

Figure S1 Schematic of experimental model and treatment paradigm. (A) PBS or KA was infused through a cannula aimed at the basolateral nucleus of the right amygdala (coordinates from bregma: AP= -1.2 mm; ML= 3.0 mm; DV= -4.5 mm) and EEG was recorded from a bipolar electrode placed into the left dorsal hippocampus (coordinates from bregma: AP = -2.0 mm; ML = -1.6 mm; DV = -1.5 mm). (B) Treatment and monitoring paradigm. Mice were treated with vehicle or 1NMPP1 for 2 weeks commencing immediately after the termination of SE, and then treatment was tapered during days 15 through 17 (see Methods). Behavior and EEG telemetry were continuously monitored (24 h/day) during weeks 1-2 and weeks 5-6 after SE.

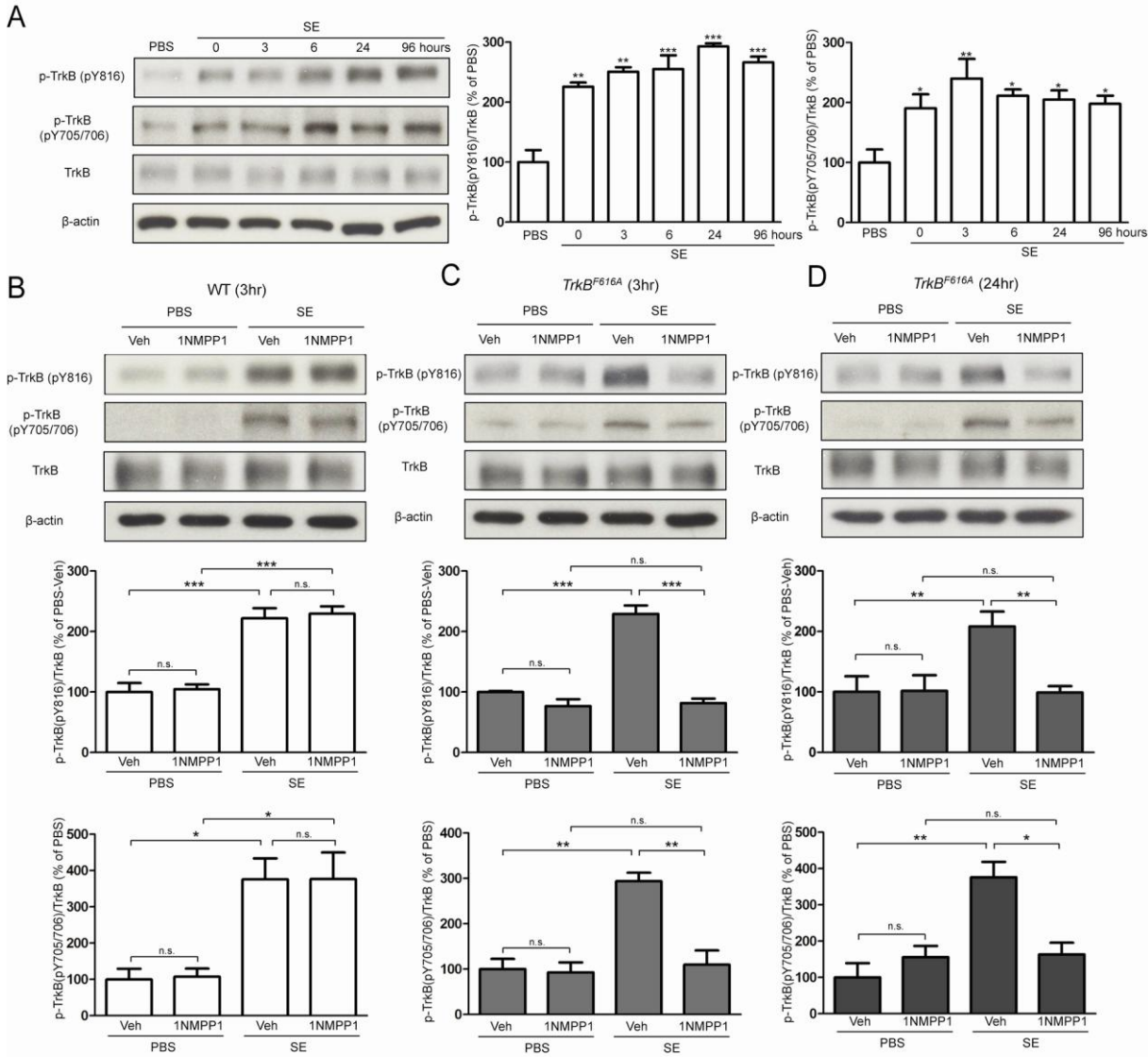


Figure S2 Activation of TrkB is increased following SE and inhibited by 1NMPP1 selectively in *TrkB^{F616A}* mice. (A) *Left*, representative Western blots of p-TrkB (pY816 and pY705/706), TrkB and β -actin immunoreactivities of WT mice infused with PBS or euthanized immediately (0) or 3, 6, 24, or 96 h after completion of SE. *Middle and right*, quantitative analyses of Western blots shows percentage increase of p-TrkB (pY816, middle and pY705/706, right) relative to TrkB is significantly higher at multiple time-points after SE compared to the PBS controls. Data are presented as mean \pm SEM of percentage increase of p-TrkB (pY816, middle and pY705/706, right) relative to TrkB in SE mice

