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Supplemental information

Lateral hypothalamic LEPR neurons drive appetitive but not consummatory behaviors

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Table 1. Statistical values and group sizes for main figures. *Related to Figures 1 - 5.*

Figure	Test	n	F or t value	p value
1D	Two-way mixed-model ANOVA	6 mice/group	F (29,290) = 5.94 ablation x day	< 0.0001
	Bonferroni post-test	6 mice/group	day 30	0.002
1E	ANCOVA (daily food intake)	6 mice/group	F (1,284) = 36.96	< 0.0001
	Student's *t* -test (cumulative food intake)	6 mice/group	t(10) = 2.41	0.037
1F	Student's *t* -test	6 mice/group	t(10) = 2.38	0.039
1G	Two-way mixed-model ANOVA	6 mice/group	F (29,290) = 0.61 ablation x day	0.95
1H	ANCOVA (daily food intake)	6 mice/group	F (1,284) = 0.017	0.9
	Student's *t* -test (cumulative food intake)	6 mice/group	t(10) = 0.11	0.91
1I	Student's *t* -test	6 mice/group	t(10) = 0.33	0.75
1K	Two-way repeated-measures ANOVA	8 YFP mice	F (4,28) = 4.78 block x CS	0.0046
		8 YFP mice	F (4,28) = 6.93 block	0.0005
		8 YFP mice	F (1,7) = 15.50 CS	0.0056
	Bonferroni post-test	8 YFP mice	block 3	0.0035
		8 YFP mice	block 4	0.0035
		8 YFP mice	block 5	< 0.0001
1K	Two-way repeated-measures ANOVA (Bonferroni post-test)	8 taCasp3 mice	F (1,7) = 19.84 CS	0.003
	Bonferroni post-test	8 taCasp3 mice	block 5	0.0004
1L	Two-way repeated-measures ANOVA	8 mice/group	F (1,14) = 0.23 group	0.64
		8 mice/group	F (4,56) = 0.83 group x block	0.51
1M	Two-way repeated-measures ANOVA	8 YFP mice	F (4,28) = 3.40 block x CS	0.0219
	Bonferroni post-test	8 YFP mice	block 4	0.0273
		8 YFP mice	block 5	0.0027
	Two-way repeated-measures ANOVA	7 taCasp3 mice	F (4,24) = 1.26 block	0.31
		7 taCasp3 mice	F (1,6) = 3.17 CS	0.13
		7 taCasp3 mice	F (4,24) = 1.53 block x CS	0.23
	Bonferroni post-test	7 taCasp3 mice	block 5	0.5
1N	Two-way repeated-measures ANOVA	7–8 mice/group	F (1,13) = 2.11 group	0.17
		7–8 mice/group	F (4,52) = 0.32 group x block	0.87
2D	Two-way mixed-model ANOVA	5 LH^{VGAT}:ChR2 and 7 LH^{VGAT}:GFP mice	F (2, 20) = 8.64 group x epoch	0.002
	Bonferroni post-test	5 LH^{VGAT}:ChR2 and 7 LH^{VGAT}:GFP mice	stim epoch	0.003
2E	Two-way mixed-model ANOVA	10 LH^{VGAT}:NpHR and 7 LH^{VGAT}:GFP	F (1, 15) = 12.87 group x epoch	0.0027
	Bonferroni post-test	10 LH^{VGAT}:NpHR and 7 LH^{VGAT}:GFP	stim epoch	0.005
2F	Two-way mixed-model ANOVA	5 LH^{LEPR}:ChR2 and 8 LH^{LEPR}:GFP	F (2,22) = 1.69 group x epoch	0.21
2G	Two-way mixed-model ANOVA	10 LH^{LEPR}:NpHR and 8 LH^{LEPR}:GFP	F (1,16) = 1.06 group x epoch	0.32
2H	Student's *t* -test	5 LH^{VGAT}:ChR2 and 7 LH^{VGAT}:GFP mice	t(10) = 6.40	<0.0001
2I	Student's *t* -test	10 LH^{VGAT}:NpHR and 7 LH^{VGAT}:GFP	t(15) = 2.19	0.045
2J	Student's *t* -test	5 LH^{LEPR}:ChR2 and 8 LH^{LEPR}:GFP	t(11) = 5.26	0.0003
2K	Student's *t* -test	10 LH^{LEPR}:NpHR and 8 LH^{LEPR}:GFP	t(16) = 3.26	0.0049
3L	Three-way ANOVA	LH^{VGAT} neurons: 107 pre-responsive, 63 cue-responsive, 152 reward-responsive; LH^{LEPR} neurons: 63 pre-responsive, 50 cue-responsive, 85 reward-responsive	F (2, 514) = 0.1048 genotype x CS	0.021
			F (2, 514) = 89.08 epoch x CS	< 0.0001
	Bonferroni post-test		cue responsive, VGAT CS- vs LEPR CS-	0.017
			within groups, CS+ vs CS-	< 0.0001
3O	Mann-Whitney *U* -test	63 LH^{VGAT} neurons, 50 LH^{LEPR} neurons	U = 1072	0.0034
4B	Two-way repeated-measures ANOVA	6 LH^{VGAT}:GFP mice	F (2, 10) = 4.220 block x CS	0.0469
			F (2, 10) = 27.80 block	< 0.0001
			F (1, 5) = 8.236 cs	0.035
	Bonferroni post-test		block 3	0.0021
	Two-way repeated-measures ANOVA	6 LH^{VGAT}:ChR2 mice	F (2, 10) = 0.2443 block x CS	0.7878
			F (2, 10) = 1.863 block	0.2052
			F (1, 5) = 0.6223 cs	0.4659
	Two-way repeated-measures ANOVA	8 LH^{VGAT}:NpHR mice	F (2, 14) = 1.761 block x CS	0.2078
			F (2, 14) = 7.677 block	0.0056
			F (1, 7) = 0.1347 cs	0.7245

