SUPPLEMENTARY INFORMATION

A Parabrachial-to-Amygdala Circuit That Determines Hemispheric Lateralization of Somatosensory Processing

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Supplemental Methods

Animals

All experiments used *Calca*$^{tm1.1(cre/EGFP)Rpa}$ (*Calca*^{Cre}) Cre-recombinase knockin mice (Jax033168; originally obtained from Dr. Richard Palmiter, University of Washington), *Calcrl*^{Cre} (obtained from Dr. Richard Palmiter, University of Washington) crossed with a *Rosa26-flox-stop-tdTomato* reporter line Ai9 (Jax Ai9- 007909) to generate *Calcrl*^{Cre}::Ai9, or wild-type C57BL/6J (Jax000664) female littermates aged 9-13 weeks of age at the time of experimentation. Wild type breeders were acquired from Jackson Laboratory and replaced every 4 generations. *Calca*^{Cre} (1) and Calcrl^{Cre} (2) mice were bred as heterozygous pairs and backcrossed to C57BL/6J mice from Jackson Labs. Genotypes were confirmed using PCR, validating the expression of the wild-type allele and transgene in Cre heterozygous animals and the lack of the wild-type allele in Cre homozygous ("CGRP-knockout") animals. Immunohistochemistry for CGRP was also used to confirm *Calca* knockout in *Calca*$^{Cre/Cre}$ homozygous animals. Transgenic animals were bred in house at Duquesne University (Pittsburgh, PA) and housed 2-5 animals per cage on a 12-h light/dark schedule (7 am-7 pm) with *ad libitum* access to food and water. For electrophysiology studies at the National Institutes of Health and behavioral studies at the University of Texas at Dallas, *Calcrl*^{Cre}::Ai9 and C57BL/6J mice were bred in-house. Behavior and physiology assays were conducted during the light cycle. All experiments were in accordance with the National Institutes of Health guidelines and were approved by the Animal Care and Use Committee at Duquesne University (protocols 1905-06 and 2006-03), University of Pittsburgh (protocol 21063864), University of Texas at Dallas (protocol 20-04) or the National Institutes of Health (protocol 1397).

Cyclophosphamide-induced cystitis

Cyclophosphamide (CYP) is a chemotherapeutic drug which is used experimentally broken into various metabolites including acrolein which causes inflammation of bladder tissue and a bladder pain-like sensitivity phenotype in rodents (3,4). Animals receive three 100 μL intraperitoneal injections of 100 mg/kg CYP (Sigma) every other day for five days prior to experimentation. CYP solutions were made fresh on the day of the injection. Control mice received 100 μL intraperitoneal injections of saline. All experiments were completed one to two days after the completion of the CYP protocol (day 6).

Stereotaxic surgeries

Viral injection

Adeno-associated viruses containing Cre-dependent optogenetic constructs (rAAV5/Ef1a-DIO-hCHR2-mCherry, rAAV5/EF1a-DIO-NpHR3.0-mCherry, or rAAV5/EF1a-DIO-mCherry) were used to manipulate activity of *Calca*-expressing fibers from the parabrachial nucleus (PBN) in the central amygdala (CeA).). *Calcrl*^{Cre} mice received a bilateral injection of a Cre-dependent fluorophore (rAAV8-hSyn-DIO-mCherry) into the CeA for slice electrophysiology experiments. *Calca*$^{Cre+/-}$ mice 6-8 weeks old were anesthetized under 2% isoflurane and placed in a stereotaxic frame; body temperature was maintained with a heating pad throughout the surgery. A craniotomy was performed over either the left, right, or bilateral PBN and 1 μL of virus was injected into the PBN (AP -5.15 mm; ML +/-1.45 mm, DV -3.45 mm) via a 2.5 μL Hamilton syringe connected to a glass pipette at a rate of 0.20μL/min using the Quintessential Stereotax Injector (Stoelting). The pipette was left in place for an additional five minutes to allow for diffusion. The experimenter was blinded to virus being injected.

Cannula/wireless LED implantation

For cannula implantation following viral injection, mice received a craniotomy over the left, right, or bilateral CeA ipsilateral to viral injection as previously described (5). Two bone screws (Stoelting) were implanted on either side of the CeA craniotomy, anterior to bregma and anterior to lambda. A stainless-steel cannula (8.01 mm long, 0.2 mm diameter) was lowered into the CeA (AP -1.45 mm, ML +/- 3.00 mm, DV -4.20 mm) and fixed in place using dental cement. Animals receiving a 4-mm wireless LED Neurolux device (Neurolux, St. Louis, MO) following viral injection had the device lowered into the CeA ipsilateral to PBN viral injection and fixed in place using superglue. The scalp was sutured over the device. Animals recovered from anesthesia on a heating pad before being returned to their homecage. Animals received topical lidocaine on scalp incisions and had free access to children's Motrin (40mg/kg/day in drinking water) for 72 hours post-surgery. Behavioral experiments were conducted 3 weeks after surgery to allow for optimal viral expression and full surgical recovery.

For animals receiving only cannulae (pharmacology experiments, ELISA experiment), a craniotomy was performed over the left, right, or bilateral CeA. In control placement experiments, cannulae were implanted over the striatum (AP -1.45 mm, M/L +/- 3.00 mm, D/V -3.50 mm). Bone screws (Stoelting) were implanted contralateral to the side of cannulation anterior to bregma and anterior to lambda. A stainless-steel cannula was lowered into the left, right, or bilateral CeA (or striatum) and fixed in place with dental cement (Stoelting). Animals

were allowed to recover from anesthesia on a heating pad before being returned to their homecage. Animals received topical lidocaine on scalp incisions and had free access to children's Motrin (40 mg/kg/day in drinking water) for 72 hours post-surgery. Behavioral experiments were conducted 1-2 weeks following cannulation.

Urinary bladder distention

Urinary bladder distention (UBD) was conducted in 9- to 13-week-old female mice and visceromotor responses (VMR) were recorded by measuring electromyography (EMG) of the external abdominal oblique muscle during noxious distention as a quantitative measure of bladder pain-like responses. UBD-VMR was performed as previously described (6) one day following the final injection of CYP (day 6). Mice were anesthetized using isoflurane in an induction chamber before being transferred to a nose cone with 2% isoflurane vaporized in 100% oxygen. A lubricated 24-guage, 14 mm catheter was inserted into the bladder via the urethra. We have previously shown that the side of body recorded does not impact brain lateralized responses (5). Thus, the left abdominal wall was exposed, and two silver wires were implanted in the left external abdominal oblique muscle. A third wire was passed through the skin of the chest to serve as a grounding wire. Body temperature was maintained at 37^{o}C throughout the experiment using a battery-operated heating pad as well as an overhead radiant heat lamp.

Following surgery, isoflurane was lowered to 1.5% and then further lowered in 0.125% steps every 10 minutes until the animal responded to a noxious toe pinch but did not ambulate of vocalize (approximately 0.875-1.0% isoflurane). Once a stable level of isoflurane was reached, animals' bladders were distended 5-10 times with 60 mmHg of compressed air administered using a custom timed-pressure regulator (Washington University School of Medicine, St. Louis, MO) to establish stable VMRs to distention. Each distention lasted 20 seconds with a 1-minute intertrial interval between distentions. EMG signals were relayed via an amplifier through a Cambridge Electronic Design (CED) 1401 module to a computer with Spike2 software. Data was exported to IgorPro where background EMG was subtracted and stimulus evoked EMGs were rectified and integrated over the 20 second pressure period using a custom script. A second similar custom script was used to analyze the pre-distention period, and all VMRs were normalized to the smallest pre-distention VMR.

Optogenetic manipulation during UBD

Following the establishment of stable VMRs to bladder distention, baseline VMRs were collected (10 distentions alternating at 30 mmHg and 60 mmHg pressure). Immediately after baseline, light (532 nm for

inhibition or 473 nm for excitation) was delivered using a low-power laser diode (Shanghai Laser and Optics Century Co, Ltd, Shanghai, China) or LED (Thor-Labs, Newton, NJ) during the “light-on” timepoint and VMRs were collected (same parameters as baseline). Immediately after the completion of the “light-on” timepoint, the light source was turned off and the post light timepoint was collected using the same pressure sequence parameters.

For inhibition experiments, 532 nm constant light stimulation (8-15 mW power) was administered. For excitation experiments, 473 nm light was pulsed (20 Hz, 5-ms pulse width, 3-20 mW power) to mimic the firing of CeA neurons following injury (7). Experimenter was blinded to virus (mCherry v ChR2/NpHR) until optogenetic manipulation was completed.

Pharmacological activation/inhibition during UBD

CGRP and CGRP(8-37) were purchased from Genscript (RP11095, RP11090) and reconstituted and diluted in aCSF. Following the collection of baseline VMRs, wild type animals received injection of aCSF, 100 nM CGRP (volume 1 μL), 100 nM CGRP(8-37), or a cocktail of 100 nM CGRP+100 nM CGRP(8-37) at a rate of 0.2 μL/min via a 32-gauge injection cannula that extended 0.1 mm beyond the tip of the cannula. The injection cannula was coupled to a Hamilton syringe via flexible plastic tubing, and the injector was left in place for an additional 5 minutes to allow for diffusion. VMRs were collected in response to UBD using the same parameters as baseline every fifteen minutes following the completion of injection for 90 minutes. Immediately following the completion of the experiment, animals were euthanized, and brains were extracted for cannula placement verification. The experimenter was blinded to drug treatment until after verification of post hoc viral expression and/or cannula placement targeting verification. For striatum placement control experiment, experimenter was blinded to experimental hypothesis, cannula placement, and treatment but not side of brain.

Combined optogenetic and pharmacological manipulation during UBD

Baseline VMRs were collected as in other UBD experiments in *Calca*^{Cre} mice. Immediately following baseline, 1 μL of aCSF, 100 nM CGRP(8-37), or a cocktail of 22 mM AP5 and 38 mM NBQX (8) was injected into either the left or right CeA as described above. Thirty minutes following injection, a fiberoptic coupled to a 473-nm laser was inserted into the cannula and pulsed at 20-Hz (15-20-mW) while VMRs were collected. Post

laser and post drug VMRs were collected 90 min following injection to allow time for drug washout. Experimenter was blinded to drug injection until viral injection site and cannula placement were verified.

For *Calca*$^{Cre/+}$ heterozygote versus knockout (*Calca*$^{Cre/Cre}$) experiments, baseline VMRs were collected as described. Following baseline, animals had either a fiberoptic coupled to a 473-nm laser for optogenetic excitation or an injector for pharmacology inserted into the cannula. Optogenetic and pharmacology experiments were performed in the same animals in a randomized order and no effect of order was found (**Supplementary Fig. 7**). Optogenetic activation of CGRP terminals in the left or right CeA was performed identical to optogenetic manipulation during UBD described above. Pharmacological activation of CGRP receptor cells in the CeA was achieved via infusion of CGRP as described above. Experimenter was blinded to genotype until viral injection site and cannula placement were verified.

von Frey abdominal sensitivity

In vivo behavioral testing was conducted one to two days following final CYP injection (day 6-7). This correlates to day 20 in experiments where animals received optogenetic stimulation of CGRP-containing PBN fibers. Animals were habituated in ventilated Plexiglas enclosures 15 cm x 15 cm on wire mesh for 1.5 hours before testing. Sixty decibel white noise was used to mask background sounds. The experimenter was present in the room for 30 min prior to the beginning of testing. Calibrated von Frey filaments (Touch Test) were used to assess abdominal sensitivity on the right and left abdomen approximately 0.5 cm from the urethra via the up-down method to calculate 50% withdrawal thresholds (9). No difference was found in abdominal sensitivity between right and left side, so withdrawal thresholds were averaged.

Optogenetic manipulation during abdominal sensitivity testing

Animals were habituated on metal mesh in Plexiglas boxes tuned for Neurolux optogenetic stimulation. Following collection of baseline 50% withdrawal thresholds, a 473-nm wireless LED Neurolux device was remotely activated (20-Hz stimulation, 5-ms pulse width, 10mW power) and 50% withdrawal threshold were collected again. Experimenter was blinded to virus (mCherry v ChR2) until after analysis of viral injection site and LED placement.

