Supporting Information

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SI Materials and Methods

Infusion Procedures. A PE-20 polyethylene tubing was connected to a 10-μl Hamilton syringe and a 30-gauge dental needle was cemented to the other end of the tubing. The infusion needle was then bent so that it extended 2 mm beyond the end of the guide cannula. The tubing was first filled with distilled water. A small air bubble was pulled in and the drug or vehicle was then pulled in. The Hamilton syringe was driven by an automated syringe pump (Sage Instruments, Boston, MA) at the rate of 0.91 μl/min (35 seconds) for NST or CB and 0.37 μl/min (32 seconds) for BLA infusions to give an infusion volume of 0.5 or 0.2 μl, respectively. The needles were then left in place for additional 35 seconds to allow the solution to diffuse. Immediately after the infusions, the animals received systemic injections of vehicle or OEA and returned to their home cage.

Histology. The rats were anesthetized with an overdose of sodium pentobarbital (100 mg·kg^{-1}, i.p.) and perfused intracardially with 0.9% saline solution followed by 4% formaldehyde solution. Subsequently, they were decapitated and the brains were removed and immersed in 4% paraformaldehyde for 2 days, then stored in 30% sucrose solution until slicing. Coronal slices of 40 μm (BLA) or 100 μm (NST, CB) were cut with a freezing microtome. Sections were mounted on gelatin-coated glass slides and stained with cresyl violet. The location of the infusion needles was determined by examining the sections under a microscope and using the standardized atlas plates of Paxinos and Watson (1). A representative section from the BLA is shown in Fig. S5. Only animals that had needle tips located within the NST, CB or BLA and had no lesions around the needle tips were included in the final analysis.

Inhibitory Avoidance Task: Drug Administration Procedures. OEA, GW7647 and capsazepine were dissolved in PEG/Tween80/saline, 5:5:90. (*R*)-1′-methyl-oleoylethanolamide (Met-OEA) was dissolved in carboxymethylcellulose/Tween80/water, 0.5:0.4:99.1. All drug solutions were freshly prepared on the day of the experiment.

a) Pretraining Administration.

1. OEA or its vehicle was administered to C57BL/6J mice (2.5–5–10 mg·kg^{-1}, i.p.) or to PPAR-alpha$^{-/-}$ and wild-type mice (5 mg·kg^{-1}, i.p.) 30 minutes before the training session (foot shock 0.3 mA, 3 seconds).

2.Wistar rats received injections of OEA (1–5−10 mg·kg^{-1}, i.p.) or its vehicle 30 minutes before the training session (foot shock 0.8 mA, 2 seconds).

b) Posttraining Administration.

1. OEA or its vehicle was administered to Sprague-Dawley rats immediately (1–10 mg·kg^{-1}, i.p.) or 3 hours (5 mg·kg^{-1}, i.p.) after the training session (foot shock 0.4 mA, 1 s).

2. GW7647 (10 mg·kg^{-1}, i.p.), met-OEA (25–100 mg·kg^{-1}, oral) or their vehicle were administered to Sprague-Dawley rats immediately after the training session (foot shock 0.4 mA, 1 second).

3. OEA (5 mg·kg^{-1}, i.p.) or its vehicle was administered to Sprague-Dawley rats immediately after the training session either alone or together with the TRPV1 antagonist capsazepine (10 mg·kg^{-1}, i.p.) or its vehicle (foot shock 0.4 mA, 1 second).

4. Lidocaine (2%, 0.5 μl) or its vehicle (PBS) was infused directly into the NST or CB of Sprague-Dawley rats immediately after the training session. OEA (5 mg·kg^{-1}, i.p.) or its vehicle was administered immediately after lidocaine infusion (foot shock 0.4 mA, 1 second).

5. Propranolol (0.5 μg) or its vehicle (sterile saline) was infused into the BLA of Sprague-Dawley rats immediately after the training session. OEA (5 mg·kg^{-1}, i.p.) or its vehicle was administered immediately after propranolol infusion (footshock, 0.5 mA, 1 second).