compared with PBS controls. Statistics were performed using one-way ANOVA with Tukey *post hoc* test, n=3 for each group. (B), (C) and (D) *Top*, representative Western blots of p-TrkB (pY816 and pY705/706), TrkB and β -actin immunoreactivities of WT (B) and *TrkB*^{F616A} (C and D) mice treated with vehicle or 1NMPP1 and euthanized 3 h (B and C) and 24 h (D) after completion of SE. Control animals underwent infusion of PBS in amygdala and were subsequently treated with vehicle or 1NMPP1. *Middle and bottom*, quantitative analyses of Western blots of three experiments shows significant increases of p-TrkB (pY816, middle and pY705/706, bottom) relative to TrkB after SE compared to PBS-vehicle controls. The enhanced TrkB activation induced by SE was inhibited by 1NMPP1 in *TrkB*^{F616A} mice (C and D) but not in WT (B) mice. Data presented in (B), (C) and (D) are mean \pm SEM of percentage increase of p-TrkB (pY816, middle and pY705/706, bottom) relative to TrkB in PBS-1NMPP1, SE-vehicle and SE-1NMPP1 groups compared with PBS-vehicle controls. Statistics were performed using two-way ANOVA with Bonferroni *post hoc* test, n=3 for each group. n.s. = not significant. *p<0.05, **p<0.01, ***p<0.001.