Real time place preference

One day prior to abdominal von Frey testing (day 6 post initial CYP injection, day 19 post-surgery), animals were habituated to the behavior room for 20 min in their home cage. Animals were habituated on day 6

to avoid a negative association of CYP injection with habituation. Sixty decibel white noise was used to mask background noise. Animals were placed in a three-chamber Plexiglas place preference apparatus (30 cm^2 x 20 cm) with distinct visual patterns and allowed to freely explore for 20 min. The animals' activity was video recorded using AnyMaze (Stoelting Co.) behavioral tracking software. The next day (day 20 post-surgery), following von Frey abdominal sensitivity testing, animals were returned to their home cage for 20 min. Animals were then placed back in the place preference apparatus where one chamber was tuned for wireless Neurolux LED stimulation. Upon entering the tuned chamber, the Neurolux device automatically started stimulation (473-nm, 20-Hz, 10mW power), which ended as soon as the animal exited the Neurolux tuned chamber. Animals' activity was video recorded for 20 min using AnyMaze. Any animal displaying more than 700 seconds in a single chamber on habituation day was excluded from the experiment. Four animals were excluded. Experimenter was blinded to virus (mCherry v ChR2) until after analysis of viral injection site and LED placement.

Immunohistochemistry

Viral Targeting

All viral constructs used in these experiments contained an mCherry sequence to allow for viral targeting in *Calca*-expressing (Cre-recombinase positive) cells in the PBN and terminals in the CeA. Following optogenetic experiments, animals were perfused using 20 mL 1x phosphate buffered saline (PBS) and 20 mL ice cold 4% paraformaldehyde (PFA). Brains were removed and stored in 4% PFA overnight at 4°C before being transferred to 30% sucrose. After 3-5 days in sucrose, brains were frozen and kept at -80°C until sectioning. Thirty µm coronal sections were collected using a cryostat and stored in PBS at 4°C. Immunohistochemistry was performed on floating PBN and CeA sections for mCherry. Sections were washed 3 times in 1x PBS, blocked for 60 minutes using 1% bovine serum albumin and 0.2% milk in 1x PBS with 0.1% Triton-X, and incubated overnight at 4°C in 1:1000 anti-mCherry (rabbit, Abcam ab167453) in blocking solution. Sections were then washed 3 times in PBS with 0.1% Triton X before being incubated in 1:1000 anti-555 (anti-rabbit Alexa Fluor 555, Invitrogen A-21249) in blocking solution at room temperature for 1 hour. Sections were then washed 3 times in PBS before being mounted onto Superfrost Plus (Fisher Scientific) microscope slides, coverslipped with Vectashield (Vector Laboratories) anti-fade mounting medium with DAPI, and imaged using a Nikon Eclipse Ti2 microscope.

CGRP quantification

C57BL/6J female mice were treated with either CYP or saline and perfused one day following the final injection (day 6). Thirty µm coronal sections were collected and stored in PBS at 4 °C until staining. Six representative sections from across the rostral-caudal axis of the CeA were picked for staining. Tissue was washed three times in 1x PBS before being blocked in 10% normal goat serum in PBS with 0.1% Triton-X for 60 minutes. Tissue was incubated with anti-CGRP (1:2000 rabbit anti CGRP, Calbiochem PC205L) in 5% normal goat serum in PBS with 0.1% Triton-X overnight at 4 °C. Tissue was then washed three times in 1x PBS with 0.1% Triton-X and incubated in fluorescent secondary antibody (goat anti-rabbit AlexaFluor 488, Life Technologies, A-11034) diluted in 5% normal goat serum in 1x PBS with 0.1% Triton-X for 60 minutes at room temperature. After three more washes in 1x PBS, tissue sections were mounted on SuperFrost Plus microscope slides and coverslipped with Vectashield anti-fade mounting medium with DAPI. Images were captured using a Nikon confocal microscope and fluorescence intensity for each channel was quantified using NIS-Elements Advanced Research software. All microscope images were acquired using settings from a negative control and settings were kept consistent. Fluorescence intensity of the 488 channel was normalized to fluorescence intensity of the DAPI channel for each image. The CeC was defined as the area 200 µm inward from BLA/CeA border (24).

***Ex-vivo* Electrophysiology**

Acute slice preparation of left and right CeA

Female *Calcrl*^{Cre}::Ai9, *Calcrl*^{Cre} mice previously injected with a Cre-dependent fluorophore (mCherry), or C57BL/6J wild-type mice (9 to 15 weeks old) were deeply anesthetized with 1.25% Avertin (0.4 mg/g). Mice were injected intraperitoneally and transcardially perfused with ice-cold cutting solution (110 mM choline chloride, 25 mM $NaHCO_3$, 1.25 mM NaH_2PO_4, 2.5 mM KCl, 0.5 mM $CaCl_2$, 7.2 mM $MgCl_2$, 25 mM D-glucose,12.7 mM L-ascorbic acid, 3.1 mM pyruvic acid, oxygenated with 95%/5% O_2/CO_2). Brains were extracted, placed in ice-cold cutting solution, and cut in coronal slices (250 µm) with a Leica VT1200 S vibrating blade microtome (Leica Microsystems Inc). CeA slices were cut in half to separate left and right hemispheres and were incubated at 33°C for 30 minutes in artificial cerebrospinal fluid (aCSF) (125 mM NaCl, 2.5 mM KCl, 1.25 mM NaH_2PO_4, 25 mM $NaHCO_3$, 2 mM $CaCl_2$, 1 mM MgCl2, 25 mM D-glucose). Slices were recovered at room temperature for at

least 20 min before recording. Chambers were continuously oxygenated with 95%/5% O_2/CO_2 during incubation and recovery.

Electrophysiological recordings

The recording chamber was perfused continuously with aCSF oxygenated with 95%/5% O_2/CO_2 (1ml/min), and all recordings were performed at 33 ± 1˚C using a recording chamber heater and in-line solution heater (Warner Instruments). Recording pipettes (2-6 MΩ resistance) were filled with internal solution (120 mM potassium methyl sulfate, 20 mM KCl, 10 mM HEPES, 0.2 mM EGTA, 8 mM $NaCl_2$, 4 mM Mg-ATP, 0.3 mM Tris-GTP, and 14 mM phosphocreatine with pH 7.3 using 5 M KOH and an osmolarity of ~300 $mosmol^{-1}$). Whole-cell current clamp recordings were collected from neurons in the capsular (CeC) subdivision of either the right or left CeA, as identified using an upright microscope (Nikon Eclipse FN1). Recordings were acquired using the Multiclamp 700B patch-clamp amplifier interfaced with a Digidata 1500 acquisition system and pCLAMP 10.7 software (Molecular Devices) on a Dell computer. Before forming a membrane-pipette seal, pipette tip potentials were zeroes. Series resistances (not exceeding 20 MΩ) were monitored throughout the recordings. Whole-cell capacitance was measured in voltage-clamp configuration, with the cell held at -70 mV, then subjected to a +/-10 mV current change of 25 ms duration.

Recordings were restricted to fluorescently labeled CeC neurons expressing the CGRP receptor (CGRPR+) in slices from *Calcrl*^{Cre}::Ai9 or injected *Calcrl*^{Cre} mice. Additionally, recordings were collected from late-firing, unlabeled neurons in slices from C57BL/6J wild-type mice within the capsular subdivision of the CeA. Late-firing neurons were defined as cells with a latency to spike higher than 100 ms at a 280 pA current amplitude as previously described (10). 500 ms depolarizing current of various amplitudes (between 20 pA and 280 pA) was injected from resting membrane potential to elicit repetitive action potentials firing under an initial aCSF bath condition and after bath application of 500 nM CGRP (11,12) for at least 5 min to allow for a full bath exchange. Only 1 cell per slice was used following CGRP bath application. Recording from control cells were obtained before and 5 min after bath exchange to aCSF. Recordings were acquired at 50 kHz and filtered at 10 kHz. At the end of each electrophysiological recording, pipette location was imaged at low magnification and the anatomical localization of each recording was determined in reference to a mouse brain atlas (13). Aliquots of CGRP were dissolved in water, stored at -20˚C, and added to 100 mL of aCSF for each experiment.

RNAscope *in situ* hybridization

RNAscope fluorescent multiplex assay v2 was used with probes for *Calcrl*, *Prkcd,* and *Sst* to determine co-localization in the CeA. Brains from perfused C57Bl/6J mice treated with either CYP or saline were postfixed in 4% PFA for 3 hours before being transferred to 30% sucrose until tissue sank. Brains were then frozen and 20 μm coronal sections were collected using a cryostat and stored in anti-freeze at -20°C. Five to six representative sections from across the rostral-caudal axis of the CeA in each animal were mounted on Superfrost Plus slides and allowed to air dry overnight at room temperature. Slides were treated twice with xylene (5 minutes each) followed by two treatments with 100% ethanol (2 min each). Tissue was treated with protease III for 20 min at 40°C in HybEZ oven. RNAscope was performed according to manufacturer's instructions (ACDBio Inc.). Briefly, probes for *Calcrl, Prkcd*, and *Sst* were hybridized for 2 hours in the HybEZ oven at 40°C. Signal was amplified using a series of AMPs according to manufacturer's instructions, followed by development of a TSA-based fluorescent label. Slides were cover slipped with Vectashield anti-fade mounting medium with DAPI. Images were captured within 48 hours using a Nikon Eclipse Ti2 microscope and images were analyzed using NIS-Elements Advanced Research software. Positive cells were identified as a DAPI-labeled nucleus surrounded by at least three puncta. Cell counts were determined blinded to treatment (CYP vs saline). Cell number and percent co-localization were averaged across all sections from the same brain.

CeA tissue collection and cAMP ELISA

Wild-type C57Bl6/J mice with bilateral cannulae were anesthetized using isoflurane in an induction chamber before being transferred to a nose cone with 1% isoflurane vaporized in 100% oxygen. CGRP purchased from Genscript (RP11095, RP11090) was reconstituted and diluted to 100nM in aCSF. Animals received bilateral injections of aCSF or 100 nM CGRP (1 μL) at a rate of 0.2 μL/min via a 32-gauge injection cannula that extended 0.1 mm beyond the tip of the cannula. The injection cannula was coupled to a Hamilton syringe via flexible plastic tubing, and the injector was left in place for an additional 5 minutes to allow for diffusion. Following infusion, mice were returned to their homecage for 40 minutes. Mice were decapitated and brains were sectioned and flash frozen to extract CeA micro punches. Left and right CeA tissue from each animal was homogenized in 1X homogenate buffer (20 mM Tris pH 7.5, 1 mM EDTA, 1 mM $Na_4P_2O_7$, 1X Halt Thermo Scientific Protease Inhibitor Single-Use Cocktail, PI78425) and centrifuged at 12,000 RPM for 20 minutes at 4°C.

Supernatant was collected and stored at -80°C until ELISA was performed. ELISA was performed according to kit instructions (Cell Biolabs, STA-500) using tissue homogenate from the left and right CeA.

Statistics and data analysis

All data analyses, including RNAscope, immunohistochemistry, behavior, and physiology, were conducted blind to treatment/virus/genotype. Data were analyzed using GraphPad Prism v9.1.2, NIS-Elements Advanced Research software v5.02, Spike2 v7.08, IgorPro v6.22A, AnyMaze (Version 9.0), and Microsoft Office v16.50. UBD data was analyzed via unpaired *t*-tests for two group comparisons, repeated-measures two-way analysis of variance (ANOVA) followed by Bonferroni or Dunnett's post hoc tests for multiple comparisons. Behavioral data was analyzed using paired *t*-tests, one-way ANOVA, or repeated measures two-way ANOVAs followed by Bonferroni or Dunnett's post hoc tests for multiple comparisons. *Ex vivo* physiology analysis was performed using Clampfit 10.7 software (Molecular Devices). Electrophysiology data were analyzed via two-way repeated measures ANOVA. RNAscope data was analyzed using two-way ANOVAs followed by Tukey post-hoc test for multiple comparisons. Statistical significance was determined at the level of $P<0.05$. Asterisks denoting P values include: *P<0.05, **P<0.01, ***P<0.001, and ****P<0.0001. All data are presented as the mean +/- standard error of the mean (SEM). Statistical information for all figures is provided in **Supplementary Table 1**.

