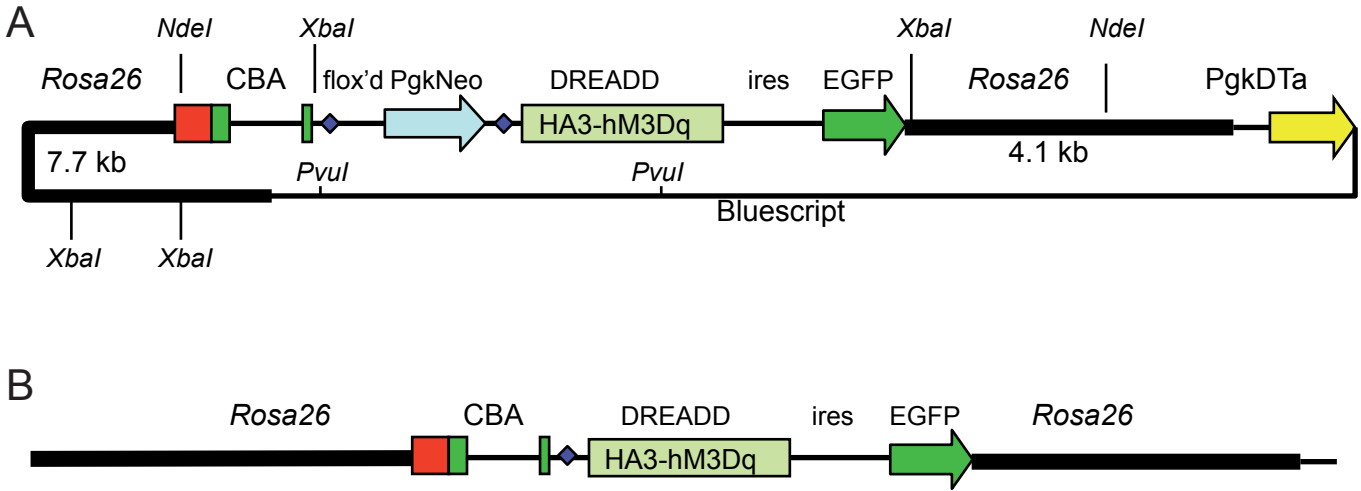

Figure S1: Gene targeting of Rosa26/CBA_hM3D, related to Figure 1. (A) The Rosa26/CBA-DREADD ires-EGFP targeting construct was prepared as described in Methods. **(B)** Rosa 26 locus after homologous recombination and removal of the PgkNeo gene.

