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Supplemental information

Complementary lateral hypothalamic populations resist hunger pressure to balance nutritional and social needs

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Supplemental information

Supplemental Figures 1-8

Figure S1

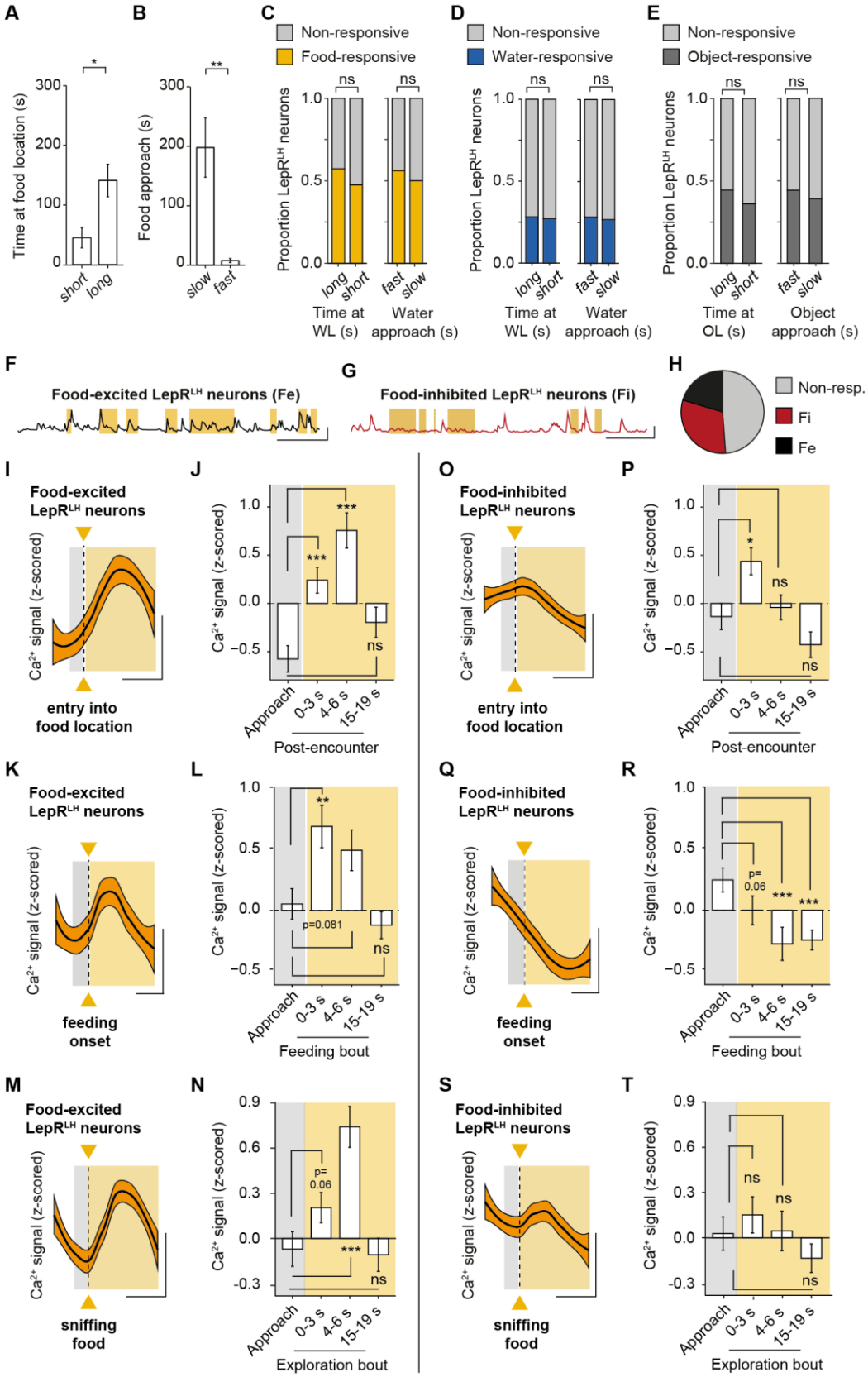
A
B
C
D
E
Time at food location (s)
Food approach (s)
short
long
slow
fast
Non-responsive
Food-responsive
Water-responsive
Object-responsive
Proportion LepR^LH neurons
Time at WL (s)
Water approach (s)
Time at OL (s)
Object approach (s)
ns
F
G
H
Food-excited LepR^LH neurons (Fe)
Food-inhibited LepR^LH neurons (Fi)
Non-resp.
Fi
Fe
I
J
O
P
Food-excited LepR^LH neurons
Food-inhibited LepR^LH neurons
Ca2+ signal (z-scored)
entry into food location
Approach
0-3 s
4-6 s
15-19 s
Post-encounter
K
L
Q
R
feeding onset
Feeding bout
p=0.081
p= 0.06
M
N
S
T
sniffing food
Exploration bout