Supplementary Table 1

Figure	Comparison	Analysis	P value, F, or t value	N
1D	Normalized VMRs to 60 mmHg distention in naïve vs CYP-treated animals	Unpaired t-test	P=0.0076 t=2.776	28 mice per group
1E	Percent change in VMR to 60 mmHg in CYP mice with mCherry or ChR2 in the left CeA before, during, and after laser stimulation	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0025, F (2, 36) = 7.132 Virus, p=0.0463, F (1, 18) = 4.582 Time x virus, p=0.0018, F (2, 36) = 7.543 mCherry: BL v laser, p=0.9922 BL v PL, p=0.9205 ChR2: BL v laser, p<0.0001 BL v PL, p>0.9999	10-11 mice per group
1F	Area under the curve for 60 mmHg pressure in CYP mice with	Unpaired t-test	P=0.0076 t=3.007	10-11 mice per group

	mCherry or ChR2 in the left CeA			
1H	Percent change in VMR to 60 mmHg in CYP mice with mCherry or ChR2 in the right CeA before, during, and after laser stimulation	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0059, F (1.909, 30.55) = 6.242 Virus, p=0.2222 F (1, 16) = 1.613 Time x virus, p=0.0043, F (2, 32) = 6.486 mCherry: BL v laser, p=0.6944 BL v PL, p=0.8047 ChR2: BL v laser, p=0.0071 BL v PL, p=0.4105	8-10 mice per group
1I	Area under the curve for 60 mmHg pressure in CYP mice with mCherry or ChR2 in the right CeA	Unpaired t-test	P=0.0505 t=2.115	8-10 mice per group
1K	Percent change in VMR to 60 mmHg in CYP mice with mCherry or NpHR in the left CeA before, during, and after laser stimulation	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0171, F (2,2 2) = 4.923 Virus, p=0.0069, F (1, 11) = 10.98 Time x virus, p=0.0023, F (2, 22) = 8.078 mCherry: BL v laser, p=0.7686 BL v PL, p=0.9967 NpHR: BL v laser, p=0.0002 BL v PL, p=0.7854	6-7 mice per group
1L	Area under the curve for 60 mmHg pressure in CYP mice with mCherry or NpHR in the left CeA	Unpaired t-test	P=0.0080 t=3.229	6-7 mice per group
1N	Percent change in VMR to 60 mmHg in CYP mice with mCherry or NpHR in the right CeA before, during, and after laser stimulation	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0002, F (1.615, 17.77) = 16.34 Virus, p=0.0008, F (1, 11) = 20.80 Time x virus, p=0.0039, F (2, 22) = 7.210 mCherry: BL v laser, p=0.8892 BL v PL, p=0.0945 NpHR: BL v laser, p=0.0017 BL v PL, p=0.7449	6-7 mice per group
1O	Area under the curve for 60 mmHg pressure in CYP mice with mCherry or NpHR in the right CeA	Unpaired t-test	P=0.0007 t=4.699	6-7 mice per group

2C	Abdominal mechanical sensitivity before and after CYP treatment	Paired t-test	P<0.0001 t=7.716	31 mice
2D	50% withdrawal thresholds in CYP mice with mCherry or ChR2 in the left CeA	Two-way RM ANOVA with Bonferroni posttest	Time, p=0.0103, F (1, 17) = 8.314 Virus, p=0.5377, F (1, 17) = 0.3959 Time x virus, p=0.4061, F (1, 17) = 0.7257 Post CYP v laser: mCherry, p=0.3989 ChR2, p=0.0209	8-11 mice per group
2E	Percent change in abdominal sensitivity in CYP mice with mCherry of ChR2 in the left CeA	Two-way RM ANOVA with Bonferroni posttest	Time, p=0.0162, F (1, 17) = 7.177 Virus, p=0.1226, F (1, 17) = 2.639 Time x virus, p=0.1226, F (1, 17) = 2.639 Post CYP v laser: mCherry, p>0.9999 ChR2,p= 0.0083	8-11 mice per group
2F	50% withdrawal thresholds in CYP mice with mCherry or ChR2 in the right CeA	Two-way RM ANOVA with Bonferroni posttest	Time, p=0.1159, F (1,11) = 2.913 Virus, p=0.5393, F (1, 11) = 0.4014 Time x virus, p=0.0327, F (1, 11) = 5.963 Post CYP v laser: mCherry, p>0.9999 ChR2, p=0.0329	6-7 mice per group
2G	Percent change in abdominal sensitivity in CYP mice with mCherry of ChR2 in the right CeA	Two-way RM ANOVA with Bonferroni posttest	Time, p=0.0022, F (1, 11) = 15.78 Virus, p=0.0001, F (1, 11) = 32.15 Time x virus, p=0.0001, F (1, 11) = 32.15 Post CYP v laser: mCherry, p=0.4745 ChR2, p<0.0001	6-7 mice per group
2I	Difference score for time spent in the laser chamber in CYP mice with mCherry or ChR2 in the left or right CeA	Two-way ANOVA	Virus, p=0.4045, F (1, 29) = 0.7157 Side, p=0.6708, F (1, 29) = 0.1844 Virus x side, p=0.5057, F (1, 29) = 0.4541	6-9 mice per group
3D	Number of spikes in left CeA neurons to increasing current steps during CGRP (500 nM) or aCSF application	Two-way ANOVA	Drug, p=0.0086, F(1,21) = 8.396 Current Injection, p<0.0001, F (1.436, 30.16) = 25.37 Drug x Current Injection, p<0.0001 F(13, 273) = 4.592	<u>aCSF</u> • 8 unidentified neurons from 7 mice • 6 CGRPR+ neurons from 5 mice <u>500 nM CGRP</u> • 4 unidentified neurons from 3 mice • 5 CGRPR+ neurons from 4 mice

3F	V_{rest} of left CeA neurons after aCSF or CGRP (500 nM) application	Paired t-test	p<0.0001 t=7.521	N=9
3H	Number of spikes in right CeA neurons to increasing current during CGRP (500 nM) or aCSF application	Two-way ANOVA	Drug, p=0.0028, F(1,27) = 10.86 Current Injection, p<0.0001, F(1.637, 44.20) = 33.98 Drug x Current Injection, p<0.0001, F(13, 351) =6.129	<u>aCSF</u> • 11 unidentified neurons from 8 mice • 9 CGRPR+ neurons from 6 mice <u>500 nM CGRP</u> • 2 unidentified neurons from 1 mouse • 7 CGRPR+ neurons from 5 mice
3J	V_{rest} of right CeA neurons after aCSF or CGRP (500 nM) application	Paired t-test	P=0.0562 t=2.231	N=9
3K	Percent change in VMRs to 60 mmHg distention after injection of drug into the left CeA of naïve mice	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0021, F (3.414, 40.97) = 5.429 Drug, p=0.0012, F (1, 12) = 17.57 Time x drug, p=0.0008, F (6, 72) = 4.363 aCSF: BL v 15, p=0.9276 BL v 30, p=0.2239 BL v 45, p=0.9954 BL v 60, p=0.0976 BL v 75, p=0.7753 BL v 90, p=0.5009 CGRP: BL v 15, p=0.2248 BL v 30, p=0.0149 BL v 45, p<0.0001 BL v 60, p=0.2936 BL v 75, p=0.8788 BL v 90, p=0.9998	7 mice per group
3L	Area under the curve for 60 mmHg distention after drug injection in the left CeA in naive mice	Unpaired t-test	P=0.0008 t=4.422	7 mice per group
3M	Percent change in VMRs to 60 mmHg distention after injection of drug into the left	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.6240, F (4.262, 144.9) = 0.6688 Drug, p<0.0001, F (3, 34) = 17.65 Time x drug, p<0.0001, F (18, 204) = 14.51	9-10 mice per group

	CeA in CYP mice		aCSF: BL v 15, p=0.3200 BL v 30, p>0.9999 BL v 45, p=0.5062 BL v 60, p=0.7185 BL v 75, p=0.0855 BL v 90, p=0.2669 CGRP: BL v 15, p=0.0016 BL v 30, p=0.0004 BL v 45, p<0.0001 BL v 60, p=0.0002 BL v 75, p=0.9028 BL v 90, p=0.1649 CGRP(8-37): BL v 15, p=0.0685 BL v 30, p=0.0011 BL v 45, p=0.0051 BL v 60, p=0.0080 BL v 75, p=0.6106 BL v 90, p=0.9093 CGRP+CGRP(8-37): BL v 15, p=0.7755 BL v 30, p=0.9807 BL v 45, p=0.9983 BL v 60, p=0.9096 BL v 75, p=0.9816 BL v 90, p=0.9717	
3N	Area under the curve for 60 mmHg distention after drug injection in the left CeA in CYP mice	One-way ANOVA with Dunnett's posttest	P<0.0001 F=22.16 R^2=0.6616 aCSF v CGRP, p=0.0152 aCSF v CGRP(8-37), p<0.0001 aCSF v CGRP +CGRP(8-37), p=0.7761	9-10 mice per group
3P	Percent change in VMRs to 60 mmHg distention after injection of drug into the right CeA of naïve mice	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0002, F (2.216, 31.03) = 10.82 Drug, p=0.0085, F (1, 14) = 9.363 Time x drug, p<0.0001, F (6, 84) = 8.119 aCSF: BL v 15, p=0.2393 BL v 30, p=0.8630 BL v 45, p=0.7829 BL v 60, p=0.9996 BL v 75, p>0.9999 BL v 90, p>0.9999 CGRP: BL v 15, p=0.0442 BL v 30, p=0.1129 BL v 45, p=0.0166 BL v 60, p=0.0446 BL v 75, p=0.9995 BL v 90, p=0.8484	8 mice per group

3Q	Area under the curve for 60 mmHg distention after drug injection in the right CeA in naive mice	Unpaired t-test	P=0.0066 t=3.151	8 mice per group
3R	Percent change in VMRs to 60 mmHg distention after injection of drug into the right CeA in CYP mice	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.3790, F (4.259, 106.5) = 1.066 Drug, p<0.0001, F (3, 25) = 45.88 Time x drug, p<0.0001, F (18, 150) = 11.97 aCSF: BL v 15, p=0.9600 BL v 30, p=0.6980 BL v 45, p=0.2404 BL v 60, p=0.8267 BL v 75, p=0.0170 BL v 90, p=0.0890 CGRP: BL v 15, p=0.0440 BL v 30, p=0.0135 BL v 45, p=0.0008 BL v 60, p=0.0005 BL v 75, p=0.8480 BL v 90, p=0.2859 CGRP(8-37): BL v 15,p=0.0244 BL v 30, p=0.0002 BL v 45, p<0.0001 BL v 60, p=0.0003 BL v 75, p=0.7076 BL v 90, p=0.9781 CGRP+CGRP(8-37): BL v 15, p=0.2740 BL v 30, p=0.3866 BL v 45, p=0.9979 BL v 60, p=0.9983 BL v 75, p=0.9999 BL v 90, p=0.7707	6-8 mice per group
3S	Area under the curve for 60 mmHg distention after drug injection in the right CeA in CYP mice	One-way ANOVA with Dunnett's posttest	P<0.0001 F=53.02 R^2=0.8642 aCSF v CGRP, p<0.0001 aCSF v CGRP(8-37), p<0.0001 aCSF v CGRP + CGRP(8-37), p=0.8942	6-8 mice per group
4B	Percent change in VMRs to 60 mmHg after injection of drug during optogenetic stimulation in the left CeA of CYP	Two-way RM ANOVA with Dunnett's posttest	Time, p<0.0001, F (1.573, 25.17) = 58.02 Drug, p=0.0003, F (2, 16) = 14.31 Time x drug, p<0.0001, F (4, 32) = 12.64 aCSF: BL v laser, p<0.0001 BL v PL, p=0.360	6-7 mice per group

	mice		CGRP(8-37): BL v laser, p=0.5061 BL v PL, p=0.5051 AP5+NBQX: BL v laser, p=0.0003 BL v PL, p=0.3714	
4C	Percent change in VMRs to 60 mmHg after injection of drug during optogenetic stimulation in the right CeA of CYP mice	Two-way RM ANOVA with Dunnett's posttest	Time, p<0.0001, F (1.167, 21.00) = 24.52 Drug, p=0.0025, F (2, 18) = 8.515 Time x drug, p=0.0008, F (4, 36) = 6.051 aCSF: BL v laser, p=0.0286 BL v PL, p=0.3955 CGRP(8-37): BL v laser, p=0.8161 BL v PL, p=0.3905 AP5+NBQX: BL v laser, p=0.0077 BL v PL, p=0.5923	6-8 mice per group
4M	Percent change in VMRs to 60 mmHg distention during a) optogenetic stimulation and b) pharmacological activation in the left CeA of CGRP heterozygous and homozygous mice treated with CYP	Two-way RM ANOVA with Bonferroni posttest	a) Time, p<0.0001, F (1.518, 22.77) = 40.12 Genotype, p=0.0709, F (1, 15) = 3.779 Time x genotype, p<0.0001, F (2, 30) = 15.06 Het v KO: Laser, p=0.0074 PL, p=0.2824 b) Time, p<0.0001, F (2.285, 34.28) = 46.89 Genotype, p=0.2353, F (1, 15) = 1.529 Time x genotype, p=0.0994, F (6, 90) = 1.844 Het v KO: 0, p=0.1301 15, p>0.9999 30, p>0.9999 45, p>0.9999 60, p>0.9999 75, p=0.2455 90, p>0.9999	8-9 mice per group
4N	Percent change in VMRs to 60 mmHg distention during a) optogenetic stimulation and b) pharmacological activation in the right CeA of CGRP heterozygous and homozygous	Two-way RM ANOVA with Bonferroni posttest	a) Time, p=0.0004, F (2, 26) = 10.62 Genotype, p=0.1312, F (1, 13) = 2.581 Time x genotype, p=0.0011, F (2, 26) = 8.979 Het v KO: Laser, p=0.0004 PL, p=0.4819 b) Time, p<0.0001, F (6, 78) = 56.74 Genotype, p=0.6945, F (1, 13) = 0.1613	7-8 mice per group