Figure	Test	n	F or t value	p value
4C	Two-way mxed-model ANOVA	6–8 mice (see above)	F (4, 34) = 1.946 block x group	0.1252
			F (2, 34) = 15.59 block	< 0.0001
			F (2, 17) = 2.215 group	0.1397
4D	Two-way mxed-model ANOVA	6–8 mice (see above)	F (2, 17) = 0.2353 CS x group	0.7929
			F (1, 17) = 5.148 CS	0.0366
			F (2, 17) = 1.522 group	0.2465
4E	Two-way repeated-measures ANOVA	8 LH^{LEPR}:GFP mice	F (2, 14) = 14.57 block x CS	0.0004
			F (2, 14) = 11.57 block	0.0011
			F (1, 7) = 1.306 CS	0.2907
	Bonferroni post-test		block 3	0.0007
	Two-way repeated-measures ANOVA	5 LH^{LEPR}:ChR2 mice	F (2, 8) = 0.1826 block x CS	0.8364
			F (2, 8) = 22.10 block	0.0006
			F (1, 4) = 1.641 CS	0.2695
	Two-way repeated-measures ANOVA	5 LH^{LEPR}:NpHR mice	F (2, 8) = 0.2432 block x CS	0.7897
			F (2, 8) = 8.756 block	0.0097
			F (1, 4) = 2.186 CS	0.2133
4F	Two-way mixed-model ANOVA	5–8 mice (see above)	F (4, 30) = 2.772 block x group	0.0451
			F (2, 30) = 60.16 block	< 0.0001
			F (2, 15) = 3.058 group	0.0769
	Bonferroni post-test		GFP CS+ vs. ChR2 CS+	0.1374
			GFP CS+ vs. NpHR CS+	0.2031
4G	Two-way mixed-model ANOVA	5–8 mice (see above)	F (2, 15) = 0.3393 CS x group	0.7176
			F (1, 15) = 9.114 CS	0.0086
			F (2, 15) = 1.003 group	0.3901
4H	Two-way repeated-measures ANOVA	6 LH^{VGAT}:GFP→VTA mice	F (2, 10) = 3.777 block x CS	0.06
			F (2, 10) = 8.120 block	0.008
			F (1, 5) = 9.170 CS	0.0291
	Bonferroni post-test		block 3	0.0167
	Two-way repeated-measures ANOVA	7 LH^{VGAT}:ChR2→VTA mice	F (2, 12) = 1.495 block x CS	0.2631
			F (2, 12) = 5.368 block	0.0216
			F (1, 6) = 2.387 CS	0.1733
	Two-way repeated-measures ANOVA	8 LH^{VGAT}:ArchT→VTA mice	F (2, 14) = 1.049 block x CS	0.3764
			F (2, 14) = 27.20 block	< 0.0001
			F (1, 7) = 4.665 CS	0.0676
4I	Two-way mixed-model ANOVA	6–8 mice (see above)	F (4, 36) = 0.6670 block x group	0.6191
			F (2, 36) = 84.31 block	< 0.0001
			F (2, 18) = 0.3933 group	0.6805
4J	Two-way mixed-model ANOVA	6–8 mice (see above)	F (2, 18) = 0.01010 CS x group	0.99
			F (1, 18) = 7.209 CS	0.0151
			F (2, 18) = 0.02622 group	0.9742
4K	Two-way repeated-measures ANOVA	7 LH^{LEPR}:GFP→VTA mice	F (2, 12) = 2.492 block x CS	0.1244
			F (2, 12) = 1.625 block	0.2374
			F (1, 6) = 19.50 CS	0.0045
	Bonferroni post-test		block 3	0.0063
	Two-way repeated-measures ANOVA	7 LH^{LEPR}:ChR2→VTA mice	F (2, 12) = 1.110 block x CS	0.3613
			F (2, 12) = 5.485 block	0.0203
			F (1, 6) = 1.418 CS	0.2786
	Two-way repeated-measures ANOVA	8 LH^{LEPR}:ArchT→VTA mice	F (2, 14) = 6.834	0.0085
			F (2, 14) = 16.31	0.0002
			F (1, 7) = 10.80	0.0134
	Bonferroni post-test		block 3	0.0047
4L	Two-way mixed-model ANOVA	7–8 mice (see above)	F (4, 38) = 1.684 block x group	0.1738
			F (2, 38) = 28.41 block	< 0.0001
			F (2, 19) = 0.1603 group	0.853
4M	Two-way mixed-model ANOVA	7–8 mice (see above)	F (2, 19) = 5.080 CS x group	0.0171
			F (1, 19) = 7.805 CS	0.0116
			F (2, 19) = 0.05602 group	0.9457
	Bonferroni post-test	8 LH^{LEPR}:ArchT→VTA mice	CS+ vs CS-	0.0032
5C	Two-way mixed-model ANOVA	7 mCherry, 5 hM3D, and 8 hM4D mice	F (4, 34) = 3.678 test x group	0.0136
			F (2, 34) = 9.364 test	0.0006
			F (2, 17) = 2.371 group	0.1235

