Supplemental Detailed Methods

Animals All experiments were performed on adult (2-4 months old) wildtype strain C57BL/6J from Jackson Labs, male mice that were group-housed in a temperature-controlled vivarium, with *ad libitum* access to food and water. They were maintained on a 12/12hr light/dark cycle, with all behavioral procedures being performed during the light cycle. All procedures used were approved by the Institutional Animal Care and Use Committee of Emory University and in compliance with National Institutes of Health (NIH) guidelines for the care and use of laboratory animals.

Drugs 7,8-DHF (Obtained both from Keqiang Ye and Tokyo Chemical Industry, ltd. cat #: D1916) was dosed systemically, intraperitoneally, (i.p.) at a 5mg/kg dose in 17% dimethylsulfoxide (DMSO) in phosphate buffered saline (PBS). Vehicle was 17% DMSO in PBS. In those experiments in which 7,8-DHF were given, mice received only a single dose 1hr before the appropriate behavioral procedure.

Immunoblotting and immunohistochemistry For western blots, mice were injected with 5 mg/kg 7,8-DHF i.p., 1hr and 2hr later were sacrificed, amygdala tissue was bilaterally collected with a 1mm punch on ice, and flash frozen on dry ice. For protein gels, the tissue was rapidly homogenated and lysed in lysis buffer, centrifuged, and the supernatant collected. The normalized proteins were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis analysis and transferred to a nitrocellular membrane. Western blotting analysis was performed with anti-TrkB Y706 (phosphorylated TrkB) (Santa Cruz, 1:200 in 5% bovine serum albumin/phosphate buffered saline) and anti-TrkB (Cell signaling, 1:500 in 5% bovine serum albumin/phosphate buffered saline), anti-P-Mitogen-activated protein kinase (MAPK) (Cell Signaling, 1:500 in 5% milk/PBS) and anti-MAPK (Cell Signaling, 1:5,000 in 5% milk/PBS). For immunohistochemistry, fixed rat brain sections from prior studies were incubated with phosphate buffered saline and Triton X-100, blocked with normal goat serum, bovine serum albumin, and Triton X-100, and incubated in a 1:500 dilution of primary TrkB rabbit polyclonal antibody (SC-12, Santa Cruz Biotechnology, Santa Cruz, CA) overnight at 4°C. Sections were then washed with PBS and bathed in a 1:500 dilution of secondary anti-rabbit biotinylated antibody (Ab) for 2 hr. Avidin–biotin complexes were amplified using a standard Vectastain Elite ABC kit and visualized with diaminobenzidine peroxidase staining.

3H- 7,8-dihydroxyflavone autoradiography For regional localization of 7,8-DHF binding to TrkB, [3H] 7,8-DHF binding was performed as previously described (18). In brief, coronal tissue sections were thawed, dried at room temperature, and preincubated in 50mM Tris (pH 7.4) for 20 min at 4°C. Then, tissue sections were incubated in 50mM Tris and 17% DMSO 40 min at 4°C. To determine level of specific vs. background binding, control slides were pre-incubated in the above solution containing non-radioactive 7,8-DHF (2.5mM). After rinse, all slides were incubated in 50mM Tris buffer containing 50nM [3H] 7,8-DHF for 40 min at 4°C. At the end of the incubation, the tissues were rinsed with ice-cold 50mM Tris–citrate buffer three times for 5 sec and washed briefly in cold distilled water and dried. Dried tissue sections were apposed to Biomax MR autoradiography film (Eastman Kodak Co., Rochester, NY) with tritium standards ([3H]Micro-scale RPA 510, Amersham) and exposed for 10-30 days.

Open field The open field was an open box (27.9cm x 27.9cm) made of Plexiglass. The mice were placed in the apparatus to explore for 10min, and then returned to home cages. Locomotor and center/surround activity data was obtained and analyzed using the Activity Software (Med Associates Inc.).