	mice treated with CYP		Time x genotype, p=0.6776, F (6, 78) = 0.6656 Het v KO: 0, p>0.9999 15, p>0.9999 30, p>0.9999 45, p>0.9999 60, p>0.9999 75, p>0.9999 90, p>0.9999	
4P	Percent change in VMRs to 60 mmHg distention during bilateral optogenetic stimulation of PBNàCeA CGRP terminals in naïve and CYP-treated mice	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.1131, F (1.620, 17.82) = 2.566 Pain, p=0.22516, F (1, 11) = 1.464 Time x pain, p=0.0265, F (2, 22) = 4.304 Naïve: BL v light, p=0.0182 BL v PL, p=0.9223 CYP: BL v light, p=0.9792 BL v PL, p=0.8198	5-8
5E	Amount of CGRP measured via fluorescence intensity in the left and right CeA of CYP and saline treated mice	Two-way ANOVA with Bonferroni posttest	Side, p=0.8664, F (1, 36) = 0.3780 Treatment, p=0.137, F (1, 36) = 6.714 Side x treatment, p=0.4701, F (1, 36) = 0.5329 CYP v saline: Left, p=0.0489 Right, p=0.3930	10 mice per group, 3-5 sections per mouse
5N	Percent of total cells expressing *Calcrl* in the left and right CeC in CYP and saline treated animals	Two-way ANOVA	Side, p=0.5810, F (1, 16) = 0.3174 Treatment, p=0.4543, F (1, 16) = 0.5882 Side x treatment, p=0.7078, F (1, 16) = 0.1456	5 mice per group, 3-6 sections per animal
5O	Percent of total cells expressing *Sst* in the left and right CeC in CYP and saline treated animals	Two-way ANOVA	Side, p=0.8736, F (1, 12) = 0.0264 Treatment, p=0.9322, F (1, 12) = 0.0076 Side x treatment, p=0.6502, F (1, 12) = 0.2163	3-5 mice per group, 3-6 sections per animal
5P	Percent of total cells expressing *Prkcd* in the left and right CeC in CYP and saline treated animals	Two-way ANOVA with Bonferroni posttest	Side, p=0.0778, F (1, 16) = 3.551 Treatment, p=0.0483, F (1, 16) = 4.571 Side x treatment, p=0.3359, F (1, 16) = 0.9845 CYP v saline: Left, p=0.8595 Right, p=0.0835	5 mice per group, 3-6 sections per animal
5Q	Percent of total cells expressing both *Sst* and *Prkcd* in the left and right CeC of saline and CYP-	Two-way ANOVA	Side, p=0.6393, F (1, 12) = 0.2312 Treatment, p=0.4155, F (1, 12) = 0.7114 Side x treatment, p=0.4847, F (1, 12) = 0.5200	3-5 mice per group, 3-6 sections/animal

	treated mice			
5R	Percent of *Calcrl*-expressing cells that also express *Sst* in the right and left CeC of mice treated with CYP and saline	Two-way ANOVA	Side, p=0.1144, F (1, 13) = 0.2863 Treatment, p=0.6049, F (1, 13) = 0.2812 Side x treatment, p=0.9858, F (1, 13) = 0.0003	3-5 mice per group, 3-6 sections/animal
5S	Percent of *Calcrl*-expressing cells that also express *Prkcd* in the right and left CeC of mice treated with CYP and saline	Two-way ANOVA with Bonferroni posttest	Side, p=0.0183, F (1, 14) = 7.125 Treatment, p=0.0015, F (1, 14) = 15.45 Side x treatment, p=0.1190, F (1, 14) = 2.758 CYP v saline: Left, p=0.2614 Right, p=0.0029 Left v right: CYP, p>0.9999 Saline, p=0.0117	5 mice per group, 3-6 sections/animal
S1A	Normalized VMRs of background EMG before, during, and after laser stimulation in CYP mice with mCherry or ChR2 in the left CeA	Two-way RM ANOVA	Time, p=0.5548, F (1.458, 24.79) =0.4998 Virus, p=0.1630, F (1, 17) = 2.126 Time x virus, p=0.4398, F (2, 34) = 0.8415	10-11 mice per group
S1B	Normalized VMRs of background EMG before, during, and after laser stimulation in CYP mice with mCherry or ChR2 in the right CeA	Two-way RM ANOVA	Time, p=0.2181, F (1.935, 30.96) = 1.602 Virus, p=0.3878, F (1, 16) = 0.7883 Time x virus, p=0.4498, F (2, 32) = 0.8193	8-10 mice per group
S1C	Normalized VMRs for each distention at 30 mmHg before, during, and after optogenetic stimulation in CYP mice with mCherry or ChR2 in the left CeA	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.1117, F (4.109, 78.07) =1.931 Virus, p=0.3216, F (1, 19)= 1.036 Time x virus, p<0.0001, F (8, 152) = 4.399 mCherry: 1 v 2, p=0.7939 1 v 3, p=0.8921 1 v 4, p=0.7407 1 v 5, p=0.9949 1 v 6, p>0.9999 1 v 7, p>0.9999 1 v 8, p=0.8505 1 v 9, p=0.9978 ChR2: 1 v 2, p=0.9685 1 v 3, p=0.9997 1 v 4, p=0.0398	10-11 mice per group

			1 v 5, p=0.0128 1 v 6, p=0.0009 1 v 7, p=0.0123 1 v 8, p=0.2902 1 v 9, p=0.6965	
S1D	Normalized VMRs for each distention at 30 mmHg before, during, and after optogenetic stimulation in CYP mice with mCherry or ChR2 in the right CeA	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0433, F (3.589, 57.43) = 2.723 Virus, p=0.8763, f (1, 16) = 0.02503 Time x virus, p=0.0958, f (8, 128) = 1.737	8-10 mice per group
S1E	Normalized VMRs for each distention at 60 mmHg before, during, and after optogenetic stimulation in CYP mice with mCherry or ChR2 in the left CeA	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0165, F (4.538, 86.21) = 3.061 Virus, p=0.1701, F (1, 19) = 2.033 Time x virus, p=0.0014, F (8, 152) = 3.374 mCherry: 1 v 2, p=0.9833 1 v 3, p=0.9996 1 v 4, p=0.7947 1 v 5, p=0.9255 1 v 6, p>0.9999 1 v 7, p=0.9920 1 v 8, p=0.8707 1 v 9, p=0.3440 ChR2: 1 v 2, p=0.9815 1 v 3, p=0.9923 1 v 4, p=0.0123 1 v 5, p=0.0290 1 v 6, p=0.0810 1 v 7, p=0.9389 1 v 8, p=0.7598 1 v 9, p=0.9996	10-11 mice per group
S1F	Normalized VMRs for each distention at 60 mmHg before, during, and after optogenetic stimulation in CYP mice with mCherry or ChR2 in the right CeA	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0554, F (4.241, 67.85) = 2.396 Virus, p=0.3716, F (1, 16) = 0.8451 Time x virus, p=0.0006, F (8,128) = 3.747 mCherry: 1 v 2, p=0.9803 1 v 3, p=0.9913 1 v 4, p=0.9679 1 v 5, p>0.9999 1 v 6, p>0.9999 1 v 7, p=0.9974 1 v 8, p>0.9999 1 v 9, p=0.9184 ChR2: 1 v 2, p=0.9998 1 v 3, p=0.8029	8-10 mice per group

			1 v 4, p=0.0649 1 v 5, p=0.0119 1 v 6, p=0.0121 1 v 7, p>0.9999 1 v 8, p>0.9999 1 v 9, p=0.9999	
S2A	Normalized VMRs of background EMG before, during, and after laser stimulation in CYP mice with mCherry or NpHR in the left CeA	Two-way RM ANOVA	Time, p=0.4154, F (1.714, 17.14) = 0.8857 Virus, p=0.1213, F (1, 10) = 2.867 Time x virus, p=0.7146, F (2, 20) = 0.3417	6-7 mice per group
S2B	Normalized VMRs of background EMG before, during, and after laser stimulation in CYP mice with mCherry or NpHR in the right CeA	Two-way RM ANOVA	Time, p=0.6038, F (2, 20) = 0.5175 Virus, p=0.1207, F (1, 10) = 2.877 Time x virus, p=0.1485, F (2, 20) = 2.101	6-7 mice per group
S2C	Normalized VMRs for each distention at 30 mmHg before, during, and after optogenetic stimulation in CYP mice with mCherry or NpHR in the left CeA	Two-way RM ANOVA with Dunnett's posttest	Time, p<0.0001, F (3.117, 34.29) = 9.387 Virus, p=0.1484, F (1, 11) = 2.416 Time x virus, p<0.0001, F (8, 88) = 10.49 mCherry: 1 v 2, p=0.9061 1 v 3, p=0.6000 1 v 4, p=0.9756 1 v 5, p=0.9994 1 v 6, p=0.9999 1 v 7, p=0.7363 1 v 8, p=0.9996 1 v 9, p=0.8980 NpHR: 1 v 2, p=0.3827 1 v 3, p=0.9998 1 v 4, p=0.0161 1 v 5, p=0.0158 1 v 6, p=0.0240 1 v 7, p=0.5989 1 v 8, p=0.9858 1 v 9, p=0.8877	6-7 mice per group
S2D	Normalized VMRs for each distention at 30 mmHg before, during, and after optogenetic	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0533, F (3.374, 37.12) = 2.705 Virus, p=0.2648, F (1, 11) = 1.380 Time x virus, p<0.0001, F (8, 88) = 5.600 mCherry:	6-7 mice per group

	stimulation in CYP mice with mCherry or NpHR in the right CeA		1 v 2, p=0.7606 1 v 3, p=0.2422 1 v 4, p=0.6577 1 v 5, p=0.9244 1 v 6, p>0.9999 1 v 7, p=7584 1 v 8, p=0.9573 1 v 9, p=0.9060 NpHR: 1 v 2, p=0.5913 1 v 3, p=0.9931 1 v 4, p=0.0185 1 v 5, p=0.0695 1 v 6, p=0.0088 1 v 7, p=0.9777 1 v 8, p=0.9534 1 v 9, p=0.6126	
S2E	Normalized VMRs for each distention at 60 mmHg before, during, and after optogenetic stimulation in CYP mice with mCherry or NpHR in the left CeA	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0055, F (2.900, 31,90) = 5.163 Virus, p=0.1262, F (1, 11) = 2.738 Time x virus, p<0.0001, F (8, 88) = 9.118 mCherry: 1 v 2, p=0.9945 1 v 3, p=0.8628 1 v 4, p=0.6908 1 v 5, p=0.8738 1 v 6, p=0.3761 1 v 7, p=0.9264 1 v 8, p=0.7806 1 v 9, p=0.9996 NpHR: 1 v 2, p=0.9931 1 v 3, p=0.9612 1 v 4, p=0.0013 1 v 5, p=0.0260 1 v 6, p=0.0092 1 v 7, p=0.8783 1 v 8, p=0.9951 1 v 9, p=0.5416	6-7 mice per group
S2F	Normalized VMRs for each distention at 60 mmHg before, during, and after optogenetic stimulation in CYP mice with mCherry or NpHR in the right CeA	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0008, F (3.581, 35.81) = 6.428 Virus, p=0.5642, F (1, 10) = 0.3556 Time x virus, p<0.0001, F (8, 80) = 5.263 mCherry: 1 v 2, p=0.3911 1 v 3, p=0.2249 1 v 4, p=0.9521 1 v 5, p=0.8652 1 v 6, p=0.6299 1 v 7, p=0.3329 1 v 8, p=0.4710 1 v 9, p=0.1536 NpHR:	6-7 mice per group