Figure S1. Encoding of feeding-related behaviors by $LepR^{LH}$ neurons. Related to Fig. 1.
(A,B) Foraging performance parameters.
(A) Time spent at food location (FL) by mice spending a "short" and those spending a "long" time at FL. Separation based on daily average time spent at FL. n = 5 mice, 3 imaging days, * P = 0.021, Student's t-test.
(B) Time to approach the FL by mice approaching "fast" and those approaching "slowly". Separation based on daily average time to approach the FL. n = 5 mice, 3 imaging days, ** P = 0.0094, Mann-Whitney U test.
(C) Proportion of food-responsive neurons. Left: in mice spending a long or short time at water location (WL); right: in mice exhibiting fast and slow water approach. Total: n = 510; food-responsive: n = 261 neurons. 5 mice, 3 imaging days, ns, Pearson's $\chi 2$ test.
(D) Proportion of water-responsive neurons in mice according to water seeking performance. n = 240 neurons, as in (C).
(E) Proportion of object-responsive neurons in mice according to object exploration performance. n = 254 neurons, as in (C).
(F,G) Representative Ca^{2+} traces of food-excited (Fe) and food-inhibited (Fi) neurons. Scale bar = 25 s.
(H) Proportion of food-responsive neurons. Fi: n = 157; Fe: n = 104 neurons. 5 mice, 3 imaging days.
(I-N) Ca^{2+} signal of food-excited neurons around the onset of feeding-related behaviors, n = 43 neurons. ns P > 0.05, ** P < 0.0098, *** P < 0.001, Mann-Whitney U test.
(O-T) Ca^{2+} signal of food-inhibited neurons around the onset of feeding-related behaviors, n = 51 neurons. ns P > 0.05, * P = 0.022, *** P < 0.001, Mann-Whitney U test.
(I-T) Scale bar: x = 5 s, y = 0.4 SD.
Data shown are mean $\pm$ SEM. ns – not significant, * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure S2

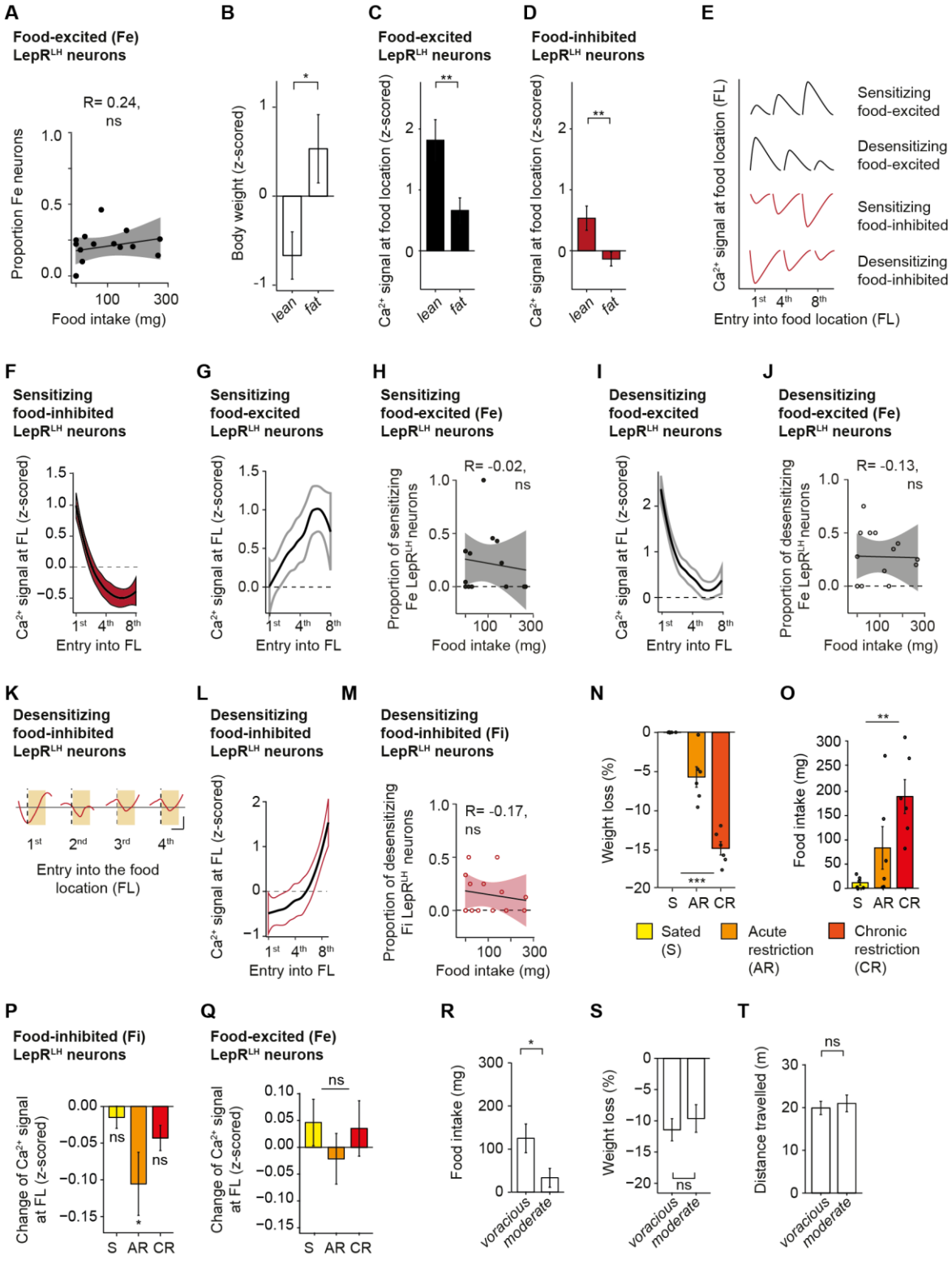
A
Food-excited (Fe)
LepR^LH neurons
R= 0.24,
ns
Proportion Fe neurons
Food intake (mg)
B
Body weight (z-scored)
lean
fat
C
Food-excited
LepR^LH neurons
Ca2+ signal at food location (z-scored)
D
Food-inhibited
LepR^LH neurons
E
Ca2+ signal at food location (FL)
Sensitizing food-excited
Desensitizing food-excited
Sensitizing food-inhibited
Desensitizing food-inhibited
Entry into food location (FL)
F
Sensitizing food-inhibited LepR^LH neurons
Ca2+ signal at FL (z-scored)
Entry into FL
G
Sensitizing food-excited LepR^LH neurons
H
Sensitizing food-excited (Fe) LepR^LH neurons
R= -0.02,
ns
Proportion of sensitizing Fe LepR^LH neurons
I
Desensitizing food-excited LepR^LH neurons
J
Desensitizing food-excited (Fe) LepR^LH neurons
R= -0.13,
ns
Proportion of desensitizing Fe LepR^LH neurons
K
Desensitizing food-inhibited LepR^LH neurons
Entry into the food location (FL)
L
Desensitizing food-inhibited LepR^LH neurons
M
Desensitizing food-inhibited (Fi) LepR^LH neurons
R= -0.17,
ns
Proportion of desensitizing Fi LepR^LH neurons
N
Weight loss (%)
S AR CR
O
Food intake (mg)
Sated (S)
Acute restriction (AR)
Chronic restriction (CR)
P
Food-inhibited (Fi) LepR^LH neurons
Change of Ca2+ signal at FL (z-scored)
Q
Food-excited (Fe) LepR^LH neurons
R
Food intake (mg)
voracious
moderate
S
Weight loss (%)
T
Distance travelled (m)