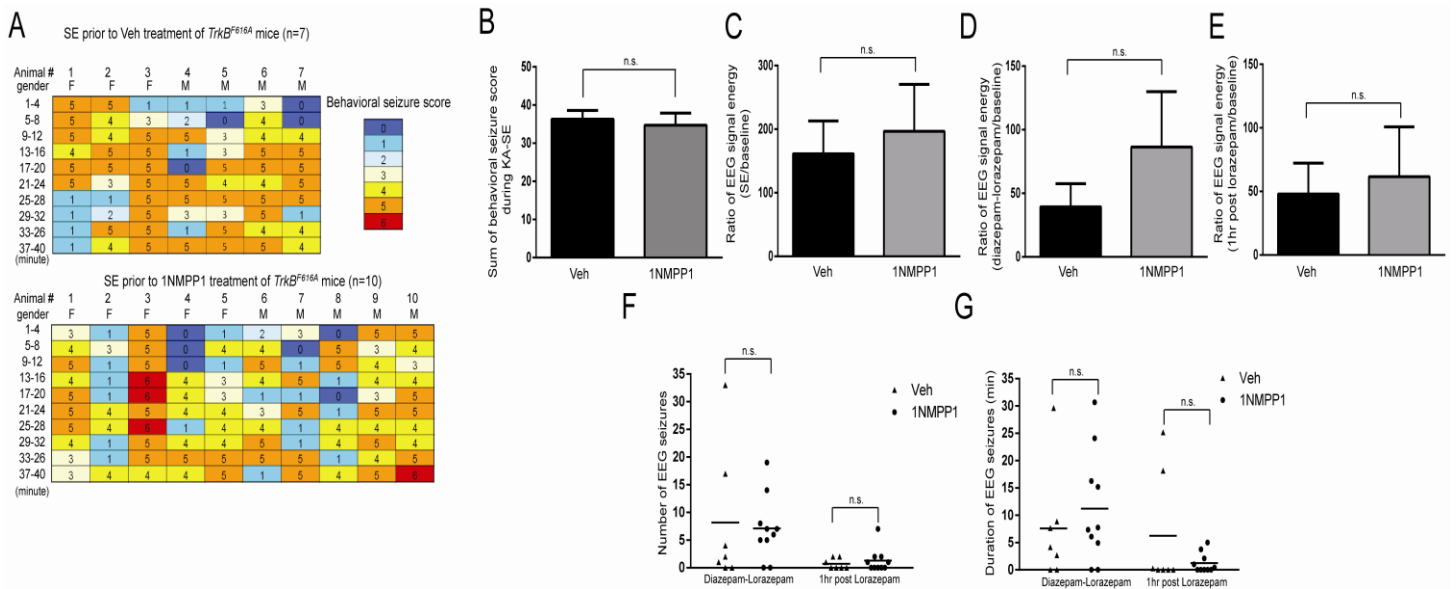


Figure S3 Equivalent severity of SE is induced by KA infusion prior to and immediately following 1NMPP1 treatment in *TrkB^{F616A}* mice. (A) Behavioral seizure severity during 40 min of SE in *TrkB^{F616A}* mice presented as a heat map. The maximum seizure score of each animal was measured every 4 min over a 40 min period. The time-scale in each box is 4 min (one mouse per column. Behavioral seizure score: Blue: 0; cyan: 1; light cyan: 2; light yellow: 3; yellow: 4; orange: 5 and red: 6). M: male, F: female. (B) Total behavioral seizure scores during 40 min of KA-induced SE. No significant difference was found between vehicle- and 1NMPP1-treated *TrkB^{F616A}* mice by Student's t-test (n=7-10). (C), (D), and (E) EEG signal energy was quantified during 40 min of SE (C), during the 1 h interval between diazepam and lorazepam treatments (D), and during the 1 h interval following lorazepam treatments normalized to baseline recording (E). No significant differences (n.s.) were found between vehicle- and 1NMPP1-treated *TrkB^{F616A}* mice by Student's t-test (n=7-10). (F) Number of EEG seizures detected during 1 h treatment of diazepam and lorazepam and during 1 h following lorazepam treatment in vehicle and 1NMPP1 treated groups. (G) Duration of EEG seizures detected during 1 h between treatment of diazepam and lorazepam and during 1 h following lorazepam treatment in vehicle and 1NMPP1 treated groups. Consistent with EEG signal energy analyses (D) and (E), no significant differences (n.s.) were

found between vehicle- and 1NMPP1-treated *TrkB^{F616A}* mice by two way ANOVA followed by post hoc Bonferroni's test (n=7-10) in seizure number (F) or duration (G). To assure objectivity, assessment of the number and duration of EEG seizures following treatment with diazepam and lorazepam was performed by an individual (J.O.M.) unaware of treatment with vehicle or 1NMPP1.