			1 v 2, p=0.3711 1 v 3, p=0.9958 1 v 4, p=0.0214 1 v 5, p=0.0271 1 v 6, p=0.0027 1 v 7, p=0.9472 1 v 8, p=0.8827 1 v 9, p>0.9999	
S3A	Percent change in VMR to 30 mmHg in CYP mice with mCherry or ChR2 in the left CeA before, during, and after laser stimulation	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0833, F (1.877, 35.66) = 2.711 Virus, p=0.0032, F (1, 19) = 11.38 Time x virus, p=0.0171, F (2, 38) = 4.536 mCherry: BL v laser, p=0.9354 BL v PL, p=0.7133 ChR2: BL v laser, p=0.0002 BL v PL, p=0.0043	10-11 mice per group
S3B	Percent change in VMR to 30 mmHg in CYP mice with mCherry or ChR2 in the right CeA before, during, and after laser stimulation	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0134, F (2, 32) = 4.947 Virus, p=0.2835, F (1, 16) = 1.232 Time x virus, p=0.1926, F (2, 32) = 1.735	10-11 mice per group
S3C	Percent change in VMR to 30 mmHg in CYP mice with mCherry or NpHR in the left CeA before, during, and after laser stimulation	Two-way RM ANOVA with Dunnett's posttest	Time, p<0.0001, F (1.951, 21.46) = 59.12 Virus, p=0.0014, F (1, 11) = 17.87 Time x virus, p<0.0001, F (2, 22) = 68.26 mCherry: BL v laser. P=0.9757 BL v PL, p=0.6281 NpHR: BL v laser, p=0.0001 BL v PL, p=0.0774	6-7mice per group
S3D	Percent change in VMR to 30 mmHg in CYP mice with mCherry or NpHR in the right CeA before, during, and after laser stimulation	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0379, F (1.584,17.42) = 4.220 Virus, p=0.0596, F (1, 11) = 4.410 Time x virus, p=0.0001, F (2, 22) = 13.94 mCherry: BL v laser, p=0.5243 BL v PL, p=0.9664 NpHR: BL v laser, p=0.0001 BL v PL, p=0.9981	6-7 mice per group
S4A	Percent change in VMRs to 30	Two-way RM ANOVA	Time, p<0.0001, F (1.825, 28.20) = 21.41	6-7 mice per group

	mmHg after injection of drug during optogenetic stimulation in the left CeA of CYP mice	with Dunnett's posttest	Drug, p=0.0162, F (2, 16) = 5.399 Time x drug, p<0.0001, F (4, 32) = 11.43 aCSF: BL v laser, p=0.0001 BL v PL, p=0.0719 CGRP(8-37): BL v laser, p=0.7882 BL v PL, p=0.1382 AP5+NBQX: BL v laser, p<0.0001 BL v PL, p=0.9509	
S4B	Percent change in VMRs to 30 mmHg after injection of drug during optogenetic stimulation in the right CeA of CYP mice	Two-way RM ANOVA with Dunnett's posttest	Time, p<0.0001, F (1.355, 25.74) = 25.78 Drug, p=0.0296, F (2, 19) = 4.260 Time x drug, p=0.0002, F (4, 38) = 7.163 aCSF: BL v laser, p=0.0020 BL v PL, p=0.6096 CGRP(8-37): BL v laser, p=0.9551 BL v PL, p=0.6912 AP5+NBQX: BL v laser, p=0.0322 BL v PL, p=0.8479	6-8 mice per group
S4C	Percent change in VMRs to 30 mmHg distention during a) optogenetic stimulation and b) pharmacological activation in the left CeA of CGRP heterozygous and homozygous mice treated with CYP	Two-way RM ANOVA with Bonferroni posttest	a) Time, p=0.0036, F (1.157, 17.35) = 10.45 Genotype, p=0.1270, F (1, 15) = 0.7993 Time x genotype, p=0.0003, F (2, 30) = 10.99 Het v KO Laser, p=0.0223 PL, p>0.9999 b) Time, p<0.0001, F (2.367, 35.50) = 21.15 Genotype, p=0.8590, F (1, 15) = 0.02365 Time x genotype, p=0.9622, F (6, 90) = 0.2397 Het v KO: 0, p>0.9999 15, p>0.9999 30, p>0.9999 45, p>0.9999 60, p>0.9999 75, p>0.9999 90, p>0.9999	8-9 mice per group
S4D	Percent change in VMRs to 30 mmHg distention during a) optogenetic stimulation and b)	Two-way RM ANOVA with Bonferroni posttest	a) Time, p=0.0044, F (2, 26) = 6.718 Genotype, p=0.2477, F (1, 13) = 1.465 Time x genotype, p=0.0058, F (2, 26) = 6.319 Het v KO:	7-8 mice per group

	pharmacological activation in the right CeA of CGRP heterozygous and homozygous mice treated with CYP		Laser, p=0.0055 PL, p=0.8418 b) Time, p<0.0001, F (6, 78) = 32.55 Genotype, p=0.1504, F (1, 13) = 1.725 Time x genotype, p=0.9629, F (6, 78) = 0.2373 Het v KO: 0, p=0.9971 15, p=0.9972 30, p>0.9999 45, p>0.9999 60, p>0.9999 75, p=0.9999 90, p=0.9945	
S5A	Percent change in VMRs to 30 mmHg distention after injection of drug into the left CeA of naïve mice	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0007, F (3.255, 39.06) = 6.650 Drug, p=0.0503, F (1, 12) = 4.731 Time x drug, p=0.0011, F (6, 72) = 4.225 aCSF: BL v 15, p=0.9997 BL v 30, p=0.8623 BL v 45, p>0.9999 BL v 60, p>0.9999 BL v 75, p=0.9959 BL v 90, p>0.9999 CGRP: BL v 15, p=0.0943 BL v 30, p<0.0001 BL v 45, p<0.0001 BL v 60, p=0.0005 BL v 75, p>0.9999 BL v 90, p=0.9997	7 mice per group
S5B	Percent change in VMRs to 30 mmHg distention after injection of drug into the right CeA of naïve mice	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.0035, F (2.929, 41.01) = 5.355 Drug, p=0.0066, F (1, 14) = 10.15 Time x drug, p=0.0005, F (6, 84) = 4.566 aCSF: BL v 15, p=0.9982 BL v 30, p=0.8354 BL v 45, p=0.2953 BL v 60, p=0.5648 BL v 75, p=0.9988 BL v 90, p=0.7415 CGRP: BL v 15, p=0.2248 BL v 30, p=0.0193 BL v 45, p=0.1057 BL v 60, p=0.1846 BL v 75, p=0.9988 BL v 90, p>0.9999	8 mice per group
S5C	Percent change in VMRs to 30	Two-way RM ANOVA	Time, p=0.0068, F (3.880, 131.9) = 3.762	9-10 mice per group

	mmHg distention after injection of drug into the left CeA in CYP mice	with Dunnett's posttest	Drug, p<0.0001, F (3, 34) = 12.95 Time x drug, p<0.0001, F (18, 204) = 7.787 aCSF: BL v 15, p=0.4836 BL v 30, p=0.5332 BL v 45, p=0.3474 BL v 60, p=0.9774 BL v 75, p=0.3924 BL v 90, p=0.1034 CGRP: BL v 15, p=0.0048 BL v 30, p=0.2077 BL v 45, p=0.0110 BL v 60, p=0.0003 BL v 75, p=0.7363 BL v 90, p=0.5535 CGRP(8-37): BL v 15,p=0.0700 BL v 30, p=0.0962 BL v 45, p=0.0134 BL v 60, p=0.0190 BL v 75, p=0.1890 BL v 90, p=0.0949 CGRP+CGRP(8-37): BL v 15, p=0.9999 BL v 30, p>0.9999 BL v 45, p=0.8021 BL v 60, p=0.9877 BL v 75, p=0.6307 BL v 90, p=0.9223	
S5D	Percent change in VMRs to 30 mmHg distention after injection of drug into the right CeA in CYP mice	Two-way RM ANOVA with Dunnett's posttest	Time, p=0.3104, F (3.666, 88.00) = 1.215 Drug, p<0.0001, F (3, 24) = 17.70 Time x drug, p<0.0001, F (18, 144) = 6.841 aCSF: BL v 15, p>0.9999 BL v 30, p=0.9963 BL v 45, p=0.7193 BL v 60, p=0.5881 BL v 75, p=0.7100 BL v 90, p=0.9996 CGRP: BL v 15, p=0.1429 BL v 30, p=0.1051 BL v 45, p=0.0536 BL v 60, p=0.0811 BL v 75, p=0.2305 BL v 90, p=0.9624 CGRP(8-37): BL v 15,p=0.0038 BL v 30, p=0.0004 BL v 45, p<0.0001 BL v 60, p=0.0118	6-8 mice per group

			BL v 75, p>0.9999 BL v 90, p=0.9999 CGRP+CGRP(8-37): BL v 15, p=0.0979 BL v 30, p=0.7222 BL v 45, p=0.6789 BL v 60, p=0.9823 BL v 75, p=0.9999 BL v 90, p=0.6980	
S6A	Normalized VMRs to 30 mmHg distention after infusion of CGRP into the left and right striatum	Two-way RM ANOVA	Time, p=0.4685, F (2.139, 12.83) = 0.8228 Side, p=0.7535, F (1, 6) = 0.1081 Time x side, p=0.2097, F (6, 36) = 1.489	4 mice per group
S6B	Percent change from baseline VMRs to 30 mmHg distention after infusion of CGRP into the left and right striatum	Two-way RM ANOVA	Time, p=0.3975, F (2.126, 13.30) = 1.012 Side, p=0.8762, F (1, 6) = 0.02643 Time x side, p=0.2080, F (6, 36) = 1.494	4 mice per group
S6C	Normalized VMRs to 60 mmHg distention after infusion of CGRP into the left and right striatum	Two-way RM ANOVA	Time, p=0.5683, F (2.010, 12.06) = 0.5940 Side, p=0.4206, F (1, 6) = 0.7472 Time x side, p=0.8036, F (6, 36) = 0.5007	4 mice per group
S6D	Percent change from baseline VMRs to 30 mmHg distention after infusion of CGRP into the left and right striatum	Two-way RM ANOVA	Time, p=0.6870, F (2.408, 14.45) = 0.9023 Side, p=0.8563, F (1, 6) = 0.03576 Time x side, p=6870, F (6, 36) = 0.6535	4 mice per group
S7A	Area under the curve for 30 mmHg distention during optogenetic activation of the left CeA in homozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.7168 t=0.3804	4 mice per group
S7B	Area under the curve for 30 mmHg distention	Unpaired t-test	P=0.8698 t=0.1710	4 mice per group

	during optogenetic activation of the left CeA in heterozygous mice treated with CYP to compare effect of order of activation on VMRs			
S7C	Area under the curve for 30 mmHg distention during optogenetic activation of the right CeA in homozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.5468 t=0.6384	3-5 mice per group
S7D	Area under the curve for 30 mmHg distention during optogenetic activation of the right CeA in heterozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.3171 t=1.077	4-5 mice per group
S7E	Area under the curve for 30 mmHg distention during pharmacological activation of the left CeA in homozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.9650 t=0.0457	4 mice per group
S7F	Area under the curve for 30 mmHg distention during pharmacological activation of the	Unpaired t-test	P=0.8906 t=0.1435	4 mice per group

	left CeA in heterozygous mice treated with CYP to compare effect of order of activation on VMRs			
S7G	Area under the curve for 30 mmHg distention during pharmacological activation of the right CeA in homozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.2901 t=1.160	3-5 mice per group
S7H	Area under the curve for 30 mmHg distention during pharmacological activation of the right CeA in heterozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.2730 t=1.190	4-5 mice per group
S7I	Area under the curve for 60 mmHg distention during optogenetic activation of the left CeA in homozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.7902 t=0.2783	4 mice per group
S7J	Area under the curve for 60 mmHg distention during optogenetic activation of the left CeA in heterozygous mice treated with	Unpaired t-test	P=0.8289 t=0.2258	4 mice per group

	CYP to compare effect of order of activation on VMRs			
S7K	Area under the curve for 60 mmHg distention during optogenetic activation of the right CeA in homozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.1997 t=1.441	3-5 mice per group
S7L	Area under the curve for 60 mmHg distention during optogenetic activation of the right CeA in heterozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.0647 t=2.190	4-5 mice per group
S7M	Area under the curve for 60 mmHg distention during pharmacological activation of the left CeA in homozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.9734 t=0.0348	4 mice per group
S7N	Area under the curve for 60 mmHg distention during pharmacological activation of the left CeA in heterozygous mice treated with CYP to compare effect of order of activation on	Unpaired t-test	P=0.3134 t=1.100	4 mice per group

