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SUPPLEMENTARY INFORMATION
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Oxytocin-receptor-expressing neurons in the parabrachial nucleus regulate fluid intake

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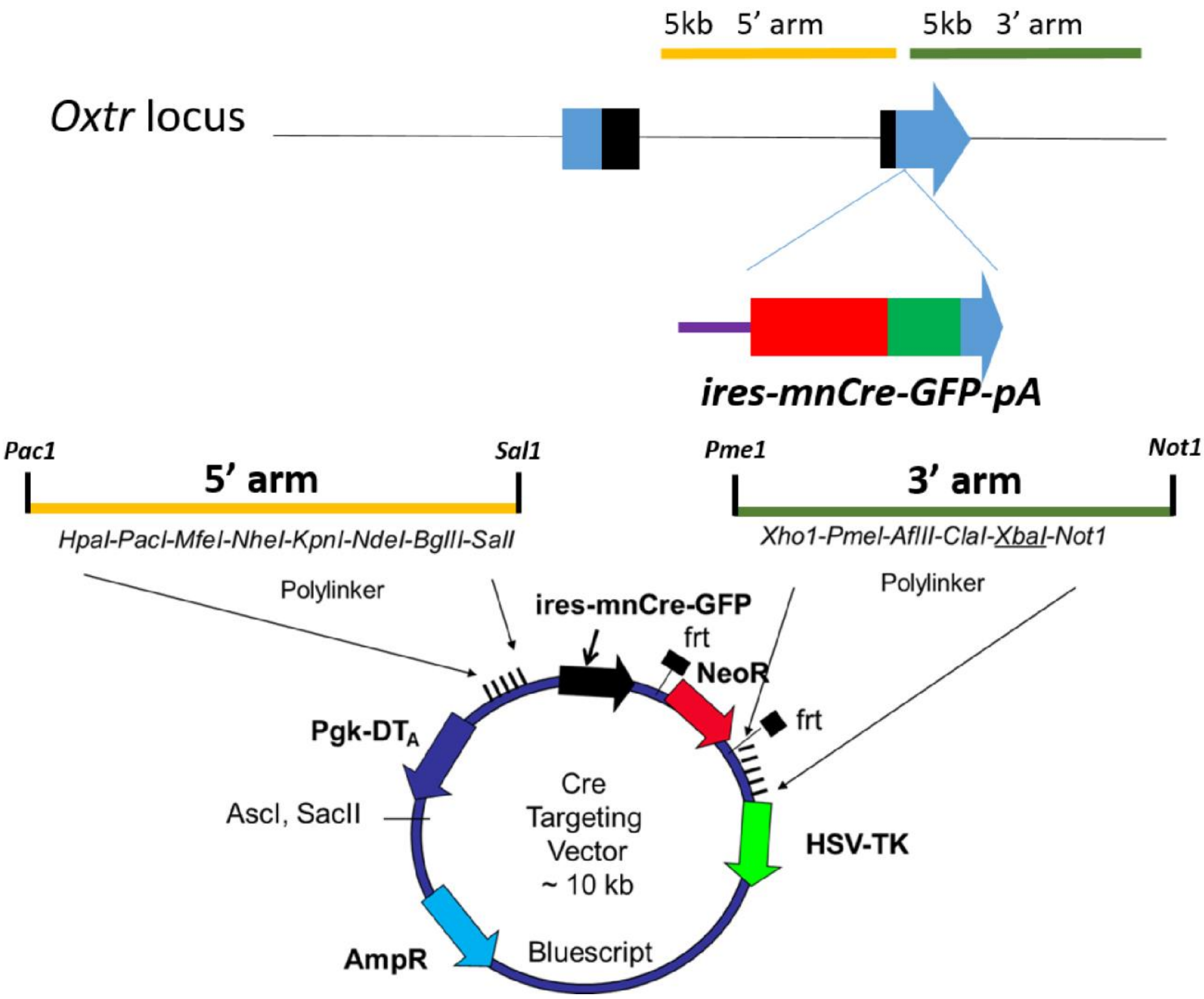
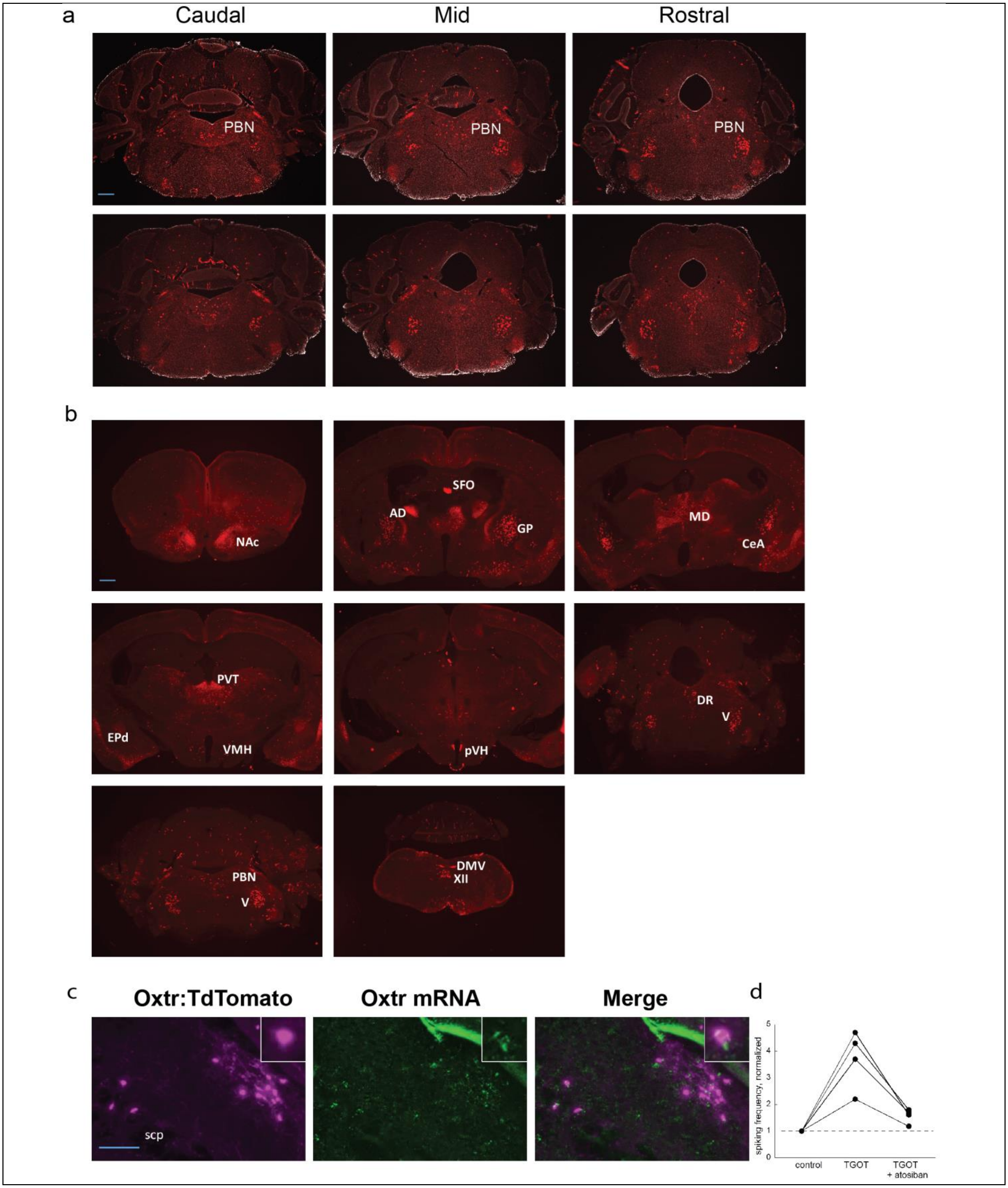


Supplementary Figure 1

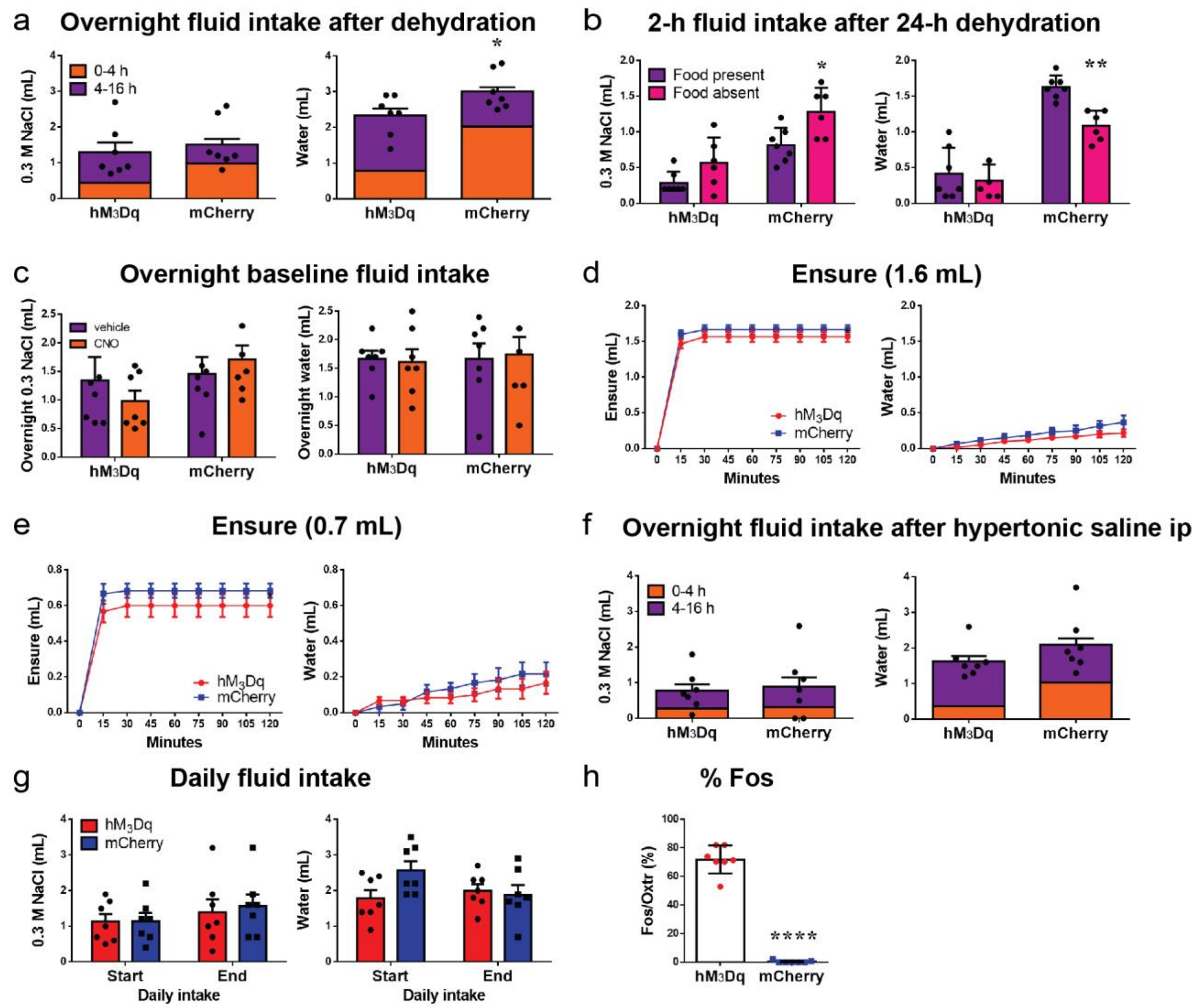
Generation of *Oxtr*$^{Cre:GFP}$ mice.