Cue Fear Conditioning Fear conditioning was conducted in startle-footshock chambers (SR-LAB, San Diego Instruments) consisting of a nonrestrictive acrylic plastic cylinder, 5.5 cm in diameter and 13 cm long, mounted on a Plexiglas platform which was located in a ventilated, sound-attenuated chamber. The footshock unconditioned stimulus was delivered through a removable stainless steel grid floor using one of four constant current shock generator (SDI, San Diego, CA) located outside the isolation chambers. A piezoelectric accelerometer mounted under each platform detected cylinder movements that were digitized and stored by an interfacing computer assembly. Shock reactivity was defined as the peak activity/accelerometer voltage that occurred during the 200 ms after the onset of the US. Response sensitivities were calibrated (SR-LAB Startle Calibration System) to be nearly identical in all startle cylinders. The tone conditioned stimulus was generated by a Tektronix function generator audio oscillator (Model CFG253, Beaverton, OR) and delivered through a high-frequency speaker (Motorola, Model 948) located 13 cm from the rear of each Sound intensities were measured by an audiometer (Radio Shack, Ft. Worth, TX, #33-2055). Stimuli presentation and data acquisition were controlled, digitized and stored by an interfacing IBM PC compatible computer using SR-LAB software.

On each of 2 days prior to training, mice were given a 10-min startle chamber exposure session to habituate mice to handling and the training context. During cued fear training, mice received 5 trials of a conditioned stimulus tone (30 s, 12 kHz, 70 db) co-terminating with a US footshock 500ms, 0.5mA in experiment 1 and 2; 500ms, 1mA in experiment 3 and 4. The training inter-trial interval was 5-min.

Fear Expression, Retention, and Extinction Testing. Mice were given fear expression, retention, and extinction testing in standard rodent modular test chambers (ENV-008-VP; Med Associates Inc. Georgia, VT) with an inside area of 30.5cm (L) x 24.1cm (W) x 21.0cm (H). The tone conditioned stimulus was generated by a Tektronix function generator audio oscillator delivered through a high-frequency speaker (Motorola, Model 948) attached to side of each chamber, as described previously (Held et al., 2007; Maguschak et al., 2008). The expression of fear was assessed 24 hr after fear conditioning in and consisted of 15 conditioned stimulus tone trials (30s each) with a 1.5min inter-trial interval. For extinction and retention testing, mice were given 30 conditioned stimulus tone trials with a 30 s inter-trial interval. Tone presentation and freezing data were controlled, stored, and analyzed with FreezeView software (Coulbourn Instruments).

Reinstatement in fear conditioning apparatus. Mice were placed in the fear extinction conditioning apparatus and 3 minutes later received a single 3s, 0.85 mA footshock. Twenty-four hours later, animals were placed in the same apparatus and freezing was evaluated with the extinction training paradigm described above.

Immobilization stress Immobilization procedures were conducted in a room separate from fear training and testing apparatus. Each animal was immobilized by gently restraining their four limbs in a prone position to metal arms attached to a wooden board for 2 hours (Panlab, Harvard apparatus, Spain). All animals of the same cage received the same treatment, immobilization or handling. After treatment, animals where returned to their home-cage where remained undisturbed until fear training.

Radioimmunoassays Plasma corticosterone levels were determined by double-antibody radioimmunoassay (RIA). Corticosterone RIA used 125I-corticosteronecarboximethyloxime-tyrosine-methyl ester (ICN-Biolink 2000, Barcelona, Spain), synthetic corticosterone (Sigma,

Barcelona, Spain) as the standard and an antibody raised in rabbits against corticosterone-carboximethyloxime-BSA kindly provided by Dr. G. Makara (Institute of Experimental Medicine, Budapest, Hungary). We followed the RIA protocol recommended by Dr. G. Makara (plasma corticosteroid-binding globulin was inactivated by low pH). All samples to be statistically compared were run in the same assay to avoid inter-assay variability. The intra-assay coefficient of
variation was 6.7% and sensitivity 0.1 ug/dl.