	VMRs			
S7O	Area under the curve for 60 mmHg distention during pharmacological activation of right CeA in homozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.5258 t=0.6733	3-5 mice per group
S7P	Area under the curve for 60 mmHg distention during pharmacological activation of the right CeA in heterozygous mice treated with CYP to compare effect of order of activation on VMRs	Unpaired t-test	P=0.9478 t=0.0678	4-5 mice per group
S8A-B S8E-F	Percent of cells expressing *Calcrl* that also express *Prkcd* across the rostral-caudal axis in the left and right CeA of saline and CYP-treated mice	Mixed-effects model with Tukey's multiple comparisons	Position, p=0.8188 F (5, 35) = 0.4382 Treatment, p=0.0009, F (1, 7) = 30.42 Side, p=0.0488, F (1, 11) = 4.907 Position x treatment, p=0.2868, F (5, 11) = 1.433 Position x side, p=0.0750, F (5, 11) = 2.755 Treatment x side, p=0.0429, F (1, 11) = 5.238 Position x treatment x side, p=0.4566, F (5, 11) = 1.009 -1.22 vs. -1.34, p=0.9936 -1.22 vs. -1.46, p>0.9999 -1.22 vs. -1.58, p=0.9997 -1.22 vs. -1.70, p=0.9660 -1.22 vs. -1.82, p=0.9993 -1.34 vs. -1.46, p=0.9714 -1.34 vs. -1.58, p=0.9481 -1.34 vs. -1.70, p=0.7173 -1.34 vs. -1.82, p=0.9479 -1.46 vs. -1.58, p>0.9999 -1.46 vs. -1.70, p=0.9884 -1.46 vs. -1.82, >0.9999 -1.58 vs. -1.70, p=0.9637 -1.58 vs. -1.82,p=0.9943 -1.70 vs. -1.82, p=0.9985	5 mice per group, 3-6 sections/animal

S8C-D S8G-H	Percent of cells expressing *Calcrl* that also express *Sst* across the rostral-caudal axis in the left and right CeA of saline and CYP-treated mice	Mixed-effects model with Tukey's multiple comparisons	Position, p=0.9762 F (5, 19) = 0.1540 Treatment, p=0.6989, F (1, 19) = 0.1542 Side, p=0.7641, F (1, 19) = 0.0927 Position x treatment, p=0.2655, F (5, 19) = 1.410 Position x side, p=0.0069, F (5, 19) = 4.531 Treatment x side, p=0.6187, F (1, 1) = 0.4660 Position x treatment x side, p=0.4145, F (5, 1) = 2.947 -1.22 vs. -1.34, p=0.9920 -1.22 vs. -1.46, p>0.9999 -1.22 vs. -1.58, p>0.9999 -1.22 vs. -1.70, p=0.9940 -1.22 vs. -1.82, p>0.9999 -1.34 vs. -1.46, p=0.9921 -1.34 vs. -1.58, p=0.9883 -1.34 vs. -1.70, p=0.9998 -1.34 vs. -1.82, p=0.9744 -1.46 vs. -1.58, p>0.9999 -1.46 vs. -1.70, p=0.9994 -1.46 vs. -1.82, >0.9999 -1.58 vs. -1.70, p=0.9989 -1.58 vs. -1.82, p>0.9999 -1.70 vs. -1.82, p=0.9956	3-5 mice per group, 3-6 sections/animal
S9	Concentration of cAMP in the left and right CeA after infusion of CGRP or aCSF	Two-way ANOVA	Hemisphere, p=0.9398, F (1, 14) = 0.005911 Drug, p=0.0002, F (1, 14) = 25.25 Hemisphere x drug, p=0.7519, F (1, 14) = 0.1040	4-5 mice per group

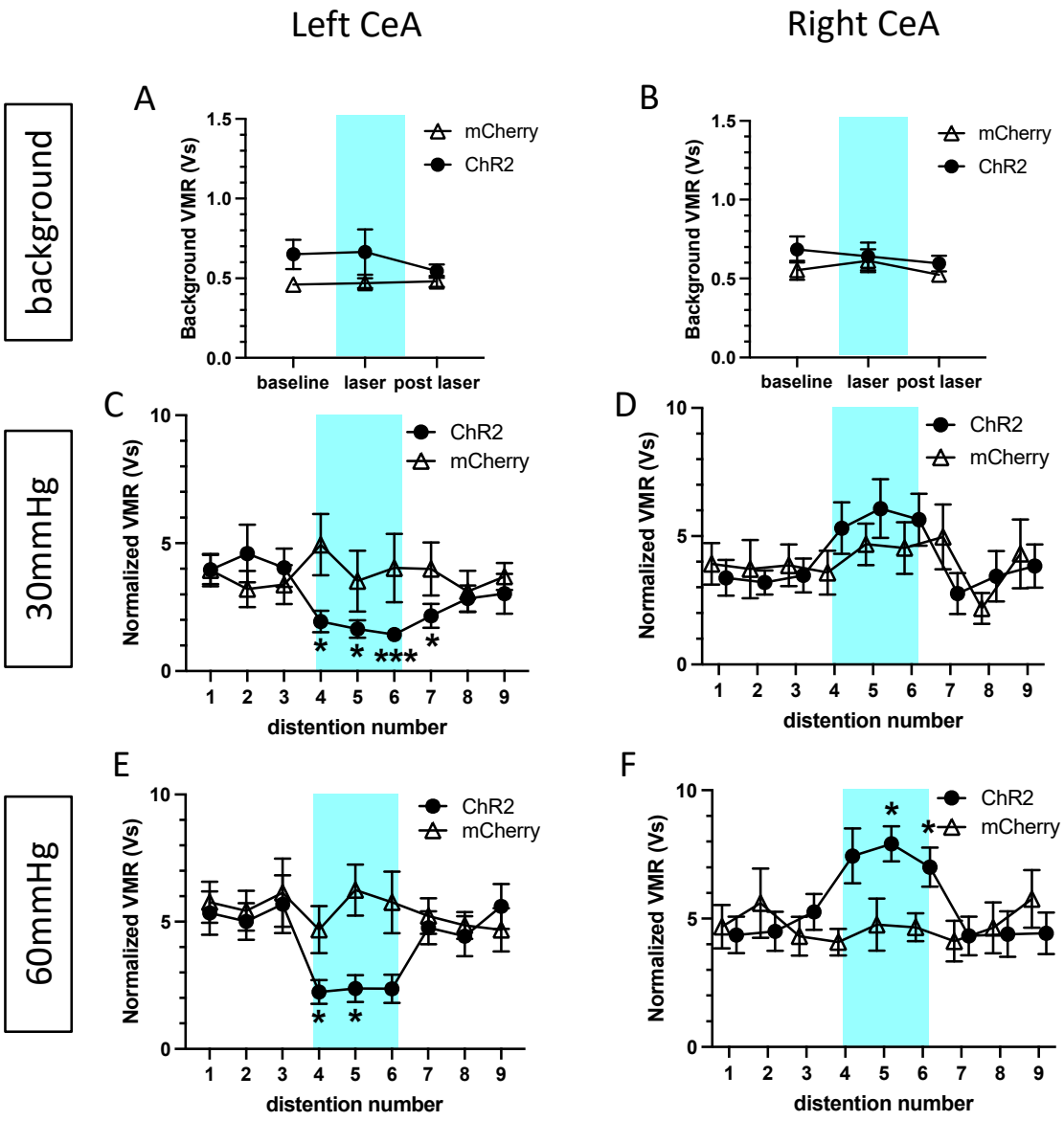

Figure S1: Background and normalized VMRs to 30 and 60 mmHg bladder distention during optogenetic activation. Background VMRs at baseline, during, and after optogenetic activation in the left (**A**) and right (**B**) CeA. Normalized VMRs to 30mmHg distentions in the left (**C**) and right (**D**) CeA. Normalized VMRs to 60mmHg distentions in the left (**E**) and right (**F**) CeA. All data are presented as mean +/- SEM and error bars represent SEM. *P<0.05 ***P<0.001. See **Supplementary Table 1** for further statistical information.

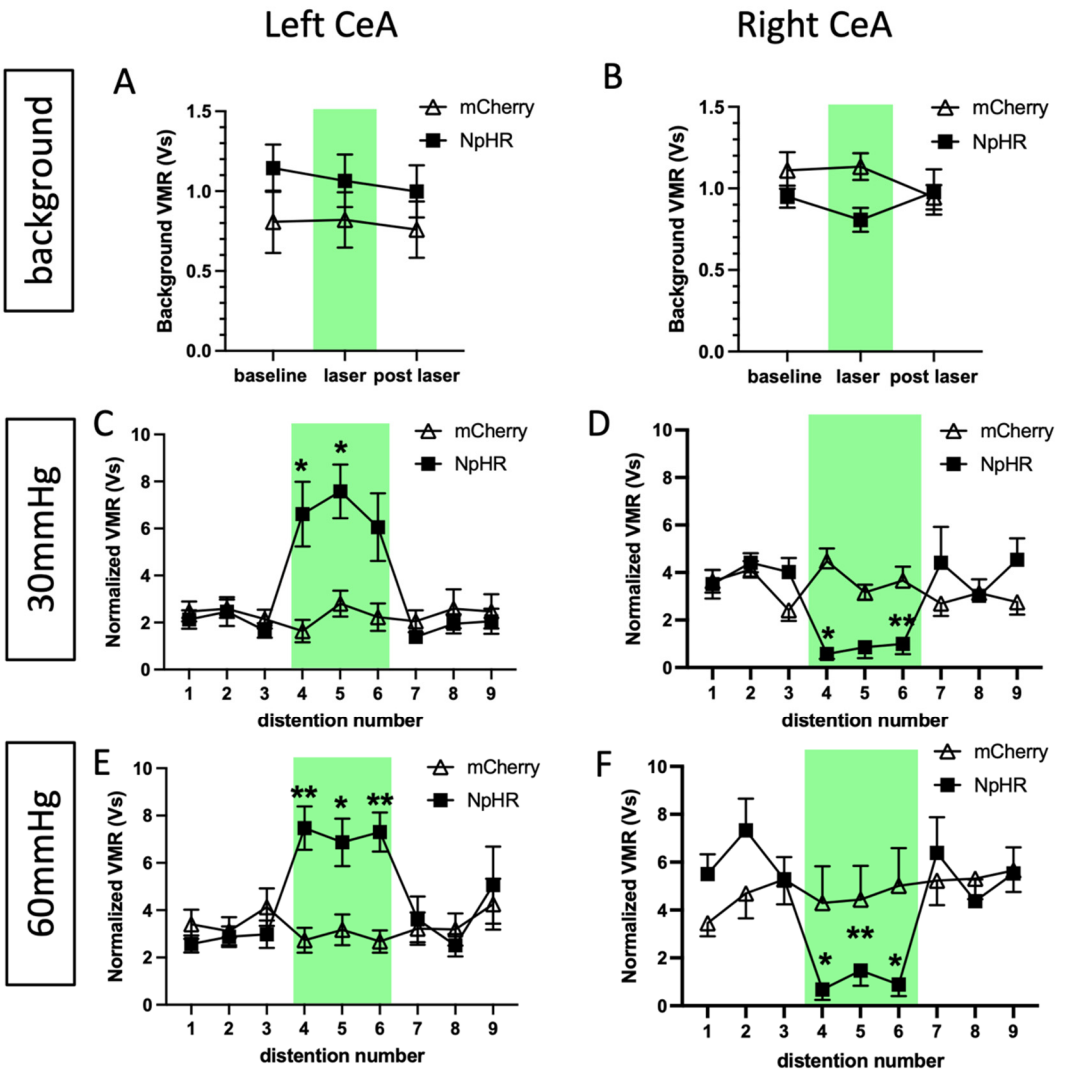

Figure S2: Background and normalized VMRs to 30 and 60 mmHg bladder distention during optogenetic inhibition. Background VMRs at baseline, during, and after optogenetic inhibition in the left (**A**) and right (**B**) CeA. Normalized VMRs to 30mmHg distentions in the left (**C**) and right (**D**) CeA. Normalized VMRs to 60mmHg distentions in the left (**E**) and right (**F**) CeA. All data are presented as mean +/- SEM and error bars represent SEM. *P<0.05 **P<0.01. See **Supplementary Table 1** for further statistical information.