Figure	Test	n	F or t value	p value
	Bonferroni post-test		LH^{LEPR}:mCherry, pre vs post 2	0.0022
			LH^{LEPR}:hM3D mice, pre vs post 2	0.0023
			LH^{LEPR}:hM4D mice, pre vs post 2	> 0.99
			LH^{LEPR}:mCherry vs LH^{LEPR}:hM4D	0.0049
			LH^{LEPR}:mCherry vs LH^{LEPR}:hM3D	0.0127
5D	Two-way mixed-model ANOVA	7 mCherry, 5 hM3D, and 8 hM4D mice	F (2, 17) = 0.3213 test x group	0.7295
			F (1, 17) = 332.2 test	< 0.0001
			F (2, 17) = 0.2684 group	0.7677
	Bonferroni post-test		all groups	< 0.0001
5E	Two-way mixed-model ANOVA	8 mCherry and 10 hM4D mice	F (3, 48) = 0.2117 test x group	0.8878
			F (3, 48) = 41.86 test	< 0.0001
			F (1, 16) = 0.2282 group	0.6393
	Bonferroni post-test		Pre (1) vs. Post (10) both groups	< 0.0001
			Post (10) vs. Ext (23) mCherry mice	0.008
			Post (10) vs. Ext (23) hM4D mice	0.0034
			Ext (23) vs. Rnst (24) mCherry mice	0.0475
			Ext (23) vs. Rnst (24) hM4D mice	0.0017
5F	Two-way mixed-model ANOVA	9 mice/group	F (3, 48) = 0.1062 cocaine x group	0.9561
			F (3, 48) = 39.54 cocaine	< 0.0001
			F (1, 16) = 2.449 group	0.1372
5G	Two-way mixed-model ANOVA	10 mice/group	F (5, 90) = 1.591 day x group	0.1706
			F (5, 90) = 7.879 day	< 0.0001
			F (1, 18) = 6.411 group	0.0209
	Bonferroni post-test		day 5	0.0426
			day 7	0.0078
5H	Two-way mixed-model ANOVA	10 mice/group	F (3, 54) = 3.356 cocaine x group	0.0254
			F (3, 54) = 118.3 cocaine	< 0.0001
			F (1, 18) = 4.938 group	0.0393
	Bonferroni post-test		32 mg/kg cocaine	0.0012
5I	Two-way mixed-model ANOVA	10 mice/group	F (3, 54) = 3.381	0.0247
			F (3, 54) = 159.7	< 0.0001
			F (1, 18) = 4.971	0.0388
	Bonferroni post-test		32 mg/kg cocaine	0.0011