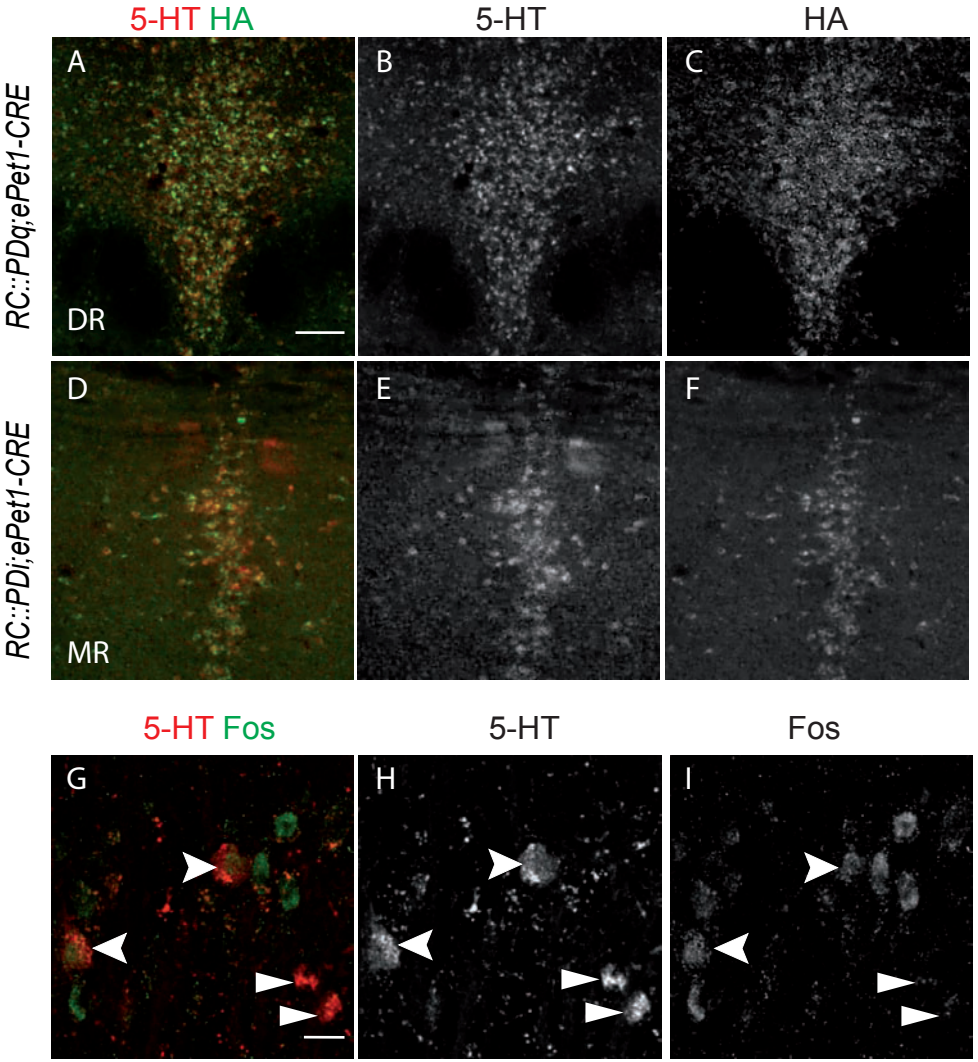

Figure S2: Expression of DREADDs and Fos in 5-HT neurons, related to Figure 1. (A-F) Immunostaining for 5-HT and HA-tag on DR of *RC::PDq;Pet1-cre* animals **(A-C)** and MR of RC::PDi;Pet1-cre animals **(D-F)** confirms the specificity and efficiency of HA staining expression in 5-HTergic neurons. **(G-I)** Exemplary images of co-immunostaining for 5-HT and Fos in the raphe. Scale bars are 100 µm in A and 20 µm in G.

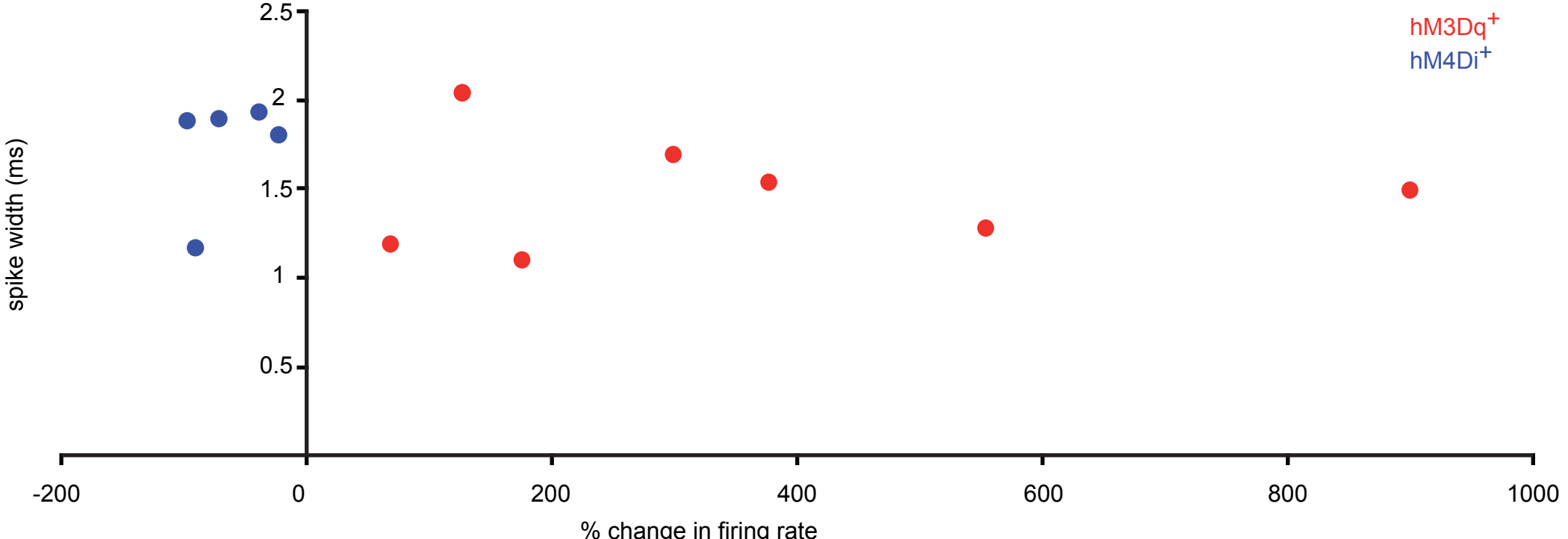

Figure S3: No correlation between spike width and change in firing rate after CNO challenge, related to Figure 1. r = -0.2788; p = 0.565.

