

## Supplementary Materials for

### **Endocannabinoid genetic variation enhances vulnerability to THC reward in adolescent female mice**

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#### **This PDF file includes:**

Fig. S1. Adolescent female mice carrying the FAAH SNP demonstrate no change in CB<sub>1</sub>R-labeled terminals forming symmetric or asymmetric synapses in the parabrachial subregion of the VTA.

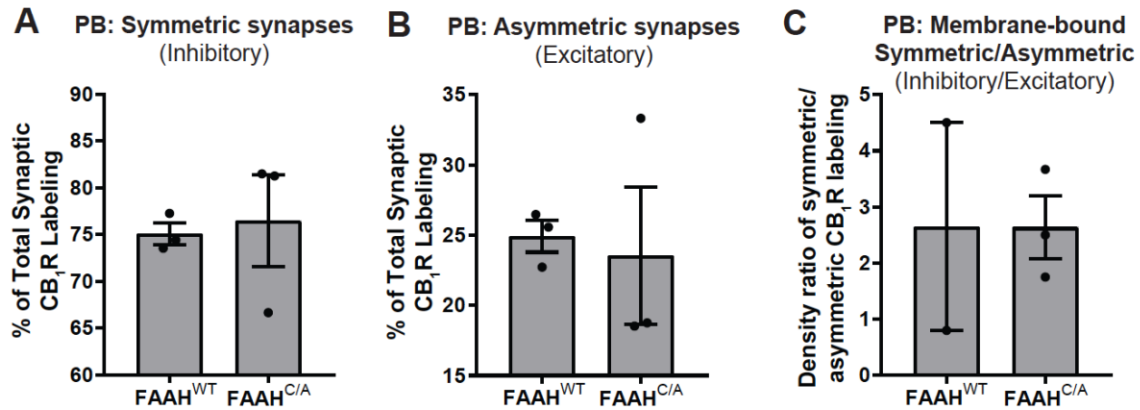
Fig. S2. THC CPP dose response in adolescent female mice carrying the FAAH SNP.

Fig. S3. Adolescent and adult FAAH<sup>C/C</sup> and FAAH<sup>C/A</sup> mice show preference for cocaine CPP.

Fig. S4. Pharmacological inhibition of FAAH in WT mice is not sufficient to reproduce rewarding effect of THC seen in adolescent female mice with the FAAH SNP.

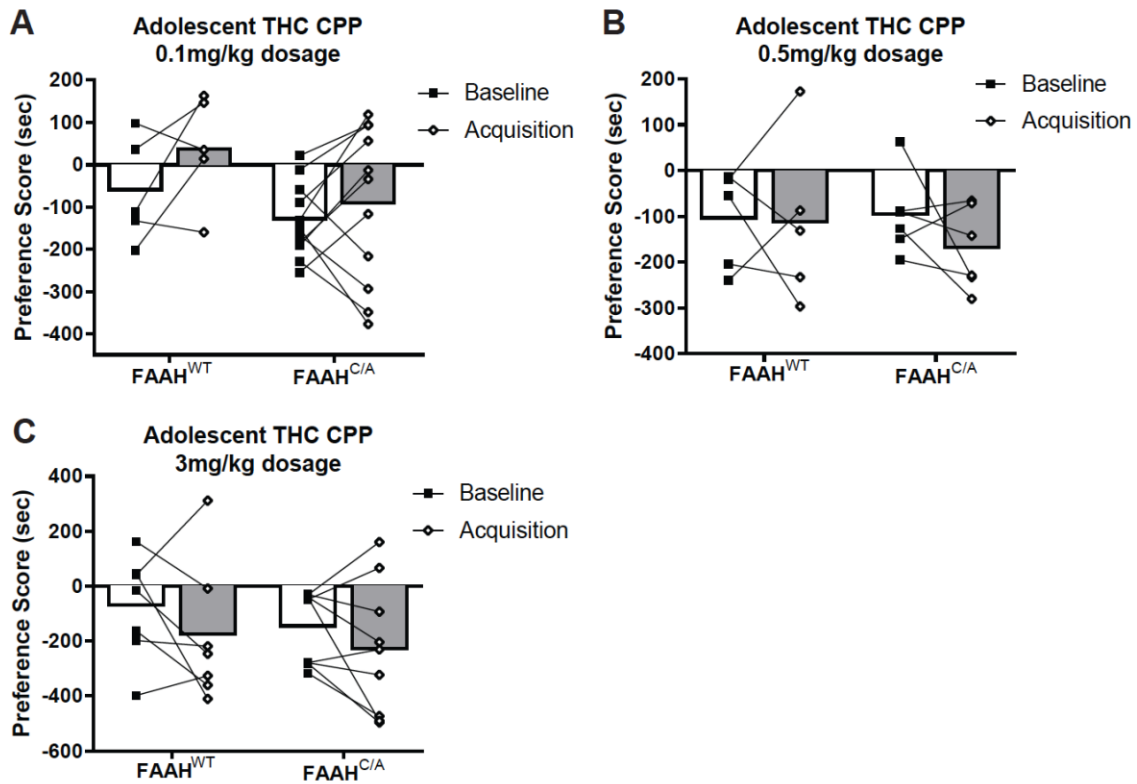
Fig. S5. THC CPP during adulthood does not result in a preference for THC in female mice carrying the FAAH SNP.

