SUPPLEMENTARY MATERIALS

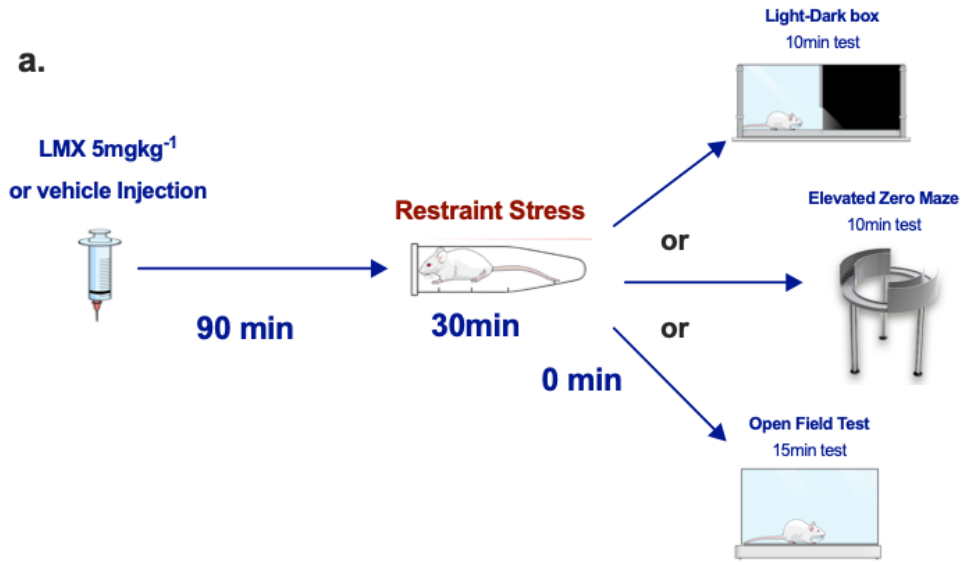

Figure S1. Experimental Design. (A) LMX 5 mg kg^{-1} or vehicle (DMSO) was administered via I.P. injection 90 minutes prior to 30 minutes of restraint stress, followed by immediate behavior testing in either LD, EZM, or OF test. No-stress groups received LMX or vehicle and remained in homecage for 120 minutes before LD, EZM, or OF testing. Separate cohorts of mice were used for LD, EZM, and OF tests.

