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Supplementary Materials for

Hippocampal cAMP regulates HCN channel function on two time scales with differential effects on animal behavior

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

Materials and Methods

Animals

Hcn2$^{R/R}$ mice were generated with the assistance of the Northwestern University Transgenic and Targeted Mutagenesis Laboratory. These mice were backcrossed onto C57BL/6J wild type mice from Jackson Labs (Bar Harbor, ME). All experiments utilizing *Hcn2*$^{R/R}$ mice were performed with littermates as controls whereas experiments that exclusively used wild type mice used mice purchased from Jackson Labs. The following primers were used for genotyping *Hcn2*$^{R/R}$ mice: Forward: 5'-CTGAGATCTGGATGGGATGGGA 3', Reverse: 5'-TGTTGCCCCACAAACCTAGAATAC 3'. The PCR reaction utilized a 50ºC annealing temperature and the PCR product was purified using a PCR cleanup kit (Qiagen, Hilden, Germany) digested using either BsmB1 or Nae1 (New England Biolabs, Ipswich, MA) with the wild type allele detected as an Nae1 digestion (producing a 400nt product) and the R591E allele detected by BsmB1 digestion (producing a 400nt product). All animal protocols were approved by the IACUC committees of Northwestern University and Vanderbilt University Medical Center. All primers were ordered from Integrated DNA Technologies (Coralville, IA). Mice aged 8-12 weeks were used for all behavioral experiments unless otherwise specified.

Viral Experiments

Viral injections were performed with minimal modifications from our previous work *(34)*. Mice aged 6-8 weeks old were anesthetized with 2-3% inhaled isoflurane and mounted on a stereotaxic apparatus (Stoelting, Wood Dale, IL). A small incision was made to visualize bregma and lambda and a craniotomy was made with a dental drill. A 5µL Hamilton syringe was then lowered to the coordinates (relative to bregma and the surface of the skull): 2.3mm A/P, +/-1.3mm M/L, -1.7mm D/V. The syringe was held there for 2 minutes prior to injecting 1µL of virus at a rate of 0.3µl/min. The syringe was then held in place for an additional 5 minutes before the syringe was slowly withdrawn. All mice were allowed to recover for 3-5 weeks prior to experimentation. All plasmids were cloned using standard methods and sequenced prior to synthesis at the University of Pennsylvania vector core. Plasmid encoding rM3D(Gs) was obtained from Addgene (Cambridge, MA) and plasmid encoding VP16-CREB *(59)* was a generous gift of Dr. Angel Barco (Universidad Miguel Hernandez-Consejo Superior de Investigaciones Científicas). AAV2/8-hSyn-TRIP8b was identical to the those used in a prior report *(34)*. This viral construct consists of TRIP8b(1a-4), the most abundant TRIP8b isoform in the hippocampus *(33)*. In experiments where a GFP control virus is used, we employed AAV8-CaMKII-GFP, obtained from Vector Biolabs (Catalog #VB1933). 1µL of each of the following titers was used for experiments: AAV8-DREADD (7.00 x 10^{12} GC/mL), AAV8-CaMKII-GFP (1.84 x 10^{12} GC/mL), AAV2/8-hSyn-TRIP8b (3.00 x 10^{12} GC/mL), AAV8-CaMKII-VP16-CREB (low titer is 9.31 x 10^{11} GC/mL, medium titer is 4.65 x 10^{12} GC/mL, and high titer is 9.31 x 10^{12} GC/mL).

Behavioral Experiments

For all behavioral experiments, animals were permitted to acclimate in the testing room for a minimum of two hours before experimentation and all experiments began at 9am (2 hours after the beginning of the light cycle). Tail suspension testing (TST) was performed as described previously *(34)*. Each mouse was individually assayed by being suspended by their tail inside of a chemical fume hood for 6 minutes. Mice were excluded from analysis if they climbed their tails at any point in the assay. For forced swim testing (FST), up to 5 mice at a time were placed into individual beakers of water inside of a chemical fume hood with dividers used to prevent the mice from seeing one another. The FST task lasted for 6 minutes, although only the final 4 minutes

were used for scoring. All experiments were video recorded, randomized, and scored by a blinded observer afterward. Open field testing (OFT) was performed using a 27cm x 27cm chamber (Med Associates).

Object location memory was performed as previously described *(18)*. On day 1 of the test, mice completed a 'training session' and were placed in a small chamber with two identical objects in the chamber and recorded using an overhead camera for 10 minutes. As specified in the main text, a subset of experiments incorporated the IP injection of either saline or CNO immediately after the training session (such as **Figure 2G**). On day 2, the mice were returned to the same chamber for 5 minutes except that one of the two identical objects in the chamber had been moved (one of the four walls of the chamber has a design on it that facilitates the determination of the moved object *(85)*). The mouse is again recorded, and the discrimination index is reported after scoring by a blinded observer as {[time spent exploring the moved object on day 2– time spent exploring the unmoved object on day 2]/total time spent exploring both objects on day 2}.

TST, FST, OLM, and OFT were performed at the Murine Neurobehavior Core lab at the Vanderbilt University Medical Center.

For experiments involving the acute administration of citalopram (Cayman Chemicals), citalopram was dissolved in 0.9% normal saline to a concentration of 2mg/mL and administered at a dose of 10μL/gram bodyweight (20mg/kg) by intraperitoneal (IP) injection. For experiments involving the acute administration of clozapine-N-oxide (CNO, ThermoFisher), CNO was dissolved in 0.9% normal saline to a concentration of 0.5mg/mL and administered at a dose of 20μL/gram bodyweight (10mg/kg) by IP injection. For control injections, an equivalent volume of saline alone was administered. In all experiments where CNO was acutely administered, it was given 40 minutes prior to the assay. In all experiments where citalopram was acutely administered, it was given 30 minutes prior to the assay.

Chronic Social Defeat (CSD)

CSD was carried out using adult male C57BL/6J mice (Jackson labs) aged 6-8 weeks at the beginning of experimentation as previously described *(60, 61)*. For a detailed description of the protocol, please see prior reports *(60, 61)*, although for completeness we detail our instantiation of the protocol here. Aggressor mice were retired CD1 breeder males aged at least 4 months and screened prior to CSD for aggressive behavior. On each day of the CSD protocol, the test C57BL/6J mice were placed into a new cage that consisted of a standard size cage with a plexiglass divider placed longitudinally. The clear plexiglass divider was arranged longitudinally in the cage and had many small holes drilled out so that mice on the two sides of the divider could not physically interact but so that sounds and smells were easily transmitted. For the first 10 minutes in a new cage, the divider was removed and the C57BL/6J mouse was permitted to interact with a novel CD1 aggressor mouse. The mice were observed during this time and any interaction that was likely to lead to an injury from which the C57BL/6J mouse could not recover was interrupted. After the 10 minute 'defeat' session, the plexiglass divider was returned to the cage. The following day, the C57BL/6J mouse was moved into a new cage with a new CD1 aggressor for a new defeat session until a total of 10 defeat sessions was completed. All mice were closely monitored during and after defeat sessions, and if at any point one of the C57BL/6J mice appeared to be injured beyond superficial abrasions, the mouse was euthanized in accordance with the animal protocol. Control C57BL/6J mice were moved from clean cage to clean cage each day without exposure to CD1 animals in order to replicate the effects of animal handling. Following CSD, all mice were individually housed.

On the day after the final defeat session, social interaction (SI) testing was carried out. During SI testing, the test mouse (C57BL/6J) is placed into an arena with an empty mesh enclosure for 150 seconds. Afterwards, the C57BL/6J is removed from the arena and a novel CD1 mouse (not previously used for defeat sessions and which has not previously been exposed to the C57BL/6J mouse) is placed in the arena in a mesh enclosure. The mesh

enclosure does not permit physical interaction with the C57BL/6J mouse but does permit the sight, smell, and vocalization of each mouse to be sensed by the other. The C57BL/6J mouse is returned to the arena for another 150 seconds and the social interaction ratio is calculated by comparing the time spent interacting with the empty enclosure with the time spent interacting with the novel CD1 mouse. Mice with a SI less than 1 were defined as 'susceptible' and those with an SI greater than 1 were defined as 'resilient' (as in other reports *(60, 61)*).

Chronic CNO experiments
3-5 weeks after viral injection, mice were assigned to receive either CNO (50mg/L) or an equivalent quantity of saline (vehicle). Given the substantial quantity of CNO used in these experiments, a custom order from ThermoFisher was placed. Water bottles were changed twice each week. Prior to behavioral testing, all animals were changed back to tap water for 2 hours to minimize the acute effects of CNO.

HEK Electrophysiology
HEK cell recordings were performed from HEK cells stably expressing HCN2, plated onto glass coverslips in a 24 well plate, and transiently transfected with 0.1µg GFP and 0.1µg rM3D(Gs) using Mirus reagents per the manufacturer's protocol (Mirus, Madison, WI). The recording setup was identical to that previously described *(34)*. The internal solution was 130mM KCl, 0.5mM $MgCl_2$, 5mM EGTA, 5mM HEPES, 2mM MgATP, and 10mM NaCl. CNO was bath applied at 1µM.