## Supplemental Figure 1



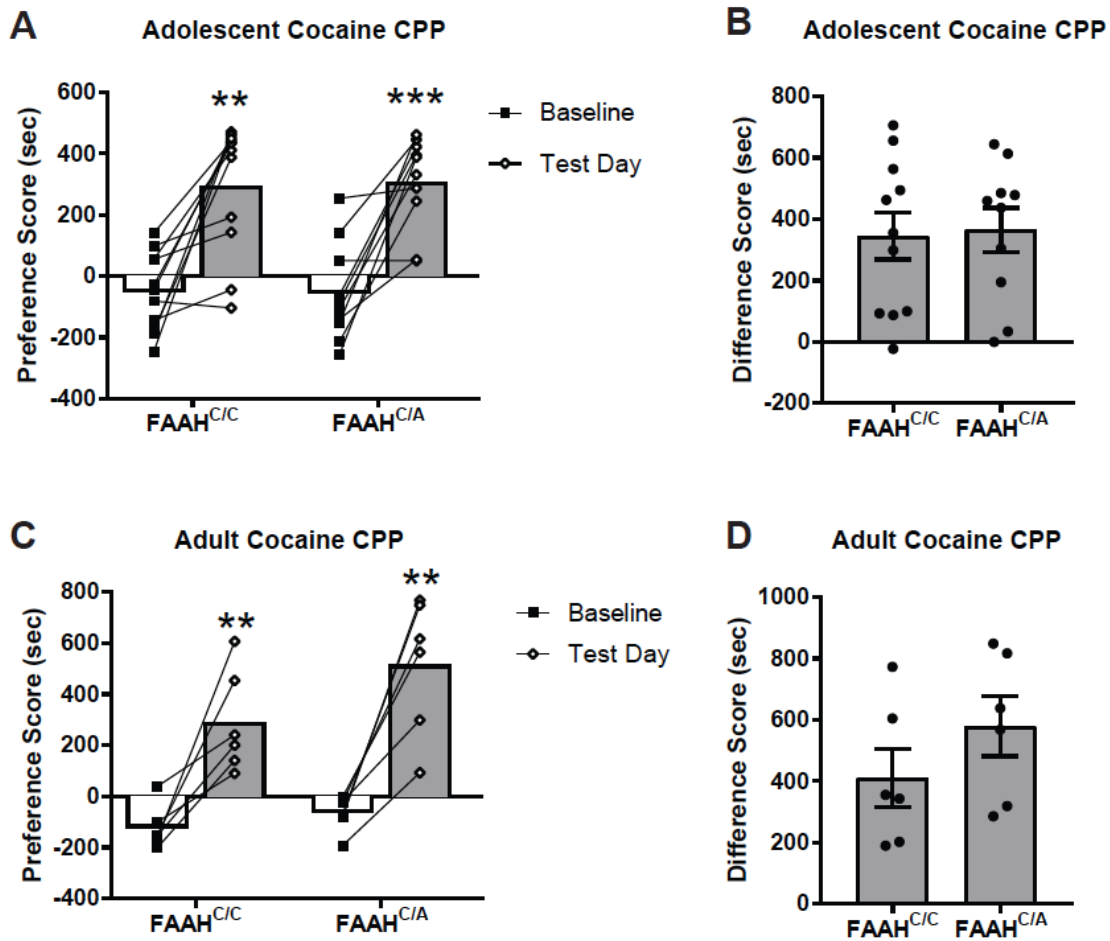
**Fig. S1. Adolescent female mice carrying the FAAH SNP demonstrate no change in CB<sub>1</sub>R-labeled terminals forming symmetric or asymmetric synapses in the parabrachial subregion of the VTA.** (A) Adolescent female FAAH<sup>C/A</sup> mice have similar percentage of CB<sub>1</sub>R-labeled terminals forming symmetric synapses compared to adolescent female FAAH<sup>C/C</sup> mice (Unpaired t-test,  $t_{(4)} = 0.2769$ ,  $p = 0.7956$ , FAAH<sup>C/C</sup>: N=3 animals, n=42-78 labels characterized/animal. FAAH<sup>C/A</sup>: N=3 animals, n=31-47 labels characterized/animal). (B) Adolescent female FAAH<sup>C/A</sup> mice have similar percentage of CB<sub>1</sub>R-labeled terminals forming asymmetric synapses compared to adolescent female FAAH<sup>C/C</sup> mice (Unpaired t-test,  $t_{(4)} = 0.2769$ ,  $p = 0.7956$ , FAAH<sup>C/C</sup>: N=3 animals, n=42-78 labels characterized/animal; FAAH<sup>C/A</sup>: N=3 animals, n=31-47 labels characterized/animal). (C) Adolescent female FAAH<sup>C/A</sup> mice have a similar ratio of membrane-bound CB<sub>1</sub>Rs on terminals forming symmetric synapses versus asymmetric synapses compared to adolescent female FAAH<sup>C/C</sup> mice (Unpaired t-test,  $t_{(3)} = 0.007143$ ,  $p = 0.9947$ , FAAH<sup>C/C</sup>: N=2 animals, n=42-78 labels characterized/animal; FAAH<sup>C/A</sup>: N=3 animals, n=31-47 labels characterized/animal).