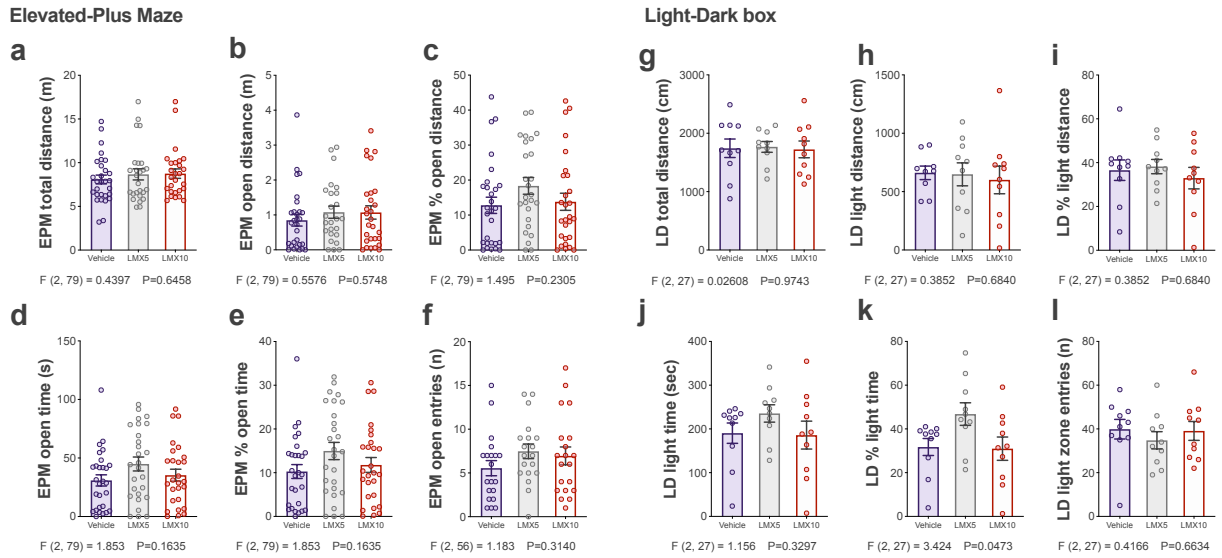

Figure S2. Behavioral effects of LMX treatment in naïve male mice. Acute LMX (5 and 10 mg kg^{-1}) did not affect baseline anxiety-like behaviors in naïve mice in EPM (A-F) or LD box (G-L) compared to vehicle (DMSO). *F*-scores from one-way ANOVAs; data are presented as individual values with Mean ± SEM, with number of subjects per group as follows: for EPM test, Vehicle n=29, LMX 5 mg kg^{-1} n=26, LMX 10 mg kg^{-1} n=27. For LD, Vehicle n=10, LMX 5mg kg^{-1} n=10, LMX 10mg kg^{-1} n=10.

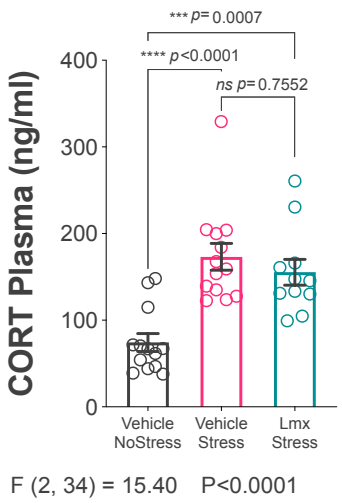

Figure S3. Plasma Corticosterone Levels after LMX treatment and restraint stress exposure. Plasma was analyzed for corticosterone levels to determine if LMX 5mg kg^{-1} treatment had any effect on HPA-axis activity after acute restraint stress. *F-score* below graph represents main effect differences between groups from One-Way ANOVA. *p-values* (above bars) represent significance levels from post-hoc Holm-Sidak pairwise comparisons. Data presented as individual values and Mean ± SEM, with number of subjects per group as follows: Vehicle-No Stress n=13, Vehicle-Stress n=13, LMX-Stress n=11.

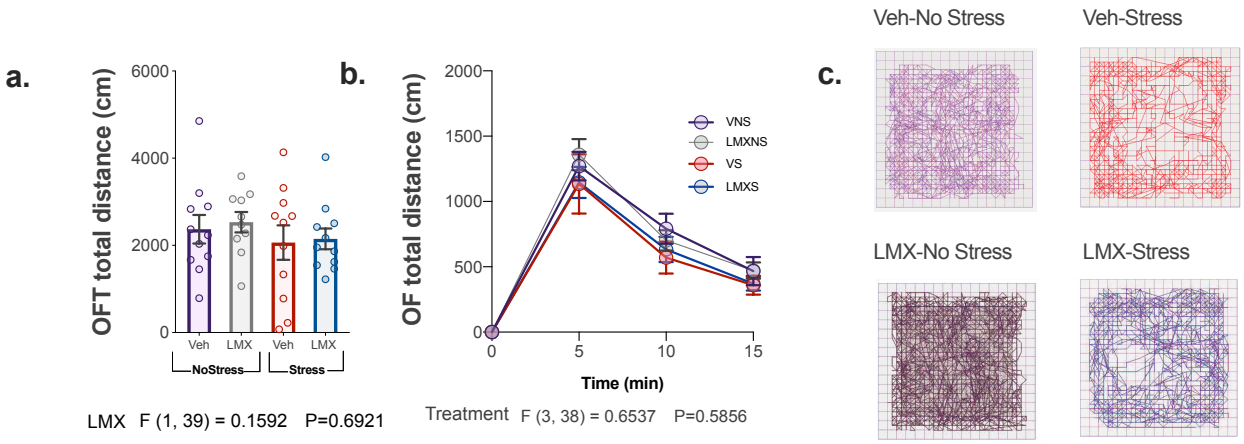

Figure S4. Effects of LMX treatment after stress in Open Field Test in male mice. LMX 5 mg kg^{-1} did not affect total distance traveled in the Open Field (A). *F*-scores from two-way ANOVAs; data are presented as individual values with Mean ± SEM, with number of subjects per group as follows: Vehicle-No Stress n=11, LMX-No Stress n=10, Vehicle-Stress n=11, LMX-Stress n=11. Track maps (C) represent total distance traveled for each group.