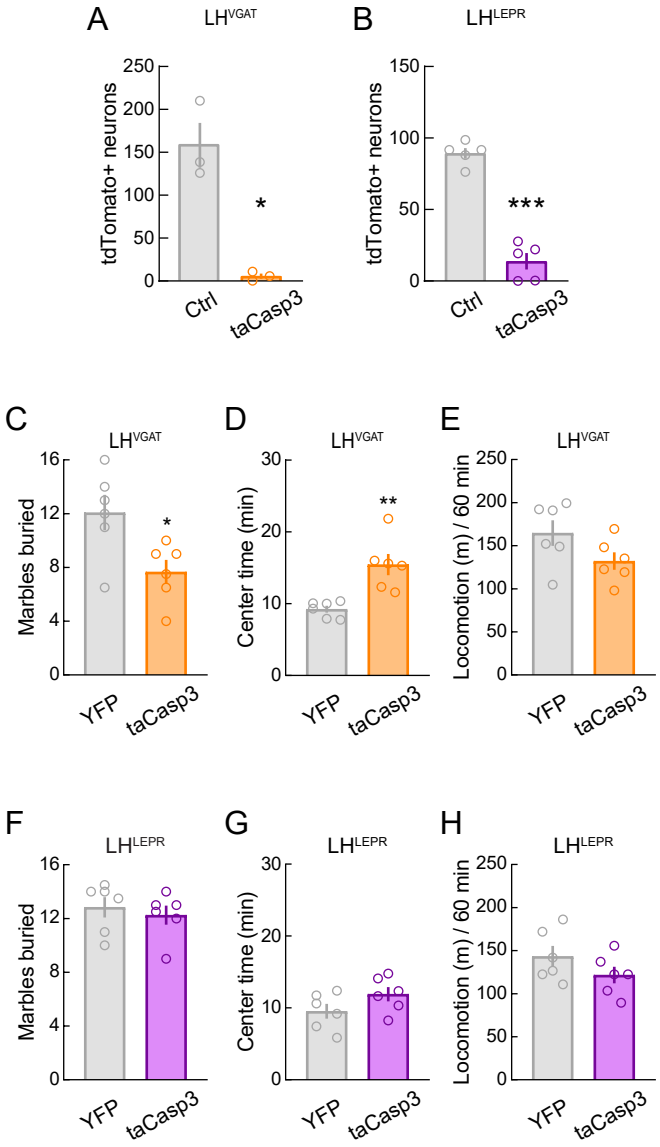

Figure S1. Caspase ablation of LH^{VGAT} and LH^{LEPR} neurons. ***Related to Figure 1.***

(A) *Slc32a1^{Cre}* mice for caspase virus validation were injected with a cocktail of Cre-dependent tdTomato and taCasp3 viruses in one hemisphere and Cre-dependent tdTomato virus diluted in PBS in the other hemisphere. Co-injection of Cre-ON viruses has been shown not to cause interference (Saunders et al., 2012). The tdTomato virus enabled visualization of intact LH^{VGAT} neurons when injected with PBS versus ablated neurons injected with taCasp3 virus. Injection of taCasp3 virus significantly reduced the number of tdTomato+ LH^{VGAT} neurons (Student's paired t-test, $t(2) = 6.542$, *p = 0.0226; n = 3 mice, 3 sections counted per mouse).

(B) *Lepr*^{Cre} mice for caspase virus validation were injected with Cre-dependent tdTomato and taCasp3 viruses in one hemisphere and tdTomato virus diluted in PBS in the other hemisphere. Note that injection of taCasp3 virus significantly reduced the number of tdTomato+ LH^{LEPR} neurons (Student's paired t-test, $t(4) = 12.44$, ***p = 0.0002; n = 5 mice, 3 sections counted per mouse).

(C) LH^{VGAT}:taCasp3 mice buried fewer marbles than control mice ($t(10) = 2.77$, p = 0.0196; n = 6 mice/group).

(D) LH^{VGAT}:taCasp3 mice spent significantly more time in the center of the open field chamber than LH^{VGAT}:YFP controls ($t(10) = 4.00$, p = 0.0025; n = 6 mice/group).