Slice Electrophysiology
Whole cell recordings from CA1 pyramidal neurons were performed on one of two electrophysiology rigs for different experiments. For characterization of $Hcn2^{R/R}$ animals, the setup was as previously described (**Figure 3** *(34)*). For investigating the effect of AAV-DREADD on electrophysiological properties, a separate rig was used (**Figures 5, 6**) as described elsewhere *(86, 87)*. In all cases, 300 micron sagittal sections were made from male and female mice with 5µM CGP52432, 2µM SR-95531, and 10µM MK-801 to block $GABA_B$, $GABA_A$, and NMDAR. ACSF was composed of 125mM NaCl, 2.5mM KCl, 25mM NaHCO3, 1.25mM Na_2PO_4, 1mM $MgCl_2$, 2mM $CaCl_2$, and 25mM glucose. The internal solution was 115mM K-gluconate, 20mM KCl, 10mM Na_2Phosphocreatinine, 2mM MgATP, and 0.3mM NaGTP. Efficacy of bath application of 20µM ZD7288 was confirmed by monitoring the sag in response to a negative current injection, which typically required 5-10 minutes of wash-in. Current clamp recordings were performed at a holding potential of -70mV. Temporal summation experiments were performed using a train of 5 stimuli at 20Hz delivered using a bipolar stimulating electrode located on the TA pathway (or SC pathway as described in the text). For some experiments, stimulus intensity was titrated to prevent action potentials (**Figure 3**), although in other experiments the stimulus intensity was varied and the fraction of stimuli trains producing action potentials was recorded (**Figure 5**).

cAMP quantification
cAMP was quantified using a commercial kit per the manufacturer's protocol (Catalog #V1501, Promega, Fitchburg, WI). Reactions were carried out in triplicate in a black 384-well microtiter plates (Corning, Lowell, MA). The luminescence measurements were obtained using a Tecan microplate reader at the Structural Biology Facility at Northwestern University, which is generously supported by NCI CCSG P30 CA060553 of the Robert H. Lurie Comprehensive Cancer Center.

Immunohistochemistry
Animals were deeply anesthetized with isoflurane and then transcardially perfused with ice-cold phosphate-buffered saline and 4% paraformaldehyde (PFA). The brain was dissected into 4%PFA and incubated at 4°C for 48-72 hours with gentle agitation before 30 micron free floating coronal sections were made using a vibratome (Leica, Buffalo Grove, IL). Tissue was stored in

0.025% Na-N_3 at 4°C for 1-90 days prior to downstream processing. For staining, antigen retrieval was performed by incubating the tissue in 10mM Na-Citrate (pH 9.0) for 10 minutes at 80°C and then letting the tissue cool to room temperature for 1 hour. Afterwards the tissue was washed 3 times for 5 minutes in PBS-T (200mL PBS with 600uL of Triton-100). The tissue was then blocked for 1 hour at room temperature in blocking buffer (47.5mL of PBS-T with 1g Bovine Serum Albumin and 2.5mL of normal goat serum). The tissue was then incubated in primary antibodies overnight at 4°C at the concentrations described below in blocking buffer. The next day the tissue was washed three times in PBS-T and then incubated for 2 hours at room temperature in secondary antibodies diluted in blocking buffer. The tissue was then washed twice in PBS-T and then incubated in DAPI before being mounted on microscope slides. After 30-60minutes, the tissue was coverslipped using ProLong antifade (Thermo Fisher Scientific, Fremont, CA).

The following antibodies were used for immunohistochemistry: Rabbit anti-GFP (1:1000, Millipore AB3080P) and custom guinea pig anti-HCN2 *(88, 89, 90)*. All secondary antibodies were raised in goat, used at 1:500, and purchased from Invitrogen.

Imaging work was performed using confocal microscopes located at the Northwestern University Center for Advanced Microscopy generously supported by NCI CCSG P30 CA060553 awarded to the Robert H Lurie Comprehensive Cancer Center as well as the Vanderbilt Cell Imaging Shared Resource (supported by NIH grants CA68485, DK20593, DK58404, DK59637, and EY08126). Quantification of images was performed as previously described *(34)* using custom written routines in MATLAB (Mathworks, Natick, MA). Images were randomized and a blinded observer drew ROIs over the stratum oriens and stratum pyramidale. A larger ROI was also drawn encompassing the stratum lacunosum moleculare and stratum radiatum which was then subdivided into ten equally spaced ROIs in order to examine the distal dendritic enrichment of HCN channels.

Western Blotting

Western blotting was performed as previously described *(34)*. Primary antibodies used were custom rabbit anti-HCN1 (1:1000) *(88, 89, 90)*, custom guinea pig anti-HCN2 (1:1000) *(88, 89, 90)*, mouse anti-TRIP8b (1:1000, Neuromab Catalog #N212/17, UC Davis, CA), mouse anti-tubulin (1:1000, Millipore Catalog #05-829), mouse anti-βIII-tubulin(1:30,000, Invitrogen vMA1-118). For chemiluminescence, secondary antibodies were purchased from Bio-Rad and ECL substrate was purchased from ThermoFisher. For immunofluorescence, antibodies were purchased from Licor (Lincoln, NE) and used per the manufacturer's protocol. Western blots were exposed on either a Licor imaging system or with a Bio-Rad ChemiDoc MP. Immunoblotting experiments were analyzed using ImageStudio (for images acquired using Licor hardware) and Image Lab (for images acquired using Bio-Rad hardware).

Co-Immunoprecipitation reactions

Our protocol was based on a previously published protocol with some modifications *(33)*. The mouse was deeply anesthetized with isoflurane, decapitated, and the hippocampi subdissected and flash frozen. Later, one hippocampus/animal was homogenized in ice cold homogenization buffer (10 mM Tris-HCl (pH7.4), 320 mM sucrose, 5 mM EDTA) supplemented with Halt protease and phosphatase inhibitor cocktail (ThermoFisher, Rockford, IL), then centrifuged for 5 min at 3000g. The pellet was resuspended in lysis buffer (10 mM Tris-HCl (pH 7.4), 320 mM sucrose, 5 mM EDTA, 150 mM NaCl , and 1% Triton) followed by sonication and centrifugation at the highest speed in a table top centrifuge at 4°C for 10min. 50µL of brain lysate was then added to 50µL of sample buffer (Bio-Rad, supplemented with DTT per the manufacturer's instructions) and set aside as an input fraction. Immunoprecipitation was carried out using 400µl of remaining lysate with a custom rabbit anti-HCN1 antibody *(35)* by overnight incubation on a nutator at 4°C. The

next day, 25µl of Pierce protein A/G agarose slurry (ThermoFisher) was washed in lysis buffer, added to the brain lysate with antibody, and incubated for 4 hours on the nutator at 4°C. After the incubation, the beads were washed with ice cold lysis buffer, then eluted in 50µl sample buffer by boiling. For western blot, 4-20% 15 well polyacrylamide precast gels from Bio-Rad were loaded with 10µl of input and 15µl of elution from beads for the experiments shown in (**Figure 6C, D**). The quantity of input and eluant were titrated in **Figures 6E, H** to produce bands in the linear range for quantification.

qPCR

Deidentified human tissue was obtained from the University of Maryland and the University of Pittsburgh through the NIH NeuroBiobank (Human Brain Collection Core, Intramural Research Program, NIMH http://www.nimh.nih.gov/hbcc) as well as the Texas Tech Southwest Brain Bank. We are grateful to the donors and their families as well as the staff members of the brain banks for their participation in this work.

Hippocampal samples from patients who did not carry a diagnosis of alcoholism were advanced for study because of a lack of sufficient controls for patients with dual diagnoses of alcoholism and MDD (there were few patients with a diagnosis of alcoholism but without MDD). The remaining hippocampal samples were subdissected to isolate CA1. RNA was extracted using the RNeasy Lipid Tissue Mini Kit (Qiagen Catalog #74804) and quantified using a Nanodrop (ThermoFisher). 2µg of RNA was used for reverse transcription performed using random hexamers and the Superscript III kit (ThermoFisher Catalog #18080051). All reactions were performed using PowerUp SYBR Green Master Mix (ThermoFisher Catalog #A25742) and a CFX96 Thermocycler (Bio-Rad). All reactions utilized 10µL of SYBR, 2µL of a 5µM stock for each primer, 4µL of dH_2O, and 2µL of cDNA (the RT reaction was diluted 160 fold in the case of mouse experiments and diluted 40-320 fold in the case of human experiments in order to generate consistent Ct values for reference genes).

All primers were generated using PrimerBank *(91, 92)*, and then validated to have an amplification efficiency between 98 and 106%. Genes with multiple splice isoforms (ie *Pex5l (35)*) had primers chosen to amplify a region common to all isoforms. Amplicons were run out on a 2% agarose gel to confirm their size and the absence of additional products. Melting curves were also used to confirm a single amplicon in each reaction. All reactions were performed in triplicate and quantified using the ΔΔCt method *(93)*.

The following human primers were used: *GAPDH* (For: GGAGCGAGATCCCTCCAAAAT, Rev: GGCTGTTGTCATACTTCTCATGG), *HCN1* (For: CATGCCACCGCTTTAATCCAG, Rev: ATTGTAGCCACCAGTTTCCGA), *HCN2* (For: CCGGGCGTCAACAAGTTCT, Rev: AGTCGCTGTACGGGTGGAT), *PEX5L* (For: ACTCAGGCTCGACTGACCAA, Rev: TCACTAGCCGAGATGGTTACTT), *KCND2* (For: GACCGTGACCCAGACATCTTC, Rev: TGCACTCGTGGCGAGGATA), *DLG4* (For: TCGGTGACGACCCATCCAT, Rev: GCACGTCCACTTCATTTACAAAC), *ACTB* (For: CATGTACGTTGCTATCCAGGC, Rev: CTCCTTAATGTCACGCACGAT).

For experiments using mouse tissue, an identical protocol was followed. The following primers were used: *Creb1(*For: AGCAGCTCATGCAACATCATC, Rev: AGTCCTTACAGGAAGACTGAACT)*, Gapdh*(For: AGGTCGGTGTGAACGGATTTG, Rev: TGTAGACCATGTAGTTGAGGTCA), *Hcn1(*For: CAAATTCTCCCTCCGCATGTT, Rev: TGAAGAACGTGATTCCAACTGG)*, Pex5l* (For: ATGGAAGCTGCAATTCTTCAGG, Rev: CTCTGGAGGGCGACAATAGC). Note that the *Creb1* primers only detect endogenous *Creb1* transcripts and do not detect AAV-VP16-CREB.