Figure S2. State-dependent food-elicited inhibition by LepR^{LH} neurons. Related to Fig. 1.
(A) Proportion of food-excited (Fe) neurons and food intake. n = 104 neurons, 5 mice, 3 imaging days, ns, Pearson's correlation.
(B) Weight categories, * P = 0.047, Student's t-test.
(C) Ca^{2+} signal at food location (FL) in Fe neurons, fat: n = 44, lean: n = 15 neurons, ** P = 0.0022, Mann-Whitney U test.
(D) As in (C) for food-inhibited (Fi) neurons, fat: n = 44, lean: n = 25 neurons, ** P = 0.0024.
(E) Schematic illustrating types of food-related responses of stimulus-responsive neurons.
(F) Sensitizing food-inhibited (Fi) population: Ca^{2+} signal during consecutive food location (FL) visits, n = 33 neurons, 5 mice, 3 imaging days, local fit.
(G,H) Sensitizing food-excited (Fe) population, n = 28 neurons, 5 mice, 3 imaging days. (G) Ca^{2+} signal as in (F). (H) Proportion and food intake, ns, Pearson's correlation.
(I,J) Desensitizing Fe neurons, n = 31 neurons, 5 mice. (I) Ca^{2+} signal as in (F). (J) As in (H), ns.
(K-M) Desensitizing Fi population, n = 11 neurons, 5 mice. (K) Example of Ca^{2+} signal during consecutive FL visits, scale bar: x = 5 s, y = 0.2 SD. (L) Ca^{2+} signal as in (F). (M) As in (H), ns.
(N) Weight loss across states. n = 6 mice, $F(2, 5) = 44.8$, *** P < 0.001, ANOVA.
(O) Food intake across states. n = 6 mice, $F(2, 5) = 8.2$, ** P = 0.0079, ANOVA.
(P) Change of Ca^{2+} signal among Fi population, overall: * P = 0.042, $F(2, 154) = 3.24$, ANOVA; *post hoc* comparisons: (S) ns, (AR) * P = 0.013, (CR) ns.
(Q) Change of Ca^{2+} signal among Fe population. ns, $F(2, 101) = 0.66$, as in (P).
(R-T) Feeding, body weight, and locomotion in "voracious" and "moderate" feeders following food restriction. Separation based on daily average food consumption, n = 5 mice, 2 imaging days.
(R) Food intake, * P = 0.028, Student's t-test. (S) Weight loss, ns. (T) Locomotion, ns.
Data shown are mean ± SEM. ns – not significant, * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure S3