Experiment 1 See Figure S1*A*. One hour prior to fear training, mice received 7,8-DHF or vehicle. During conditioned stimulus (12kHz tone) + unconditioned stimulus (0.5mA footshock) training, shock reactivity to the 0.5mA intensity unconditioned stimulus footshock was measured. Twenty-four hours later animals were tested in a distinct fear testing apparatus. Testing consisted of 15 conditioned stimulus presentations during which conditioned freezing was measured.

Experiment 2 See Figure S1*B*. Mice were given cue dependent fear conditioning in the startle training apparatus by receiving 5 presentations of tone (30 sec, 12kHZ) co-terminating with a shock (0.5mA, 500ms). Animals received a matching session 24h after training. This matching session consisted in a 15 conditioned stimulus presentation in the testing chambers (a new context). Twenty-four and 48 hours later mice received an extinction test that consisted in a 30 conditioned stimulus presentation.

7,8-DHF was given 1 hour before the first extinction test. Twenty-four hours later freezing was evaluated again in the same paradigm but animals did not receive injection. Twenty-four hours after the second extinction test, animals were given reinstatement training.

Experiment 3 See Figure S1*C*. Twenty-four hours after the two days of habituation to the fear conditioning chamber, half of the animals were immobilized for 2hr and the other received standard compensatory handling. 6 days later mice were trained for cue-dependent fear conditioning with a 1mA shock intensity. Twenty-four, 48, 72, and 96 hours later were extinction trained with 30 conditioned stimulus in the modular chambers to assess conditional freezing.

Experiment 4 See Figure S1*D*. The day after pre-exposure habituation to the startle training apparatus, half of the animals were immobilized and the other half had a compensatory standard handling. 12 days later animals were given fear conditioning in the startle training apparatus. Twenty-four hours later mice fear expression was assessed by presenting 15

conditioned stimulus trials in the modular testing chambers. Twenty-four hours later, a single injection of 7,8-DHF or vehicle was given 1 hr before extinction training, consisting of 30 conditioned stimulus tone presentations. Animals were tested again in the absence of drug 24hr later.

Statistical Analyses Fear acquisition, expression, and extinction data were analyzed by 1 or 2-way ANOVA with repeated measures. All other behavioral tests were analyzed by ANOVA, where appropriate. Statistically significant main effects or interactions by ANOVA were followed by post hoc least squares difference (LSD) tests for multiple comparisons. Data are presented as mean and SD and statistical significance was set at $P < .05$. Tests for homogeneity of variance were performed, and when necessary, square root transformations were used. Detection of outliers was performed, and when necessary, removed from analyses.

Figure S1. Organization of Behavioral Experiments

Timeline for fear acquisition and extinction in wildtype and wildtype immobilized mice. All animals were handled and habituated to the fear conditioning chambers for 2 sessions on 2 consecutive days before fear conditioning. All animals were fear conditioned to a tone in the startle apparatus and freezing was evaluated in the modular testing chambers (a new chamber/context).

A) 7,8-DHF or vehicle was given 1hr before training in fear conditioning, 5 shocks (0,5mA 500ms) and 24hr later extinction training was evaluated in the modular testing chambers.

B) 24hr after fear conditioning (5 shocks, 0,5mA 500ms), animals were matched to the same freezing levels. 24hr later 7,8-DHF was given 1hr before extinction training in the modular chamber. 24hr later the same extinction training was given but no drug was administered. 24hr after reinstatement and extinction training was given.

C) Mice were immobilized for 2hr and undisturbed in the vivarium for 6 days, and then were fear conditioned with 5 shocks (1mA 500ms). Twenty-four, 48, 72, and 96 hours later animals received an extinction test in the modular chambers.

D) Mice were immobilized for 2hr and undisturbed for 12 days and then were fear conditioned with 5 shocks (1mA 500ms). Twenty-four hours later were tested in the modular chamber to asses conditioned freezing and groups were matched according to their freezing. Twenty-four hours later received 7,8-DHF or vehicle 1hr before training in the extinction test.