Figure S17 was created using BioRender.com

Supplemental Figures

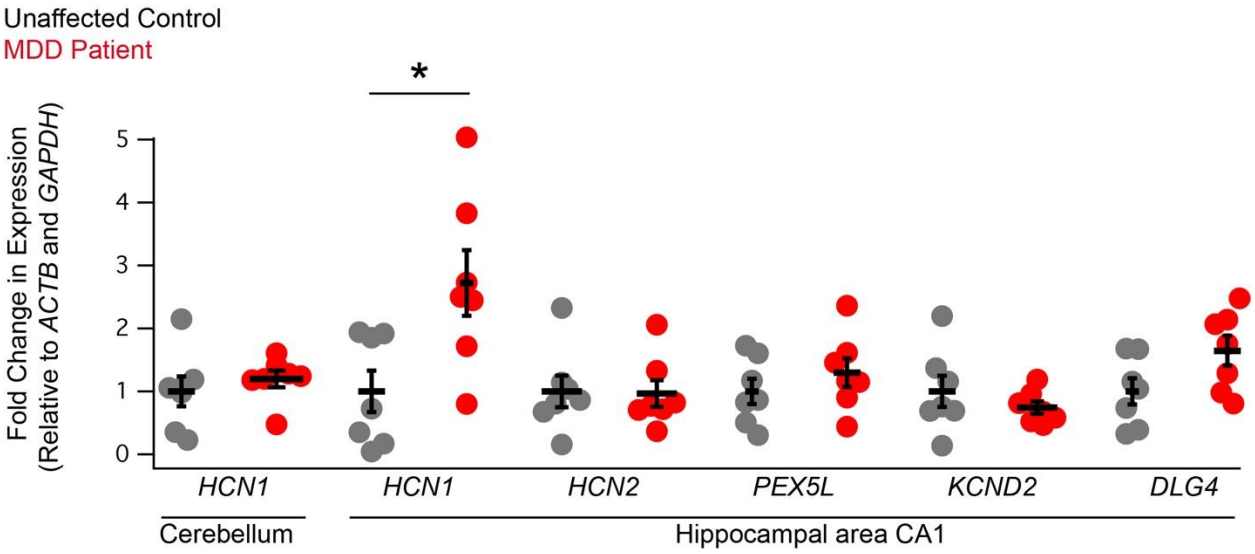

Fig. S1. qPCR of human samples with cerebellar controls
To determine if our results could be attributed to differences in sample preparation, storage, or handling conditions, we examined samples obtained from the cerebellum in a subset of the samples for which this data was available (males and females were pooled because of the smaller sample size available). Data corresponding to CA1 expression of each gene was reexamined using only these n=7,7 subjects as indicated along the bottom. (*p<0.05). See **table S2** for statistics.

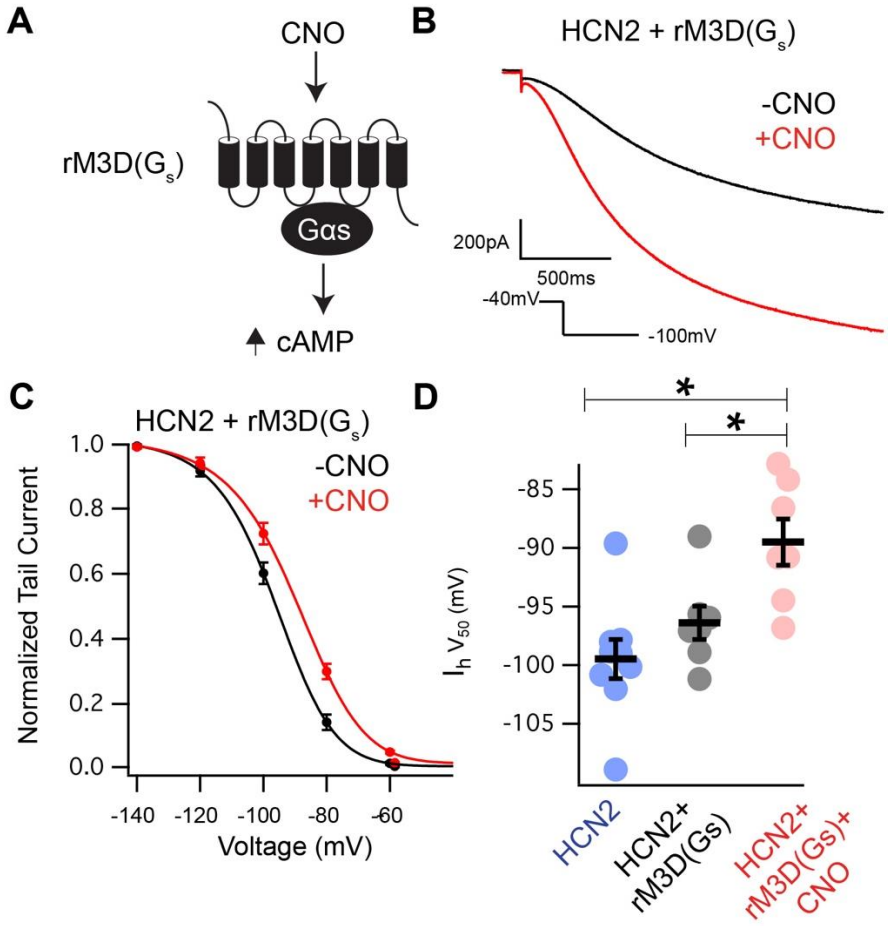

Fig. S2. rM3D(Gs) activation leads to an increase in HCN channel function

A) Schematic showing rM3D(Gs) activity. **B)** HEK293T cells stably expressing HCN2 were used to examine the effect of rM3D(Gs) mediated cAMP production on I_h, the current mediated by HCN channels. **C)** Activation curves for HCN2 in the presence or absence of CNO. **D)** Quantification of the half activation potential (V_{50}) in HEK cells stably expressing HCN2 (blue), HCN2+rM3D(Gs) in the absence of CNO, and HCN2+rM3D(Gs) with extracellular CNO. Shapiro-Wilks tests for untransfected: W=0.8573, p=0.0802, pre-CNO: W=0.8679, p=0.1514, with CNO: W=0.7205, p=0.949. Levene's test (df(2,20)= 0.6185, p=0.5487). One way ANOVA was performed (F(2,20)=8.9028, p<0.01). N= 9,7,7 for Untransfected (blue), rM3D(Gs) without CNO (black), and rM3D(Gs) with CNO (red).

*Denotes p<0.05 by Tukey's post-hoc test.

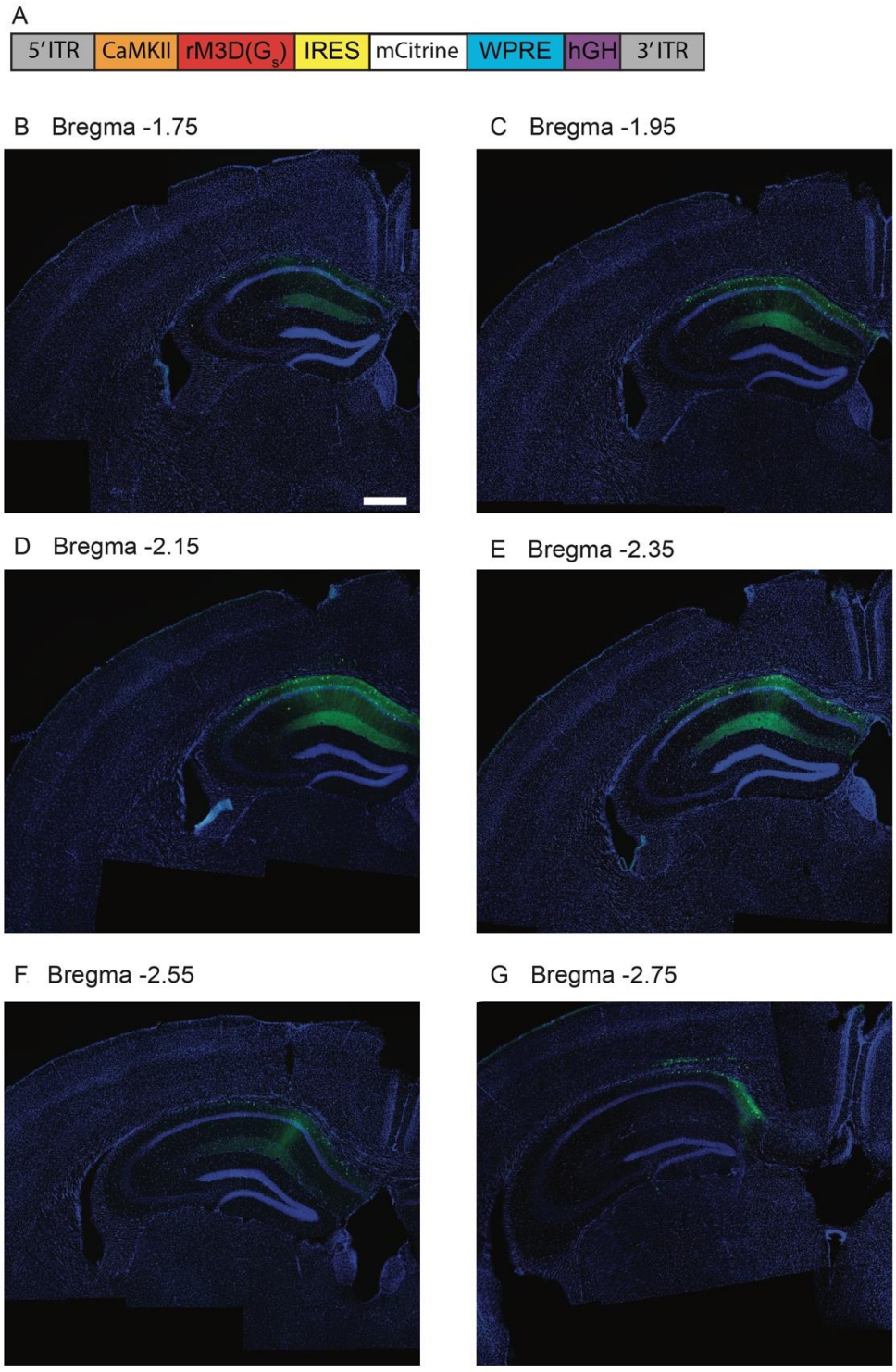

Fig. S3. Extent of AAV-DREADD viral spread
A) Schematic of AAV-DREADD virus. **B)** Series of images to illustrate extent of viral spread. Location relative to Bregma is based on comparison with Allen Brain Atlas. Scale bar is 500microns.