Statistical Analyses. Inhibitory avoidance data were subjected to nonparametric analysis. Kruskal-Wallis analysis of variance was applied to evaluate the main effect of treatment, and the Mann-Whitney *U* test was used to assess the significance of differences between treatment groups, using the Bonferroni's correction for multiple comparisons (*post hoc* comparisons). Water-maze training data were analyzed with a one-way ANOVA with the six acquisition trials as repeated measure. Quadrant search times on the probe trial were analyzed with a two-way ANOVA. Tukey's *post hoc* test was performed on the treatment × time interaction to determine the source of detected significances. One-way ANOVA was performed on data of each parameter measured in the open field and the elevated plus maze tests. A probability level of <0.05 was accepted as statistically significant.

1. Paxinos G, Watson C (1997) in *The rat brain in stereotaxic coordinates* (Academic, San Diego).

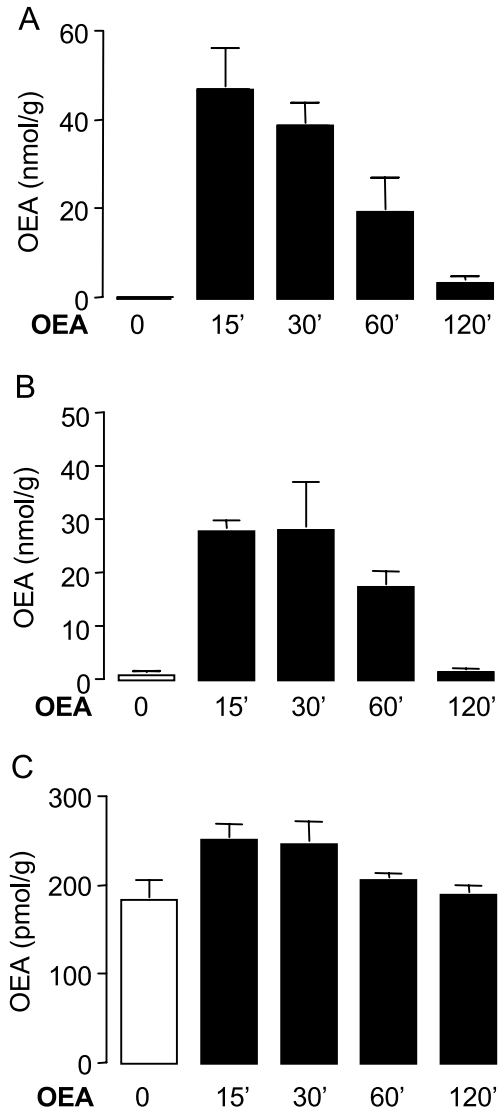

Fig. S1. Time-course of OEA levels in (*A*) duodenum, (*B*) jejunum, and (*C*) brain of rats following administration of OEA (10 mg·kg^{-1}, i.p.). Values (mean ± SEM, $n = 4$) are expressed as nmol·g^{-1} (*A*, *B*) or pmol·g^{-1} (*C*).