Diagram showing: top, insertion of ires-mnCre:GFP construct just 3' of the termination codon of the *Oxtr* gene; bottom, the targeting vector. Some key restriction enzymes sites used for cloning are shown. See Methods for details.



a
Caudal
Mid
Rostral
PBN
PBN
PBN
b
NAc
SFO
AD
GP
MD
CeA
PVT
EPd
VMH
pVH
DR
V
PBN
V
DMV
XII
c
Oxtr:TdTomato
Oxtr mRNA
Merge
scp
d
spiking frequency, normalized
0
1
2
3
4
5
control
TGOT
TGOT
+ atosiban

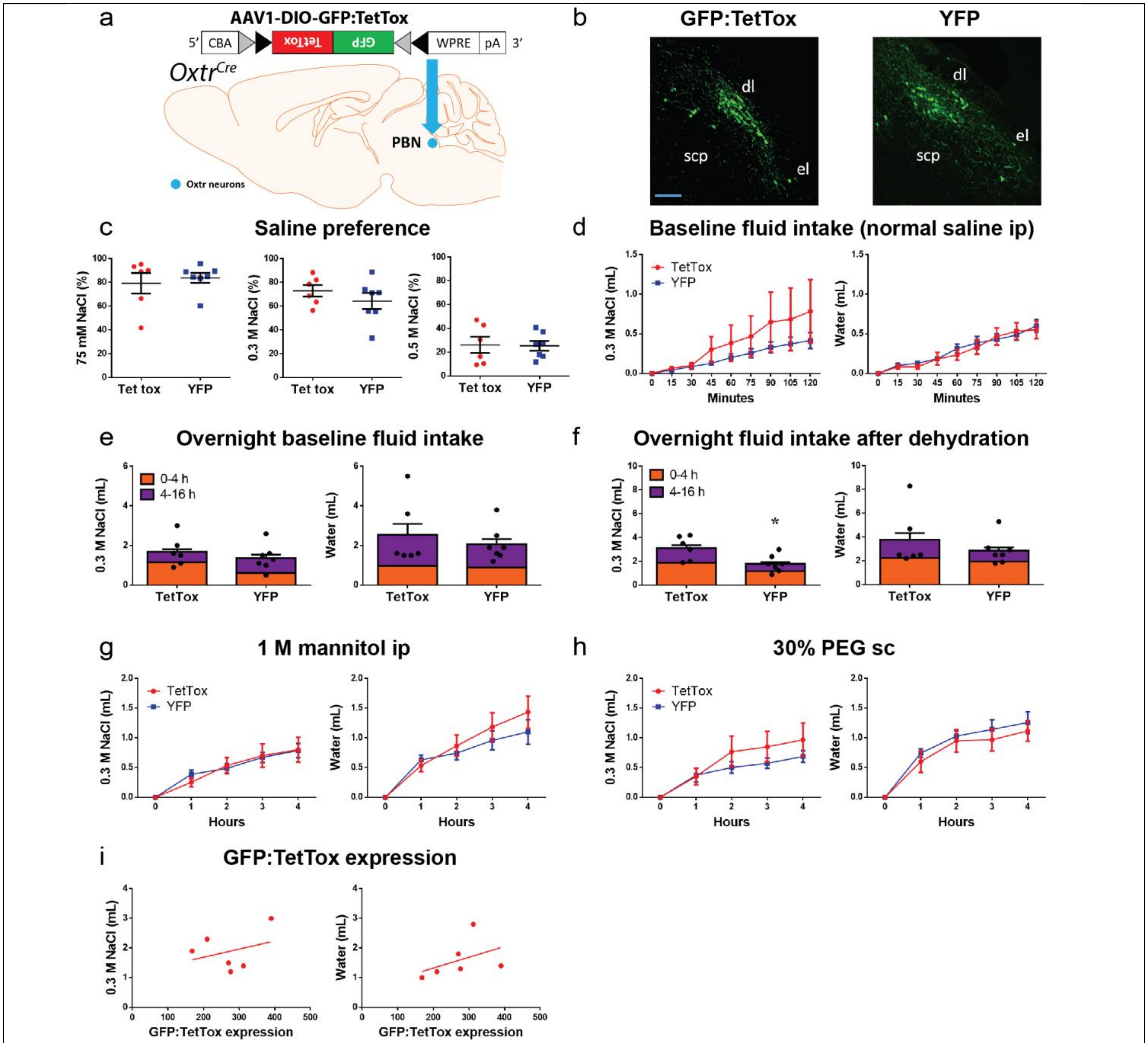
Supplementary Figure 2
Oxytocin receptor expression in the parabrachial nucleus.
(**a**) Coronal sections 90 µm apart from *Oxtr*$^{Cre/+}$*::*Ai14 mouse demonstrating oxytocin receptor (Oxtr) expression in the parabrachial nucleus (PBN) from bregma -5.1 to -5.5; scale bar, 500 µm. (**b**) Selection of brain images demonstrating robust Oxtr expression; AD, anterodorsal thalamic nucleus; CeA, central nucleus of amygdala; EPd, dorsal endopiriform nucleus; DMV, dorsal motor nucleus of the vagus; DR, dorsal raphé nucleus; GP, globus pallidus; XII, hypoglossal nucleus; MD, mediodorsal thalamic nucleus; NAc, nucleus accumbens; PBN, parabrachial nucleus; PVT, paraventricular thalamic nucleus; pVH, periventricular nucleus of the hypothalamus; SFO, subfornical organ; V, trigeminal motor nucleus; VMH, ventromedial hypothalamic nucleus; scale bar, 500 µm (n = 3). (**c**) Representative RNAscope® image of PBN demonstrating co-expression of *Oxtr* mRNA in 80 ± 3% Oxtr:TdTomato-expressing neurons (n = 3). Scale bar, 100 µm; scp, superior cerebellar peduncle. (**d**) Oxtr agonist, TGOT, increased spiking frequency in Oxtr^{PBN} neurons by 3.7 ± 0.55-fold, which was inhibited by Oxtr antagonist, atosiban to 1.6 ± 0.14 fold (n = 4/4 Oxtr^{PBN} neurons). Data were normalized to spiking frequency prior to TGOT application.



Supplementary Figure 3

Chemogenetic activation of Oxtr^{PBN} neurons decreases fluid intake acutely.