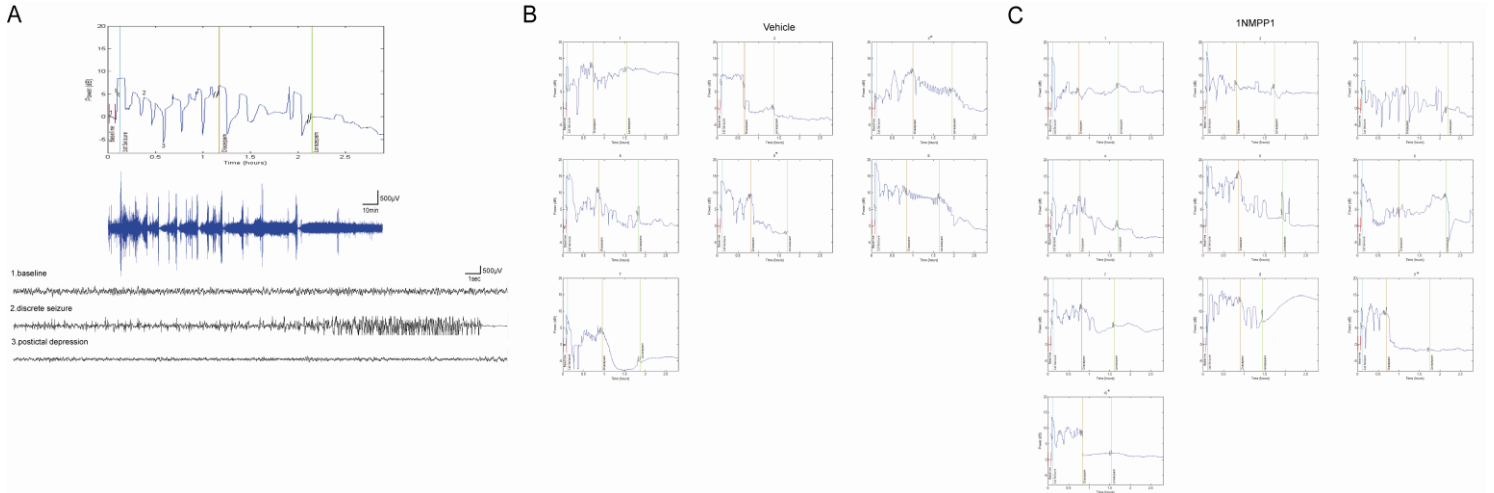


Figure S4 Quantitative assessment of electrographic seizure activity induced by infusion of KA in amygdala of vehicle and 1NMPP1 treated *TrkB^{F616A}* mice. (A) Representative plot of \log_{10} power of an EEG recording adjusted to baseline power prior to KA infusion (top). This plot is that of animal #3 in Figure S4 C. The EEG trace from which this power analysis is obtained is presented immediately below, using a similar time scale. EEG traces (expanded time scales) of baseline, electrographic seizure, and suppression following electrographic seizure corresponding to points 1, 2, and 3 of the \log_{10} power plot are presented at bottom of A. Note that increases and decreases of power in the \log_{10} plots correspond to electrographic seizure and post-seizure suppression of EEG activity. **(B)** and **(C)** \log_{10} power plots of EEG recordings of each vehicle (B) and 1NMPP1 (C) treated *TrkB^{F616A}* animal presenting baseline, period from first EEG seizure to diazepam, and the 1 h following lorazepam treatment. Number of each animal corresponds to number of animal in heat map presented in Figure 1 C and D and Figure S3A. Note that persistent elevation of power evident in some animals following lorazepam treatment (e.g. vehicle animal #1 and 1NMPP1 animal #8) is due to persistent interictal spiking. The absence of EEG data in animal #5 in vehicle treated group during the 1h after lorazepam is due to electrode being disconnected.

Extended Experimental Procedures

Animals

TrkB^{F616A} and WT mice in a C57BL/6 background (Charles River) were housed under a 12-hour light/dark cycle with food and water provided *ad libitum*. Animals were handled according to the National Institutes of Health Guide for the Care and Use of the Laboratory Animals and the experiments were conducted under an approved protocol by the Duke University Animal Care and Use Committee.

Surgery and Amygdala Kainic Acid (KA) Microinfusion

Adult male and female mice (20-25g) were anesthetized and placed in a stereotaxic frame. A guide cannula (Plastics One) was inserted above the right amygdala (coordinates from bregma: AP=-1.2 mm; L= 3.0 mm (Fig S1A)). A bipolar electrode was placed into the left dorsal hippocampus (coordinates from bregma: AP=-2.0 mm; L=-1.6 mm; and D=-1.5 mm below dura). After a 7-day postoperative recovery, animals were gently restrained and an infusion cannula (Plastics One) was inserted into the right amygdala through the guide cannula to a depth of 3.7 mm below the dura. Either KA (0.3 µg in 0.5 µl phosphate-buffered saline, PBS) or vehicle (0.5 µl of PBS) was infused into the right basolateral amygdala at the rate of 0.11 µl/min. The infusion cannula was left in the right amygdala for two additional min. Subsequently, animals were housed individually for long-term electroencephalogram (EEG) telemetry and video monitoring.

SE and EEG and Video-Monitoring

Continuous hippocampal EEG telemetry (Grass Instrument Co.) and time-locked video-monitoring were performed using Harmonie software (Stellate Systems). Monitoring started 15 min before amygdala KA

infusion for recording baseline EEG and behavioral activity. SE was typically evident electrographically and behaviorally (Mouri et al., 2008) 8-12 min after KA infusion. Forty minutes after onset of KA-induced SE, diazepam (10 mg/kg, i.p.) was administered to suppress SE and this was followed by lorazepam (6 mg/kg, i.p.) 1 h later. Mortality was less than 5%. Behavioral and EEG seizures during 40 min of SE were quantified to verify severity of SE in individual animals before initiating treatment.