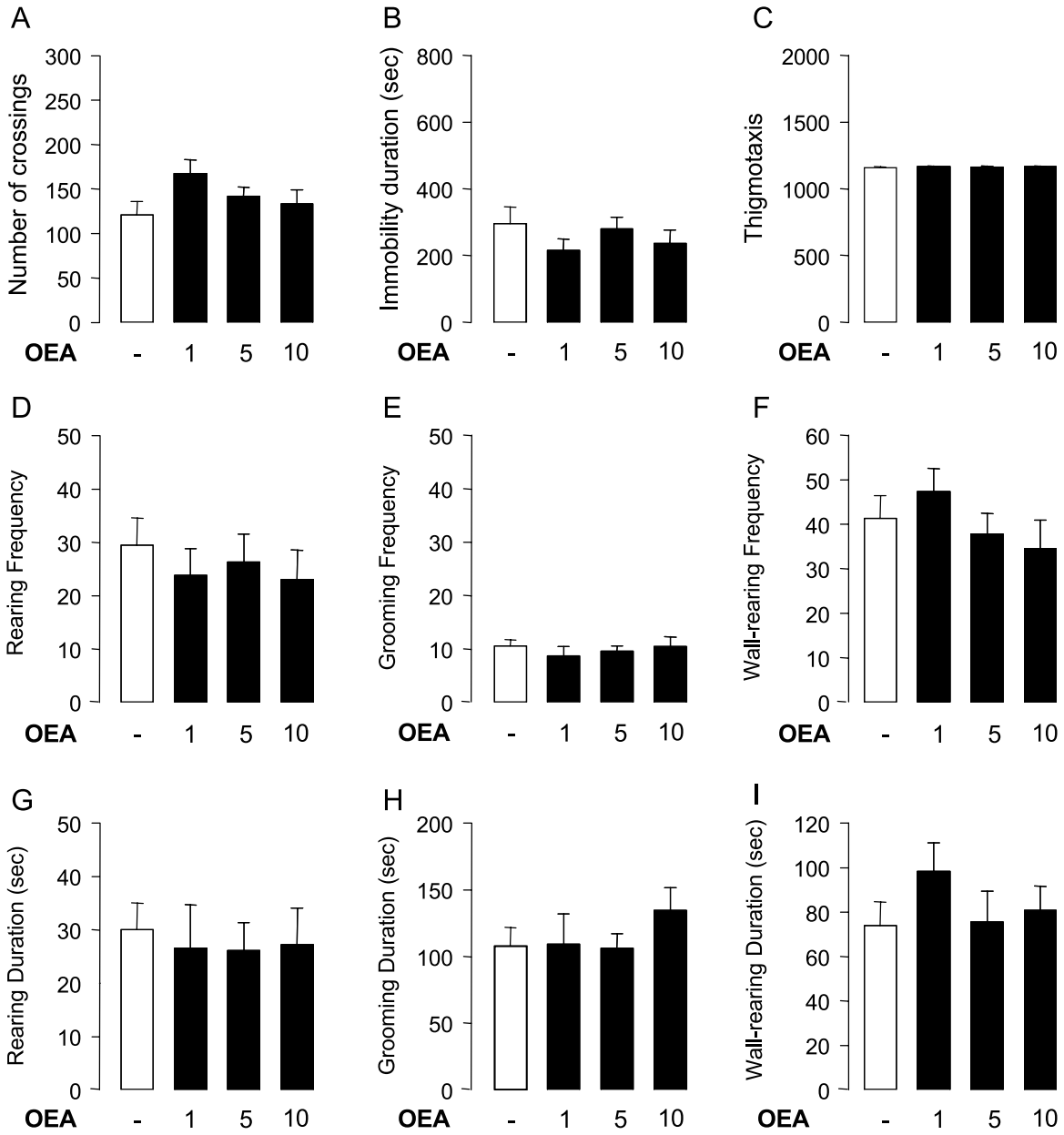

Fig. S2. OEA does not affect the behavior of Wistar rats in the open field test. No significant effects were found when OEA (1–10 mg·kg^{-1}, i.p.) was injected 30 minutes before the test on (*A*) the number of square limit crossings with both forepaws, (*B*) thigmotaxis (time spent near walls), (*C*) immobility duration, (*D*, *G*) frequency and duration of rearing (standing with the body inclined vertically, forequarters raised), (*E*, *H*) wall-rearing (standing on the hind-limbs and touching the walls of the apparatus with the forelimbs) and (*F–I*) grooming (rubbing the body with paws or mouth and rubbing the head with paws). Results are expressed as mean ± SEM, $n = 12$. One-way ANOVA revealed no statistical differences for crossings, immobility, thigmotaxis, and for frequency and duration of rearing, grooming and wall rearing ($F_{3,44} = 1.737$, $F_{3,44} = 0.737$, $F_{3,44} = 0.309$, $F_{3,44} = 0.347$, $F_{3,44} = 0.933$, $F_{3,44} = 0.0917$; $F_{3,44} = 0.675$, $F_{3,44} = 0.757$, NS, respectively).