## Supplemental Figure 2



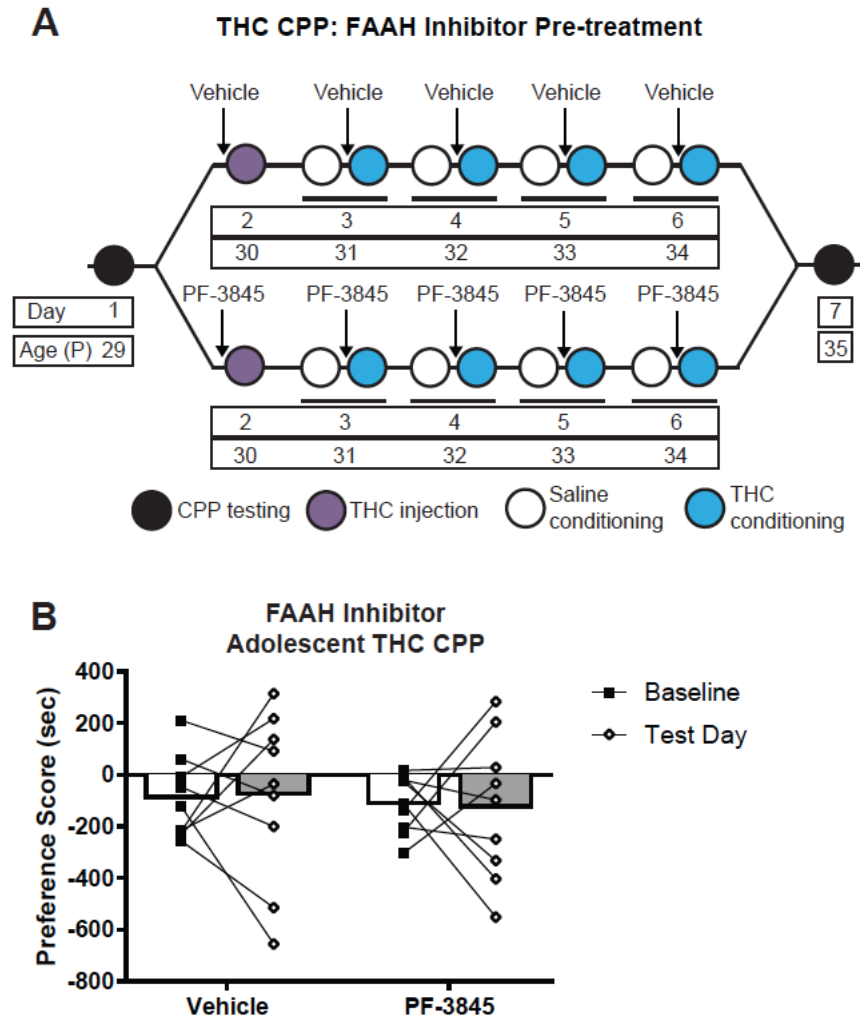
**Fig. S2. THC CPP dose response in adolescent female mice carrying the FAAH SNP. (A-C)** Adolescent female FAAH<sup>C/C</sup> and FAAH<sup>C/A</sup> mice show no change in preference for the THC-paired chamber on the test day compared to the baseline test when trained with 0.1mg/kg THC (A) (FAAH<sup>C/C</sup>: Paired t-test,  $t_{(4)} = 1.551$ ,  $p = 0.1959$ ,  $n=5$ ; FAAH<sup>C/A</sup>: Paired t-test,  $t_{(10)} = 0.7506$ ,  $p = 0.4702$ ,  $n=11$ ), 0.5mg/kg THC (B), (FAAH<sup>C/C</sup>: Paired t-test,  $t_{(4)} = 0.1058$ ,  $p = 0.921$ ,  $n=5$ ; FAAH<sup>C/A</sup>: Paired t-test,  $t_{(5)} = 1.32$ ,  $p = 0.244$ ,  $n=6$ ) or 3mg/kg THC (C), FAAH<sup>C/C</sup>: Paired t-test,  $t_{(6)} = 1.179$ ,  $p = 0.2830$ ,  $n=7$ ; FAAH<sup>C/A</sup>: Paired t-test,  $t_{(8)} = 1.27$ ,  $p = 0.2398$ ,  $n=9$ ).

### Supplemental Figure 3



**Fig. S3. Adolescent and adult FAAH<sup>C/C</sup> and FAAH<sup>C/A</sup> mice show preference for cocaine CPP.