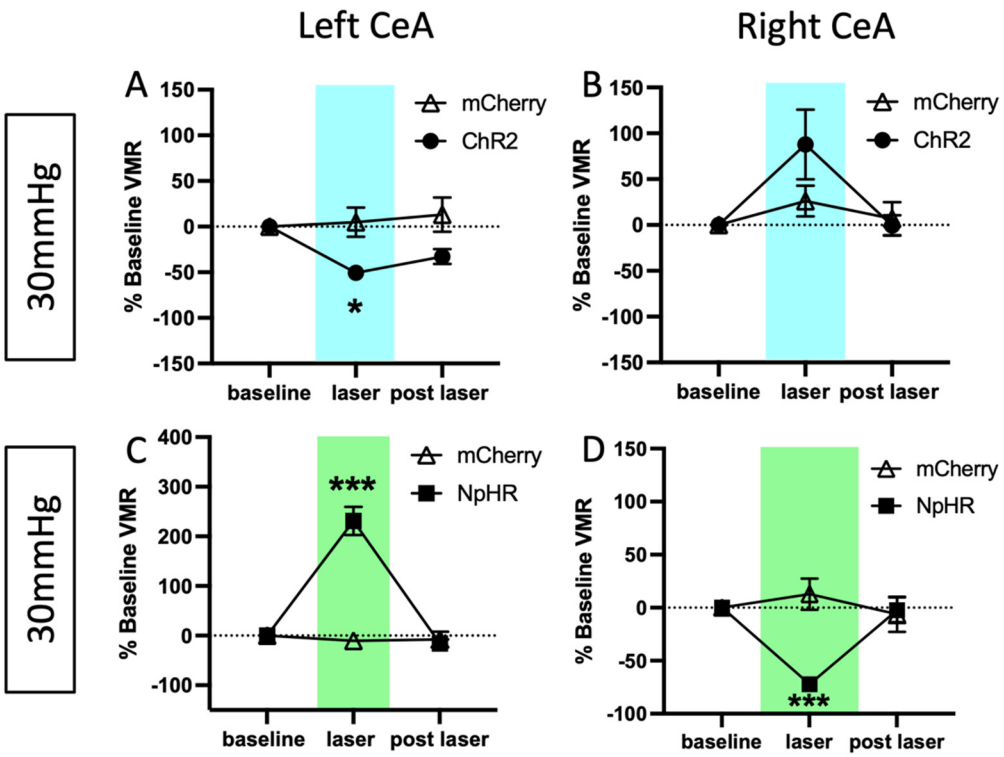

Figure S3: Optogenetic stimulation of CGRP terminals in the left or right CeA has opposing effects on to low pressure distention. Percent change from baseline VMRs during and after optogenetic activation of CGRP terminals in the left (**A**) and right (**B**) CeA. Percent change from baseline VMRs during and after optogenetic inhibition of CGRP terminals in the left (**C**) and right (**D**) CeA. All data are presented as mean +/- SEM and error bars represent SEM. *P<0.05, **P<0.01 ***P<0.001. See **Supplementary Table 1** for further statistical information.

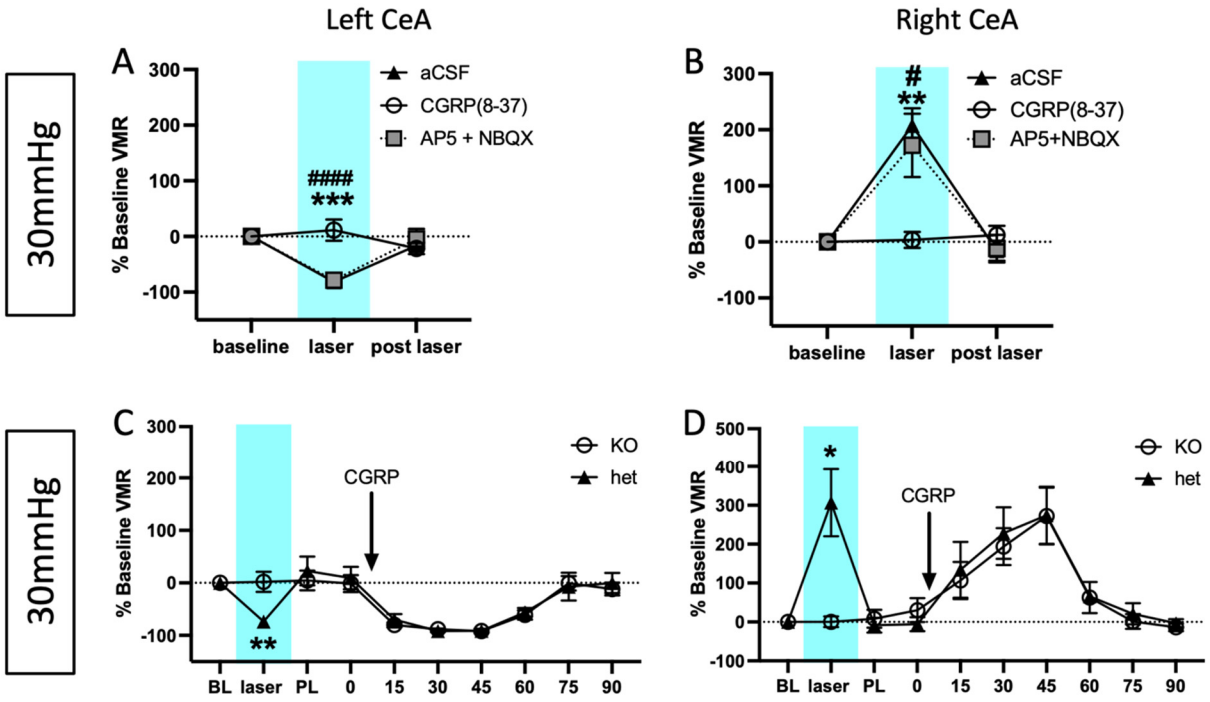

Figure S4: Effects of optogenetic activation of CGRP terminals in the CeA during low pressure distention is due to parabrachial CGRP signaling. Percent change from baseline VMRs to 30mmHg distention during optogenetic activation of parabrachial CGRP terminals and pharmacological inhibition of various receptor cells in the left (**A**) and right (**B**) CeA (# APv+NBQX, *aCSF). Percent change from baseline VMRs to 30mmHg distention during optogenetic and activation (left) of parabrachial Cre positive terminals and pharmacological activation (right) of CGRP receptor cells in the left (**C**) and right (**D**) CeA of *Calca*-Cre heterozygous and homozygous CYP mice. All data are presented as mean +/- SEM and error bars represent SEM. *P<0.05, **P<0.001 ***P<0.001. See **Supplementary Table 1** for further statistical information.

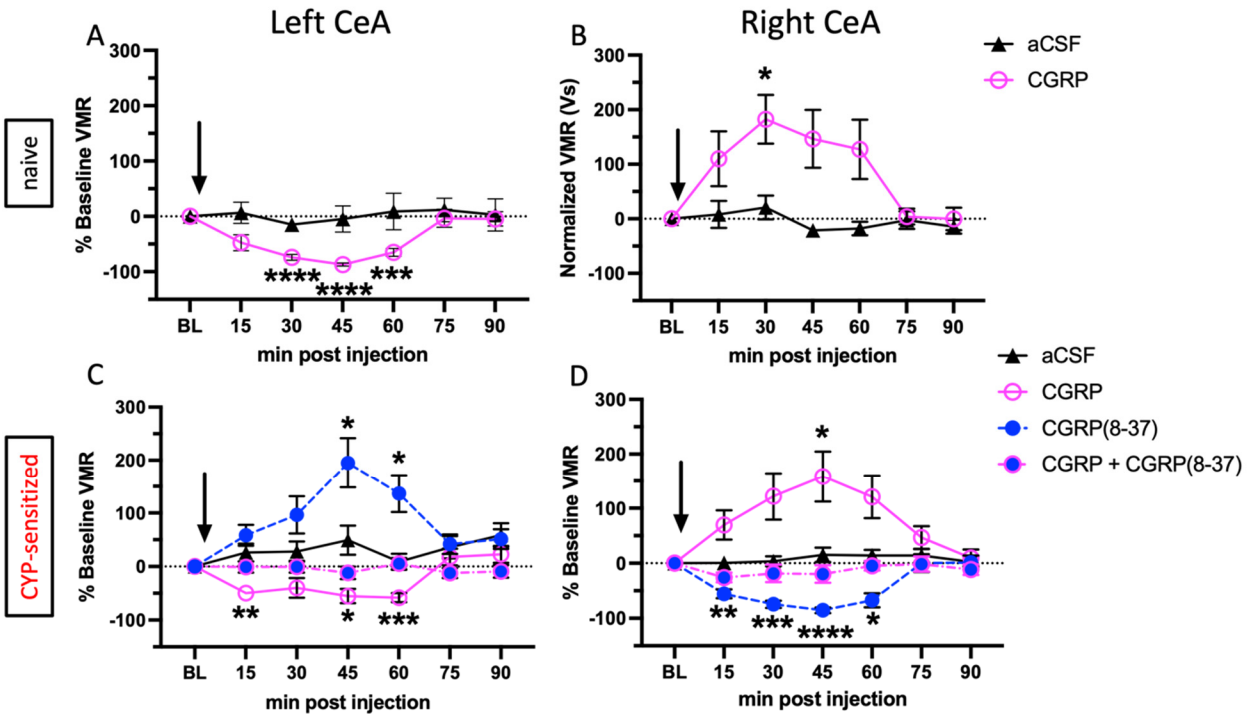

Figure S5: CGRP pharmacology in the left and right CeA has opposing effects to low pressure distention. Percent change from baseline VMRs to 30mmHg distention during pharmacological stimulation of CGRP receptor cells in the left (**A**) and right (**B**) CeA of naïve animals. Percent change from baseline VMRs to 30mmHg distention during pharmacological activation of CGRP receptor cells in the left (**C**) and right (**D**) CeA of CYP mice. All data are presented as mean +/- SEM and error bars represent SEM. *P<0.05, **P<0.01, ***P<0.001 ****P<0.0001. See **Supplementary Table 1** for further statistical information.

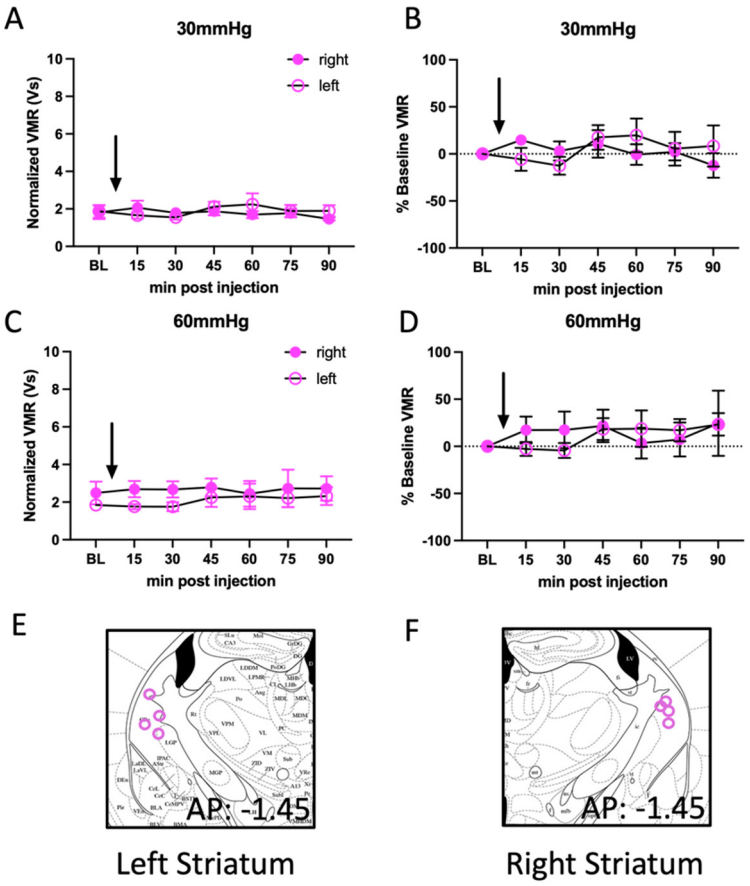

Figure S6: CGRP pharmacology in the left and right striatum has no effect on VMRs. A) Normalized VMRs to 30 mmHg after infusion of CGRP into the left and right striatum. **B**) Percent change from baseline VMRs to 30 mmHg distention in the left and right striatum after infusion of CGRP. **C**) Normalized VMRs to 60 mmHg distention after infusion of CGRP into the left and right striatum. **D**) Percent change from baseline VMRs to 60 mmHg distention in the left and right striatum after infusion of CGRP. **E, F**) Targeting of cannulae in the left (**E**) and right (**F**) striatum.All data are presented as mean +/- SEM and error bars represent. See **Supplementary Table 1** for further statistical information.