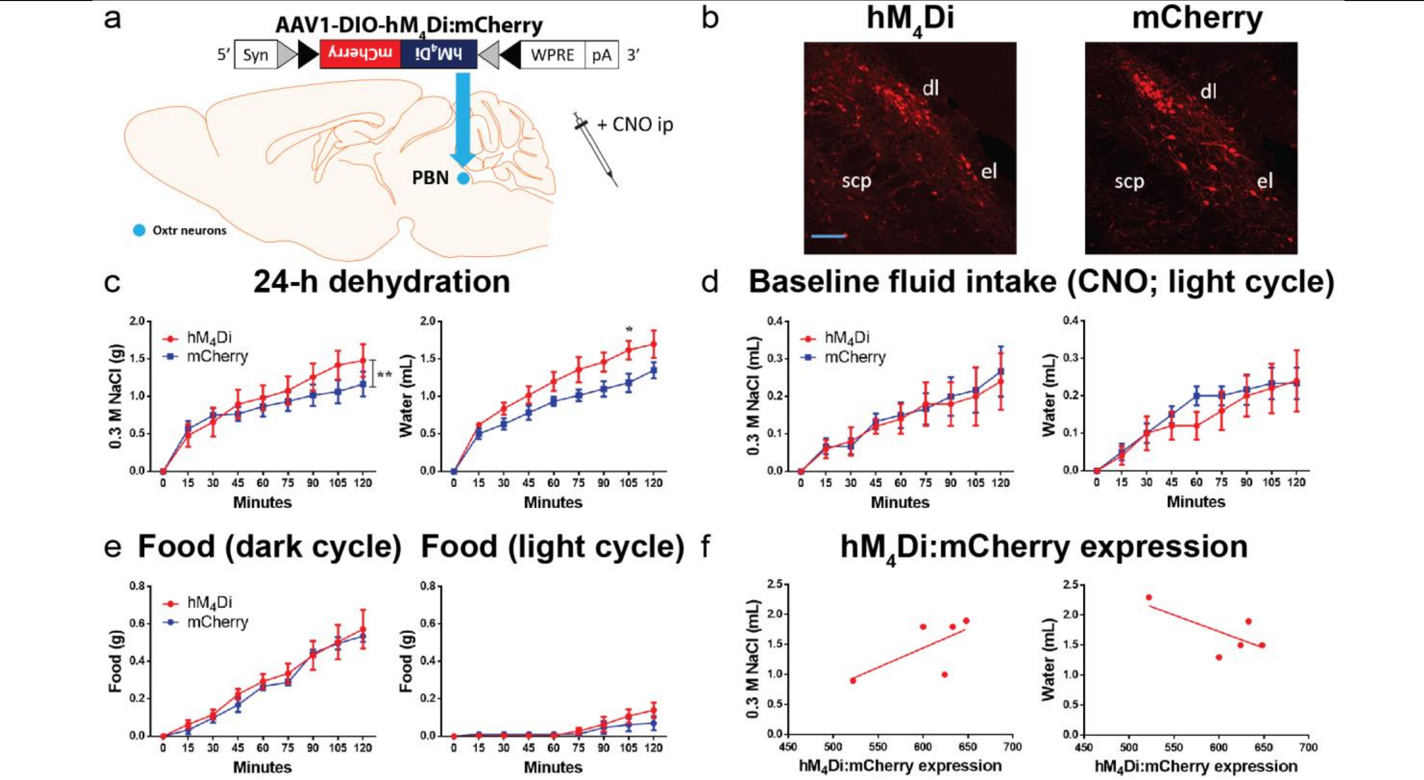
(**a**) Overnight NaCl was not significantly different, but overnight water intake remained slightly decreased following CNO injection in hM$_3$Dq-injected vs control mCherry-injected mice after 24-h dehydration (n = 7/group; unpaired 2-tailed Student's t-test; NaCl: $t(12) = 0.5656$; $p = 0.5821$; water: $t(12) = 2.311$; $p = 0.0394$). (**b**) Control mCherry-injected mice consumed more NaCl and less water when food was absent; but there was no significant difference in fluid intake after CNO injection in hM$_3$Dq-injected mice (n = 7/group in food present; 6/group in food absent; 2-way ANOVA; NaCl: interaction $F(1,22) = 0.7401$; $p = 0.3989$; main effect of viral genotype $F(1,22) = 32.44$; $p < 0.0001$; main effect of food availability $F(1,22) = 11.77$; $p = 0.0024$; water: interaction $F(1,22) = 4.946$; $p = 0.0367$). (**c**) Overnight baseline fluid intake was not significantly different following vehicle or CNO injection (n = 7/group; 2-way RM ANOVA; NaCl: interaction $F(1,12) = 1.806$; $p = 0.2038$; water: interaction $F(1,12) = 0.1977$; $p = 0.6645$). **d,e,** Acute Oxtr^{PBN} activation demonstrated no significant difference in Ensure or water intake when Ensure was limited to (**d**) ~1.6 mL (n = 6/group; 2-way RM ANOVA; Ensure: interaction $F(8,80) = 1.168$; $p = 0.3285$; water: interaction $F(8,80) = 1.027$; $p = 0.4228$) or (**e**) ~0.7 mL (n = 6/group; 2-way RM ANOVA; Ensure: interaction $F(8,80) = 1.182$; $p = 0.3202$; water: interaction $F(8,80) = 1.313$; $p = 0.2489$). (**f**) Overnight fluid intake was not significantly different after hypertonic saline ip injection in hM$_3$Dq- or vs mCherry-injected mice (n = 7/group; unpaired 2-tailed Student's t-test; NaCl: $t(12) = 0.2868$; $p = 0.7791$; water: $t(12) = 1.354$; $p = 0.2008$). (**g**) Daily intake of NaCl and water was not significantly different between the start and end of experimentation (n = 7/group; 2-way RM ANOVA; NaCl: interaction $F(1,12) = 0.1273$; $p = 0.7275$; water: interaction: $F(1,12) = 4.243$; $p = 0.0618$). (**h**) Percentage Fos in Oxtr^{PBN} neurons in hM$_3$Dq-injected vs mCherry-injected *Oxtr$^{Cre/+}$* mice (n = 7/group; unpaired 2-tailed Student's t-test; $t(12) = 19.28$; $p < 0.0001$). Data expressed as mean ± s.e.m. ****$p < 0.0001$; **$p < 0.01$; *$p < 0.05$. See Supplementary Information for statistical analyses.



Supplementary Figure 4

Chronic Oxtr^PBN neuron inactivation does not affect fluid intake at baseline or following various homeostatic challenges.

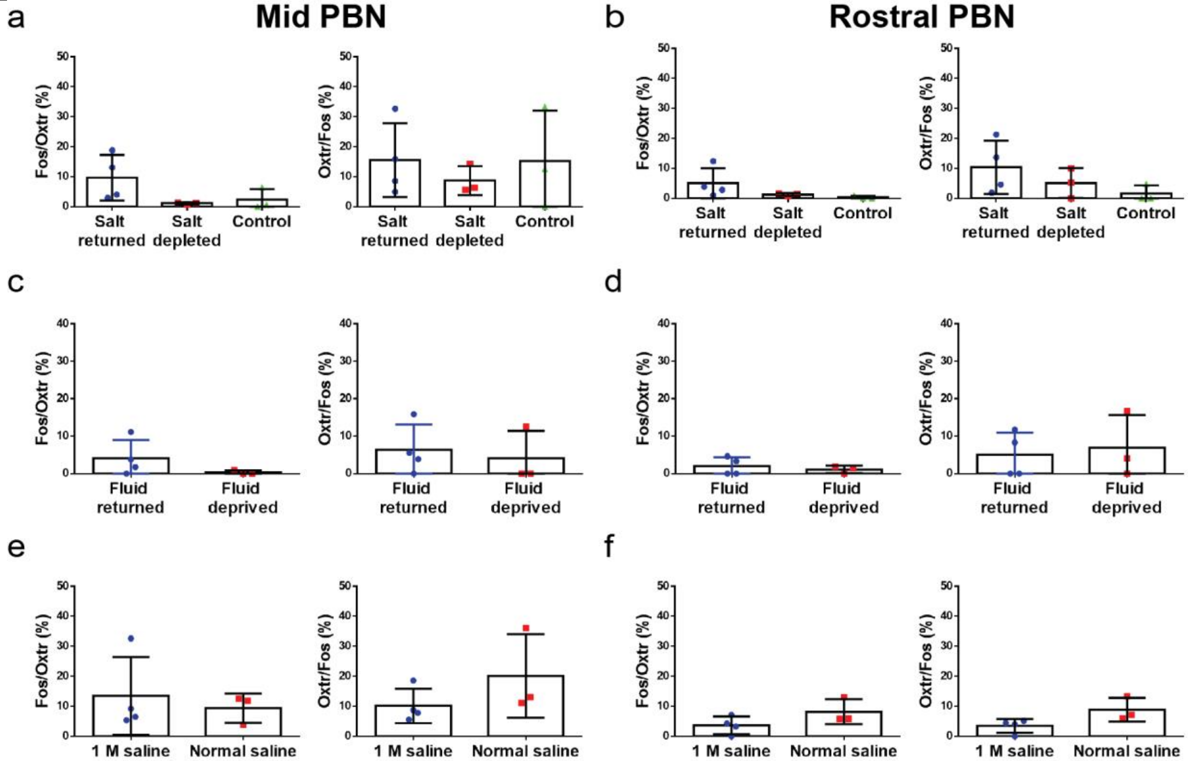
(**a**) Injection of AAV-DIO-GFP:TetTox in Oxtr^PBN neurons. (**b**) Representative images of GFP expression in Oxtr^PBN neurons in GFP:TetTox- vs control YFP-injected mice; scp, superior cerebellar peduncle; dl, dorsolateral; el, external lateral; scale bar 100 µm. (**c**) Chronic Oxtr^PBN inactivation does not significantly alter baseline saline preference at different concentrations (0.075, 0.3, 0.5 M) (n = 6 TetTox, 7 YFP; unpaired 2-tailed Student's t-test; 0.075 M NaCl: $t(11) = 0.4967$; $p = 0.6292$; 0.3 M NaCl: $t(11) = 1.005$; $p = 0.3364$; 0.5 M NaCl: $t(11) = 0.09961$; $p = 0.9224$), and does not significantly change fluid intake following normal saline ip, either (**d**) acutely (n = 6 TetTox, 7 YFP; 2-way RM ANOVA; NaCl: interaction $F(8,88) = 0.7729$; $p = 0.6275$; water: interaction $F(8,88) = 0.4721$; $p = 0.8728$) or (**e**) overnight (n = 6 TetTox, 7 YFP; unpaired 2-tailed Student's t-test; NaCl: $t(11) = 0.8014$; $p = 0.4398$; water: $t(11) = 0.6858$; $p = 0.5070$). (**f**) Overnight NaCl intake remained significantly increased in TetTox-injected mice (n = 6 TetTox, 7 YFP; unpaired 2-tailed Student's t-test; NaCl: $t(11) = 2.760$; $p = 0.0186$; water: $t(11) = 0.8881$; $p = 0.3935$). Chronic Oxtr^PBN inactivation does not significantly change fluid intake following (**g**) 1 M mannitol ip (*n* = 6 TetTox; 7 YFP; 2-way RM ANOVA; NaCl: interaction $F(4,44) = 0.4638$; $p = 0.7619$; water: interaction $F(4,44) = 1.524$; $p = 0.2118$) or (**h**) 30% PEG sc (*n* = 6 TetTox; 7 YFP; 2-way RM ANOVA; NaCl: interaction $F(4,44) = 1.494$; $p = 0.2203$; water: interaction $F(4,44) = 0.3461$; $p = 0.8453$). (**i**) There was no significant correlation between the level of GFP:TetTox expression and the amount of fluid intake after 24-h dehydration (*n* = 6; Pearson product-moment correlation; NaCl: $r = 0.3167$; $p = 0.5408$; water: $r = 0.4335$; $p = 0.3905$). Data are expressed as mean ± s.e.m. $^*p < 0.05$. See Supplementary Information for statistical analyses.



Supplementary Figure 5

Acute Oxtr^{PBN} neuron inhibition increases fluid intake after dehydration, but does not affect baseline food or fluid intake.