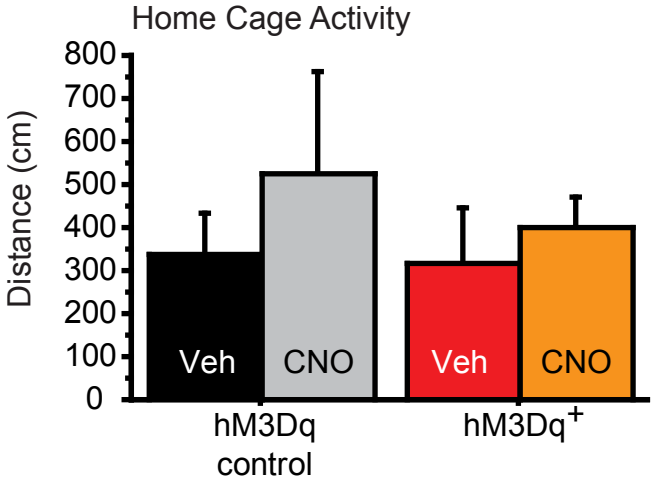

Figure S4: Pharmacogenetic activation of 5-HTergic neurons does not alter activity in the home cage, related to Figure 2. Distance travelled for 15min in the home cage. We did not detect a GXTx interaction ($F(1,21) = 0.182$, $p = 0.6739$) or a main effect for genotype ($F(1,21) = 0.365$, $p = 0.5538$). n = 5-8. Graphs represent mean ± SEM.

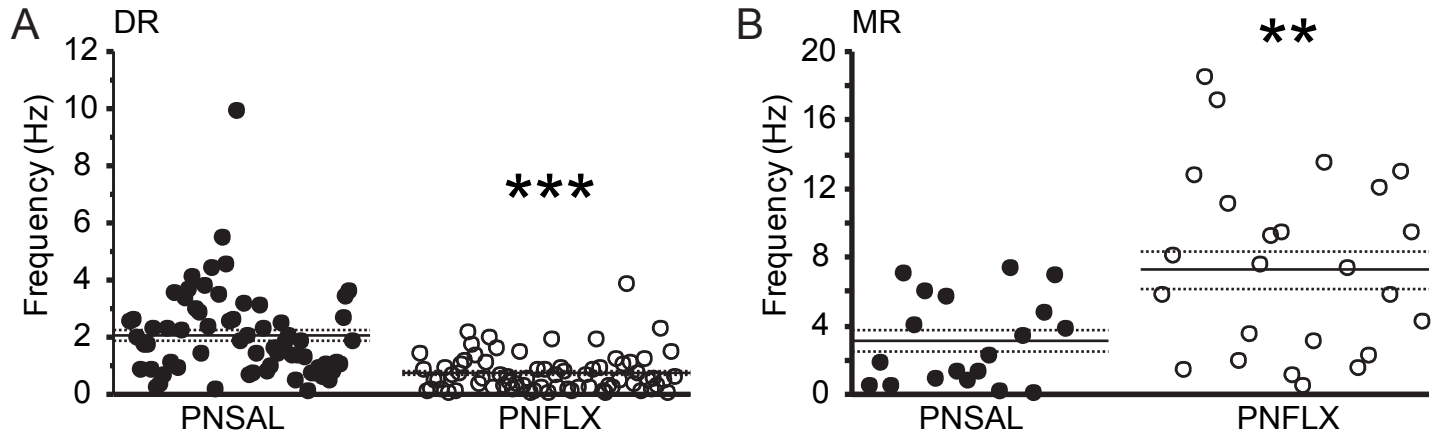

Figure S5: Serotonergic activity is unbalanced in PNFLX raphé nuclei, related to Figure 6. Scattergrams represent mean firing rates of putative serotonergic single neurons in the DR (A) a nd MR (B) of PNSAL (black plots) and PNFLX (white plots) adult animals. Horizontal lines indicate means (solid lines) ± SEM (dashed lines). DR, dorsal raphé; MR, median raphé.
** $p < 0.01$, *** $p < 0.001$