(E) Similar 1 h open field locomotion was observed between LH^{VGAT}:taCasp3 and LH^{VGAT}:YFP mice ($t(10) = 1.81$, p = 0.10; n = 6 mice/group).

(F) Marble burying in LH^{LEPR}:taCasp3 mice was similar to LH^{LEPR}:YFP mice ($t(10) = 0.56$, p = 0.59; n = 6 mice/group).

(G) LH^{LEPR}:taCasp3 mice did not differ from LH^{LEPR}:YFP mice in open field center time ($t(10) = 1.66$, p = 0.13; n = 6 mice/group).

(H) No differences in locomotion between the LH^{LEPR}:taCasp3 and LH^{LEPR}:YFP mice ($t(10) = 1.40$, p = 0.19; n = 6 mice/group).

Data represented as mean ± SEM.

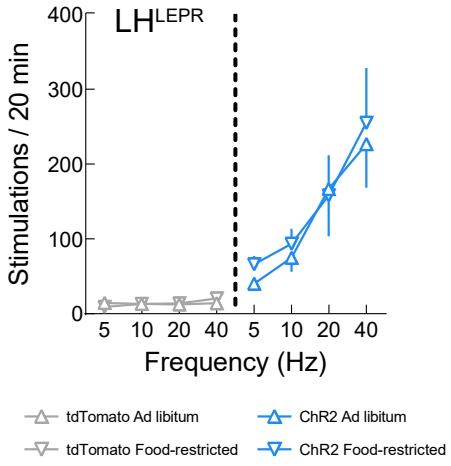

Figure S2. Activation of LH^{LEPR} neurons maintains operant self-stimulation. ***Related to Figure 2.***

Photostimulation of LH^{LEPR} neurons maintained operant self-stimulation responding (n = 8 mice per group). Three-way ANOVA revealed significant main effects of frequency ($F(3,42) = 9.93$, $p < 0.0001$) and group ($F(1,14) = 12.68$, $p = 0.003$) but not feeding status ($p = 0.29$), with a significant frequency × group interaction ($F(3,42) = 8.92$, $p = 0.0001$). Data represented as mean ± SEM.