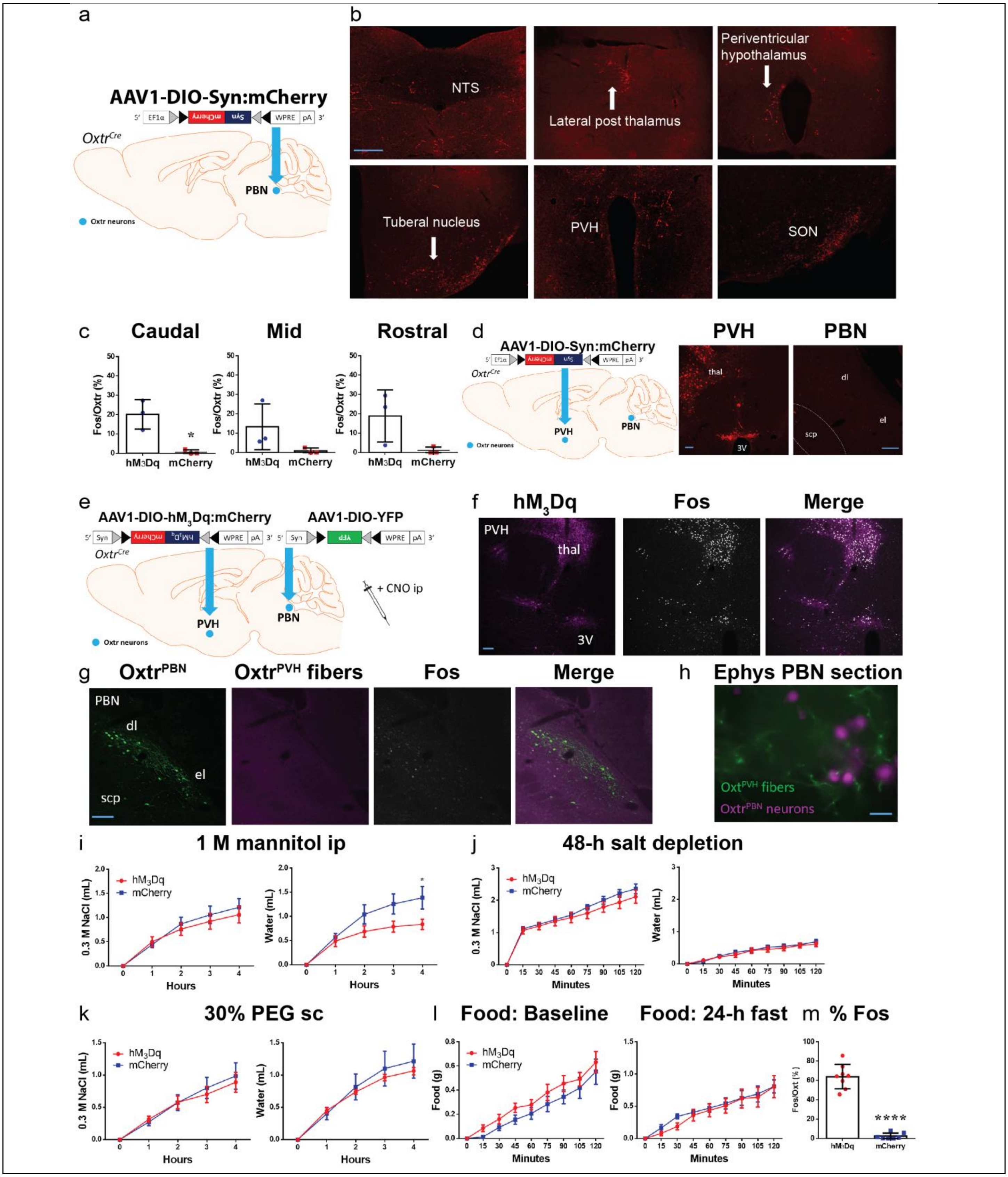
(**a**) Injection of AAV-DIO-hM$_4$Di:mCherry in Oxtr^{PBN} neurons. (**b**) Representative images of hM$_4$Di and mCherry expression in Oxtr^{PBN} neurons; scp, superior cerebellar peduncle; dl, dorsolateral; el, external lateral; scale bar 100 µm. (**c**) Acute Oxtr^{PBN} inhibition increases NaCl and water intake following 24-h dehydration (n = 5 hM$_4$Di, 6 mCherry; 2-way RM ANOVA; NaCl: interaction $F(8,72) = 2.962$; p = 0.0064; water: interaction $F(8,72) = 2.289$; p = 0.0304), but (**d**) does not significantly alter fluid intake at baseline during the light cycle (n = 5 hM$_4$Di, 6 mCherry; 2-way RM ANOVA; NaCl: interaction $F(8,72) = 0.1264$; p = 0.9979; water: interaction $F(8,72) = 0.6017$; p = 0.7734). (**e**) Oxtr^{PBN} inhibition does not significantly alter food intake during the dark or light cycle (n = 5 hM$_4$Di, 6 mCherry; 2-way RM ANOVA; baseline food: interaction $F(8,72) = 0.2429$; p = 0.9811; post 24-h fast: interaction $F(8,72) = 1.040$; p = 0.4510). (**f**) There was no significant correlation between the level of hM$_4$Di:mCherry expression and the amount of fluid intake after 24-h dehydration (n = 5; Pearson product-moment correlation; NaCl: r = 0.6649; p = 0.2208; water: r = -0.6857; p = 0.2013). Data are expressed as mean ± s.e.m. **$p < 0.01$; *$p < 0.05$. See Supplementary Information for statistical analyses.



Supplementary Figure 6

Oxtr^{PBN} neurons in mid and rostral PBN show no significant difference in Fos expression.

a,c,e, Quantification of mid Oxtr^{PBN} co-expression of Fos and Oxtr in (**a**) salt depletion (*n* = 4 salt returned, 4 salt depleted, 3 control; 1-way ANOVA; Fos/Oxtr: interaction $F(2,7) = 2.727$; $p = 0.1331$; Oxtr/Fos: interaction $F(2,7) = 0.3097$; $p = 0.7432$), (**c**) fluid deprivation (*n* = 4 fluid returned, 3 fluid deprived; unpaired 2-tailed Student's t-test; Fos/Oxtr: $t(5) = 1.335$; $p = 0.2394$; Oxtr/Fos: $t(5) = 0.4049$; $p = 0.7023$) and (**e**) and hypertonic saline experiments (*n* = 4 for 1 M saline, 3 normal saline; unpaired 2-tailed Student's t-test; Fos/Oxtr: $t(5) = 0.5084$; $p = 0.6328$; Oxtr/Fos: $t(5) = 1.319$; $p = 0.2444$). **b,d,f,** Quantification of rostral Oxtr^{PBN} co-expression of Fos and Oxtr in (**b**) salt depletion (*n* = 4 salt returned, 4 salt depleted, 3 control; 1-way ANOVA; Fos/Oxtr: interaction $F(2,7) = 2.008$; $p = 0.2046$; Oxtr/Fos: interaction $F(2,7) = 1.582$; $p = 0.2711$), (**d**) fluid deprivation (*n* = 4 fluid returned, 3 fluid deprived; unpaired 2-tailed Student's t-test; Fos/Oxtr: $t(5) = 0.5977$; $p = 0.5761$; Oxtr/Fos: $t(5) = 0.3396$; $p = 0.7479$) and (**f**) and hypertonic saline (*n* = 4 for 1 M saline, 3 normal saline; unpaired 2-tailed Student's t-test; Fos/Oxtr: $t(5) = 1.716$; $p = 0.1469$; Oxtr/Fos: $t(5) = 2.300$; $p = 0.0698$). Data are expressed as mean ± s.e.m. See Supplementary Information for statistical analyses.

