on day 2 when the rats receive JZL 184 on the recall day prior to already establishing that association between the box and the shock on day 1: how is the low endocannabinoid (on day 1) affecting the retrieval of that memory and translating that into freezing behavior?

## References

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