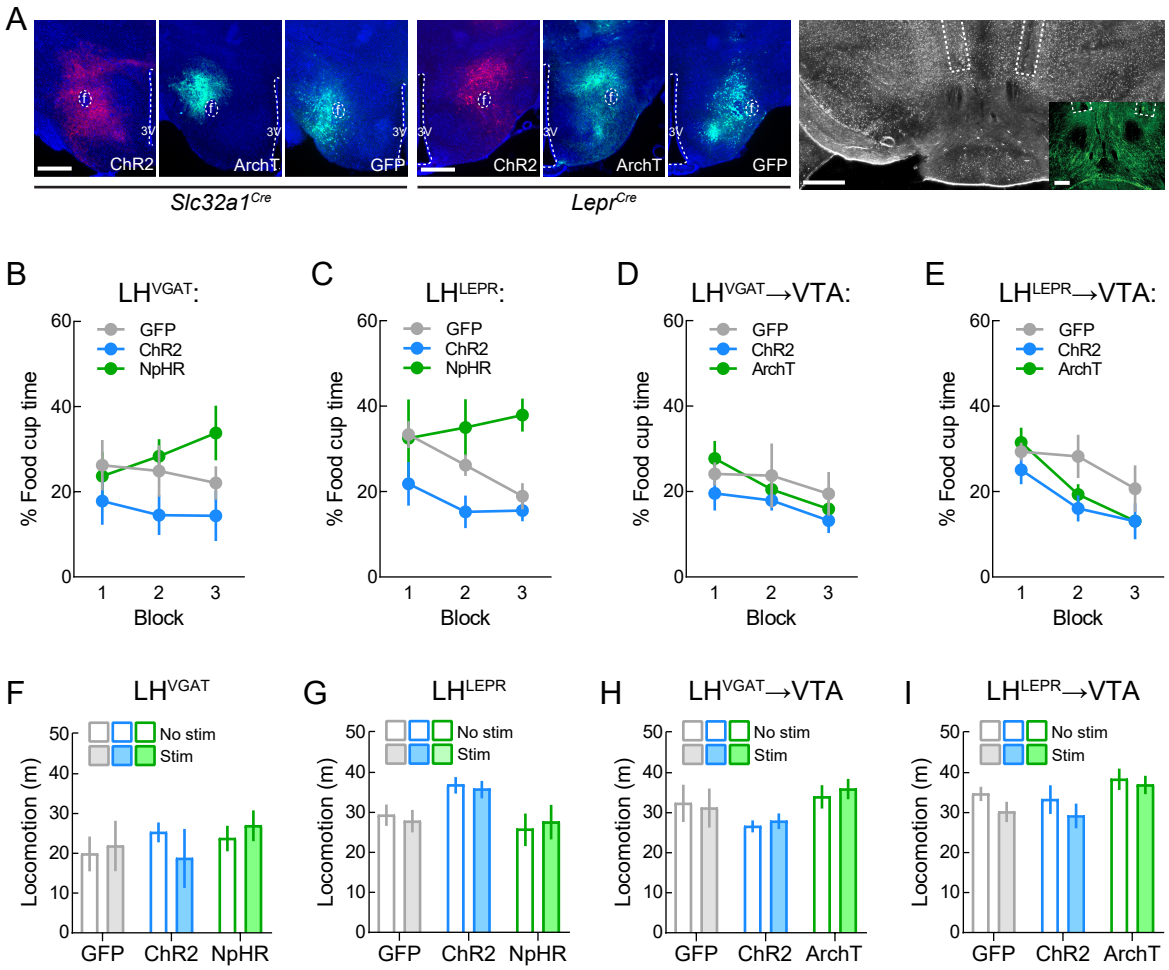

Figure S3. Pre-CS responding and locomotion of mice used in Pavlovian conditioning experiments with optogenetics. *Related to Figure 4.*

(A) *Left,* representative images of ChR2, ArchT, and GFP in the LH of *Slc32a1*^{Cre} and *Lepr*^{Cre} mice; Scale bars = 500 µm. *Right*, representative image of optical fiber location above the VTA; Scale bars = 500 µm and (inset) 200 µm.

(B) Average food cup responding in the pre-CS period for LH^{VGAT} mice (n = 6 LH^{VGAT}:GFP mice, n = 6 LH^{VGAT}:ChR2 mice, and n = 8 LH^{VGAT}:NpHR mice, two-way mixed-model ANOVA group × block interaction, p = 0.20).

(C) Average food cup responding in the pre-CS period for LH^{LEPR} mice (n = 8 LH^{LEPR}:GFP mice, n = 5 LH^{LEPR}:ChR2 mice, and n = 5 LH^{LEPR}:NpHR mice, two-way mixed-model ANOVA group × block interaction, p = 0.14).

(D) Average food cup responding in the pre-CS period for LH^{VGAT}→VTA mice (n = 6 LH^{VGAT}:GFP→VTA mice, n = 7 LH^{VGAT}:ChR2→VTA mice, and n = 8 LH^{VGAT}:ArchT→VTA mice, two-way mixed-model ANOVA group × block interaction, p = 0.57).

(E) Average food cup responding in the pre-CS period for LH^{LEPR}→VTA mice (n = 7 LH^{LEPR}:GFP→VTA mice, n = 7 LH^{LEPR}:ChR2→VTA mice, and n = 8 LH^{LEPR}:ArchT→VTA mice, two-way mixed-model ANOVA group × block interaction, p = 0.29).

(F) Mice connected to patch cords were placed in novel open field chambers for 30 min and received photostimulation or photoinhibition in alternating, counterbalanced 3 min epochs. LH^{VGAT} neuronal activation or inhibition did not evoke changes in locomotor activity (n = 6 LH^{VGAT}:GFP mice, n = 6 LH^{VGAT}:ChR2 mice, and n = 8 LH^{VGAT}:NpHR mice, two-way mixed-model ANOVA group × stimulation interaction, p = 0.24).

(G) LH^{LEPR} activation or inhibition did not trigger changes in locomotor activity (n = 8 LH^{LEPR}:GFP mice, n = 5 LH^{LEPR}:ChR2 mice, and n = 5 LH^{LEPR}:NpHR mice, two-way mixed-model ANOVA group × stimulation interaction, p = 0.59).

(H) LH^{VGAT}→VTA activation or inhibition did not evoke changes in locomotor activity (n = 6 LH^{VGAT}:GFP→VTA mice, n = 7 LH^{VGAT}:ChR2→VTA mice, and n = 8 LH^{VGAT}:ArchT→VTA mice, two-way mixed-model ANOVA group × stimulation interaction, p = 0.76).