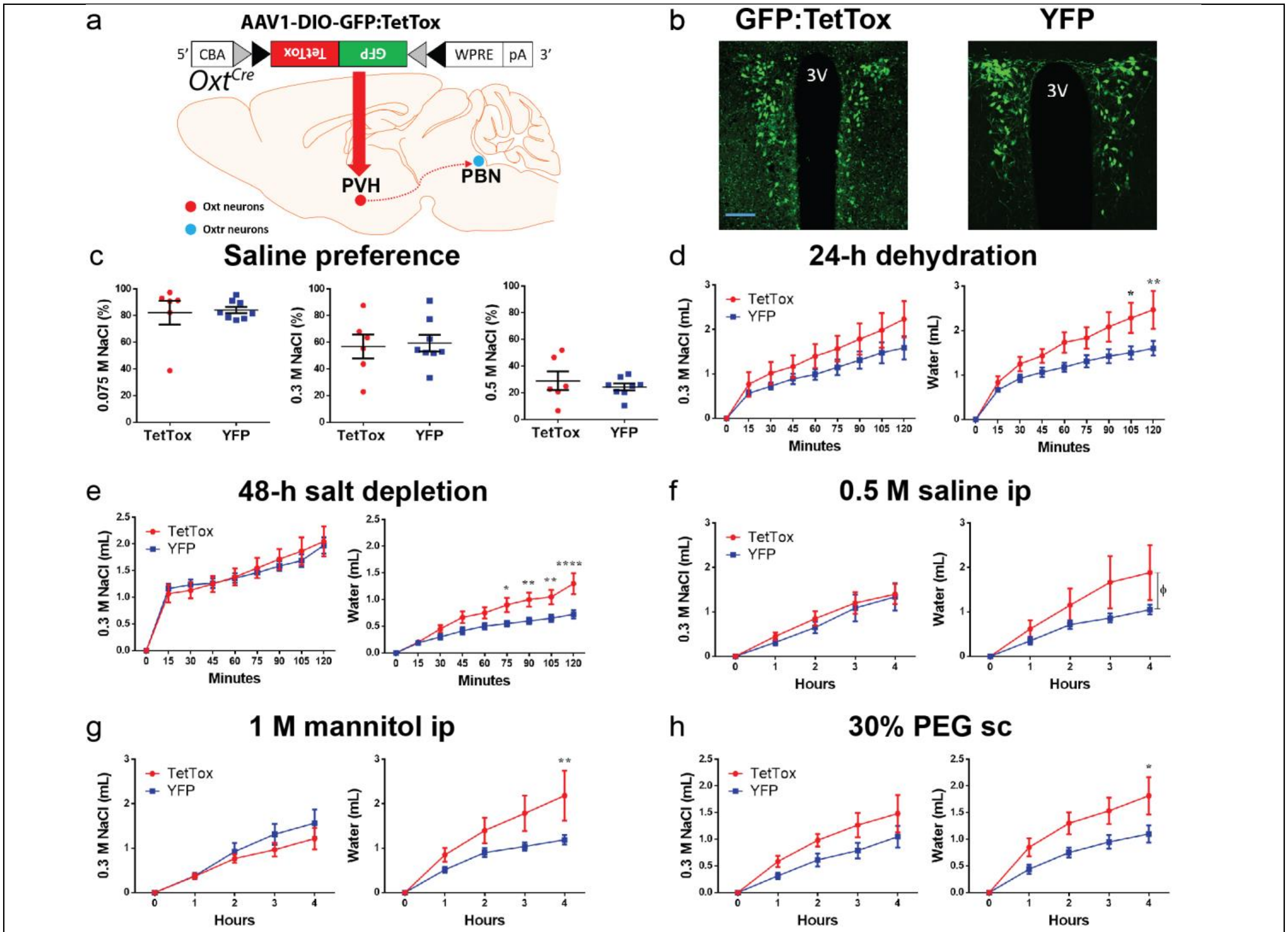


a
AAV1-DIO-Syn:mCherry
Oxtr^Cre
PBN
Oxtr neurons
b
NTS
Lateral post thalamus
Periventricular hypothalamus
Tuberal nucleus
PVH
SON
c
Caudal
Mid
Rostral
Fos/Oxtr (%)
hM3Dq
mCherry
d
AAV1-DIO-Syn:mCherry
PVH
PBN
thal
3V
dl
el
scp
e
AAV1-DIO-hM3Dq:mCherry
AAV1-DIO-YFP
+ CNO ip
f
hM3Dq
Fos
Merge
g
Oxtr^PBN
Oxtr^PVH fibers
Fos
Merge
h
Ephys PBN section
Oxt^PVH fibers
Oxtr^PBN neurons
i
1 M mannitol ip
0.3 M NaCl (mL)
Water (mL)
Hours
j
48-h salt depletion
Minutes
k
30% PEG sc
l
Food: Baseline
Food: 24-h fast
Food (g)
m
% Fos
Fos/Oxt (%)

Supplementary Figure 7

Downstream and upstream projections of $Oxtr^{PBN}$ neurons.

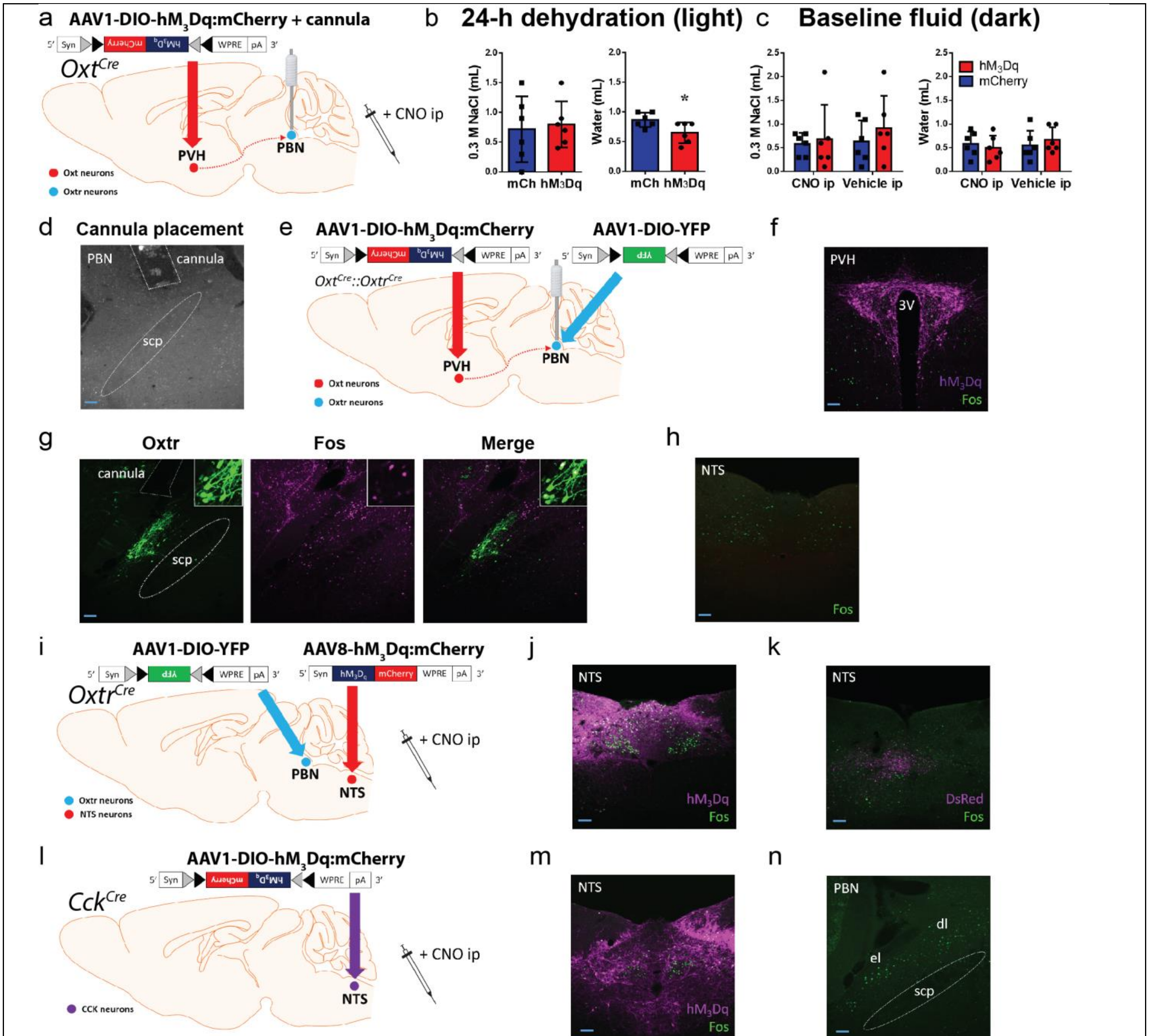
(**a**) Injection of AAV-DIO-synaptophysin:mCherry in $Oxtr^{PBN}$ neurons demonstrates less prominent downstream projections to (**b**) the nucleus of the solitary tract (NTS), lateral posterior thalamus, periventricular hypothalamus, tuberal nucleus, paraventricular nucleus of the hypothalamus (PVH) and supraoptic nucleus (SON); (*n* = 2) scale bar, 200 µm. (**c**) Percentage Fos in caudal, mid and rostral $Oxtr^{PBN}$ neurons in $Oxt^{Cre/+}$::$Oxtr^{Cre/+}$ mice injected with hM_3Dq or mCherry in PVH (*n* = 3/group; unpaired 2-tailed Student's t-test; caudal: *t*(4) = 4.385; *p* = 0.0118; mid: *t*(4) = 1.807; *p* = 0.1451; rostral: *t*(4) = 2.278; *p* = 0.0850). (**d**) Injection of AAV1-DIO-synaptophysin:mCherry in PVH of $Oxtr^{Cre/+}$ mice demonstrates no visible projections in PBN (*n* = 3); 3V, third ventricle; thal, thalamic nuclei; scp, superior cerebellar peduncle; dl, dorsolateral PBN; el, external lateral PBN; scale bar, 100 µm. (**e**) Injection of AAV-DIO-hM_3Dq into the PVH and AAV-DIO-YFP into the PBN of $Oxtr^{Cre/+}$ mice. (**f**) Following CNO, Fos is robustly expressed in the PVH and thalamus, but (**g**) there was minimal expression in the PBN (*n* = 3). (**h**) Representative live image of the lateral PBN prior to electrophysiological recordings demonstrating Oxt^{PVH} fibers and $Oxtr^{PBN}$ neurons; scale bar, 200 µm. (**i**) Acute Oxt^{PVH} stimulation decreases water intake following 1 M mannitol ip (*n* = 7 hM_3Dq, 8 mCherry; 2-way RM ANOVA; NaCl: interaction *F*(4,52) = 0.6187; *p* = 0.6512; water: interaction *F*(4,52) = 4.120; *p* = 0.0057), but (**j**) does not significantly alter fluid intake following 48-h salt appetite (*n* = 7 hM_3Dq, 8 mCherry; 2-way RM ANOVA; NaCl: interaction *F*(8,104) = 1.056; *p* = 0.3999; water: interaction *F*(8,104) = 0.6962; *p* = 0.6941) or (**k**) 30% PEG sc (*n* = 7 hM_3Dq, 8 mCherry; 2-way RM ANOVA; NaCl: interaction *F*(4,52) = 0.3265; *p* = 0.8589; water: interaction *F*(4,52) = 0.5231; *p* = 0.7191), or (**l**) food intake at baseline or after a 24-h fast (*n* = 7 hM_3Dq, 8 mCherry; 2-way RM ANOVA; baseline food: interaction *F*(8,104) = 0.3734; *p* = 0.9325; post 24-h fast: interaction *F*(8,104) = 0.2928; *p* = 0.9670). (**m**) Percentage Fos in Oxt^{PVH} neurons in hM_3Dq-injected vs mCherry-injected $Oxt^{Cre/+}$ mice (*n* = 7 hM_3Dq, 8 mCherry; unpaired 2-tailed Student's t-test; *t*(13) = 12.50; *p* < 0.0001). Data are expressed as mean ± s.e.m. ****$p < 0.0001$; *$p < 0.05$. See Supplementary Information for statistical analyses.



Supplementary Figure 8

Chronic Oxt^{PVH} inactivation increases water intake following dehydration, salt depletion, mannitol and PEG.

