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 (CMSU), an animal model of depression, downregulates hippocampal CB1 receptors in young adult male rats and upregulates CB1 receptors in female rats (Reich et al., 2009).

CMSU 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 CMSU 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 CMSU) 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 CMSU protocol (Table 1) or the non-stress protocol (handled daily). The complete regimen lasted 7 days/week for 3 wks. Individually, no stressor was present, and the unpredictability of the protocol is thought to constitute much of the stress.

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 2 sec.

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).

Figure 1. CMS 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).

Figure 2

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

1) ACEA_Veh: Conditioning 24 hrs Recall/Extinction 24 hrs Recall Test No Injections

2) Vehicle,ACEA: Conditioning Recall/Extinction Recall Test No Injections

3) Vehicle, ACEA: Conditioning Recall/Extinction Recall Test No Injections

B: 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 NS females!

CB1 activation does not facilitate or may impair Short-Term Extinction in both Non-Stress of Stress females!

Figure 3

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

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

Summary and Conclusions

- CMSU 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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