(I) No changes in locomotor activity were observed during LH^{LEPR}→VTA activation or inhibition (n = 7 LH^{LEPR}:GFP→VTA mice, n = 7 LH^{LEPR}:ChR2→VTA mice, and n = 8 LH^{LEPR}:ArchT→VTA mice, two-way mixed-model ANOVA group × stimulation interaction, p = 0.41).

Data represented as mean ± SEM.

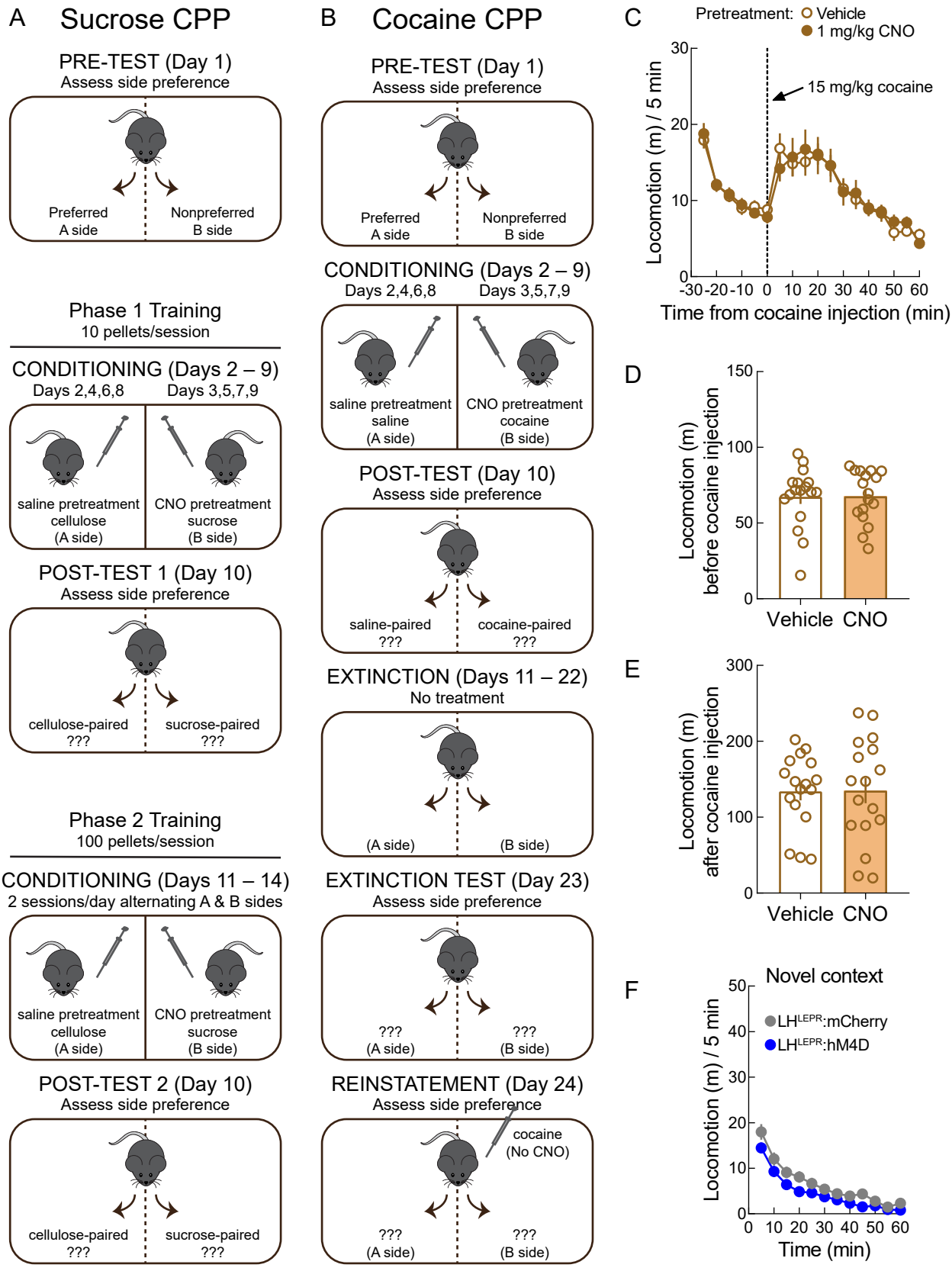
A
Sucrose CPP
PRE-TEST (Day 1)
Assess side preference
Preferred
A side
Nonpreferred
B side
Phase 1 Training
10 pellets/session
CONDITIONING (Days 2 – 9)
Days 2,4,6,8
Days 3,5,7,9
saline pretreatment
cellulose
(A side)
CNO pretreatment
sucrose
(B side)
POST-TEST 1 (Day 10)
Assess side preference
cellulose-paired
???
sucrose-paired
???
Phase 2 Training
100 pellets/session
CONDITIONING (Days 11 – 14)
2 sessions/day alternating A & B sides
saline pretreatment
cellulose
(A side)
CNO pretreatment
sucrose
(B side)
POST-TEST 2 (Day 10)
Assess side preference
cellulose-paired
???
sucrose-paired
???
B
Cocaine CPP
PRE-TEST (Day 1)
Assess side preference
Preferred
A side
Nonpreferred
B side
CONDITIONING (Days 2 – 9)
Days 2,4,6,8
Days 3,5,7,9
saline pretreatment
saline
(A side)
CNO pretreatment
cocaine
(B side)
POST-TEST (Day 10)
Assess side preference
saline-paired
???
cocaine-paired
???
EXTINCTION (Days 11 – 22)
No treatment
(A side)
(B side)
EXTINCTION TEST (Day 23)
Assess side preference
???
(A side)
???
(B side)
REINSTATEMENT (Day 24)
Assess side preference
cocaine
(No CNO)
???
(A side)
???
(B side)
C
Pretreatment: Vehicle
1 mg/kg CNO
15 mg/kg cocaine
Locomotion (m) / 5 min
Time from cocaine injection (min)
D
Locomotion (m)
before cocaine injection
Vehicle
CNO
E
Locomotion (m)
after cocaine injection
Vehicle
CNO
F
Novel context
LHLEPR:mCherry
LHLEPR:hM4D
Locomotion (m) / 5 min
Time (min)