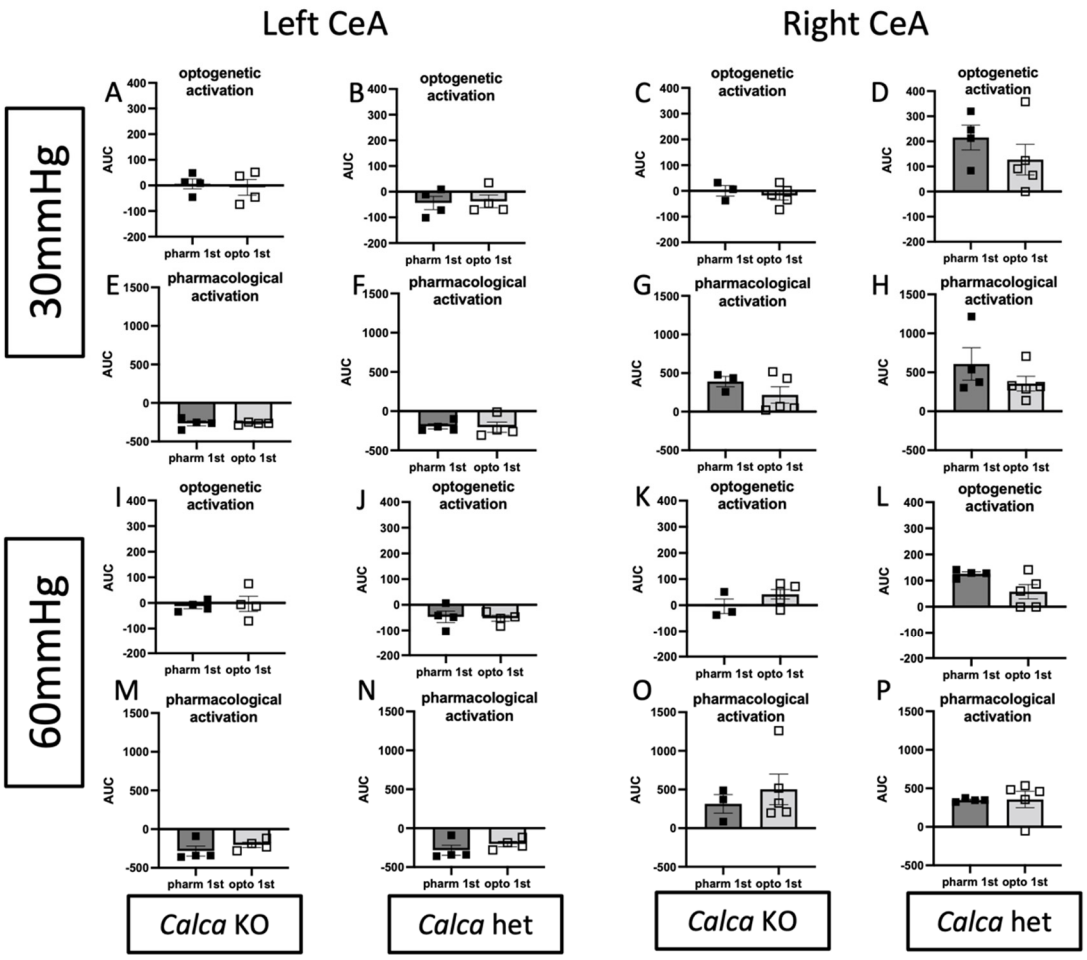

Figure S7: Order of optogenetic or pharmacological activation has no effect on magnitude of VMR responses. AUC for optogenetic activation in the left CeA during 30mmHg distention in *Calca* knockout (**A**) and *Calca* heterozygous (**B**) animals. AUC for optogenetic activation in the right CeA during 30mmHg distention in *Calca* knockout (**C**) and *Calca* heterozygous (**D**) animals. AUC for pharmacological activation in the left CeA during 30mmHg distention in *Calca* knockout (**E**) and *Calca* heterozygous (**F**) animals. AUC for pharmacological activation in the right CeA during 30mmHg distention in *Calca* knockout (**G**) and *Calca* heterozygous (**H**) animals. AUC for optogenetic activation in the left CeA during 60mmHg distention in *Calca* knockout (**I**) and *Calca* heterozygous (**J**) animals. AUC for optogenetic activation in the right CeA during 60mmHg distention in *Calca* knockout (**K**) and *Calca* heterozygous (**L**) animals. AUC for pharmacological activation in the left CeA during 60mmHg distention in *Calca* knockout (**M**) and *Calca* heterozygous (**N**) animals. AUC for pharmacological activation in the right CeA during 60mmHg distention in *Calca* knockout (**O**) and *Calca* heterozygous (**P**) animals. All data are presented as mean +/- SEM and error bars represent SEM. See **Supplementary Table 1** for further statistical information.

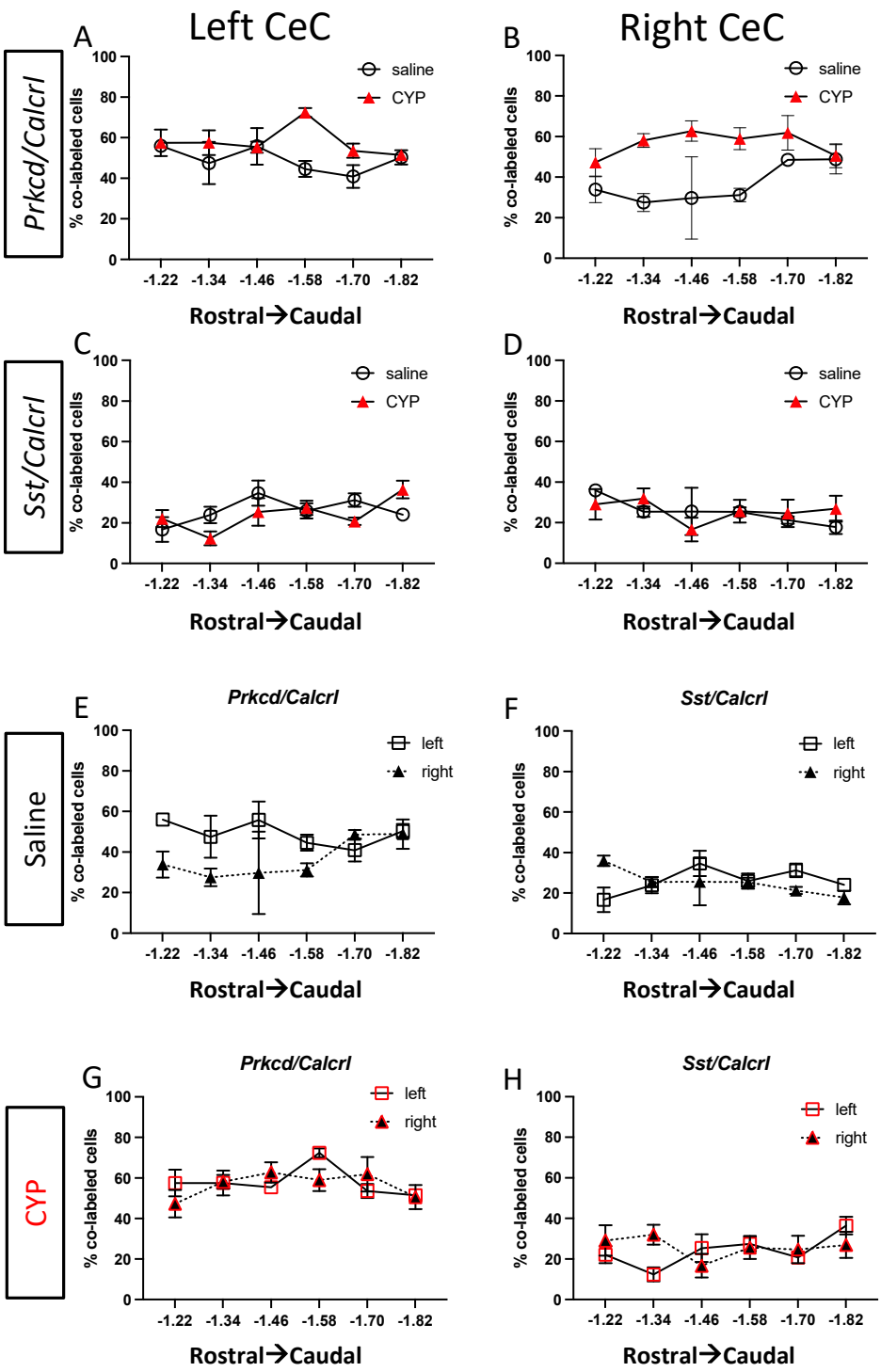

Figure S8: Co-localization of *Sst* and *Prkcd* with *Calcrl* in the CeC based on rostral-caudal position. Comparison of *Calcrl* co-localized with *Prkcd* between pain and non-pain animals in the left (**A**) and right (**B**) CeC. Comparison of *Calcrl* co-localized with *Sst* between pain and non-pain animals in the left (**C**) and right (**D**) CeC. Comparison of *Calcrl* co-expressed with *Prkcd* in the left and right CeC of saline (**E**) and CYP (**G**) treated mice. Comparison of *Calcrl* co-expressed with *Sst* in the left and right CeA of saline (**F**) and CYP (**H**) treated mice. All data are presented as mean +/- SEM and error bars represent SEM. See **Supplementary Table 1** for further statistical information.

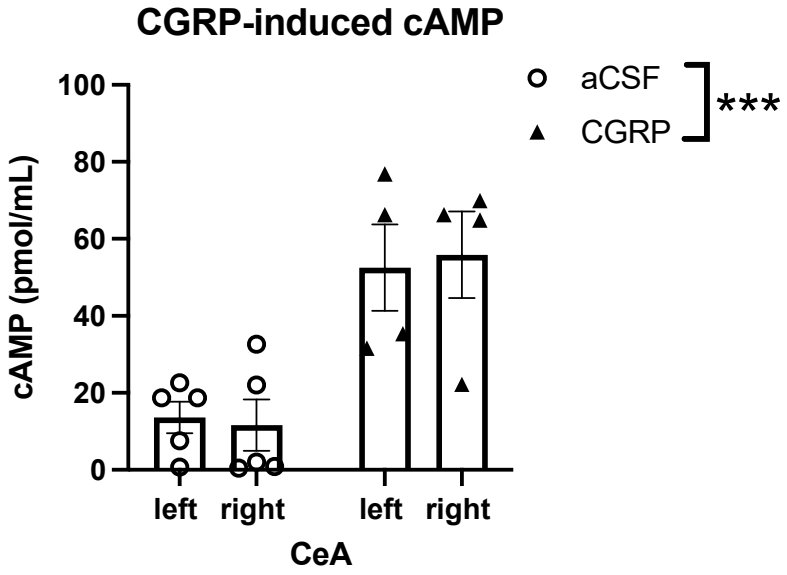

Figure S9: Quantification of CGRP-induced cAMP in the left and right CeA. CGRP infusion into both the left and right CeA increases cAMP compared to aCSF infusion. All data are presented as mean +/- SEM and error bars represent SEM. ***P<0.001 See **Supplementary Table 1** for further statistical information.

Supplemental References

1. Carter ME, Soden ME, Zweifel LS, Palmiter RD (2013): Genetic identification of a neural circuit that suppresses appetite. *Nature* 503: 111–116.
2. Han S, Soleiman M, Soden M, Zweifel L, Palmiter RD (2015): Elucidating an Affective Pain Circuit that Creates a Threat Memory. *Cell* 162: 363–374.
3. Boudes M, Uvin P, Kerselaers S, Vennekens R, Voets T, De Ridder D (2011): Functional characterization of a chronic cyclophosphamide-induced overactive bladder model in mice. *Neurourol Urodyn* 30: 1659–1665.
4. Stillwell TJ, Benson RC (1988): Cyclophosphamide-induced hemorrhagic cystitis: A review of 100 patients. *Cancer* 61: 451–457.
5. Sadler KE, McQuaid NA, Cox AC, Behun MN, Trouten AM, Kolber BJ (2017): Divergent functions of the left and right central amygdala in visceral nociception. *Pain* 158: 747–759.
6. Sadler KE, Stratton JM, Kolber BJ (2014): Urinary bladder distention evoked visceromotor responses as a model for bladder pain in mice. *Journal of Visualized Experiments*. https://doi.org/10.3791/51413
7. Ji G, Neugebauer V (2009): Hemispheric Lateralization of Pain Processing by Amygdala Neurons. *J Neurophysiol* 102: 2253–2264.
8. Tye KM, Prakash R, Kim SY, Fenno LE, Grosenick L, Zarabi H, *et al.* (2011): Amygdala circuitry mediating reversible and bidirectional control of anxiety. *Nature* 471: 358–362.
9. Chaplan SR, Bach FW, Pogrel JW, Chung JM, Yaksh TL (1994): Quantitative assessment of tactile allodynia in the rat paw. *Journal of Neuroscience Methods*, vol. 53.
10. Adke AP, Khan A, Ahn HS, Becker JJ, Wilson TD, Valdivia S, *et al.* (2021): Cell-type specificity of neuronal excitability and morphology in the central Amygdala. *eNeuro* 8: 1–28.
11. Okutsu Y, Takahashi Y, Nagase M, Shinohara K, Ikeda R, Kato F (2017): Potentiation of NMDA receptor-mediated synaptic transmission at the parabrachial-central amygdala synapses by CGRP in mice. *Mol Pain* 13: 1–11.
12. Han JS, Adwanikar H, Li Z, Ji G, Neugebauer V (2010): Facilitation of synaptic transmission and pain responses by CGRP in the amygdala of normal rats. *Mol Pain* 6. https://doi.org/10.1186/1744-8069-6-10
13. Paxinos G, Franklin KB (2019): *Paxinos and Franklin's The Mouse Brain in Stereotaxis Coordinates*. Academic Press.