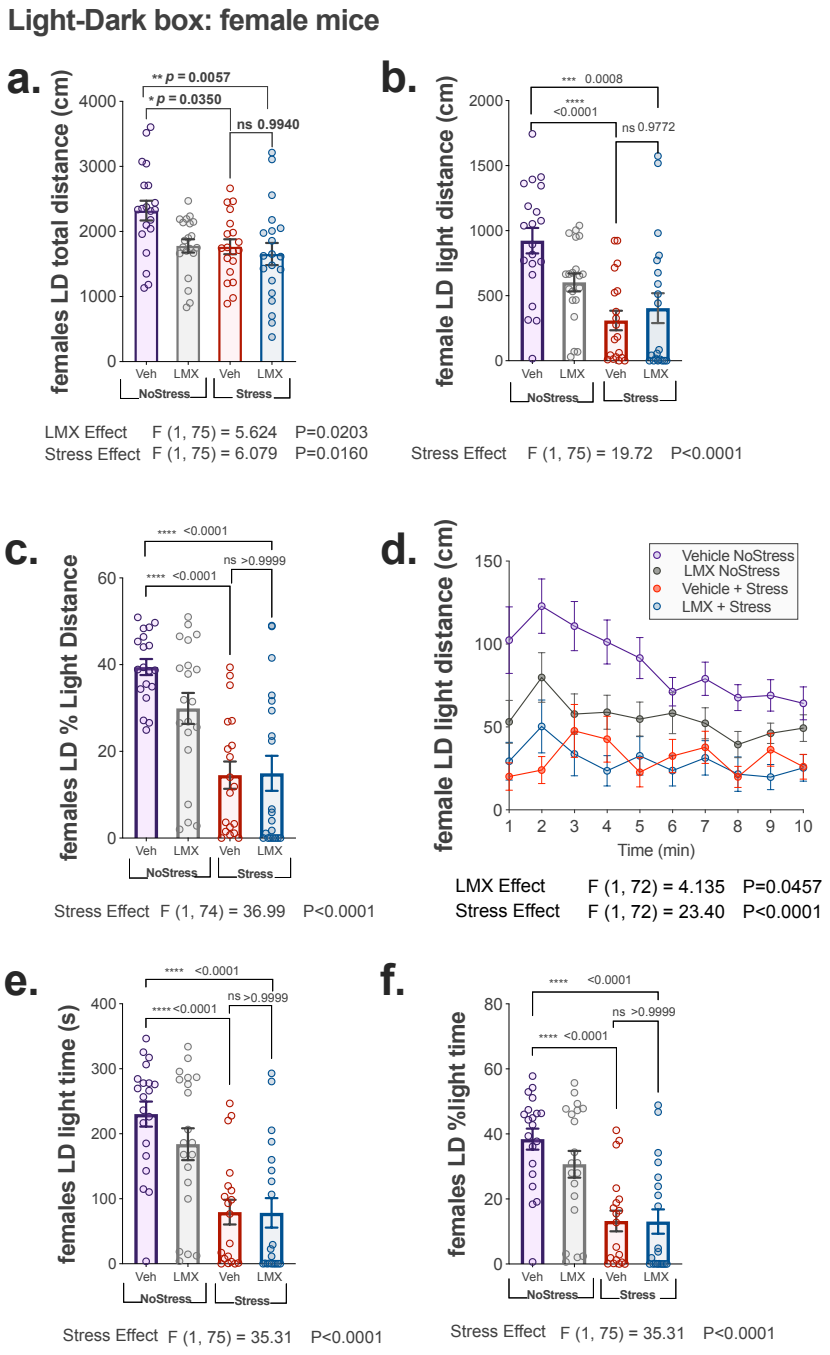

Figure S5. Effects of LMX on anxiety-like behavior after acute restraint stress in female mice. (A-F) In female ICR mice, LMX 5 mg kg^{-1} did not prevent anxiety-like behavior in the LD Box test induced by acute restraint stress. *F*-scores are from two-way ANOVAs; *p*-values (above bars) from Sidak pairwise comparisons. Data presented as individual values and Mean ± SEM, with number of subjects per group as follows: Vehicle-No Stress n=20, LMX-No Stress n=20, Vehicle-Stress n=20, LMX-Stress n=20.