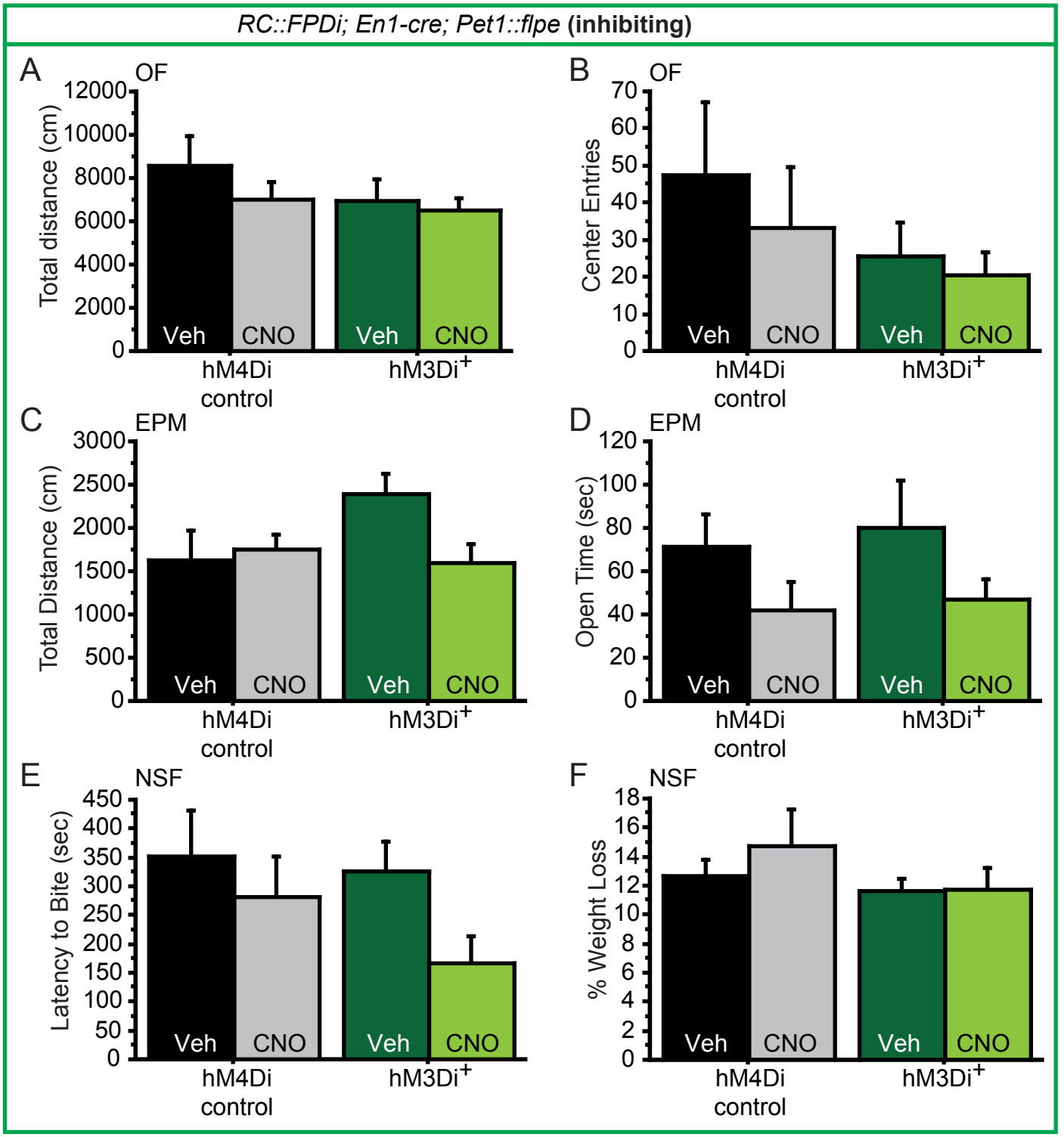

Figure S6: Pharmacogenetic inhibition of r1 5-HT neurons does not alter anxiety-like behavior, related to Figure 7. (A, B) No difference could be detected between En1-mutants and their controls when exposed to Veh or CNO in the distance travelled **(A)** or in the number of entries into the center **(B)** of the OF. n = 5-7 per genotype and treatment. **(C, D)** No difference could be detected between En1-mutants and their controls in the distance travelled **(C)** or time spent in the open arm **(D)** of the EPM. n = 5-7 per genotype and treatment. **(E, F)** No difference could be detected between En1-mutants and their controls when exposed to Veh or CNO in the latency to feed **(A)** or in the % weight loss **(B)** in the NSF test. n = 5-7 per genotype and treatment. EPM: elevated plus maze, NSF: novelty-suppressed feeding, OF: open field. Graphs represent mean ± SEM.

