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Supplemental Information

Anandamide Metabolites Protect against Seizures through the TRP Channel Water Witch in *Drosophila melanogaster*

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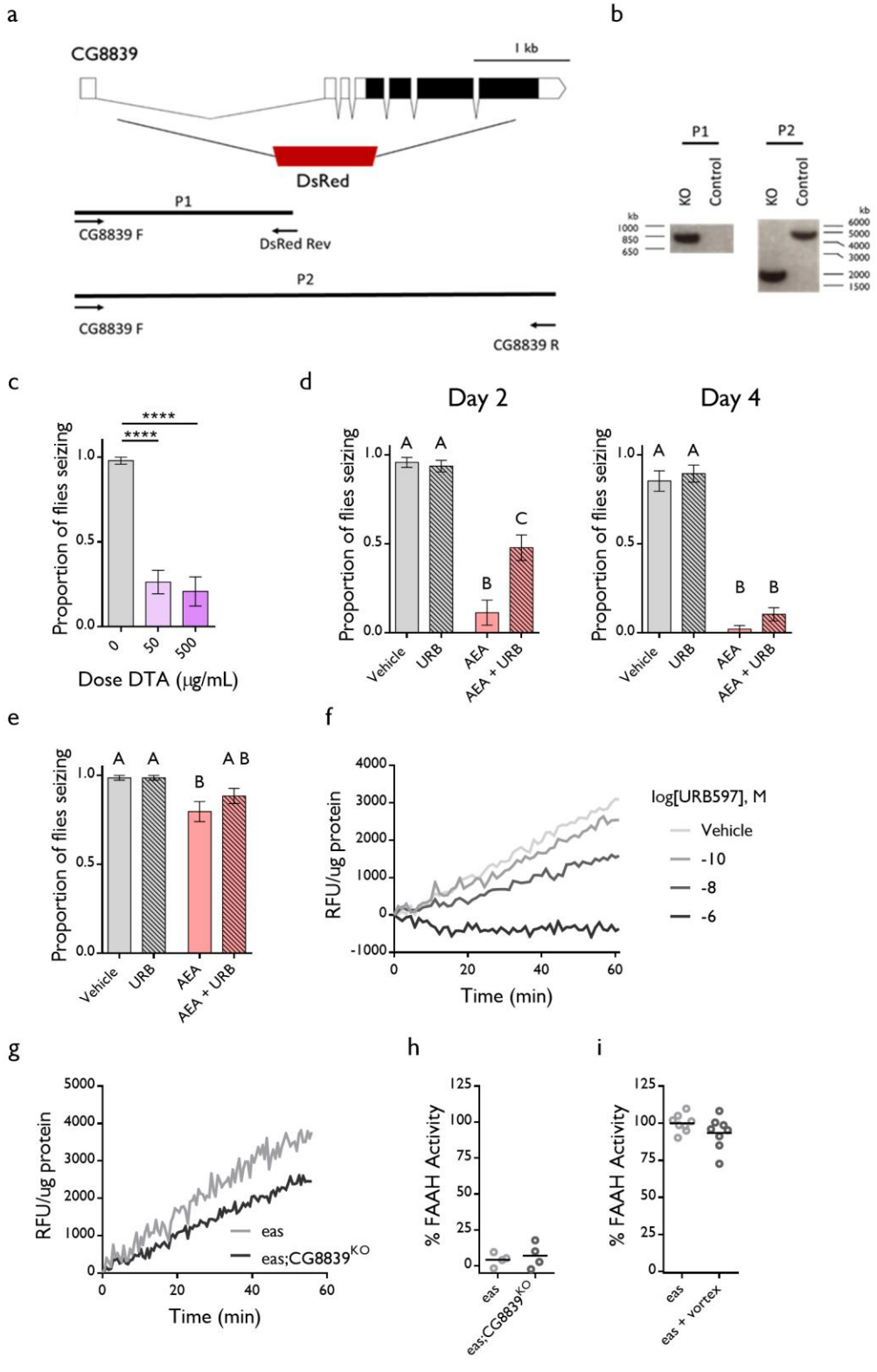
a
CG8839
1 kb
DsRed
P1
CG8839 F
DsRed Rev
P2
CG8839 F
CG8839 R
b
P1
P2
KO
Control
KO
Control
kb
1000
850
650
kb
6000
5000
4000
3000
2000
1500
c
****
****
Proportion of flies seizing
1.0
0.5
0.0
0
50
500
Dose DTA (μg/mL)
d
Day 2
Day 4
Proportion of flies seizing
A
A
B
C
Vehicle
URB
AEA
AEA + URB
e
Proportion of flies seizing
A
A
B
A B
Vehicle
URB
AEA
AEA + URB
f
RFU/ug protein
4000
3000
2000
1000
0
-1000
0
20
40
60
Time (min)
log[URB597], M
Vehicle
-10
-8
-6
g
RFU/ug protein
5000
4000
3000
2000
1000
0
0
20
40
60
Time (min)
eas
eas;CG8839KO
h
% FAAH Activity
125
100
75
50
25
0
eas
eas;CG8839KO
i
% FAAH Activity
125
100
75
50
25
0
eas
eas + vortex