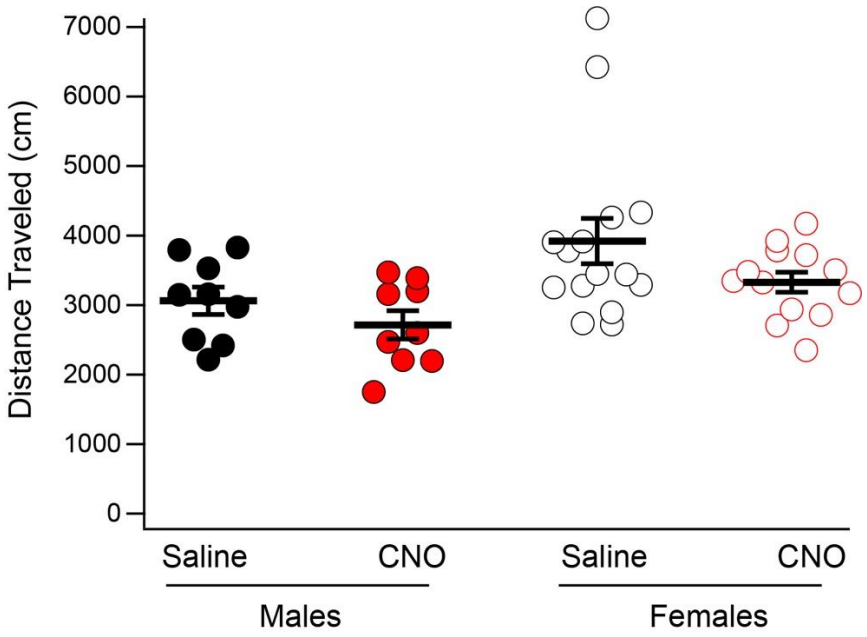

Fig. S4. AAV-DREADD activation in wild type mice does not affect open field result
Adult male and female mice were bilaterally injected with AAV-DREADD into their dorsal hippocampi and then later IP injected with either saline (NS) or CNO. Shapiro-Wilks tests were performed for Male NS: W=0.930, p=0.485; Male CNO: W=0.924, p=0.430, Female NS: W=0.776, p=0.002, Female CNO: W=0.986, p=0.997. Levene's test was performed (F(3,42)=1.7519, p=.171). 2x2 ANOVA of aligned rank values revealed an effect of sex (F(1,42)=11.62, p=0.001) but not of NS/CNO (F(1,42)=3.66, p=0.06) or an interaction term (F(1,42)=0.000, p=0.979). NS: Saline, CNO: Clozapine-N-Oxide.

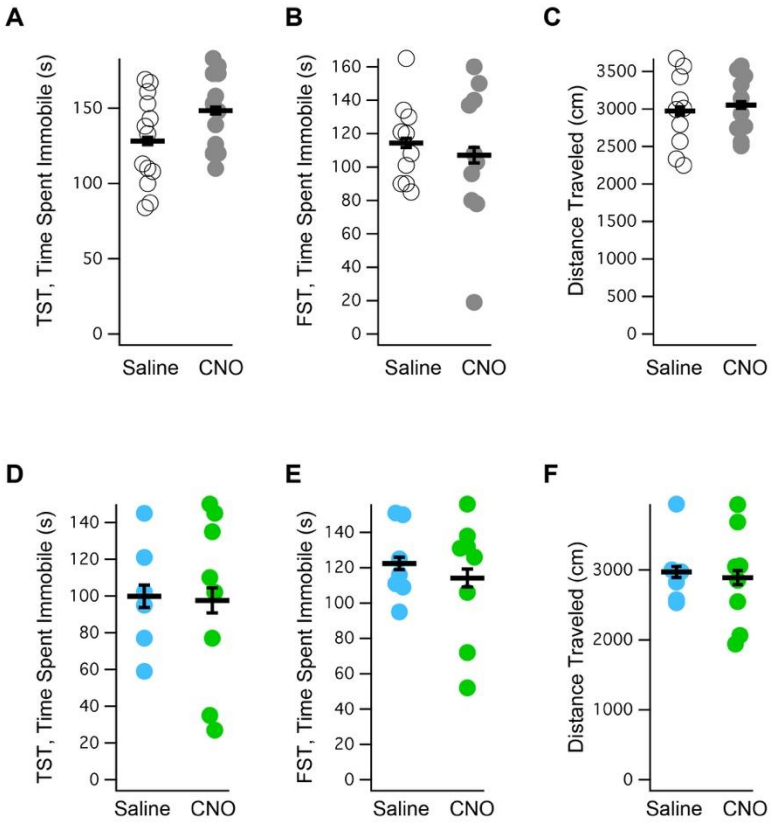

Fig. S5. IP injection of CNO does not produce changes in locomotion or motivated behavior **A)** Wild type male mice were IP injected with saline (NS) or CNO prior to TST (NS: 128.1±8.2s, CNO: 148.4±7.3s, t(23)=1.825, p=0.081, n=13, 12). **B)** A similar paradigm was used to examine FST after IP injection of either NS or CNO (NS: 114.4±7.8s, CNO: 107.1±3.1s, t(18)=0.47, p=0.6434, n=10, 10). **C)** Open field testing was also performed after IP injection of NS or CNO in wild type mice (NS: 2973.9±156.6, CNO: 3052.7±127.3, t(18)= -.390, p>0.700, n=10, 10). **D)** Wild type male mice were bilaterally injected in the dorsal hippocampus with a control virus (AAV-GFP). 4 weeks later they were IP injected with NS or CNO 40 minutes prior to TST (AAV-GFP/NS: 99.8±12.5s, AAV-GFP/CNO: 97.6±16.8s, t(12)=0.098, p=0.923, n=6,8). **E)** Mice injected with AAV-GFP were subjected to FST 40 minutes after IP injection of NS or CNO (AAV-GFP/NS: 122.4±7.9s, AAV-GFP/CNO: 114.1±12.5s, t(13)=0.54, p=0.598, n=7,8). **F)** IP NS or CNO was followed by open field testing in mice injected with AAV-GFP (AAV-GFP/NS: 2971.0±176.8cm, AAV-GFP/CNO: 2890.3±249.4cm, Mann-Whitney Ranksum=64, p=.7323, n=7,8).

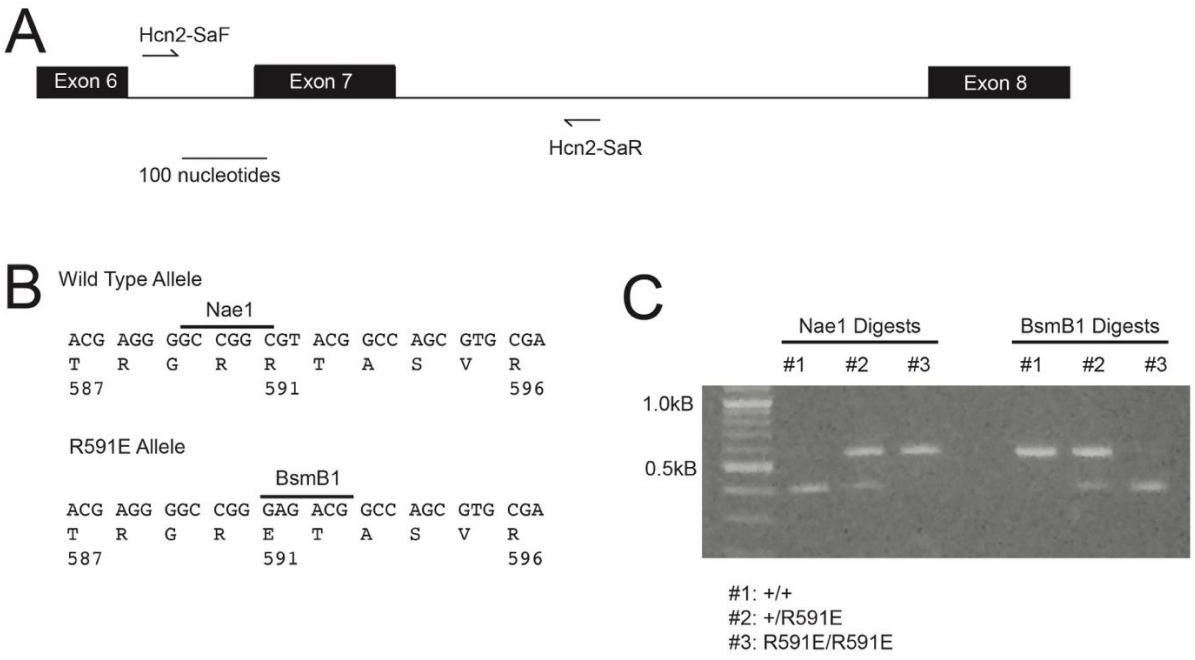

Fig. S6. Genotyping strategy for Hcn2$^{R/R}$ mice

A) Schematic showing the location of primer annealing sites. **B)** Location of restriction sites in the two alleles. **C)** Representative agarose gel showing the results of digests with Nae1 and BsmB1.