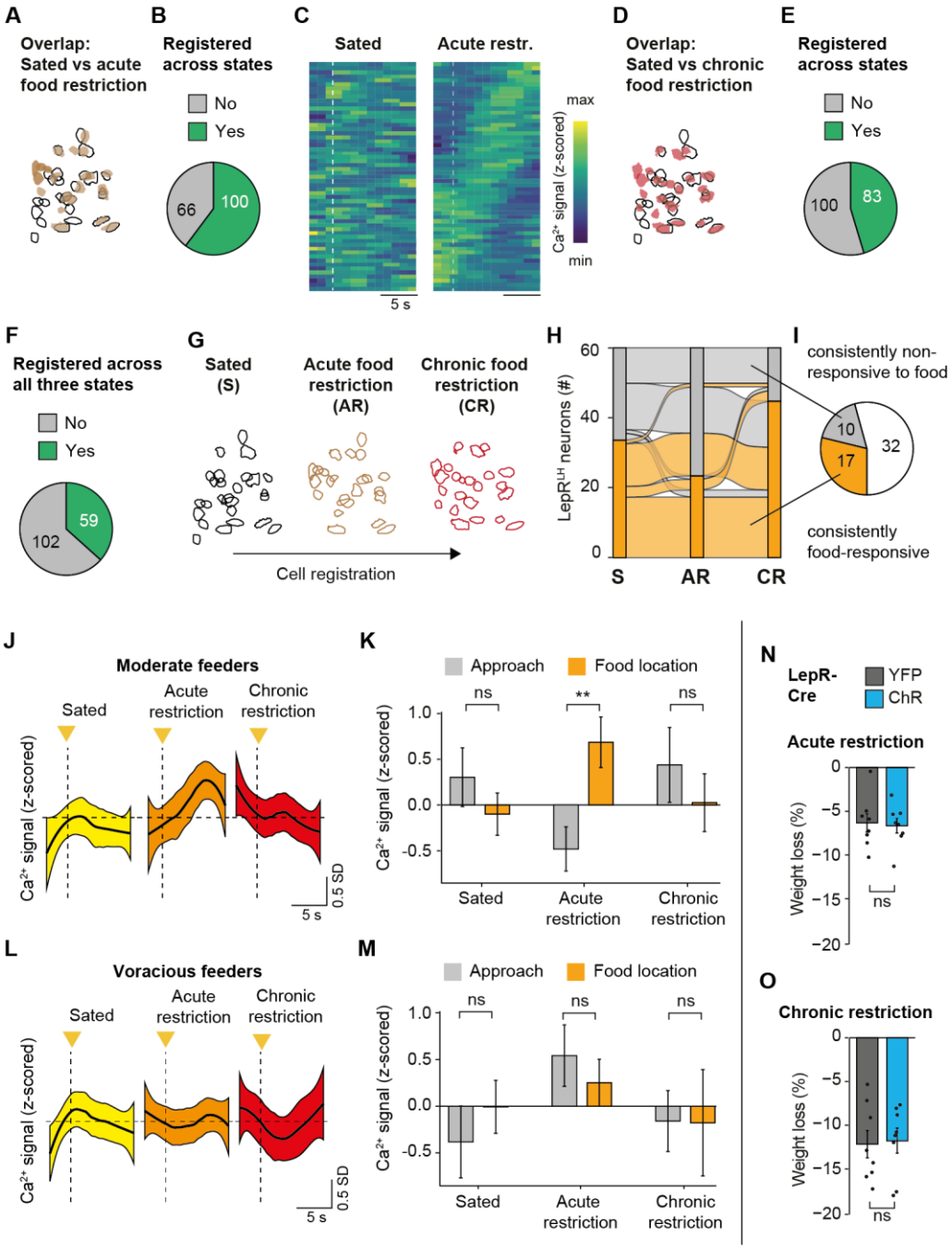
A
Overlap:
Sated vs acute
food restriction
B
Registered
across states
No
Yes
66
100
C
Sated
Acute restr.
max
min
Ca2+ signal (z-scored)
5 s
D
Overlap:
Sated vs chronic
food restriction
E
Registered
across states
No
Yes
100
83
F
Registered across
all three states
No
Yes
102
59
G
Sated
(S)
Acute food
restriction
(AR)
Chronic food
restriction
(CR)
Cell registration
H
LepRLH neurons (#)
60
40
20
0
S
AR
CR
I
consistently non-
responsive to food
10
17
32
consistently
food-responsive
J
Moderate feeders
Sated
Acute
restriction
Chronic
restriction
Ca2+ signal (z-scored)
0.5 SD
5 s
K
Approach
Food location
ns
**
ns
1.0
0.5
0.0
-0.5
Ca2+ signal (z-scored)
Sated
Acute
restriction
Chronic
restriction
L
Voracious feeders
Sated
Acute
restriction
Chronic
restriction
Ca2+ signal (z-scored)
0.5 SD
5 s
M
Approach
Food location
ns
ns
ns
1.0
0.5
0.0
-0.5
Ca2+ signal (z-scored)
Sated
Acute
restriction
Chronic
restriction
N
LepR-
Cre
YFP
ChR
Acute restriction
Weight loss (%)
0
−5
−10
−15
−20
ns
O
Chronic restriction
Weight loss (%)
0
−5
−10
−15
−20
ns

Figure S3. State-dependent food-related excitation of LepR^{LH} neurons restrains feeding. Related to Fig. 1.