Supplemental Figure S1. Metabolites mediate the anticonvulsant effect of AEA. Related to Figure 2. **a)** CG8839 KOs were generated by replacing the CG8839 coding region with a DsRed marker. **b)** PCR of genomic DNA confirms that DsRed was incorporated into deleted region of CG8839 by HDR in *CG8839*^{KO} flies but not in controls. See materials and methods for primer sequences. **c)** 50 and 500 μg/mL DTA feeding protects against seizures in *eas* flies. n=12 vials/group. ANOVA with Tukey's post-hoc analysis. ****P≤0.0001 **d)** Co-feeding 50 μg/mL URB597 with 20 μg/mL AEA reduces the protective effect of AEA in *tko*^{25t} flies after 2 days but not after 4 days of feeding. n=11-12 vials/group. ANOVA with Tukey's post-hoc analysis. Means with different letters are significantly different (*P≤0.05). **e)** Co-feeding 50 μg/mL URB597 with 200 μg/mL AEA does not reduce the protective effect of AEA in *bss*^{1} flies after 4 days of feeding. n=11-12 vials/group. ANOVA with Tukey's post-hoc analysis. Means with different letters are significantly different (*P≤0.05). **f)** Representative data from FAAH experiments analyzed in Fig. 2e. **g)** Representative data from FAAH experiments analyzed in Fig. 2f. **h)** 1 μM URB597 inhibits FAAH activity in both control and CG8839^{KO} flies. % FAAH activity normalized to vehicle treated homogenates for each sample. n=4 samples/genotype, 2 technical replicates/sample. Unpaired t-test. **i)** FAAH activity is not changed 15 minutes after seizure induction. % FAAH activity normalized to no vortex control FAAH activity for each experiment. n=8 samples/group, 3 technical replicates/sample. Unpaired t-test. Bar graph data are presented as mean ± s.e.m.