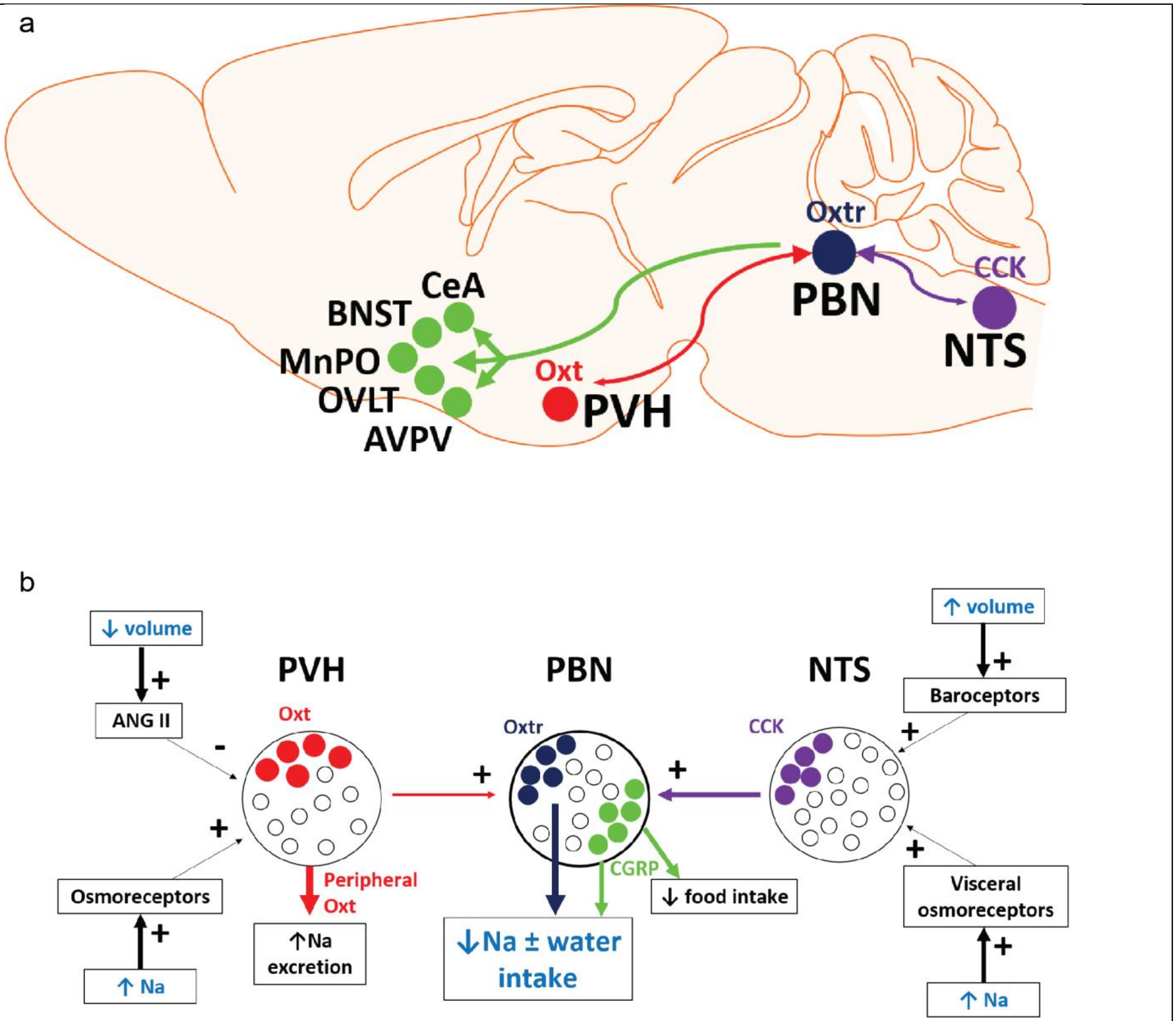
(**a**) Injection of AAV-DIO-GFP:TetTox in Oxt^{PVH} neurons. (**b**)**,** Representative images of GFP expression in Oxt^{PVH} neurons in GFP:TetTox- vs control YFP-injected mice; 3V, third ventricle; (n = 6 TetTox, 8 YFP) scale bar 100 µm. (**c**) Chronic Oxt^{PVH} inactivation does not significantly alter baseline saline preference at different concentrations (0.075, 0.3, 0.5 M) (n = 6 TetTox, 8 YFP; unpaired 2-tailed Student's t-test; 0.075 M NaCl: interaction $t(12) = 0.2346$; $p = 0.8184$; 0.3 M NaCl: interaction $t(12) = 0.2509$; $p = 0.8061$; 0.5 M NaCl: interaction $t(12) = 0.6848$; $p = 0.5065$), but (**d**) but significantly increases water following 24-h dehydration (n = 6 TetTox, 8 YFP; 2-way RM ANOVA; NaCl: interaction $F(8,96) = 1.098$; $p = 0.3712$; water: interaction $F(8,96) = 4.073$; $p = 0.0003$), (**e**) 48-h salt depletion (n = 6 TetTox, 8 YFP; 2-way RM ANOVA; NaCl: interaction $F(8,96) = 0.7341$; $p = 0.6612$; water: interaction $F(8,96) = 6.866$; $p < 0.0001$), (**f**) demonstrates a trend to increase water following 0.5 M saline ip (n = 6 TetTox, 8 YFP; 2-way RM ANOVA; NaCl: interaction $F(4,48) = 0.1292$; $p = 0.9711$; water: interaction $F(4,48) = 2.183$; $p = 0.0850$), and (**g**) increases water following 1 M mannitol ip (n = 6 TetTox, 8 YFP; 2-way RM ANOVA; NaCl: interaction $F(4,48) = 0.8378$; $p = 0.5080$; water: interaction $F(4,48) = 3.628$; $p = 0.0116$), and (**h**) and 30% PEG sc (n = 6 TetTox, 8 YFP; 2-way RM ANOVA; NaCl: interaction $F(4,48) = 1.296$; $p = 0.2848$; water: interaction $F(4,48) = 3.617$; $p = 0.0118$). Data are expressed as mean ± s.e.m. ****$p < 0.0001$; **$p < 0.01$; *$p < 0.05$; ϕ $p = 0.085$. See Supplementary Information for statistical analyses.



Supplementary Figure 9

Characterization of upstream projections to Oxtr^{PBN} neurons.

(**a**) Injection of AAV-DIO-hM$_3$Dq:mCherry in Oxt^{PVH} neurons of *Oxt$^{Cre/+}$* mice and bilateral cannula into PBN. (**b**) Acute Oxt^{PVH} activation by CNO ip decreases 2-h water consumption after 24-h dehydration during the light cycle (n = 6/group; unpaired 2-tailed Student's t-test; NaCl: interaction $t(10)$ = 0.3017; p = 0.7690; water: interaction $t(10)$ = 2.484; p = 0.0323), but (**c**) demonstrates no significant change in 2-h NaCl or water consumption at baseline (n = 6/group; 2-way RM ANOVA; NaCl: interaction $F(1,10)$ = 0.9098; p = 0.3627; water: interaction $F(1,10)$ = 2.118; p = 0.1763). (**d**) Representative histological image demonstrating cannula placement in PBN (n = 6/group). (**e**) Injection of AAV-DIO-hM$_3$Dq:mCherry in Oxt^{PVH} neurons and AAV-DIO-YFP in Oxtr^{PBN} neurons of *Oxt$^{Cre/+}$::Oxtr$^{Cre/+}$* mice and bilateral cannula into PBN. (**f**) Following intra-PBN CNO, we saw some Fos expression in Oxt^{PVH} neurons, (**g**) in Oxtr^{PBN} neurons, and (**h**) some scattered Fos in the NTS (n = 3). (**i**) Injection of AAV-hM$_3$Dq:mCherry in NTS and AAV-DIO-YFP in Oxtr^{PBN} neurons of *Oxtr$^{Cre/+}$* mice. (**j**) Following CNO ip, we observed robust Fos expression in hM$_3$Dq-expressing NTS neurons (n = 6) and (**k**) minimal Fos expression in control DsRed-expressing NTS neurons (n = 5). (**l**) Injection of AAV-DIO-hM_3Dq:mCherry in CCK^{NTS} neurons of *Cck*$^{Cre/+}$ mice. **m,n**, Following CNO ip, we observed Fos in the (**m**) NTS and (**n**) PBN of hM_3Dq-injected mice (n = 5 hM_3Dq, 4 mCherry). Data are expressed as mean ± s.e.m. dl, dorsolateral PBN; el, external lateral PBN; scp, superior cerebellar peduncle. *$p < 0.05$. Scale bars, 100 μm. See Supplementary Information for statistical analyses.



Supplementary Figure 10

Model of neural circuits that suppress fluid intake.

(**a**) Model of $Oxtr^{PBN}$ neural circuitry illustrating Oxt^{PVH} projections to $Oxtr^{PBN}$ neurons, likely projections from CCK^{NTS} neurons to $Oxtr^{PBN}$ neurons, and major $Oxtr^{PBN}$ projections to CeA, BNST, OVLT, AVPV and MnPO. (**b**) $Oxtr^{PBN}$ neurons are proposed to decrease or prevent hypernatremia ± hypervolemia by decreasing NaCl ± water intake. Inputs to $Oxtr^{PBN}$ neurons arise from Oxt^{PVH} and NTS neurons. NTS neurons receive signals about volume and osmolarity status from peripheral baroceptors and visceral osmoreceptors (and likely from oropharyngeal and upper gastrointestinal receptors); while Oxt^{PVH} neurons receive signals from forebrain osmoreceptors and angiotensin II (ANG II). Oxt^{PVH} neurons project to PBN and can also release oxytocin peripherally to increase renal NaCl excretion. NTS neurons likely project to both $Oxtr^{PBN}$ and $CGRP^{PBN}$ neurons, which can decrease NaCl, water and food intake.

Neuronal subtype	n	2-h NaCl	2-h water	2-h total fluid	4-h NaCl	4-h water	4-h total fluid
Oxtr^{PBN}	hM_3Dq = 7; mCh = 7	35%	25%	29%	45%	39%	41%
Oxt^{PVH}	hM_3Dq = 8; mCh = 7	n.s.	73%	n.s.	n.s.	71%	81%
CGRP^{PBN}	hM_3Dq = 8; mCh = 7	n.s.	34%	56%	n.s.	39%	67%
CCK^{NTS}	hM_3Dq = 5; mCh = 4	61%	51%	55%	63%	51%	53%
NTS	hM_3Dq = 6; DsRed = 5	14%	11%	12%	13%	16%	14%

n.s., not significant; mCh, mCherry.

Supplementary Table 1

Average percentage fluid intake after stimulating different neuronal populations.

Average percentage fluid intake after chemogenetic activation of different neuronal populations, demonstrating a range of effects on fluid consumption. Results show the percentage of fluid intake of hM_3Dq-injected mice compared to control (mCherry/DsRed-injected) mice after 24-h dehydration at 2-h and 4-h timepoints.

Supplementary Table 2
Supplementary Statistics
Summary of statistical analyses from each figure, including test used, *n*, P value, degrees of freedom and post hoc multiple comparisons test.