(A-C) Registration of cells recorded in the sated state and following acute food restriction.
(A) Overlap of representative cell maps.
(B) Proportion and (C) heatmap of food-elicited responses of cells registered across two states, dashed line indicates entry into the food location, sorted based on peak activity following acute food restriction.
(D,E) Registration of cells recorded in sated state and following chronic food restriction.
(F,G) Registration of cells recorded in three states: sated state, following acute food restriction, and following chronic food restriction.
(H,I) Change of food responses of individual neurons across all three states, n = 59 cells, 5 mice.
(H) Alluvial diagram. (I) Proportion of consistently food-responsive neurons.
(J,K) Food-elicited responses of cells that were food-inhibited during sated state in "moderate" feeders. (J) Local fit, n = 11 neurons, 5 mice. (K) Ca^{2+} signal during approach and after entry into food location (FL), ns $P > 0.05$, ** $P = 0.0097$, Mann-Whitney U test.
(L,M) Food-elicited responses of cells that were food-inhibited during sated state in "voracious" feeders. (L) Local fit, n = 9 neurons, 5 mice. (M) Ca^{2+} signal, ns.
(N,O) Weight loss preceding optogenetic stimulation. YFP: n = 8 mice; ChR: n = 8 mice.
(N) Following acute food restriction, ns, Student's t-test. (O) Following chronic food restriction, ns.
Data shown are mean $\pm$ SEM. ns – not significant, * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Figure S4