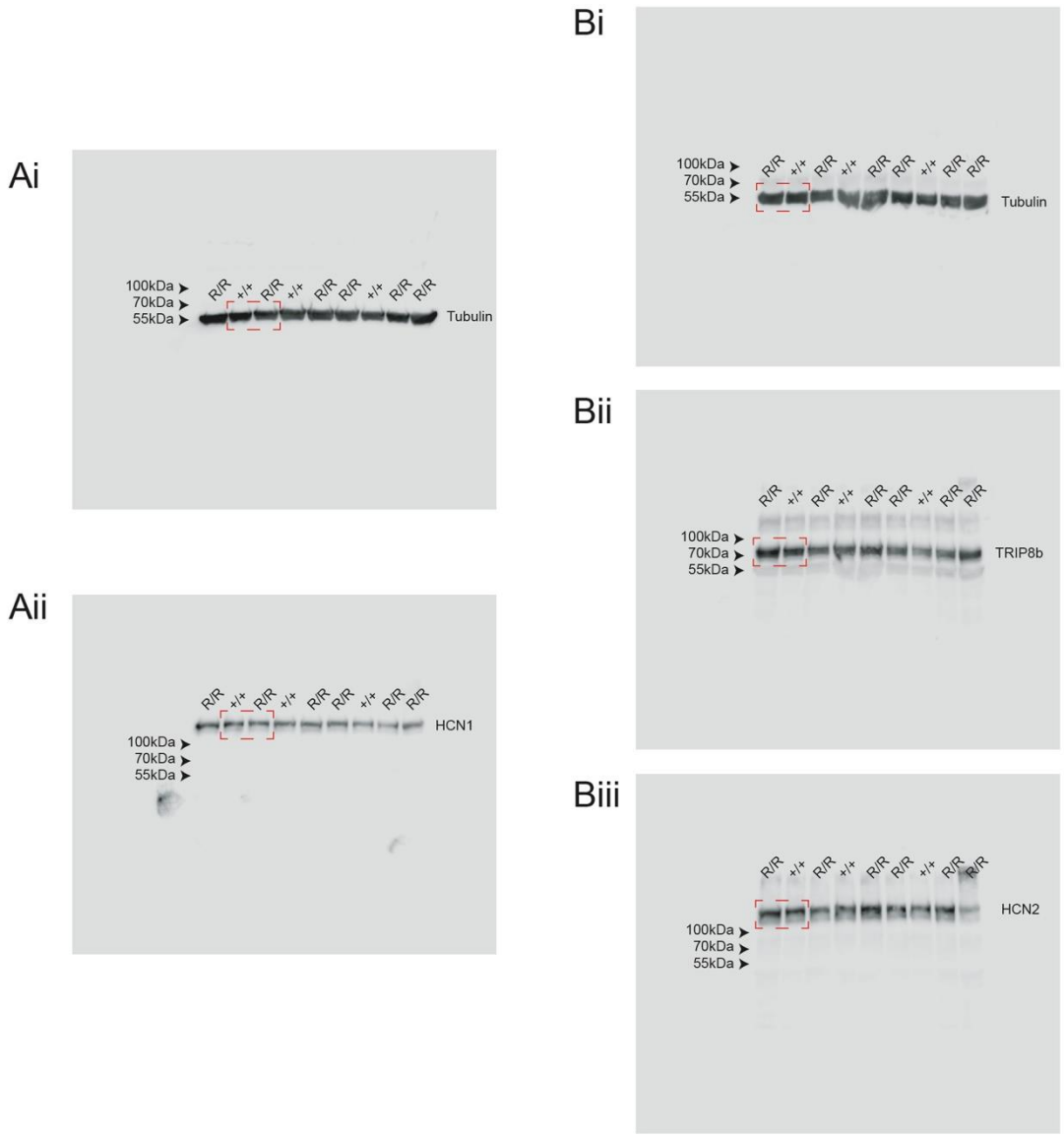

Fig. S7. Uncropped western blot images corresponding to Figure 3
A) Uncropped western blots stained for tubulin (**Ai**) and HCN1 (**Aii**). **B)** Uncropped western blots stained for tubulin (**Bi**), TRIP8b (**Bii**), and HCN2 (**Biii**). Note that dashed red boxes highlight the bands shown as representative in **Figure 3.**

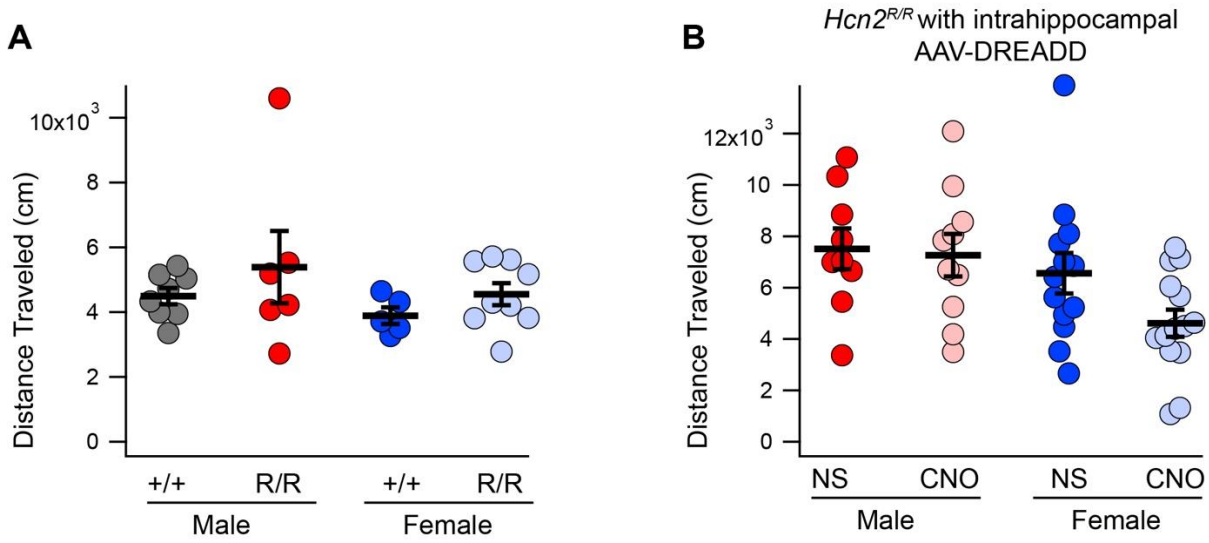

Fig. S8. Hcn2$^{R/R}$ mice show no change in open field testing
A) *Hcn2*$^{+/+}$ and *Hcn2*$^{R/R}$ mice male and female mice were subjected to 30 minutes of open field testing (Male +/+: 4492.9±251.1cm, Male R/R: 5387.6±1116.9cm, Female +/+ 3889.5±256.8cm, Female R/R: 4552.39±339.5cm, n=8,6,5,9, Effect of genotype $F(1,24)=2.33$, $p>0.05$, Effect of Sex $F(1,24)=1.39$, $p>0.05$, Interaction $F(1,24)=0.11$, $p>0.05$). **B)** Male and female *Hcn2*$^{R/R}$ mice were bilaterally injected with AAV-DREADD. They then received IP saline or CNO prior to 60 minutes of open field testing (Male AAV-DREADD/NS: 7513.08±792.30cm, Male AAV-DREADD/CNO: 7265.65±828.73cm, Female AAV-DREADD/NS: 6560.76±787.83cm, Female AAV-DREADD/CNO: 4615.24±527.57cm, n=9,10,13,14). See **table S7** for details.

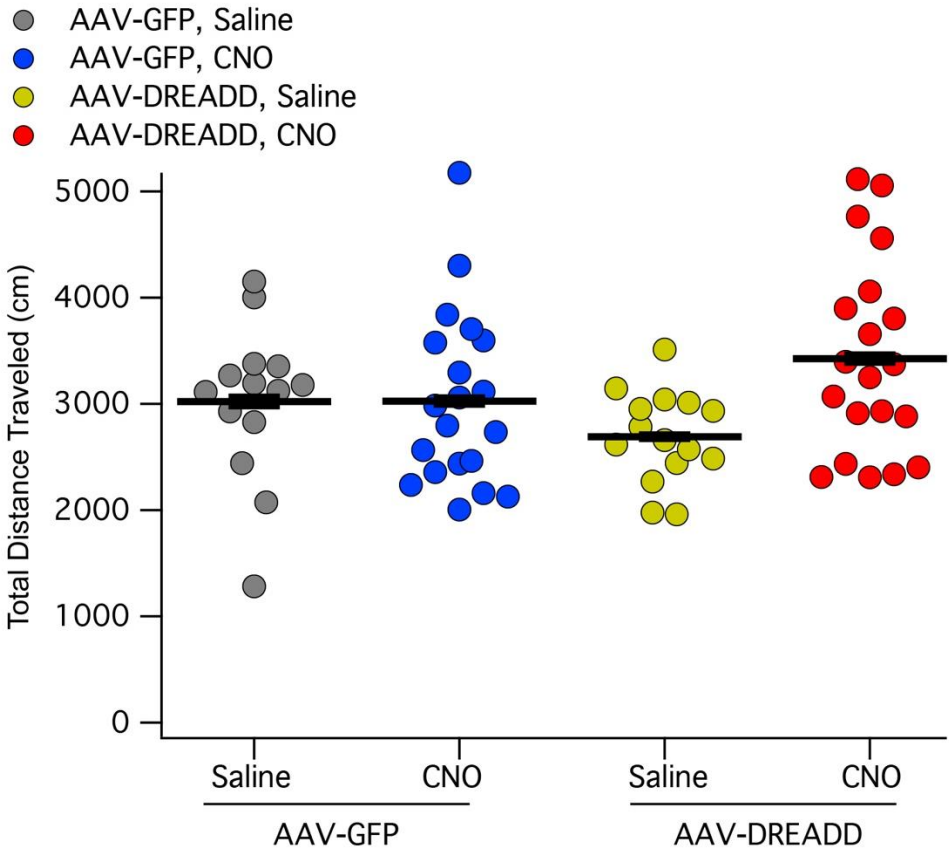

Fig. S9. Open field results in mice receiving chronically elevated cAMP
Male mice were bilaterally injected into the dorsal hippocampus with either AAV-GFP or AAV-DREADD and then 4 weeks later received 3 weeks of either saline (vehicle) or CNO in their drinking water. The animals then had their drinking water changed back to tap water prior to open field testing. AAV-GFP/NS: 3022±52cm, AAV-GFP/CNO: 3026±40.9s, AAV-DREADD/NS: 2690±28cm, AAV-DREADD/CNO: 3426±45cm. See **table S10** for statistics.