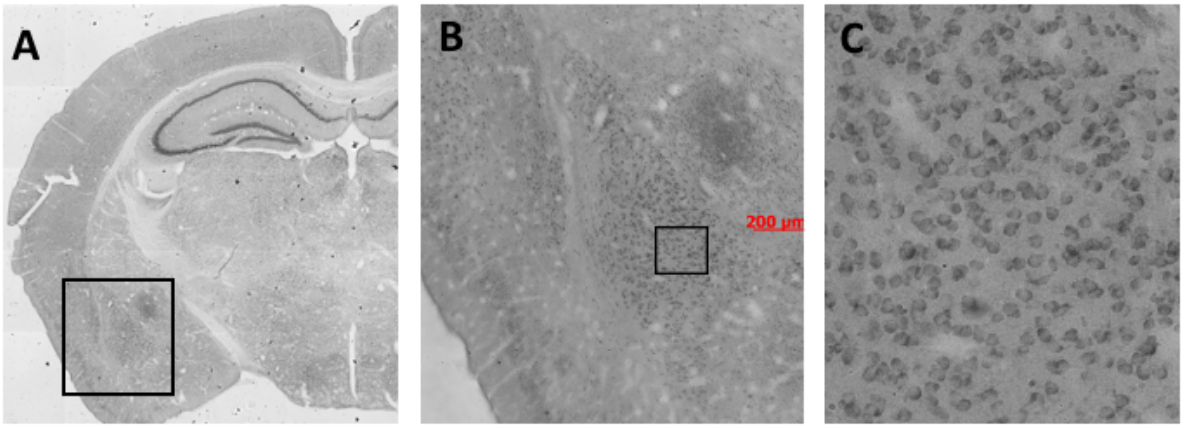

Figure S6. COX-2 immunostaining. (A) Low magnification image showing high COX-2 immunoreactivity within the hippocampus and amygdala. (B) higher magnification of boxed area in A showing strong COX-2 immunoreactivity in the basolateral and central amygdala. (C) Picture of boxed inset shown in B demonstrating pyramidal-like neurons in the BLA expressing strong COX-2 immunoreactivity.

Detailed Materials and Methods

Animals

Male ICR (CD-1) mice were used for all behavioral experiments (Envigo; Indianapolis, IN), with the exception of a single female cohort in *Supplemental Figure 5*. Mice were group housed in animal care facilities at Vanderbilt University (Nashville, TN), where colony rooms are climate-controlled and maintained at 21 ± 2˚C, 30% ± 10% relative humidity on a 12L:12D cycle, with lights on at 0700h. Food and water were provided *ad libitum* for the duration of the experiments. All studies were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals and were approved by the Vanderbilt University Institutional Animal Care and Use Committee (#M1600213-01).

Drugs and Restraint stress model

Lumiracoxib 5 or 10 mg kg^{-1} (Selleck Chemicals; TX, USA) in dimethyl sulfoxide (DMSO; vehicle) at a volume of 1 mL kg^{-1} was administered via I.P. route. The restraint stress procedure was conducted for 30 minutes exactly as described previously (Bedse et al., 2017).

Stereotaxic surgery: virus and chronic optical fiber implantation

Four-week old male ICR mice were deeply anesthetized with isoflurane (initial anesthesia dose 3% isoflourane, followed by surgery maintenance dose of 1.5%). The skull was exposed to allow small bilateral craniotomy holes. A targeted stereotaxtic intracranial microinjection needle containing 200–300nl of GCaMP7 AAV construct (product #104488-rg; Addgene; Watertown, MA) was lowered (30 seconds) intracranially into the basolateral amygdala [AP: 0.0, ML: ±3.5, DV: 5.00;(Paxinos and Franklin, 2004)]. Following bilateral injections, stainless steel threaded

ferrules (Shenzhen Perfect Precision Product Company LTD; Shenzhen, Guangdong, China) containing a multimode fiber (0.22 N.A., 300 μm diameter core/cladding, Thorlabs Inc.; Newton, N.J.) were implanted 20μm above the injection site, using a base layer of Metabond (C&B Metabond Clear L-Powder; Parkell, Edgewood, NJ), and a cranial cap of Fuji CEM 2 dental cement (Patterson Dental; Saint Paul, MN). The virus was allowed to express for 4 weeks before calcium photometry experiments.