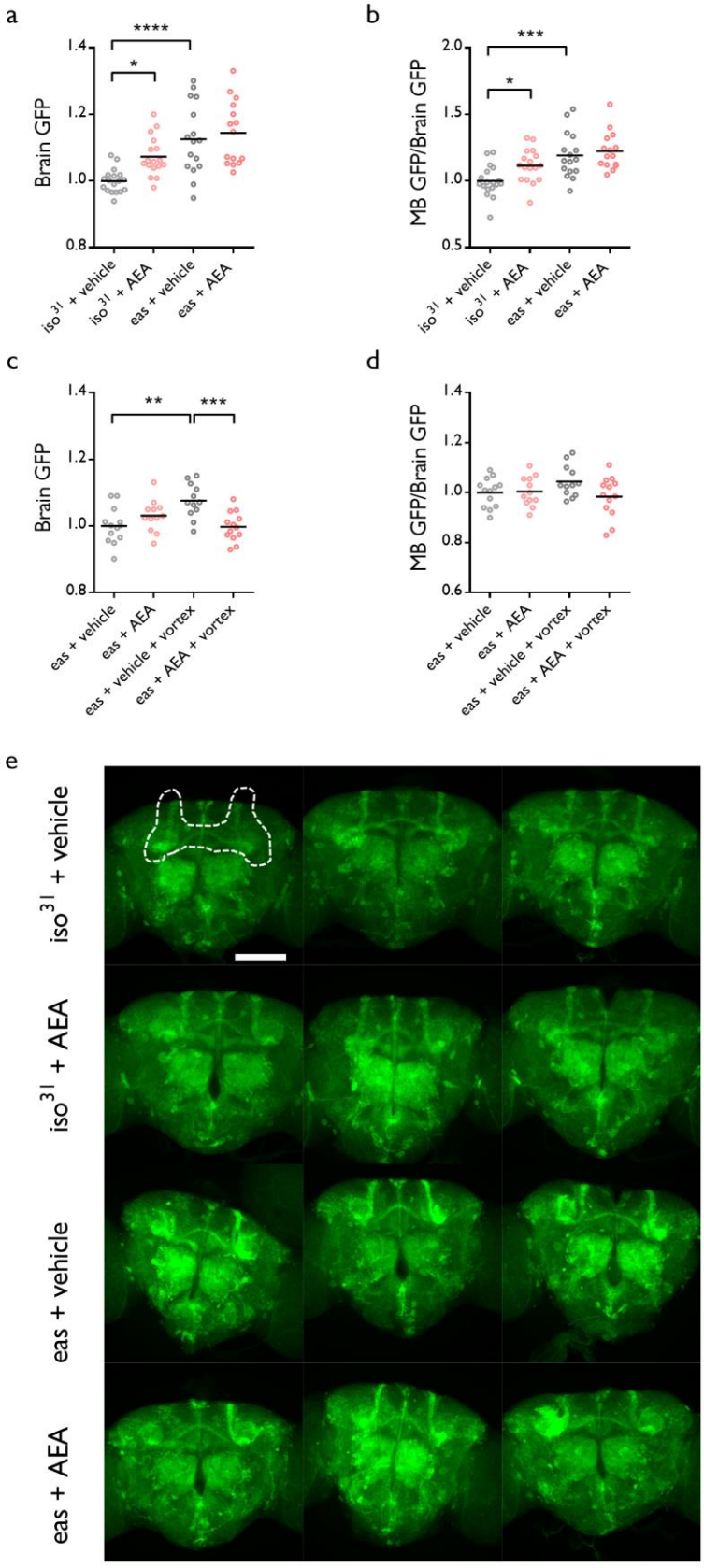
a
Brain GFP
1.4
1.2
1.0
0.8
****
*
iso31 + vehicle
iso31 + AEA
eas + vehicle
eas + AEA
b
MB GFP/Brain GFP
2.0
1.5
1.0
0.5
***
*
iso31 + vehicle
iso31 + AEA
eas + vehicle
eas + AEA
c
Brain GFP
1.4
1.2
1.0
0.8
**
***
eas + vehicle
eas + AEA
eas + vehicle + vortex
eas + AEA + vortex
d
MB GFP/Brain GFP
1.4
1.2
1.0
0.8
0.6
eas + vehicle
eas + AEA
eas + vehicle + vortex
eas + AEA + vortex
e
iso31 + vehicle
iso31 + AEA
eas + vehicle
eas + AEA