Figure S4. Schematics and control experiments for conditioned place preference and locomotor sensitization. ***Related to Figure 5.***

(A) Schematic representation of the sucrose conditioned place preference experiment. Following the pre-test session, mice received "phase 1 training," consisting of one training session per day for eight days with the center door closed and only one chamber accessible; these sessions were not recorded. On even days, mice received an injection of saline (i.p.) and were immediately placed on 'side A' for 30 min with access to ten 20-mg calorie-free, flavorless cellulose pellets. During odd days, mice received a 1 h pretreatment with 1 mg/kg CNO (i.p.) and were placed on 'side B' for 30 min with access to ten 20-mg sucrose pellets. After these eight conditioning sessions, a 15-min post-test was performed. Mice then received "phase 2 training," which was similar to phase 1 training except that 100 sucrose pellets were offered during sucrose training sessions. After these eight conditioning sessions, a second 15-min post-test was performed.
(B) Schematic representation of the cocaine conditioned place preference experiment. During conditioning, pretreatment with CNO occurred 1 h before cocaine administration. After the pre-test session, mice received one training session per day for eight days with the center door closed and only one chamber accessible. On even days, mice received an injection of saline (i.p.) and were immediately placed on 'side A' for 30 min. During odd days, mice received a 1 h pretreatment with 1 mg/kg CNO (i.p.) before injection with 15 mg/kg cocaine (i.p.) and placed on 'side B' for 30 min. On day 10, untreated mice were placed back in the testing arena with free access to both chambers. For CPP extinction, mice were placed into the apparatus with access to both chambers for twelve 30-min sessions. Then, a 15-min extinction test was performed to verify a decrease in group preference for the cocaine-paired side to under 50%. The next day, for CPP reinstatement, 15 mg/kg cocaine (i.p) was injected immediately prior to placing the mice in the apparatus with free access to both chambers.
(C) Time course of novel open field locomotion and locomotion induced by cocaine (15 mg/kg, i.p.) in wild type mice (n = 17 mice per group). CNO (1 mg/kg, i.p.) was administered 30 min prior to being placed in the novel chamber, and thus 60 min prior to cocaine administration.
(D) CNO did not affect novel open field locomotion (Student's unpaired t-test, $t(32) = 0.065$, $p = 0.95$) or **(E)** cocaine-induced locomotion ($t(32) = 0.052$, $p = 0.96$) in wild-type mice.

(F) Chemogenetic inhibition of LH^{LEPR} neurons did not affect novel open field locomotion (n = 10 mice per group). Two-way mixed-model ANOVA revealed a significant main effect of time ($F(11,198) = 85.39$, $p < 0.0001$) but not group ($p = 0.10$) or group × time interaction ($p = 0.48$). Data represented as mean ± SEM.