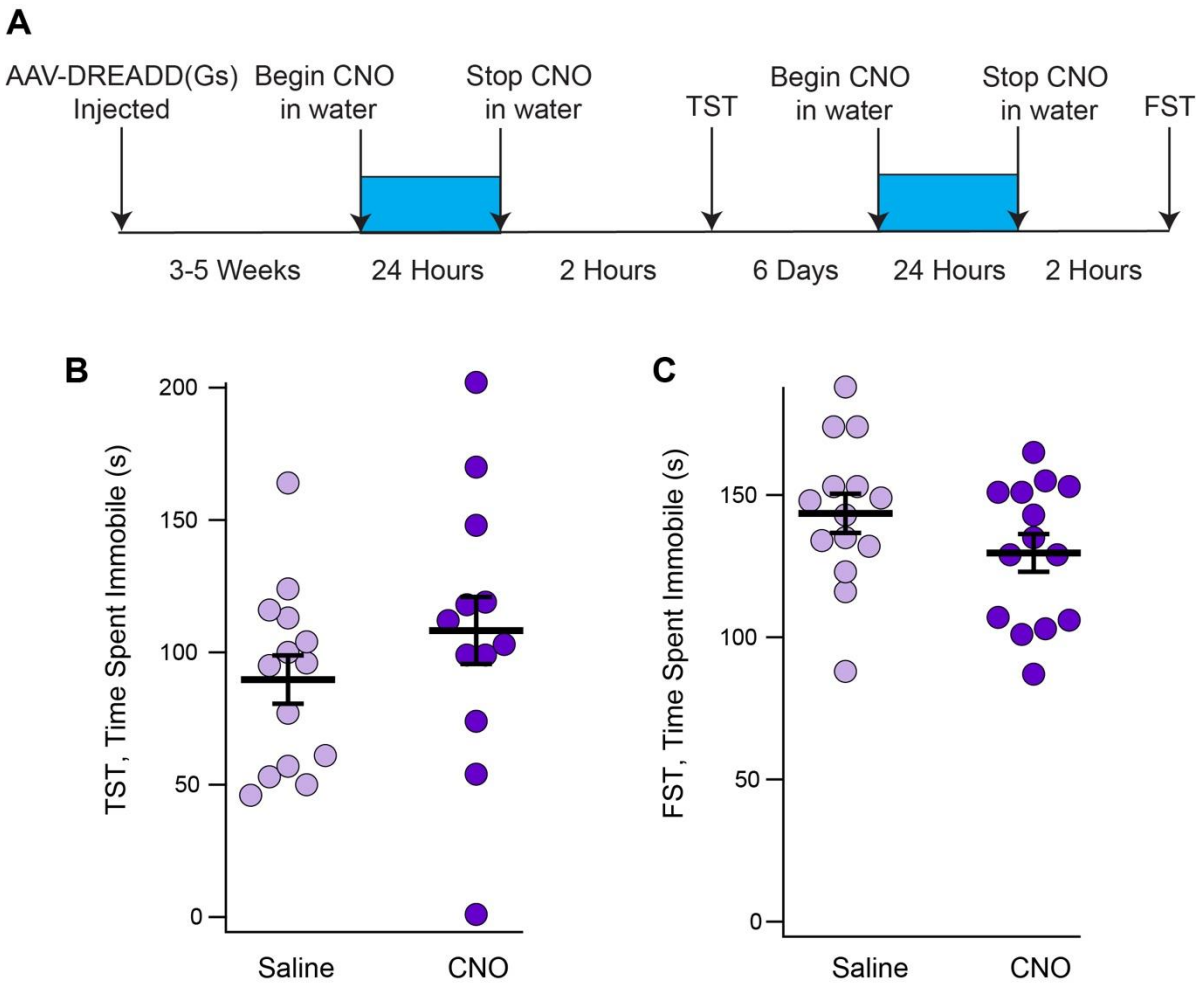

Fig. S10. 24 hours of elevated hippocampal cAMP does not produce changes in motivated behavior

Male mice were bilaterally injected in the hippocampus with AAV-DREADD. Several weeks later the drinking water was changed to include either saline or CNO for 24 hours (**A**). After 24 hours the water was changed to tap water for 2 hours prior to TST (**B**, AAV-DREADD/NS: 89.7±9.1s, AAV-DREADD/CNO: 118±12.6s, n=14, 11). **C)** One week later the drinking water was again changed to either saline or CNO for 24 hours and then changed back to tap water for 2 hours prior to FST (AAV-DREADD/NS: 143.5±6.8s, AAV-DREADD/CNO 129.6±6.6s, n=14,14). See **table S11** for statistics.

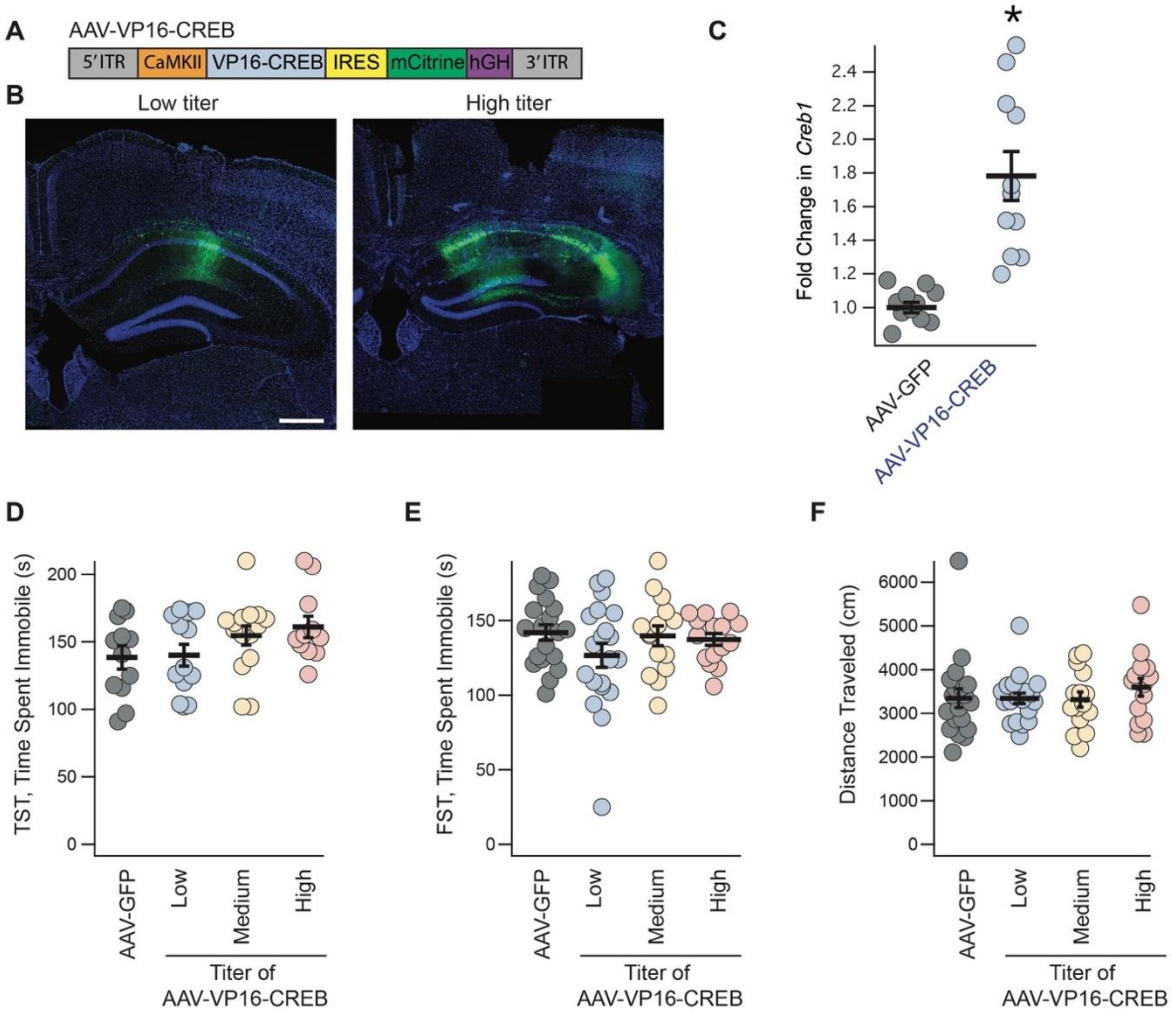

Fig. S11. Overexpression of constitutively active CREB in the dorsal CA1 does not increase motivated behavior