Restraint Stress and in-vivo recordings

Following 30 minutes of habituation to the fiber optic cables for 3 consecutive days, mice were habituated to the photometry room only for a half hour and then given I.P. injection of either LMX or vehicle, and returned to homecages for 90 minutes. Following this, calcium recordings began as mice were connected to the fiber optic photometry cable and placed into an empty housing cage for three minutes (referred to as "pre-stress"). This was followed by 10 minutes of restraint stress in a custom slotted plastic tube of the same diameter as the conical tubes used in other restraint procedures (Vanderbilt Machine Shop; Nashville, TN). Photometry recordings continued for 3 minutes after restraint stress, wherein mice freely roamed the empty housing cage ("post-stress").

Calcium imaging and analysis

Calcium detection with fiber photometry was collected using the laser-based, ChiSquare χ^2-202 System (ChiSquare Bioimaging) as previously described (Harris et al., 2018). In brief, blue light from a 473 nm picosecond-pulsed laser 50 MHz; BDL-473-MC, Becker & Hickl) was directed onto a GFP dichroic filter (Thorlabs) and coupled using a FC/PC fiber coupler (PM480, Lasos) into a multimode fiber patch cord terminating in the ferrule implant described above.

Fluorescence emission was collected and filtered through a 550 nm broadpass filter (Semrock; Rochester, NY) and dispersed into a spectra by a polychromator (PML-SPEC, Becker & Hickl). Individual photons were detected by a time-correlated single photon counting (TCSPC) module. Raw photon counts (50Hz sampling rate) were entered into ClampFit 10.5 software (Axon Conventional Electrophysiology; San Jose, CA) and filtered using a Chebyshev 8 pole low-pass filter (5Hz), followed by manual baseline correction.

Next, trace spikes were identified using the MLspike program in MATLAB (Deneux et al., 2016) (MATLAB; The Mathworks, Inc.). The frequency of transients was analyzed using peaks exceeding 4 SD in amplitude, relative to baseline noise. The spikes and spike z-scores were plotted across 16 minutes (3 minute "pre-stress" epoch, ten minutes of restraint stress, and 3 minutes "post-stress" epoch). Area Under the Curve (AUC) analysis was conducted using Prism Graphpad 7 (San Diego, CA, USA). AUC was set at the highest baseline point for each trace. For statistical comparison of spike frequency and AUC between mice, individual subjects' data was normalized to pre-stress baseline for spike frequency and AUC values.

Behavior testing and data analysis

Elevated-plus and -zero mazes (EPM and EZM), Open-field test (OFT), and Light-dark box (LD) were performed exactly as described previously (Morgan et al., 2019). Data were analyzed via 2-way ANOVA followed by Sidaks post hoc multiple analysis tests. *F-scores and p-values* for ANOVAs and post hoc *p-values* are shown in relevant panels and graphs.

Corticosterone blood plasma assay

Mice were injected with vehicle (DMSO) or LMX 5mg kg^{-1} and returned to home cages. 120 minutes later, Vehicle-No Stress mice underwent a 5 min EZM test and were immediately sacrificed for trunk blood collection. 90 minutes after injection, Vehicle-Stress and LMX-Stress mice were restrained for 30 minutes, tested for 5 minutes in the EZM test, and sacrificed for trunk blood samples. Blood samples were immediately centrifuged (2,000 × *g* for 15 min at 4°C) to produce serum which was removed and stored at −80°C until testing. Plasma samples were diluted 1:40 for corticosterone level measurement, using an enzyme immunoassay (ELISA) kit (Enzo Life Sciences, Farmingdale, NY, USA) using 10 µL serum as per kit instructions.

COX-2 immunohistochemistry

Briefly, peroxidase IHC was used to visualize COX-2 in 40µm BLA slices of 5 week old male ICR mice. Sections were incubated with a rabbit anti-COX-2 antibody (#160126, 1:500; 48h at 4°C; Cayman; Ann Arbor, Michigan) followed by a biotinylated donkey anti-rabbit IgG secondary antibody (1:1000; 1.5h at room temperature; Jackson, Inc.; West Grove, PA, USA) and horseradish peroxidase-conjugated streptavidin (1:1600; 1h; Jackson, Inc.). A nickel-cobalt intensified diaminobenzidine solution with 0.3% hydrogen peroxide was used to cause blue–black cytoplasmic labeling of COX-2 positive cells.