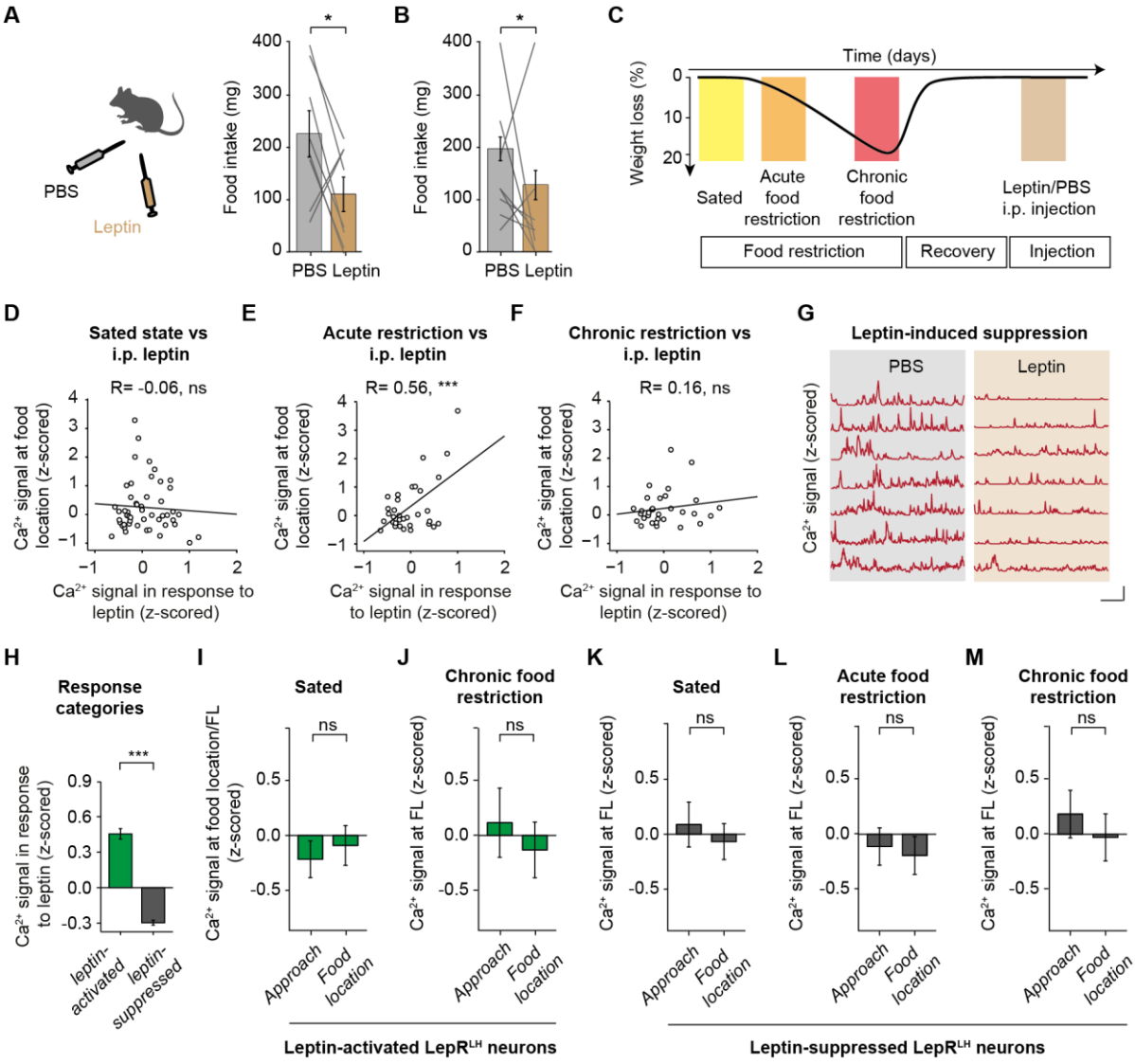

Figure S4. Relationship between food encoding and leptin sensitivity of LepR^{LH} neurons. Related to Fig. 2.

(A,B) Food intake 30 min (A) and 120 min (B) following i.p. leptin injection, paired Wilcoxon signed-rank test, n = 7 mice.

(C) Experimental regime.

(D-F) Response to leptin injection and Ca^{2+} signal at food location (FL) following (D) *ad libitum* access to food, n = 49 neurons, ns, (E) acute food restriction, n = 33 neurons,

*** P < 0.001, or (F) chronic food restriction, n=32 neurons, 5 mice, ns, Pearson's correlation.

(G) Representative Ca^{2+} traces. Scale bar: x = 25 s, y = 0.5 SD.

(H) Leptin response categories, *** P < 0.001, Mann-Whitney U test.

(I,J) Food-elicited responses of leptin-activated neurons recorded following (I) *ad libitum* access to food, n = 23 neurons, or (J) chronic food restriction, n = 12 neurons, 5 mice, ns, paired Wilcoxon signed-rank test.

(K-M) Food-elicited responses of leptin-suppressed neurons recorded following (K) *ad libitum* access to food, n = 26 neurons, (L) acute food restriction, n = 20 neurons, (M) chronic food restriction, n = 20 neurons, 5 mice, ns, paired Wilcoxon signed-rank test.

Data shown are mean ± SEM. ns – not significant, * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure S5