A) Schematic of the constitutively active CREB virus. **B)** Representative images of low titer (left) and high titer (right) AAV-VP16-CREB. Scale bar is 500 microns. **C)** qPCR was performed using primers to detect endogenous *Creb1* expression (but not AAV-VP16-CREB) and *Creb1* expression was examined (AAV-GFP: 1.00±0.03, AAV-VP16-CREB, low titer: 1.78±0.14 n=11,11). **D)** A range of titers of AAV-VP16-CREB were examined for their effect on TST (AAV-GFP 138.5±8.6s, AAV-VP16-CREB, low titer: 140.0±8.0s, medium titer: 154.6±7.0s, high titer: 161.0±7.9s, n=12,13,15,11). **E)** The influence of AAV-VP16-CREB expression on FST was also examined (AAV-GFP 141.9±5.2s, AAV-VP16-CREB, low titer: 126.6±8.0s, medium titer: 139.7±6.66, high titer: 137.3±3.9s, n=19,20,15,15) as well as on open field testing (**F**, AAV-GFP 3346±217cm, AAV-VP16-CREB, low titer: 3344±120cm, medium titer: 3315±172cm, high titer: 3599±202cm, n=19,20,15,15). *p<0.05

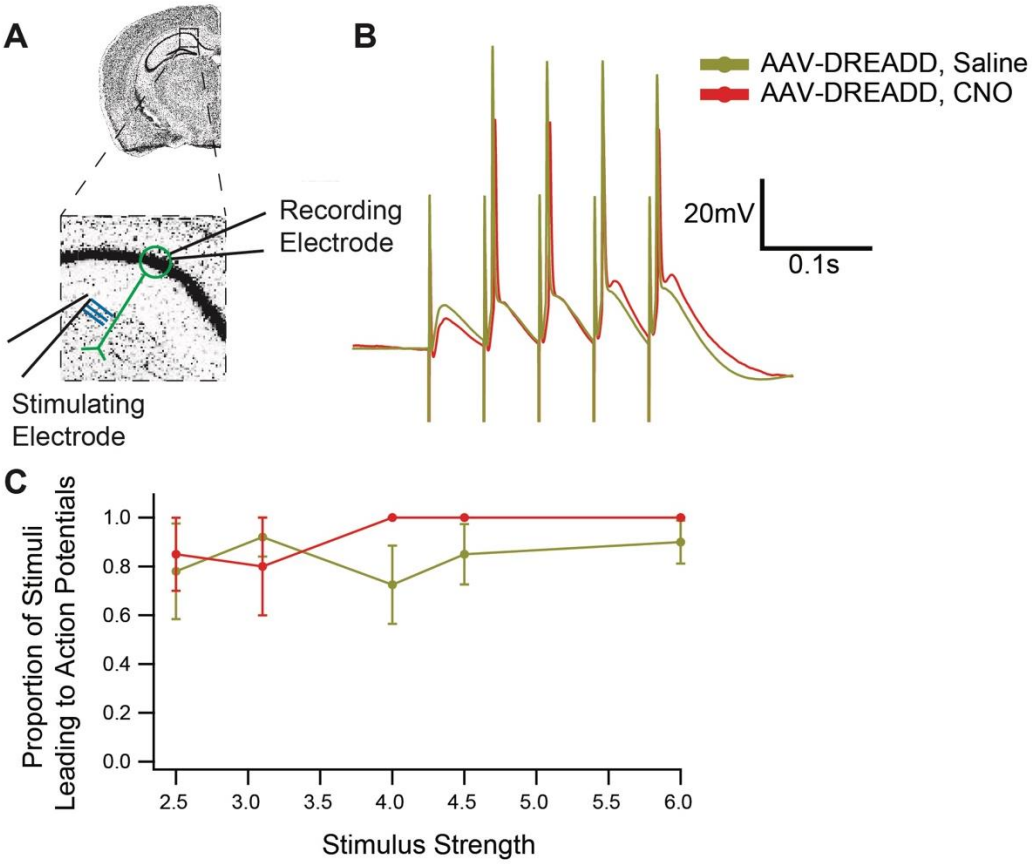

Fig. S12. Chronically elevated cAMP does not affect the SC pathway
A) Schematic for the recording configuration used to study the Schaffer collateral pathway. Whole cell recordings from GFP positive CA1 pyramidal neurons (in green) with a bipolar stimulating electrode placed on the SC pathway (in pink). **B)** Sample traces obtained from AAV-DREADD/Saline (yellow) and AAV-DREADD/CNO (red). **C)** Quantification of action potentials produced in response to a range of SC pathway stimulation intensities. See **tables S15-S16** for statistics.

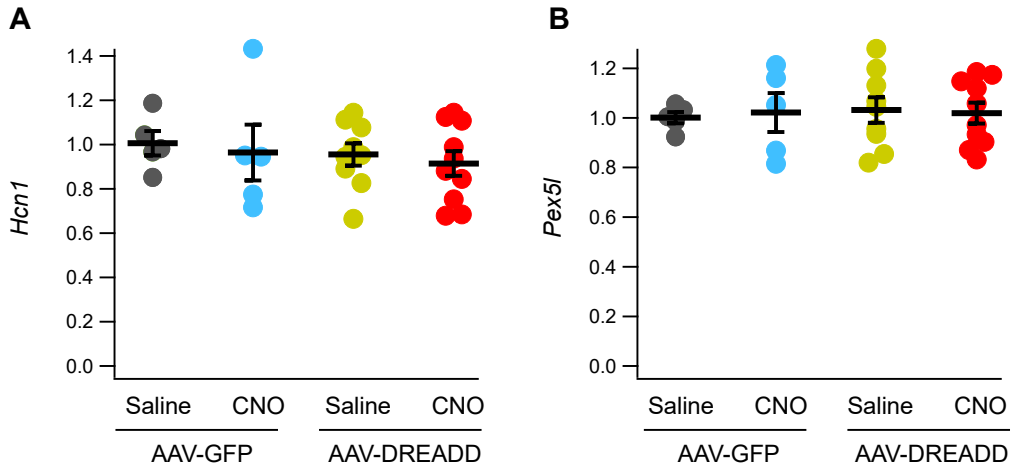

Fig. S13. Chronically elevated cAMP does not affect the expression of *Hcn1* or *Pex5l,* the gene encoding TRIP8b

Mice were bilaterally injected with AAV-GFP or AAV-DREADD and then received saline (NS) or CNO in their drinking water for 3 weeks. The mice were then sacrificed for qPCR and qPCR performed to examine *Hcn1* (AAV-GFP/NS: 1.00±0.05, AAV-GFP/CNO: 0.96±0.12, AAV-DREADD/NS: 0.95±0.05, AAV-DREADD/CNO: 0.91±0.05) and *Pex5l* (AAV-GFP/NS: 1.00±0.02, AAV-GFP/CNO: 1.02±0.07, AAV-DREADD/NS: 1.03±0.05, AAV-DREADD/CNO: 1.01±0.042). See **table S17** for statistics.

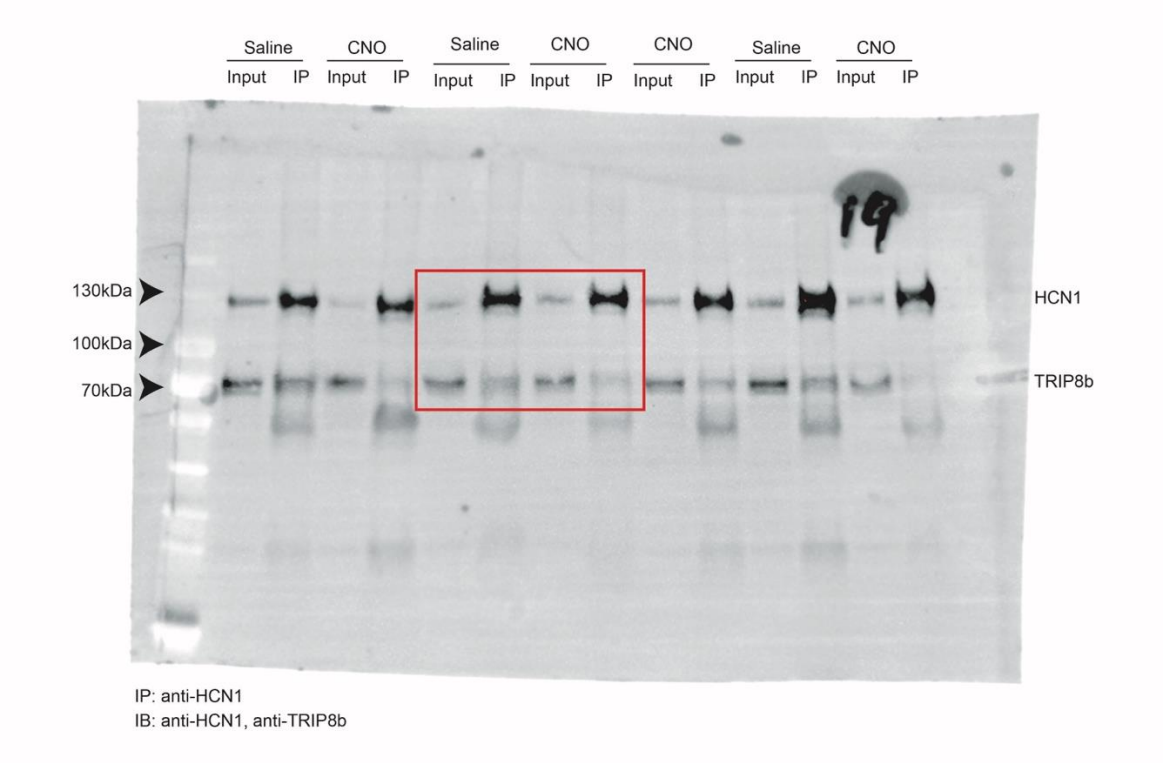

Fig. S14. Uncropped blot corresponding to Figure 6C
CO-IP reaction was performed using anti-HCN1 antibodies and hippocampal lysate from animals injected with hippocampal AAV-DREADD and then receiving 3 weeks of either saline or CNO in their drinking water. 'Input' refers to the original hippocampal lysate set aside prior to addition of anti-HCN1 antibody and 'IP' refers to the protein eluted from beads. Note that the red box denotes the cropped image presented in **Figure 6C**.