Figure number	Test used	n	Litters	Descriptive stats	P value	Degrees of freedom & F/t/r/etc value	Post hoc multiple comparisons test
1e panel 1	two-way RM ANOVA	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9678	F(8,96) = 2.901	
1e panel 2	two-way RM ANOVA	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.3424	F(8,96) = 0.3424	
1f panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,96) = 12.63	15 min: P = 0.0271 30 min: P = 0.0094 45 min: P = 0.0005 60 min: P = 0.0002 75 min: P < 0.0001 90 min: P < 0.0001 105 min: P < 0.0001 120 min: P < 0.0001
1f panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,96) = 39.75	15 min: P < 0.0001 30 min: P < 0.0001 45 min: P < 0.0001 60 min: P < 0.0001 75 min: P < 0.0001 90 min: P < 0.0001 105 min: P < 0.0001 120 min: P < 0.0001
1g panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,80) = 8.173	30 min: P = 0.0494 45 min: P = 0.0222 60 min: P = 0.0020 75 min: P = 0.0052 90 min: P = 0.0020 105 min: P = 0.0011 120 min: P = 0.0015
1g panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,80) = 22.31	15 min: P = 0.0004 30 min: P < 0.0001 45 min: P < 0.0001 60 min: P < 0.0001 75 min: P < 0.0001 90 min: P < 0.0001 105 min: P < 0.0001 120 min: P < 0.0001
1h panel 1	three-way mixed design ANOVA (with Greenhouse-Geisser correction).	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.210	F(2.875,34.501) = 1.593	

1h panel 2	three-way mixed design ANOVA (with Greenhouse-Geisser correction). To investigate simple 2-way interactions, statistical significance was accepted at Bonferroni-adjusted alpha level of 0.025. Sidak's multiple comparisons test was used.	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.021 2-way interactions: For hM_3Dq: P < 0.0001 For mCherry: P = 0.524	F(2.099,25.183) = 4.464 2-way interactions: For hM_3Dq: F(8,48) = 8.303 For mCherry: F(8,48) = 0.900	Post hoc tests for hM_3Dq-injected mice: CNO vs vehicle: 60 min: P = 0.0099 75 min: P < 0.0001 90 min: P < 0.0001 105 min: P < 0.0001 120 min: P < 0.0001
1i panel 1	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9282	F(8,88) = 0.3809	
1i panel 2	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.8505	F(8,88) = 0.5037	
2a	two-way RM ANOVA and Sidak's multiple comparisons test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0002	F(4,44) = 7.143	2h: P = 0.0047 3h: P = 0.0037 4h: P = 0.0011
2b panel 1	two-way RM ANOVA	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.2028	F(8,96) = 1.408	
2b panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,96) = 35.57	30 min: P = 0.0051 45 min: P < 0.0001 60 min: P < 0.0001 75 min: P < 0.0001 90 min: P < 0.0001 105 min: P < 0.0001 120 min: P < 0.0001
2c panel 1	two-way RM ANOVA	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9853	F(4,48) = 0.08955	
2c panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(4,48) = 11.37	3 h: P = 0.0003 4 h: P < 0.0001
2d panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0114	F(4,48) = 3.638	4 h: P = 0.0118
2d panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(4,48) = 24.56	1 h: P = 0.0049 2 h: P < 0.0001 3 h: P < 0.0001 4 h: P < 0.0001
2e panel 1	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.8046	F(4,44) = 0.4042	

2e panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(4,44) = 11.54	2 h: P = 0.0037 3 h: P < 0.0001 4 h: P < 0.0001
3b panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0009	F(4,44) = 5.704	3 h: P = 0.0167 4 h: P = 0.0139
3b panel 2	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.5342	F(4,44) = 0.7961	
3c panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0011	F(4,44) = 5.497	3 h: P = 0.0079 4 h: P = 0.0014
3c panel 2	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9693	F(4,44) = 0.1334	
3d panel 1	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9100	F(8,88) = 0.4136	
3d panel 2	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.5230	F(8,88) = 0.8966	
3f panel 1	three-way mixed design ANOVA (with Greenhouse-Geisser correction). To investigate simple 2-way interactions, statistical significance was accepted at Bonferroni-adjusted alpha level of 0.025. Sidak's multiple comparisons test was used.	5,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.018 2-way interactions: For hM_4Di: P < 0.0001 For mCherry: P = 0.7775	F(2.644, 23.797) = 4.305 2-way interactions: For hM_4Di: F(8,32) = 6.213 For mCherry: F(8,40) = 0.5932	Post hoc tests for hM_4Di-injected mice: CNO vs vehicle: 30 min: P = 0.0002 45 min: P < 0.0001 60 min: P < 0.0001 75 min: P < 0.0001 90 min: P < 0.0001 105 min: P < 0.0001 120 min: P < 0.0001
3f panel 2	three-way mixed design ANOVA (with Greenhouse-Geisser correction).	5,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.436	F(1.451, 13.062) = 0.790	
4b	one-way ANOVA and Tukey's multiple comparisons test.	4,3,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.0063	F(2,7) = 11.41	Salt returned vs Salt depleted: P = 0.0180 Salt returned vs Control: P = 0.0086
4c	one-way ANOVA and Tukey's multiple comparisons test.	4,3,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.0006	F(2,7) = 26.02	Salt returned vs Salt depleted: P = 0.0025 Salt returned vs Control: P = 0.0007

4d	unpaired two-tailed Student's t-test	4,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.0078	t(5) = 4.285	
4e	unpaired two-tailed Student's t-test	4,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.0186	t(5) = 3.430	
4f	unpaired two-tailed Student's t-test	4,3	mice from 2 litters	Error bars are mean ± SEM	P = 0.0100	t(5) = 4.033	
4g	unpaired two-tailed Student's t-test	4,3	mice from 2 litters	Error bars are mean ± SEM	P = 0.2341	t(5) = 1.353	
4m	two-way RM ANOVA and Sidak's multiple comparisons test	94, 85, 80	mice from 2 litters	Error bars are mean ± SEM	P < 0.0001	F(2,257) = 17.01	For Water: before vs after bout: P < 0.0001
4n	one-way RM ANOVA and Tukey's multiple comparisons test	83	mice from 1 litters	Error bars are mean ± SEM	P < 0.0001	F(8,656) = 134.7	5 vs 10: P < 0.0001 5 vs 15: P < 0.0001 5 vs 20: P < 0.0001 5 vs 25: P < 0.0001 5 vs 30: P < 0.0001 5 vs 45: P < 0.0001 5 vs 60: P < 0.0001 5 vs 120: P < 0.0001 10 vs 15: P < 0.0001 10 vs 20: P < 0.0001 10 vs 25: P < 0.0001 10 vs 30: P < 0.0001 10 vs 45: P < 0.0001 10 vs 60: P < 0.0001 10 vs 120: P < 0.0001 15 vs 120: P = 0.0126 60 vs 120: P = 0.0376
5c panel 1	three-way mixed design ANOVA (with Greenhouse-Geisser correction).	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.406	F(1.876, 24.391) = 0.921	
5c panel 2	three-way mixed design ANOVA (with Greenhouse-Geisser correction). To investigate simple 2-way interactions, statistical significance was accepted at Bonferroni-adjusted alpha level of 0.025. Sidak's multiple comparisons test was used.	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.023 2-way interactions: For hM_3Dq: P = 0.0205 For mCherry: P = 0.075	F(2.629, 34.177) = 3.768 2-way interactions: For hM_3Dq: F(8,56) = 2.518 For mCherry: F(8,56) = 1.941	Post hoc tests for hM_3Dq-injected mice: CNO vs vehicle: 120 min: P = 0.0003
5d panel 1	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.6395	F(8,104) = 0.07589	

5d panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,104) = 22.22	15 min: P = 0.0004 30 min: P < 0.0001 45 min: P < 0.0001 60 min: P < 0.0001 75 min: P < 0.0001 90 min: P < 0.0001 105 min: P < 0.0001 120 min: P < 0.0001
5e panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,104) = 5.789	30 min: P = 0.0477 45 min: P = 0.0187 60 min: P = 0.0064 75 min: P = 0.0028 90 min: P = 0.0002 105 min: P = 0.0003 120 min: P = 0.0011
5e panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,104) = 14.01	45 min: P = 0.0213 60 min: P = 0.0005 75 min: P = 0.0003 90 min: P < 0.0001 105 min: P < 0.0001 120 min: P < 0.0001
7b panel 1	three-way mixed design ANOVA (with Greenhouse-Geisser correction).	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.468	F(1.749, 22.735) = 0.748	
7b panel 2	three-way mixed design ANOVA (with Greenhouse-Geisser correction).	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.305	F(2.480, 32.236) = 1.250	
7c panel 1	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9922	F(8,104) = 0.1871	
7c panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0014	F(8,104) = 3.456	90 min: P = 0.0256 105 min: P = 0.0401 120 min: P = 0.0136
7d panel 1	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.1007	F(4,52) = 2.052	
7d panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0006	F(4,52) = 5.763	3 h: P = 0.0007 4 h: P = 0.0005
7f panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0489	F(8,96) = 2.046	No significant differences in post hoc tests.
7f panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,96) = 5.036	75 min: P = 0.0346 90 min: P = 0.0087 105 min: P = 0.0063 120 min: P = 0.0005
7h panel 1	two-way RM ANOVA	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9975	F(8,80) = 0.1334	