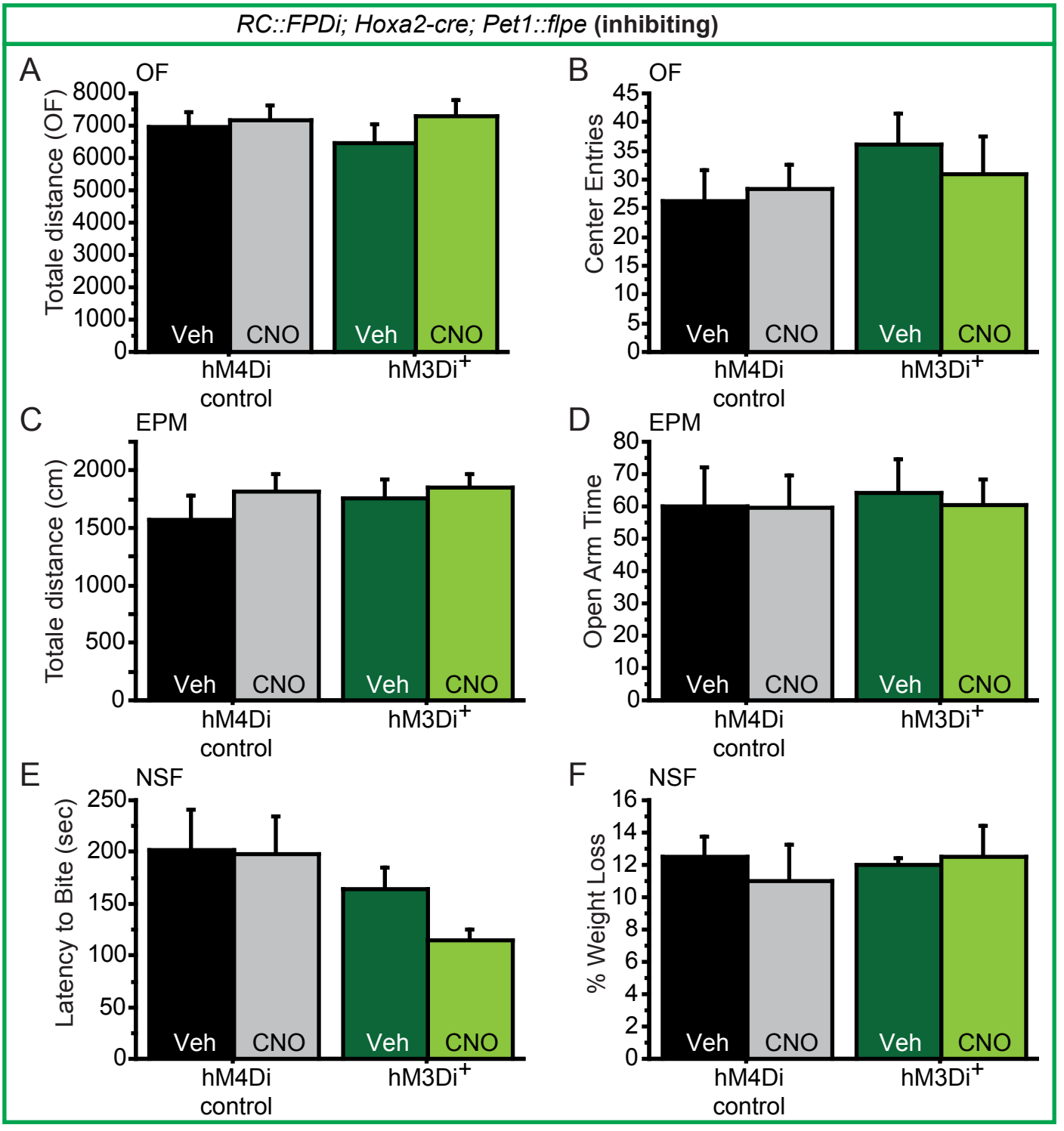

Figure S7: Pharmacogenetic inhibition of r2 5-HTergic neurons does not alter anxiety-like behavior, related to Figure 7. (A, B) No difference could be detected between Hoxa2-mutants and their controls when exposed to Veh or CNO in the distance travelled **(A)** or in the number of entries to the center **(B)** of the OF. n = 9-11 per genotype and treatment. **(C, D)** No difference could be detected between Hoxa2-mutants and their controls in the distance travelled **(C)** and time spent in the open arm **(D)** of the EPM. n = 11-15 per genotype and treatment. **(E, F)** No difference could be detected between Hoxa2-mutants and their controls when exposed to Veh or CNO in the latency to feed **(A)** or in the percentage of weight loss **(B)** in the NSF test. n = 6-12 per genotype and treatment. EPM: elevated plus maze, NSF: novelty-suppressed feeding, OF: open field. Graphs represent mean ± SEM.

SUPPLEMENTAL EXPERIMENTAL PROCEDURES:

Generation of the RC::PDq line:

The targeting constructs for RC::PDq has a cassette including: the cytomegalovirus-chicken *β-actin* (CBA) promoter followed by a flox'd Pgk-Neomycin resistance gene, a hemagglutanin (HA, 3 copies)-tagged DREADD receptor, an internal ribosome entry site (ires), and enhanced green fluorescent protein (EGFP), targeted to the transcriptional start site of the mouse Gt(ROSA)26 Sor locus (see also **Figure S5**). The targeting construct also has a Pgk-diphtheria toxin (DT)A gene for negative selection. The linearized (PvuI) gene targeting construct was electroporated into G4 hybrid 129/Sv x C57Bl/6 embryonic stem cells. Correct targeting was identified by Southern blot of DNA digested with Nde1 and 32-labeled probe beyond the 5' arm of the targeting vector. Positive clones were injected into blastocysts of C57Bl/6 recipient embryos. Agouti pups were bred with C57Bl/6 mice and genotyped by PCR to detect pups that carried the targeted allele. Positive pups were bred with $Mox2^{Cre/+}$ mice to remove the flox'd Pgk-Neo cassette.

Drug administration:

Fluoxetine (FLX; ANAWA Trading) was dissolved in 0.9% NaCl at a concentration of 2 mg/ml, to achieve 10 mg/kg when administered intraperitoneally (ip) at 5 ml/kg body weight. 8-OH-DPAT (Sigma-Aldrich) was administered dissolved in 0.9% NaCl at a concentration of 0.1 mg/ml, to achieve 1 mg/kg when administered ip at 10 ml/kg body weight. Clozapine-N-oxyde was dissolved in 0.4% DMSO and 0.9% NaCl at a concentration of 0.2 mg/ml to achieve 1 mg/kg when administered ip at 5 ml/kg body weight. All CNO injections occurred 30 min before the onset of tests, as suggested by our HPLC (Figure1) and electrophysiology results as well as published data (Alexander et al., 2009)

Postnatal drug administration:

Postnatal drug treatments were performed as described previously (Rebello et al., 2014). In short, on postnatal day 2 (P2), litters were randomly assigned to vehicle (Veh; 0.9% NaCl, 5 ml/kg, ip) and FLX (10 mg/kg, 5 ml/kg, ip) treatments. Mice were treated once per day from P2 to P11. After weaning, mice were housed separately according to sex and randomly mixed for treatment.

Behavioral testing:

For mood-related behavioral testing, male and female mice were investigated, and all animals were exposed to the same series of behavioral paradigms starting at 3 months of age (P90). The tests were administered in the following order: open field, elevated plus maze, novelty-suppressed-feeding (for PNFLX and PNSAL cohorts), and forced swim test, with a minimum of 5 days between each test. All behavioral testing took place during the light cycle between 12 am and 7 pm. To eliminate odor cues, each apparatus was thoroughly cleaned after each animal and testing of males and females were conducted separately. Animals were injected 30min before the test with Clozapine-N-oxide (CNO, 0.2 mg/ml CNO + 0.4% DMSO + 0.9% NaCl) and Vehicle (Veh, 0.4% DMSO + 0.9% NaCl) injected as 3 ml/kg.

Open field test: Exploration and reactivity to a novel environment was assessed using the open field test as previously described (Yu et al., 2014). In brief, square Plexiglas activity chambers equipped with infrared detectors to track animal movement in the horizontal and vertical planes were used as the novel environment. The conflicting innate tendencies to avoid bright light and open spaces but explore novel environments influence locomotion. Mice were placed into the center of the open field and activity was recorded for 30 min. Testing took place under bright ambient light conditions. Total distance, total ambulatory time and vertical activity were automatically recorded using a video tracker (Kinder scientific software, Kinder scientific.).