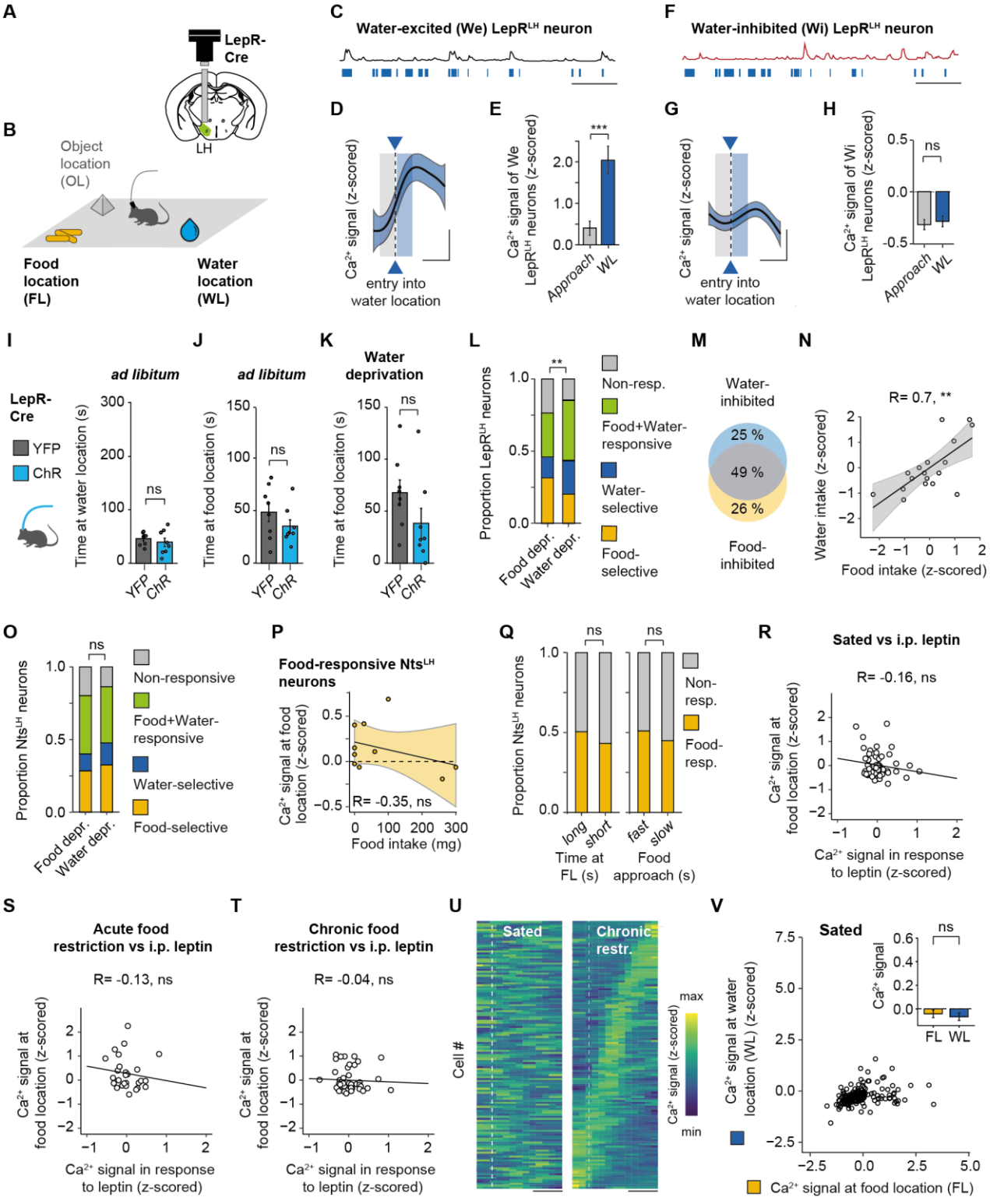
A
LepR-Cre
LH
B
Object location (OL)
Food location (FL)
Water location (WL)
C
Water-excited (We) LepR^LH neuron
D
Ca2+ signal (z-scored)
entry into water location
E
Ca2+ signal of We LepR^LH neurons (z-scored)
***
Approach
WL
F
Water-inhibited (Wi) LepR^LH neuron
G
Ca2+ signal (z-scored)
entry into water location
H
Ca2+ signal of Wi LepR^LH neurons (z-scored)
ns
Approach
WL
I
ad libitum
LepR-Cre
YFP
ChR
Time at water location (s)
ns
YFP
ChR
J
ad libitum
Time at food location (s)
ns
YFP
ChR
K
Water deprivation
Time at food locaiton (s)
ns
YFP
ChR
L
Proportion LepR^LH neurons
**
Food depr.
Water depr.
Non-resp.
Food+Water-responsive
Water-selective
Food-selective
M
Water-inhibited
25 %
49 %
26 %
Food-inhibited
N
R= 0.7, **
Water intake (z-scored)
Food intake (z-scored)
O
Proportion Nts^LH neurons
ns
Food depr.
Water depr.
Non-responsive
Food+Water-responsive
Water-selective
Food-selective
P
Food-responsive Nts^LH neurons
Ca2+ signal at food location (z-scored)
R= -0.35, ns
Food intake (mg)
Q
Proportion Nts^LH neurons
ns
ns
Non-resp.
Food-resp.
long
short
fast
slow
Time at FL (s)
Food approach (s)
R
Sated vs i.p. leptin
R= -0.16, ns
Ca2+ signal at food location (z-scored)
Ca2+ signal in response to leptin (z-scored)
S
Acute food restriction vs i.p. leptin
R= -0.13, ns
Ca2+ signal at food location (z-scored)
Ca2+ signal in response to leptin (z-scored)
T
Chronic food restriction vs i.p. leptin
R= -0.04, ns
Ca2+ signal at food location (z-scored)
Ca2+ signal in response to leptin (z-scored)
U
Sated
Chronic restr.
Cell #
max
Ca2+ signal (z-scored)
min
V
Sated
ns
Ca2+ signal
FL
WL
Ca2+ signal at water location (WL) (z-scored)
Ca2+ signal at food location (FL)

Figure S5. Need-dependent and need-resistant regulation of drinking by $LepR^{LH}$ and Nts^{LH} neurons. Related to Figs. 3 and 4.

(A) Schema of lens and microendoscope placement.

(B) Enclosure (30 x 50 cm) with free access to a food (FL), water (WL), and object (OL) location.

(C-E) Water-excited (We) $LepR^{LH}$ neurons following acute water deprivation.

(C) Representative example. Scale bar: x = 25 s, y = 0.4 SD. (D) Ca^{2+} signal around entry (dashed line) into WL, GAM fit. Scale bar: x = 5 s, y = 0.4 SD, n = 34 neurons, 5 mice. (E) Ca^{2+} signal during approach and after entry into WL. *** P < 0.001, paired Wilcoxon signed-rank test.

(F-H) As in (C-E) for water-inhibited (Wi) $LepR^{LH}$ neurons. Scale bar: x = 25 s, y = 0.2 SD. (F) As in C. (G) As in (D), n = 65 neurons, 5 mice. (H) Same as (E), ns.

(I-K) Time at WL and FL during optogenetic activation following *ad libitum* access to water or acute water deprivation. YFP: n = 8 mice; ChR: n = 8 mice. ns, Mann-Whitney U test.

(L) Stimulus selectivity of $LepR^{LH}$ population, ** P = 0.0048, Pearson's $\chi 2$ test.

(M) Functional overlap between water-inhibited and food-inhibited $LepR^{LH}$ neurons recorded following water or food deprivation and registered across both states, n = 53 neurons.