7h panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0444	F(8,80) = 2.108	No significant differences in post hoc tests.
8b panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	6,5	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,72) = 11.88	45 min: P = 0.0352 60 min: P = 0.0046 75 min: P = 0.0014 90 min: P = 0.0014 105 min: P = 0.0003 120 min: P < 0.0001
8b panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	6,5	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,72) = 17.94	30 min: P = 0.0117 45 min: P = 0.0023 60 min: P = 00002 75 min: P = 0.0001 90 min: P < 0.0001 105 min: P < 0.0001 120 min: P < 0.0001
8c	two-way RM ANOVA and Sidak's multiple comparisons test	6,5	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(4,36) = 91.69	1 h: P < 0.0001 2 h: P < 0.0001 3 h: P < 0.0001 4 h: P < 0.0001
8f panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	5,4	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0065	F(8,56) = 3.047	No significant differences in post hoc tests.
8f panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	5,4	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,56) = 5.693	75 min: P = 0.0277 90 min: P = 0.0134 105 min: P = 0.0050 120 min: P = 0.0028
Suppl 3a panel 1	Unpaired 2-tailed Student's t-test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.5821	t(12) = 0.5656	
Suppl 3a panel 2	Unpaired 2-tailed Student's t-test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0394	t(12) = 2.311	
Suppl 3b panel 1	two-way ANOVA and Sidak's multiple comparisons test	7,6,7,6	mice from at least 2 litters/group	Error bars are mean ± SEM	Interaction: P = 0.3989 Main effect of virus: P < 0.0001 Main effect of food availability: P = 0.0024	Interaction: F(1,22) = 0.7401 Main effect of virus: F(1,22) = 32.44 Main effect of food availability: F(1,22) = 11.77	Food present vs Food absent: For hM_3Dq: P = 0.1588 For mCherry: P = 0.0122
Suppl 3b panel 2	two-way ANOVA and Sidak's multiple comparisons test	7,6,7,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0367	F(1,22) = 4.946	Food present vs Food absent: For hM_3Dq: P = 0.7499 For mCherry: P = 0.0018
Suppl 3c panel 1	two-way RM ANOVA	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.2038	F(1,12) = 1.806	
Suppl 3c panel 2	two-way RM ANOVA	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.6645	F(1,12) = 0.1977	

Suppl 3d panel 1	two-way RM ANOVA	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.3285	F(8,80) = 1.168	
Suppl 3d panel 2	two-way RM ANOVA	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.4228	F(8,80) = 1.027	
Suppl 3e panel 1	two-way RM ANOVA	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.3202	F(8,80) = 1.182	
Suppl 3e panel 2	two-way RM ANOVA	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.2489	F(8,80) = 1.313	
Suppl 3f panel 1	Unpaired 2-tailed Student's t-test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.7791	t(12) = 0.2868	
Suppl 3f panel 2	Unpaired 2-tailed Student's t-test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.2008	t(12) = 1.354	
Suppl 3g panel 1	two-way RM ANOVA	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.7275	F(1,12) = 0.1273	
Suppl 3g panel 2	two-way RM ANOVA	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0618	F(1,12) = 4.243	
Suppl 3h	Unpaired 2-tailed Student's t-test	7,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	t(12) = 19.28	
Suppl 4c panel 1	Unpaired 2-tailed Student's t-test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.6292	t(11) = 0.4967	
Suppl 4c panel 2	Unpaired 2-tailed Student's t-test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.3364	t(11) = 1.005	
Suppl 4c panel 3	Unpaired 2-tailed Student's t-test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9224	t(11) = 0.09961	
Suppl 4d panel 1	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.6275	F(8,88) = 0.7729	

Suppl 4d panel 2	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.8728	F(8,88) = 0.4721	
Suppl 4e panel 1	Unpaired 2-tailed Student's t-test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.4398	t(11) = 0.8014	
Suppl 4e panel 2	Unpaired 2-tailed Student's t-test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.5070	t(11) = 0.6858	
Suppl 4f panel 1	Unpaired 2-tailed Student's t-test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0186	t(11) = 2.760	
Suppl 4f panel 2	Unpaired 2-tailed Student's t-test	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.3935	t(11) = 0.8881	
Suppl 4g panel 1	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.7619	F(4,44) = 0.4638	
Suppl 4g panel 2	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.2118	F(4,44) = 1.524	
Suppl 4h panel 1	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.2203	F(4,44) = 1.494	
Suppl 4h panel 2	two-way RM ANOVA	6,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.8453	F(4,44) = 0.3461	
Suppl 4i panel 1	Pearson-product-moment correlation	6	mice from at least 2 litters	Error bars are mean ± SEM	P = 0.5408	r(4) = 0.3167	
Suppl 4i panel 2	Pearson-product-moment correlation	6	mice from at least 2 litters	Error bars are mean ± SEM	P = 0.3905	r(4) = 0.4335	
Suppl 5c panel 1	two-way RM ANOVA and Sidak's multiple comparisons test	5,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0064	F(8,72) = 2.962	No significant differences in post hoc tests.
Suppl 5c panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	5,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0304	F(8,72) = 2.289	105 min: P = 0.0308

Suppl 5d panel 1	two-way RM ANOVA	5,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9979	F(8,72) = 0.1264	
Suppl 5d panel 2	two-way RM ANOVA	5,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.7734	F(8,72) = 0.6017	
Suppl 5e panel 1	two-way RM ANOVA	5,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9811	F(8,72) = 0.2429	
Suppl 5e panel 2	two-way RM ANOVA	5,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.4150	F(8,72) = 1.040	
Suppl 5f panel 1	Pearson-product-moment correlation	5	mice from 2 litters	Error bars are mean ± SEM	P = 0.2208	r(3) = 0.6649	
Suppl 5f panel 2	Pearson-product-moment correlation	5	mice from 2 litters	Error bars are mean ± SEM	P = 0.2013	r(3) = -0.6857	
Suppl 6a panel 1	one-way ANOVA	4,3,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.1331	F(2,7) = 2.727	
Suppl 6a panel 2	one-way ANOVA	4,3,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.7432	F(2,7) = 0.3097	
Suppl 6b panel 1	one-way ANOVA	4,3,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.2046	F(2,7) = 2.008	
Suppl 6b panel 2	one-way ANOVA	4,3,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.2711	F(2,7) = 1.582	
Suppl 6c panel 1	Unpaired 2-tailed Student's t-test	4,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.2394	t(5) = 1.335	
Suppl 6c panel 2	Unpaired 2-tailed Student's t-test	4,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.7023	t(5) = 0.4049	
Suppl 6d panel 1	Unpaired 2-tailed Student's t-test	4,3	mice from 1 litter	Error bars are mean ± SEM	P = 0.5761	t(5) = 0.5977	

Suppl 6d panel 2	Unpaired 2-tailed Student's t-test	4,3	mice from 1 litter	Error bars are mean ± SEM	$P = 0.7479$	$t(5) = 0.3396$	
Suppl 6e panel 1	Unpaired 2-tailed Student's t-test	4,3	mice from 2 litters	Error bars are mean ± SEM	$P = 0.6328$	$t(5) = 0.5084$	
Suppl 6e panel 2	Unpaired 2-tailed Student's t-test	4,3	mice from 2 litters	Error bars are mean ± SEM	$P = 0.2444$	$t(5) = 1.319$	
Suppl 6f panel 1	Unpaired 2-tailed Student's t-test	4,3	mice from 2 litters	Error bars are mean ± SEM	$P = 0.1469$	$t(5) = 1.716$	
Suppl 6f panel 2	Unpaired 2-tailed Student's t-test	4,3	mice from 2 litters	Error bars are mean ± SEM	$P = 0.0698$	$t(5) = 2.300$	
Suppl 7c panel 1	Unpaired 2-tailed Student's t-test	3,3	mice from 1-2 litters/group	Error bars are mean ± SEM	$P = 0.0118$	$t(4) = 4.385$	
Suppl 7c panel 2	Unpaired 2-tailed Student's t-test	3,3	mice from 1-2 litters/group	Error bars are mean ± SEM	$P = 0.1451$	$t(4) = 1.807$	
Suppl 7c panel 3	Unpaired 2-tailed Student's t-test	3,3	mice from 1-2 litters/group	Error bars are mean ± SEM	$P = 0.0850$	$t(4) = 2.278$	
Suppl 7i panel 1	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	$P = 0.6512$	$F(4,52) = 0.6187$	
Suppl 7i panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	$P = 0.0057$	$F(4,52) = 4.120$	4 h: $P = 0.0270$
Suppl 7j panel 1	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	$P = 0.3999$	$F(8,104) = 1.056$	
Suppl 7j panel 2	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	$P = 0.6941$	$F(8,104) = 0.6962$	
Suppl 7k panel 1	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	$P = 0.8589$	$F(4,52) = 0.3265$	

Suppl 7k panel 2	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.7191	F(4,52) = 0.5231	
Suppl 7l panel 1	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9325	F(8,104) = 0.3734	
Suppl 7l panel 2	two-way RM ANOVA	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9670	F(8,104) = 0.2928	
Suppl 7m	Unpaired 2-tailed Student's t-test	8,7	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	t(13) = 12.50	
Suppl 8c panel 1	Unpaired 2-tailed Student's t-test	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.8184	t(12) = 0.2346	
Suppl 8c panel 2	Unpaired 2-tailed Student's t-test	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.8061	t(12) = 0.2509	
Suppl 8c panel 3	Unpaired 2-tailed Student's t-test	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.5065	t(12) = 0.6848	
Suppl 8d panel 1	two-way RM ANOVA	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.3712	F(8,96) = 1.098	
Suppl 8d panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0003	F(8,96) = 4.073	105 min: P = 0.0250 120 min: P = 0.0089
Suppl 8e panel 1	two-way RM ANOVA	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.6612	F(8,96) = 0.7341	
Suppl 8e panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P < 0.0001	F(8,96) = 6.866	75 min: P = 0.0356 90 min: P = 0.0097 105 min: P = 0.0097 120 min: P < 0.0001
Suppl 8f panel 1	two-way RM ANOVA	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.9711	F(4,48) = 0.1292	
Suppl 8f panel 2	two-way RM ANOVA	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0850	F(4,48) = 2.183	

Suppl 8g panel 1	two-way RM ANOVA	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.5080	F(4,48) = 0.8378	
Suppl 8g panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0116	F(4,48) = 3.628	4 h: P = 0.0099
Suppl 8h panel 1	two-way RM ANOVA	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.2848	F(4,48) = 1.296	
Suppl 8h panel 2	two-way RM ANOVA and Sidak's multiple comparisons test	6,8	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0118	F(4,48) = 3.617	4 h: P = 0.0142
Suppl 9b panel 1	Unpaired 2-tailed Student's t-test	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.7690	t(10) = 0.3017	
Suppl 9b panel 2	Unpaired 2-tailed Student's t-test	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.0323	t(10) = 2.484	
Suppl 9c panel 1	two-way RM ANOVA	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.3627	F(1,10) = 0.9098	
Suppl 9c panel 2	two-way RM ANOVA	6,6	mice from at least 2 litters/group	Error bars are mean ± SEM	P = 0.1763	F(1,10) = 2.118	