Unless specified otherwise, following SE animals underwent continuous video-EEG monitoring 24 h/day, 7 days/week during weeks 1-2 and weeks 5-6 post-SE. Spontaneous recurrent seizures (SRS) were identified by review of video-EEG files by two independent trained readers blinded to both genotype and treatment of mice. The consistency of identifying SRS between readers was ~95%; in instances in which readers disagreed, the events were excluded from this study. SRS was defined electrographically as high frequency (>5Hz), high amplitude (>2 X baseline) rhythmic epileptiform activity with a minimal duration of 5 s and behaviorally with video-monitoring (see behavioral-seizure scoring).

Behavioral Seizure Scoring

Behavioral seizures were classified according to a modification of the Racine scale for mice (Borges et al., 2003): 0, normal activity; 1, arrest and rigid posture; 2, head nodding; 3, partial body clonus (unilateral forelimb clonus); 4, rearing with bilateral forelimbs clonus; 5, rearing and falling (loss of postural control); 6, tonic-clonic seizures with running or jumping. All EEG SRS were confirmed by corresponding behavioral seizures documented by time-locked video review.

Quantitative Analysis of EEG Energy Content

Quantitative analysis of EEG energy content (Figure S4 B, C) was performed using the method

described by Lehmkuhle et al. (2009). Results from each animal for the 40 min of SE, 60 min interval between treatment with diazepam and lorazepam, and 60 min interval after lorazepam were normalized to 5 min of baseline collected prior to KA infusion. Insufficient duration of artifact-free baseline activity prior to infusion of KA necessitated using 5 minutes of EEG activity one day following KA infusion as a measure of baseline activity of two vehicle (#s 3 and 5) and two 1NMPP1 (#s 9 and 10) treated animals (* in Figure S4 B, C). Experimental design necessitated handling the animals (e.g. KA microinfusion, treatment with diazepam and lorazepam) which resulted in EEG artifacts. EEG records were reviewed to detect these artifacts and these portions of recording were removed. Variable durations of these artifacts resulted in some differences in intervals in plots of individual animals. To assure objectivity, detection and removal of such artifacts from power plots and corresponding EEG were performed without knowledge of treatment with vehicle or 1NMPP1. In addition, the values of the filtered and smoothed energy function, $d(k)$, were summed over each of the intervals (using the formula below) and normalized to the sum of the baseline interval to give ratios representing gross activity (Figure S3 C, D, E).

$$E_d = \sum_{k=T_1}^{T_2} d(k)$$

Treatment

1NMPP1 (1-(1,1-dimethylethyl)-3-(1-naphthalenylmethyl)-1H-pyrazolo[3,4-d]pyrimidin-4-amine) was dissolved in dimethyl sulfoxide (DMSO) at 100 mM and stored at -20°C until use. 1NMPP1 was diluted in solubilization buffer containing 0.9% NaCl and 2.5% Tween-20 prior to each administration. Following KA-induced SE, the first dose of 1NMPP1 (16.6 µg/g, i.p.) was injected immediately after giving diazepam. A second dose of 1NMPP1 (16.6 ng/g) was injected subcutaneously immediately after administration of lorazepam 1 h after the diazepam treatment. A third dose of 1NMPP1 (16.6 µg/g, i.p.) was injected approximately 12 h post-SE. For the first 2-weeks post-SE, 1NMPP1 was administered daily (16.6 µg/g, i.p.) and also included in drinking water (25 µM). At the end of second week, treatment was

tapered: on Day 15 post-SE, ½ dose (8.3 µg/g, i.p.) was administered and treatment continued in drinking water; on Day 16 post-SE, ¼ dose (4.15 µg/g, i.p.) was administered and treatment continued in the drinking water; on Day 17 post-SE, i.p. injections were discontinued and treatment continued in the drinking water; on Day 18 post-SE, all treatment was terminated. WT mice and *TrkB*^{F616A} mice injected under the same regimen with DMSO in solubilization buffer (i.p. and in drinking water) served as controls.

