Effects of Chronic Mild Stress and CB1 Receptor Signaling on Trace Fear Conditioning in Adolescent Female Rats

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Background

Endocannabinoids (eCBs) mediate a variety of behavioral phenomena including learning and memory, mood, aggression, stress and feeding (Martin et al., 2002).

Chronic-Mild-Unpredictable-Stress (CMUS), an animal model of depression, *downregulates* hippocampal CB1 receptors (CB1) in young adult male rats and *upregulates* CB1 receptors in female rats (Reich et al., 2009).

CMUS enhances hippocampal-dependent fear conditioning in adolescent male rats. Exogenous CB1 activation rescues stress-induced fear enhancement and facilitates extinction in both stress and non-stress male rats. (Reich et al., 2013)

We now hypothesize that CMUS will enhance fear conditioning in female rats, however exogenous CB1 activation will be ineffective in acquisition, recall and extinction in reducing stress-enhanced fear due to the sex differences in CB1 expression.

Methods

Subjects

Adolescent female (35 days old at start of CMUS) Sprague-Dawley rats (Charles River, Boston, MA) were group-caged (3) in hanging plastic cages upon arrival in the Ramapo College animal facility for 5 days.

Chronic Mild Stress

Animals were subjected to either the CMUS protocol (Table 1) or the non-stress protocol (handled daily). The complete regimen lasted 7 days/wk for 3 wks. Individually, no stressor was severe, and the unpredictability of the protocol are thought to constitute much of the stress.

Week #	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday	Sunday
1	Strobe light, Food deprivation	Restraint, Social Isolation	Cage Rotation, Forced Swim	Strobe, Restraint, Food and Water Deprivation	Cage Rotation, Social Isolation	Strobe, Restraint, Water Deprivation	Restraint, Cage Rotation
2	Strobe light, Food deprivation	Restraint, Social Isolation	Cage Rotation, Forced Swim	Strobe, Restraint, Food and Water Deprivation	Cage Rotation, Social Isolation	Strobe, Restraint, Water Deprivation	Restraint, Cage Rotation
3	Strobe light, Food deprivation	Restraint, Social Isolation	Cage Rotation, Forced Swim	Strobe, Restraint, Food and Water Deprivation	Cage Rotation, Social Isolation	Strobe, Restraint, Water Deprivation	Restraint, Cage Rotation

Fear Conditioning

Twenty-four hours after the last stressor, all animals underwent fear conditioning. Animals were placed into one of three classical conditioning chambers with a stainless-grid floor. Animals were monitored for freezing behavior as an index of a fear response via individual CCD cameras (Coulbourn Instruments, White Hall, PA). Freezing was defined as absence of all movement except respiration for ≥ 3 sec.

Trace: Acquisition consisted of 3 trials with 15 sec, CS tone (1000 Hz, 80 dB), 30 sec trace interval and a 2 sec US footshock (0.6 mA). Inter-trial-interval (ITI) was 3 min. Recall/Extinction was assessed 24 hours after acquisition by placing animals in altered chambers (plexiglass floor, triangle shape, different visual and olfactory cues. Trials consisted of 5 CS-only presentations with a 3 min ITI.

Pharmacological Treatment: Subgroups of animals were either injected with the CB1 agonist ACEA (0.1 mg/kg, i.p., Tocris) or Vehicle (physiological saline and DMSO, 3:1 ratio) solution 20 min prior to experimental procedures. Statistical comparisons were performed using one-way ANOVAs or repeated measures MANOVA (SPSS).

CMS Modulates Trace Fear Conditioning

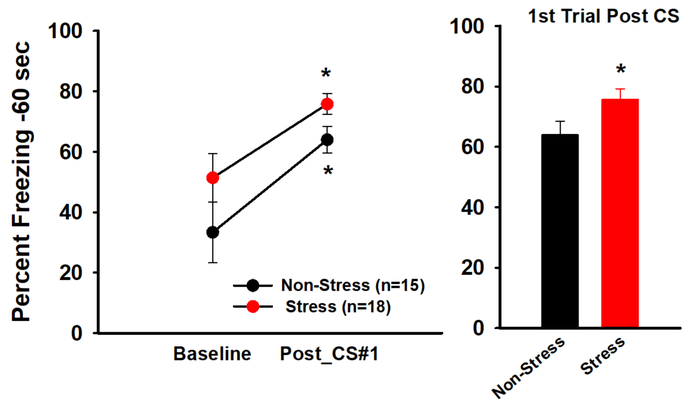

Figure 1. CMUS Enhances Generalized (Baseline) and Cued Freezing in Female Rats.

* - indicate differences between Stress (S) and Non-Stress (NS) or between Baseline and Cued Freezing during the first recall trial,

($p < 0.05$).