** (A) Adolescent female FAAH<sup>C/C</sup> and FAAH<sup>C/A</sup> mice show a preference for the cocaine-paired chamber on the test day compared to the baseline test (FAAH<sup>C/C</sup>: Paired t-test,  $t_{(10)} = 4.526$ ,  $p = 0.0011$ ,  $n=11$ ; FAAH<sup>C/A</sup>: Paired t-test,  $t_{(9)} = 5.12$ ,  $p = 0.0006$ ,  $n=10$ ). (B) Adolescent female FAAH<sup>C/C</sup> and FAAH<sup>C/A</sup> mice show similar difference score in cocaine CPP (Unpaired t-test,  $t_{(19)} = 0.1913$ ,  $p = 0.8503$ , FAAH<sup>C/C</sup>:  $n=11$ , FAAH<sup>C/A</sup>:  $n=10$ ) (C) Adult female FAAH<sup>C/C</sup> and FAAH<sup>C/A</sup> mice show a preference for the cocaine-paired chamber on the test day compared to the baseline test (FAAH<sup>C/C</sup>: Paired t-test,  $t_{(5)} = 4.347$ ,  $p = 0.0074$ ,  $n=6$ ; FAAH<sup>C/A</sup>: Paired t-test,  $t_{(5)} = 5.928$ ,  $p = 0.0019$ ,  $n=6$ ). (D) Adolescent female FAAH<sup>C/C</sup> and FAAH<sup>C/A</sup> mice show similar difference score in cocaine CPP (Unpaired t-test,  $t_{(10)} = 1.236$ ,  $p = 0.2448$ , FAAH<sup>C/C</sup>:  $n=6$ , FAAH<sup>C/A</sup>:  $n=6$ ).