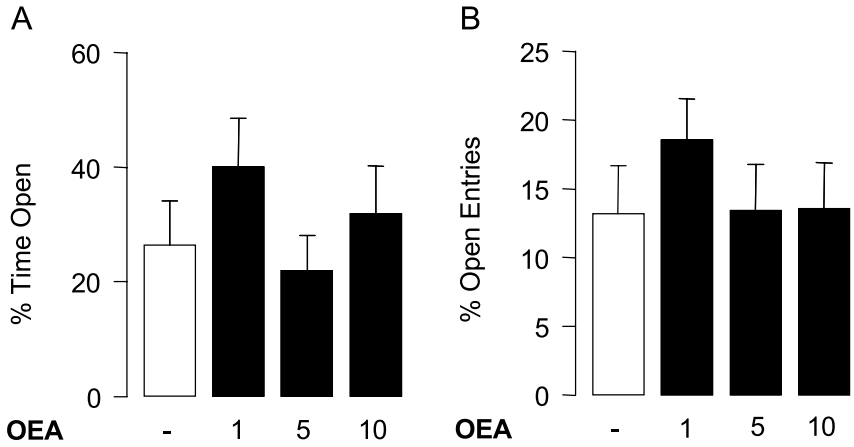

Fig. S3. OEA (1–10 mg·kg^{-1}, i.p.) does not exert anxiolytic or anxiogenic effects in the elevated plus maze test. (*A*) Percent time spent in the open arms; and (*B*) Percent entries into the open arms. Results are expressed as mean ± SEM, $n = 12$. One-way ANOVA revealed no statistical differences for percent of time spent in the open arms and percent of open entries ($F_{3,36} = 1.033$, $F_{3,36} = 0.953$, NS, respectively).

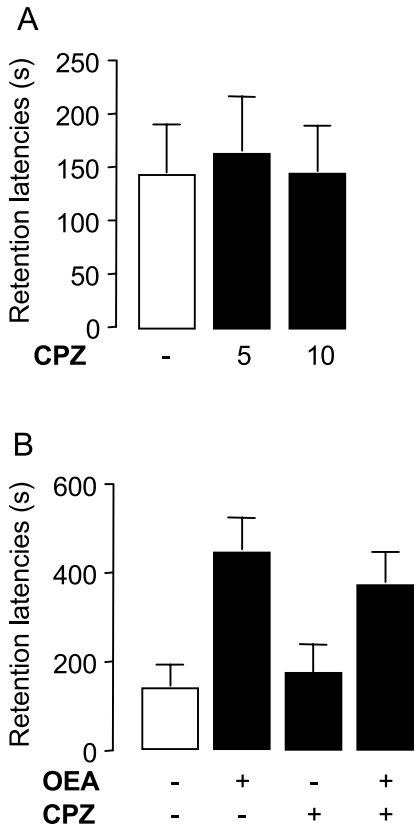

Fig. S4. Capsazepine administration (CPZ) does not prevent the memory-enhancing effects of OEA (10 mg·kg^{-1}, i.p.) in the rat inhibitory avoidance task. (*A*) CPZ (5, 10 mg·kg^{-1}, i.p.) does not alter retention latencies when administered alone. (*B*) CPZ does not significantly alter the effect of OEA on retention latency when administered together with OEA. Kruskal-Wallis ANOVA showed a significant difference among groups (H = 10.608, df = 3, $P = 0.014$). Particularly, systemic post-training administration of OEA enhanced retention latencies ($P < 0.05$ vs. vehicle). This effect was still present in animal that also received capsazepine injections ($P < 0.05$ vs. vehicle).

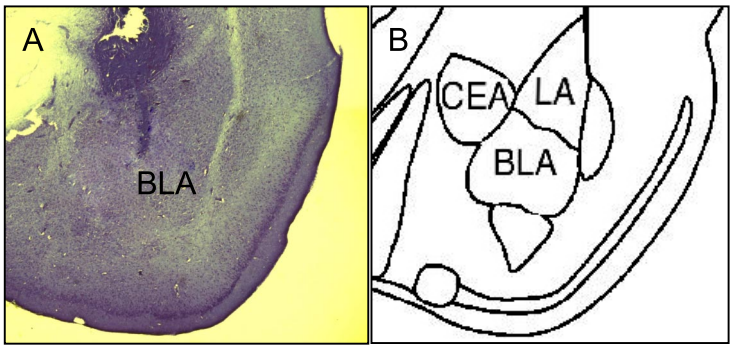

Fig. S5. Representative histological section (cresyl violet) (*A*) and diagram (*B*) showing the position of a guide cannula in the rat BLA. CEA, central nucleus of the amygdala; LA, lateral nucleus of the amygdala.