Figure S2. Anandamide increases baseline calcium and protects against stimulus-induced calcium elevations. Related to Figure 3. **a)** 20 μg/mL AEA feeding increases mean GFP signal in iso^{31} fly brains. Brains from vehicle-fed *eas* flies have higher GFP than brains from vehicle-fed iso^{31} flies. n=15-18 brains/group. ANOVA with Sidak's multiple comparison. *P≤0.05 ****P≤0.0001. **b)** 20 μg/mL AEA feeding increases MB-specific GFP in iso^{31} fly brains. Brains from vehicle-fed *eas* flies have higher MB-specific GFP than brains from vehicle-fed iso^{31} flies. n=15-18 brains/group. ANOVA with Sidak's multiple comparison. *P≤0.05 ***P≤0.001. **c)** 10-second vortex induces an increase in GFP in brains from vehicle-fed *eas* flies but not in brains from AEA-fed *eas* flies. n=12-13 brains/group. ANOVA with Sidak's multiple comparison. *P≤0.05 **P≤0.01. **d)** MB-specific GFP is similar in all *eas* groups. n=12-13 brains/group. ANOVA with Sidak's multiple comparison. **e)** Representative images of fly brains from Fig. S2a-b. White dotted line outlines MB. White scale bar indicates 100 μm.

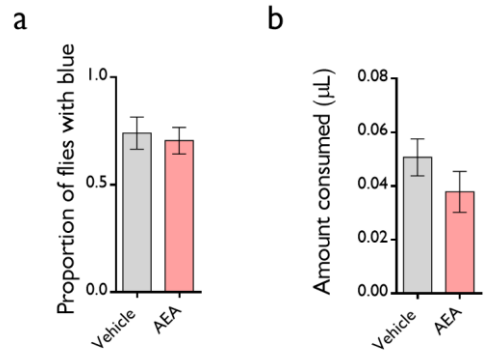

Figure S3. Flies consume AEA during acute exposure. Related to Figure 4. **a)** A similar proportion of flies consume vehicle and 2 µg/mL AEA food during an acute, 1-hour exposure after 16 hours of starvation. n=10 vials/group. Unpaired t-test. **b)** 2 µg/mL AEA does not affect total amount consumed. n=10 vials/group. Unpaired t-test. Bar graph data are presented as mean ± s.e.m.

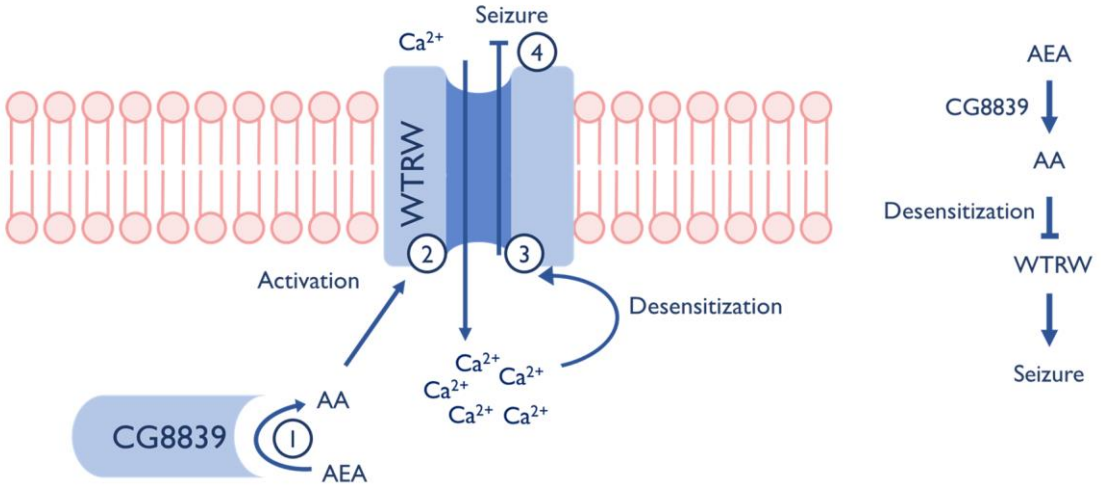

Figure S4. Hypothesized model of seizure protection by AEA metabolites. Related to Figures 1-4. **1**) AA is produced by catabolism of AEA by CG8839. **2**) AA activates WTRW causing Ca^{2+} influx. **3**) Chronic activation desensitizes WTRW. **4)** Desensitization of WTRW blocks seizure initiation.