CB1 Activation Impairs Long-Term Extinction (LTE) in Females

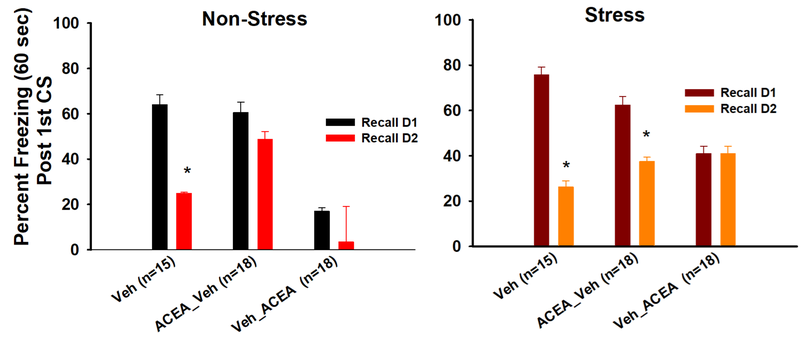

Figure 3. LTE is observed in both NS and S: Veh females, however, ACEA appears to impair extinction in NS and S: ACEA_Veh) and Veh_ACEA groups.

* indicate differences between Recall Day 1 (D1) and Recall Day 2 (D2), ($p < 0.05$).

The CB1 agonist ACEA (0.1 mg/kg) modulates Generalized and Cued Freezing Responses

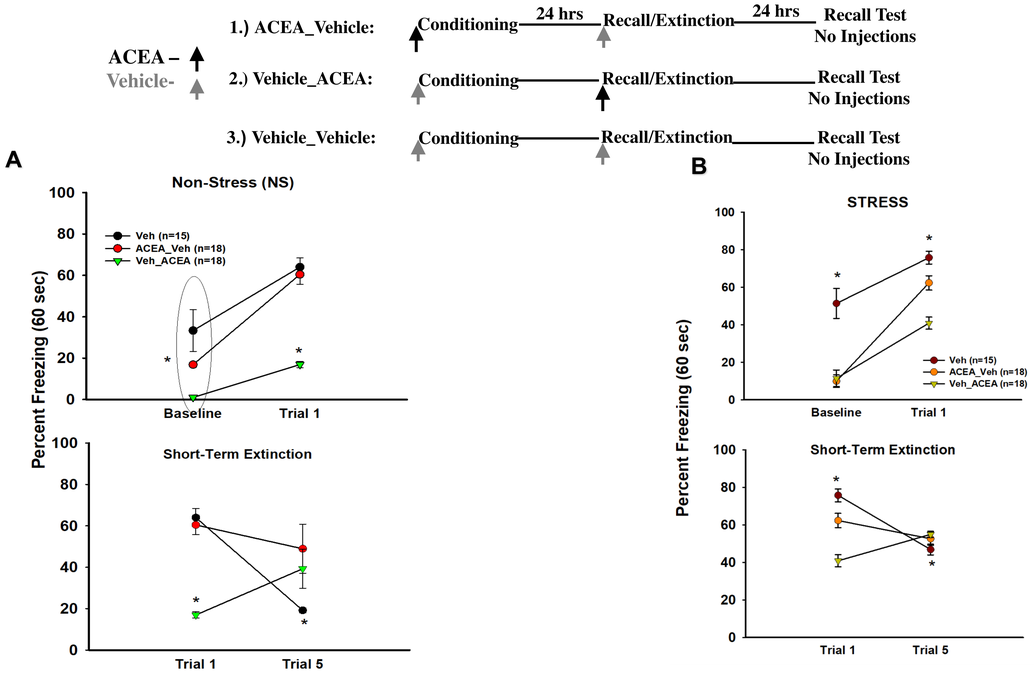

Figure 2A and 2B: ACEA decreases both Generalized Fear and Cued Fear Recall (Veh_ACEA) in both NS and S females. *However, ACEA only decreases Cued Fear Acquisition (ACEA_Veh) in S females!* CB1 activation does not facilitate and may impair Short-Term Extinction in either NS or S females! * indicate differences between drug groups or between Trial 1 and Trial 5, ($p < 0.05$).

Summary and Conclusions

- CMUS *enhances* Generalized and Trace-cued freezing in adolescent female rats.
- CB1 agonist ACEA decreases Generalized fear in both Non-Stress and Stress females.
- ACEA administered prior to cued fear recall impairs freezing in Non-Stress females. ACEA prior to acquisition does not affect freezing.
- ACEA administered prior to trace acquisition in Stress females modestly attenuates cued freezing during a recall test.
- ACEA does not facilitate or may impair Short-Term Extinction in both Non-Stress or Stress females.
- ACEA IMPAIRS Long-Term-Extinction in both Non-Stress or Stress females!! This is in stark contrast to male rats.

References

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Reich CG, Taylor, ME, McCarthy, MM. (2009) Differential effects of chronic unpredictable stress on hippocampal CB1 receptors in male and female rats. Behav. Brain Res. 203(2): 264-69.

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Acknowledgements

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