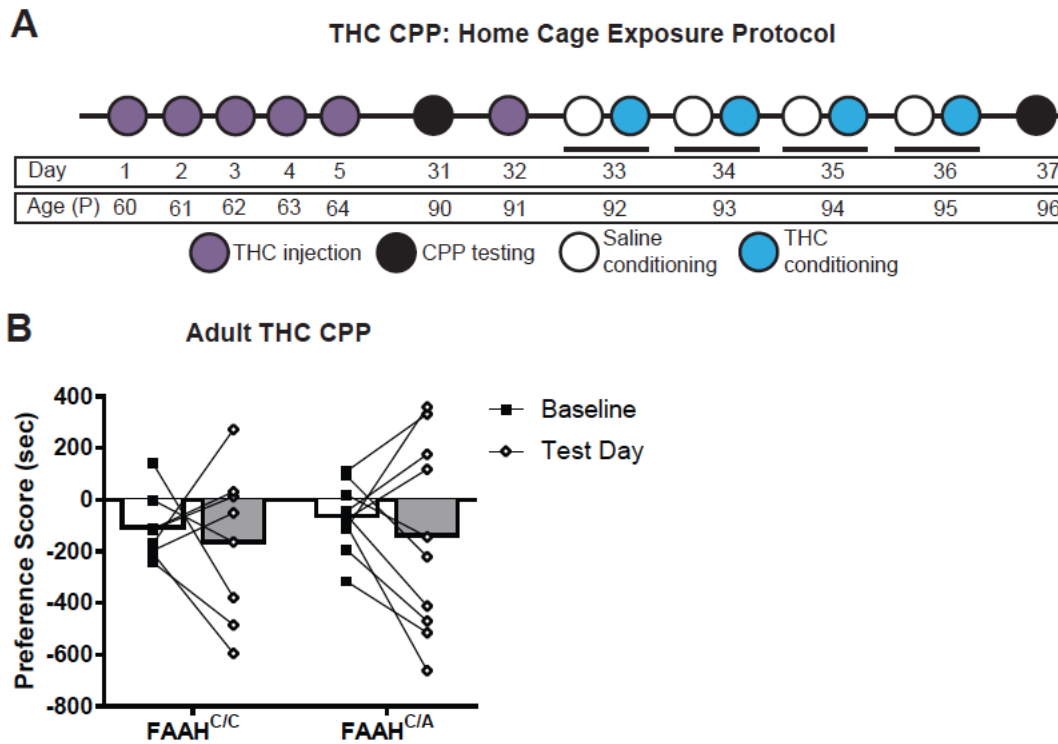
## Supplemental Figure 4



**Fig. S4. Pharmacological inhibition of FAAH in WT mice is not sufficient to reproduce rewarding effect of THC seen in adolescent female mice with the FAAH SNP. (A)**

Experimental timeline for THC CPP and the FAAH inhibitor PF-3845 treatment. **(B)** Female C57BL/6J WT adolescent mice pretreated with vehicle or PF-3845 prior to each THC exposure in the THC CPP paradigm showed no THC CPP response as measured by a lack of change in preference score from baseline test to test day (Vehicle: Paired t-test,  $t_{(8)} = 0.102$ ,  $p = 0.9213$ ,  $n=9$ ; PF-3845: Paired t-test,  $t_{(8)} = 0.1331$ ,  $p = 0.8974$ ,  $n=9$ ).

## Supplemental Figure 5



**Fig. S5. THC CPP during adulthood does not result in a preference for THC in female mice carrying the FAAH SNP.** (A) Experimental timeline for home cage exposure of THC. Mice were treated during adulthood and trained and tested in THC CPP 30 days later. (B) Female  $FAAH^{C/C}$  and female  $FAAH^{C/A}$  mice exposed to THC in their home cage during adulthood did not induce a preference for THC when trained and tested in THC CPP thirty days later ( $FAAH^{C/C}$ : Paired t-test,  $t_{(7)} = 0.4961$ ,  $p = 0.6350$ ,  $n=8$ ;  $FAAH^{C/A}$ : Paired t-test,  $t_{(9)} = 0.7296$ ,  $p = 0.4842$ ,  $n=10$ ).