Elevated plus maze test: The maze is a plus-cross-shaped apparatus, with two open arms (45cm) and two arms closed by walls linked by a central platform 50 cm above the floor. Mice were individually put in the center of the maze facing an open arm and were allowed to

explore the maze during 5 min. The time spent in and the numbers of entries into the open arms were used as an anxiety index. All parameters were measured using a video tracker (AnyMaze, Stoelting Co.).

Novelty-suppressed feeding test: The novelty-suppressed feeding test is a behavior conflict paradigm that is sensitive to chronic but not acute antidepressant treatment as well as acute benzodiazepine treatment. The test was performed as described previously (Yu et al., 2014) the testing apparatus consisted of a plastic box (50 × 50 × 20 cm). The floor was covered with 2 cm of corn bedding. Twenty-four hours before behavioral testing, animals were deprived of food in the home cage. Each mouse was weighed right before food deprivation and before testing to determine the % weight lost. At the time of testing, two food pellets were placed on a round filter paper (12-cm diameter) positioned in the center of the box. Animals were placed in a corner of the box and the latency to approach the pellet and begin feeding was recorded (maximum time, 10 min). After the trial, we measured the weight of pellet consumption into the home cage upon 5 min. All parameters were measured manually.

Porsolt Forced Swim Test: We performed the behavioral despair test using 30X16 cm acrylic glass cylinder filled with water (25°C), from which mice cannot escape. Mice were exposed 3 times to the test, each time for 6 min. During the first day of trial, they were exposed without any injection and activity was recorded for 6minutes as training. During the second day of trial, mice were injected 30 min before the test with CNO or SAL, balancing genotype, sex, litters and treatments. During the third trial, they were exposed to the reversed injections of CNO or SAL. The vertical activity of 5 animals was simultaneously tracked using View Point (ViewPoint Construction software). Animals were then put back in home cage and exposed to a bright light until dry.

Electrophysiological recording:

Adult mice were anesthetized with chloral hydrate (400 mg/kg, ip) and placed in a stereotaxic holder. Supplemental doses of chloral hydrate (up to 100 mg/kg) were given so as to

maintain an adequate level of anesthesia. A tungsten wire electrode was positioned at 4.5-5 mm posterior to the bregma for DR and advanced into the DR, typically found at a depth 2.5-3 mm relative to bregma (Hof PR et al., 2000: Comparative Cytoarchitectonic Atlas of the C57BL/6 and 129/Sv Mouse Brains, San Diego, CA: Elsevier). For MR, electrode was positioned at 5-5.5 mm posterior to the bregma and advanced into the MR, typically found at a depth 4-5mm relative to bregma according to Paxinos Atlas (Paxinos G, 2001: The Mouse Brain in Stereotaxic Coordinates, Second Edition, San Diego, CA: Academic Press). Putative 5-HT$^+$ neurons were discriminated according to the shape, the duration of the repolarization (>2 ms) (Urbain et al., 2006; Vandermaelen and Aghajanian, 1983). Putative serotonergic neurons were identified according to their tri-phasic shape and the total width of the spike (1-2ms). No differences were observed for the spike-width between neurons recorded in the DR of hM3Dq$^+$ versus hM4Di$^+$ animals (unpaired t test, $p = 0.5314$) or within the same neuron before and after CNO exposure (hM3Dq$^+$: $p = 0.2405$; hM4Di$^+$: $p = 0.4748$). Similarly, no difference was detected in spike-width between the putative serotonergic neurons recorded between PNSAL and PNFLX animals, neither in the DR ($p = 0.901356$), nor in the MR ($p = 0.981313543$). These results indicate high homogeneity within the population of recorded neurons. For recordings associated with HPLC, 3 min of each putative 5-HTergic neuron was recorded for baseline simultaneously to the first 30 min of probe sampling. Then, the first next putative 5-HTergic neuron was recorded for 3 min before CNO was injected and recording of the same neuron was further a maintained for as long as possible (maximum 30min). Then 3 min recordings were pooled for 0-15 min and 15-30 min post CNO injection according to their time of recording. The change in firing rate (FR) for each neuron was calculated as the difference in the FR for a 10-20min period right after and right before CNO injection, divided by the variance in firing rate before CNO injection in *RC::PDq;Pet1-cre* and *RC::PDi;Pet1-cre* animals. Means and variance in FR were calculated from 10 measures of FR/min randomly distributed within the period of interest. We observed no correlation between FR change and spike width (**Figure S3**). For PNFLX versus PNSAL neuronal recordings, each neuron was recorded for 3 min and, after 4 - 5 neurons were collected,

animal was injected ip with 8-OH-DPAT (1mg/kg) to test for autoreceptor inhibition (Calizo et al., 2011).