Western Blotting

Animals were euthanized and decapitated, and the head was quickly immersed in liquid nitrogen for 4 sec to rapidly cool the brain. The hippocampi were rapidly dissected on ice and homogenized in isotonic sucrose buffer (4 mM HEPES, 0.32M sucrose, pH 7.4, supplemented with 1 mM sodium orthovanadate, 1mM phenylmethanesulfonylfluoride [PMSF] and one complete Mini protease inhibitor tablet [Mini, Roche]/10 ml). Following centrifugation at 1000 x g for 10 min at 4°C, the supernatants were removed and centrifuged at ~200,000 x g for 15 min at 4°C. The pellet (crude membrane fraction) was resuspended in RIPA buffer (20 mM Tris, pH 7.5, 137 mM sodium chloride, 1% NP40, 0.25% sodium deoxycholate, 1 mM sodium orthovanadate, 1 mM PMSF, and one complete Mini protease inhibitor cocktail tablet [Roche]/10 ml), incubated on ice for 15 min, and centrifuged at ~200,000 x g for 15 min at 4°C. The supernatant was collected and stored at -80°C until further analysis. Western blotting was conducted as previously described(He et al., 2010) by probing p-Trk (pY816, 1:1000; Abcam,), p-Trk (pY705/pY706, 1:1000; Santa Cruz), TrkB (1:2000; Cell Signaling), and β-actin (1:10000; Sigma). The immunoreactivity of individual bands on Western blots was measured by ImageJ software (National Institutes of Health) and normalized to TrkB or β-actin contents; similar results were obtained with the

two methods. Shown are representative results of immunoblotting from at least three independent experiments.

Behavioral Tests

After EEG and behavioral monitoring, all KA-infused mice were examined for spontaneous activity in the open field and anxiety-like behavior in the light/dark box at 8 weeks post-SE. PBS-infused (amygdala) WT or *TrkB*^{F616A} mice treated with vehicle or 1NMPP1 were behaviorally tested at 8 weeks post-infusion and they served as controls. *Open Field Test*. The open field (40 x 40 x 40 cm) was subdivided into sixteen 10 x 10 cm squares demarcated by lines marked on the floor. Mouse was placed in the center of the open field and given free-exploration of the apparatus for 5 min under ~300 lx illumination. *Light-Dark Emergence Test*. The apparatus consisted of a darkened (20 x 40 x 40 cm,) and a lighted compartment (40 x 40 x 40 cm, illuminated at 600 lx) that were separated by a divider with a 5.5 x 5.5 cm opening at the floor-level. Mice were placed into the darkened side and were given full access to both compartments for 5 min. During both behavioral tests, a video camera was mounted directly above the apparatus to monitor animal activity and movement. An investigator blinded to the genotype and treatment conditions reviewed the video and recorded for the open field: 1) line crossing (frequency of crossing grid lines with all four paws) and the frequency of rearing (standing on their hind legs or leaning against the walls of the arena); and for the light-dark box: 2) the latency to first entry into lighted compartment (entering the lighted side with all four paws) and the total time spent on the lighted side.

Neuropathology

At 10 weeks post-SE, mice were perfused with PBS containing heparin (5U/ml) followed by 4% paraformaldehyde. Brains were removed, frozen by slow immersion in isopentane chilled in dry ice, cryoprotected, and sectioned. Serial 20 µm coronal sections were cut through the forebrain spanning the

entire hippocampus. Adjacent sections at two levels relative to bregma (~1.82mm posterior designated “midlevel”) or caudal (~2.92 mm posterior designated “caudal”) were subjected to immunofluorescent staining as described (Mouri et al., 2008). Neurons and astrocytes were visualized using antibodies against neuronal nuclei (mouse monoclonal to NeuN, 1:500; Millipore) and glial fibrillary acidic protein (rabbit polyclonal to GFAP, 1:500; Sigma) detected with Alexafluor 488 coupled anti-mouse and Alexafluor 594 coupled anti-rabbit secondary antibodies (Molecular Probes), respectively. Images were captured using a Leica TCS SL confocal microscopy system equipped with a 63x oil-immersion objective lens. NeuN-positive cell counting was performed by an investigator blinded to the genotype and treatment conditions with ImageJ software (Ferreira and Rasband, 2011) in a 260 X 260 μm field within the CA3b pyramidal cell layer. Mean counts were obtained from two adjacent midlevel and two adjacent caudal hippocampal sections ipsilateral to the infused amygdala.

Data Analysis

All data are presented as the mean \pm standard error of the mean (SEM). Unless otherwise stated, comparisons between two groups were analyzed using unpaired Student’s t-tests, while multi-group comparisons were analyzed using two-way ANOVA followed by Bonferroni’s *post-hoc* tests. A $p < 0.05$ was considered significant.