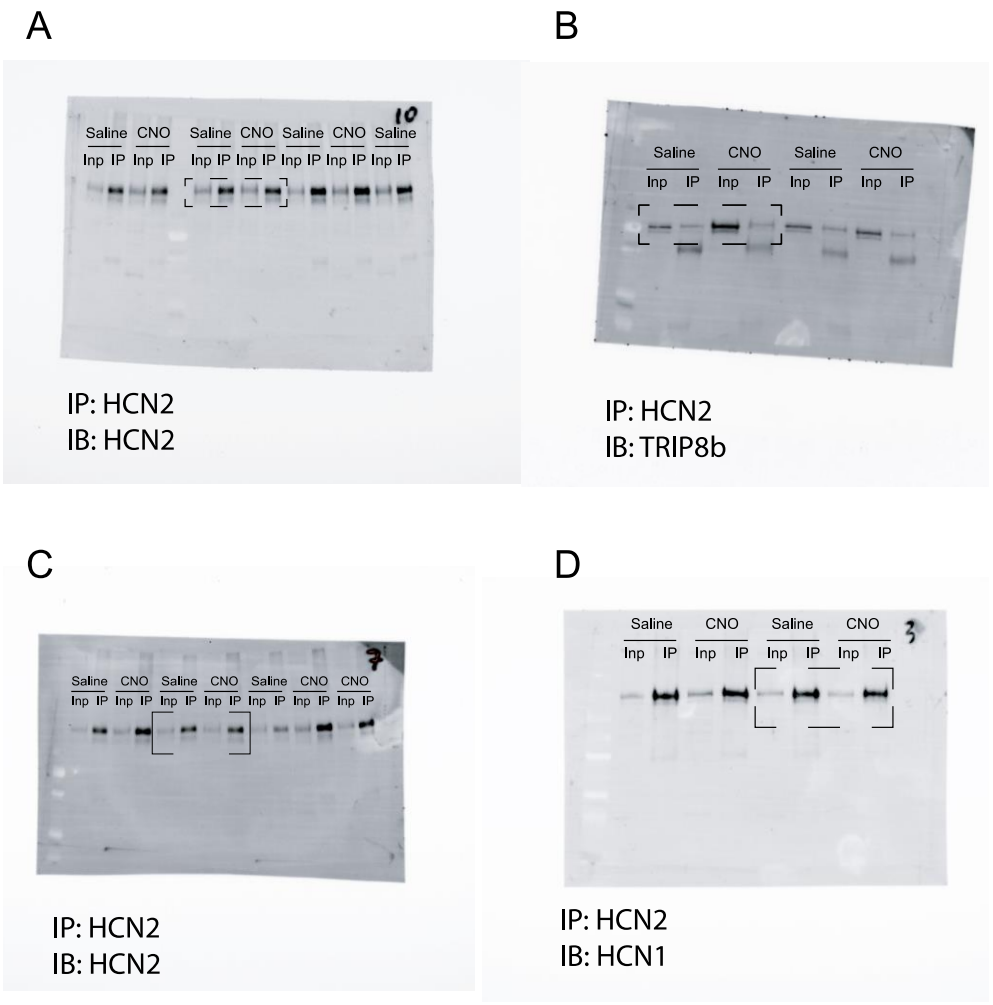

Fig. S15. Uncropped blot corresponding to Figure 6E and 6G
Co-immunoprecipitation was carried out with anti-HCN2 antibodies using hippocampal lysate of mice bilaterally injected with hippocampal AAV-DREADD and then receive 3 weeks of either saline or CNO in their drinking water. As in **figure S14**, 'Input' refers to the original hippocampal lysate set aside prior to addition of antibody and 'IP' refers to the protein eluted from beads. **A/B)** Samples cropped for images shown in **Figure 6E** with dashed lines highlighting the same samples on two different membranes. **C/D)** Samples cropped for images shown in **Figure 6G**, with dashed lines highlighting the same samples on each membrane.

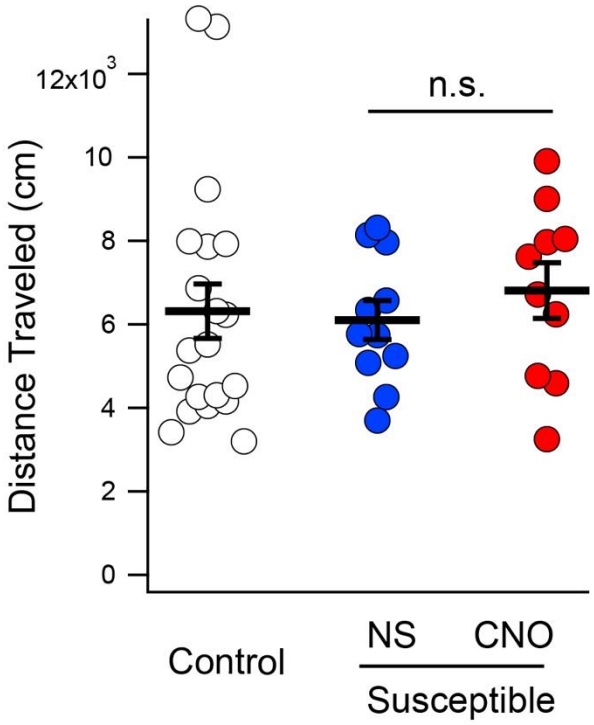

Fig. S16. Susceptible mice injected with AAV-DREADD do not show a change in locomotion after CNO/NS

Mice were injected with AAV-DREADD into the bilateral hippocampi and then subjected to CSD (see **Figure 7** in the main text). Susceptible animals received either CNO or saline in their drinking water and were subjected to open field testing (Distance traveled for NS: 6101.78±467.15cm, CNO: 6809.93±667.40cm, n=11, 10, 2 Tail T test t(19)=-0.882, p=0.388)

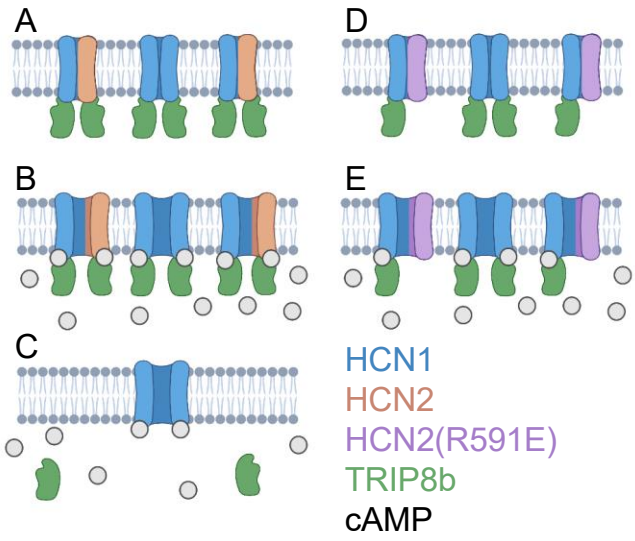

Fig. S17. Schematic for the role of cAMP in influencing TRIP8b-mediated HCN channel trafficking.
A) HCN1 (blue) and HCN2 (orange) heterotetratmers are bound in a 1:1 ratio by TRIP8b (green). **B)** In the presence of cAMP (gray), both HCN1 and HCN2 are bound by cAMP and there is an increase in I_h (occurring because of activation of HCN2 given that HCN1's V_{50} shifts only minimally in response to cAMP binding). **C)** Chronically elevated cAMP leads to a disruption of the TRIP8b-HCN interaction and a loss of surface HCN channels. Although these channels are still bound by cAMP, there are fewer of them so that there is a net loss of I_h. **D)** HCN2(R591E) heterotetramerizes with HCN1 to form functional channels (purple/blue heterotetramers). HCN2(R591E) is not bound by TRIP8b, but HCN2(R591E) binds to HCN1 subunits that are bound by TRIP8b and hence HCN2(R591E) is transported into the dendrites. **E)** In the presence of cAMP, there is no effect on I_h because cAMP does not bind HCN2(R591E) and HCN1 does not meaningfully shift its V_{50} in response to cAMP binding.

SUPPLEMENTAL TABLES

Table S1. Patient demographics for Figure 1
Table of subjects from the NIH Neurobiobank and Southwest Tissue bank used for qPCR.

Subject #	Sex	Age	Race	Psychiatric Category	Cause of Death (Where Available)
1	Male	67	Hispanic	Control	
2	Male	69	White	Control	
3	Male	50	Hispanic	Control	
4	Male	84	White	Control	
5	Male	68	White	Control	
6	Male	73	Hispanic	Control	
7	Male	39	Hispanic	Control	
8	Male	20	Black	Control	
9	Male	49	Black	Control	Cardiac Tamponade
10	Male	28	White	Control	
11	Male	52	White	MDD	
12	Male	75	White	MDD	
13	Male	57	Other	MDD	
14	Male	53	White	MDD	
15	Male	47	Hispanic	MDD	
16	Male	19	White	MDD	Suicide
17	Male	23	White	MDD	
18	Male	19	White	MDD	Suicide
19	Male	46	Black	MDD	Suicide
20	Female	78	White	Control	
21	Female	63	White	Control	
22	Female	54	White	Control	
23	Female	52	Hispanic	Control	
24	Female	25	Black	Control	Pulmonary Embolism
25	Female	77	White	Control	Myocardial Infarction
26	Female	39	White	Control	Pulmonary Embolism
27	Female	18	White	Control	Cardiac Arrythmia
28	Female	75	White	MDD	
29	Female	52	Other	MDD	
30	Female	53	White	MDD	
31	Female	18	White	MDD	Suicide
32	Female	26	Asian	MDD	Hemorrhage
33	Female	19	Black	MDD	

Tables S2-S18: provided as separate Excel file

Datafile S1: Raw data (provided as separate Excel file)