Surgery for micro dialysis:

Adult mice were anesthetized with chloral hydrate (400 mg/kg, ip) and placed in a stereotaxic holder. Supplemental doses of chloral hydrate (100 mg/kg) were given so as to maintain an adequate level of anesthesia. After a hole was drilled at 2.5–3.0 mm anterior to bregma and 2.0 mm lateral to the midline, a Microdialysis Probe MBR-2-5 (BASi, MB-2212) with confirmed *in vitro* recovery of > 5% was slowly inserted into the right mPFC at the rate of ~1 µm/10 min during 1 h and until 1.7 mm ventral from a point on the dural surface located 2.9 mm anterior to bregma and 1.7 mm lateral to the midline. The probe was then perfused with an artificial cerebral spinal fluid (aCSF; a modified Dulbecco's buffer with 5 mM glucose) at a rate of 1 µL/min and left for 90 min prior to collecting the first sample, approximately 150 min prior to the reference baseline collection used for analysis. During the 90 min left for probe recovery, a hole was drilled for the placement of the electrodes in the raphé and electrodes were inserted until 2.5 mm from a point on the dural surface located 4.5 mm posterior to bregma and 0 mm lateral to the midline. Following the equilibration period, dialysis samples (flow rate of 1.0 µl/min) were collected (tubing length: 20 cm; tubing volume: 1.5 µm/10 cm) into vials containing 5 µl of HeGA preservative every 30 min. After collection of 3 baseline samples every 30 min, CNO (1 mg/kg, ip) was injected and 4 more 30 min samples were collected. Dialysis samples were immediately analyzed (see below). Simultaneous to microdialysis sampling, single-unit extracellular recordings of raphe neurons were performed as described above.

Serotonin levels analysis:

5-HT in each dialysate was quantified by high-performance liquid chromatography (HPLC) with electrochemical detection. Immediately after collection, samples were transferred to HPLC vials kept at 4°C, and precise volumes were ascertained via glass syringe. 5-HT (and

metabolites) were then measured using a 100 mm long C8-silica bonded column (i.d. 2.0 mm; particle size 3 µm (Agilent Technologies) and a mobile phase consisting of HPLC grade chemicals: 10% acetonitrile, 2% methanol, 75 mM sodium phosphate monobasic monohydrate, 25 mM citric acid, 25 µM EDTA, 0.5 M 1-octanesulfonic acid soap, 100 µL/L triethylamine. Oxidation peaks were detected via an electrochemical cell (ESA, model 5020) coupled to a CoulArray detector (ESA, model 5600A) with CoulArray software. Multiple set of external standards prepared in the same aCSF prepared for the *in vivo* samples were interspersed with samples. Serotonin peaks were measured by integrating the area under the curve and quantified using an external standard curve that bounded *in vivo* values.

Immunohistochemistry:

To perform Fos and 5-HT immuno-labeling, animals were injected with CNO (1 mg/kg), 2 h before perfusion. They were anesthetized using a mixture of ketamine and xylazine before perfusion by a chase of PBS followed by 10 min of paraformaldheyde (PFA) 4% in phosphate buffer (PB). Brain were dissected and post fixed overnight in PFA 4% in PB. Then when later rinsed in PBS and transferred in sucrose solution (30%) for 2 days for cryoprotection. They were then cut in 60-µM thick coronal sections at the cryostat before double immuno-labeling was performed overnight using rabbit anti-Fos (Millipore, PC38) and goat anti-5-HT (LifeSpan Biosciences C75755-100). Cy3 anti-rabbit and Alexa 488 anti-goat (Jackson Laboratory) were used as secondary. Images were acquired using Leica SP5 confocal.

Cell counting:

Fos and 5-HT co-labeling counts were performed using ImageJ software. Mosaic images from raphe were split according to color. 5-HT+ neurons were labeled and their position was then copy past on the Fos channel to assess double labeling. Cell counts were done blind to treatment using animal numbers.

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