(N) Food and water intake. Cumulative intake over 7 h, n = 18 mice, ** P = 0.0013, Pearson's correlation.

(O) Stimulus selectivity of Nts^{LH} population. ns, Pearson's $\chi 2$ test.

(P) Food intake and average activity of food-responsive Nts^{LH} neurons. n = 307 neurons, 4 mice, 3 imaging days. ns, Pearson's correlation.

(Q) Proportion of food-responsive Nts^{LH} neurons. Left: in mice spending a long and short time at food location (FL); right: in mice exhibiting fast and slow food approach. n = 307 neurons, 4 mice, 3 imaging days. ns, Pearson's $\chi 2$ test.

(R-T) Response to leptin injection and Ca^{2+} signal at FL following (R) *ad libitum* access to food, n = 55 neurons, (S) acute food restriction, n = 27 neurons, or (T) chronic food restriction, n = 48 neurons, 5 mice. ns, Pearson's correlation.

(U) Heatmap of water-elicited responses of cells registered across sated and acute food restriction state, sorted by peak response following chronic food restriction, dashed line indicates entry into the food location, n = 135 neurons, scale bar = 5 s.

(V) Ca^{2+} signal of individual Nts^{LH} neurons in response to food or water in sated state. n = 277 neurons. ns, paired Wilcoxon signed-rank test.

Data shown are mean $\pm$ SEM. ns – not significant, * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure S6

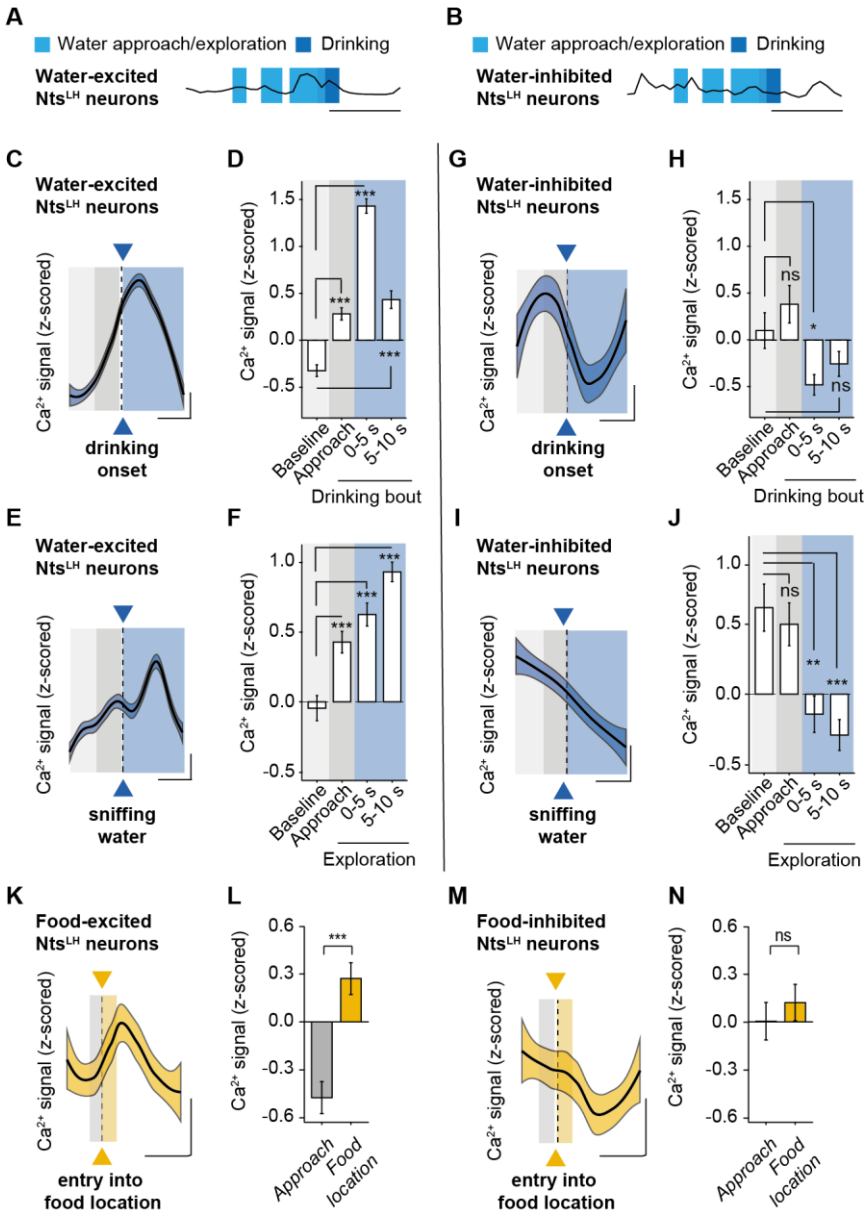

Figure S6. Encoding of drinking-related behaviors by Nts^{LH} neurons. Related to Fig. 5.
(A,B) Representative Ca^{2+} traces. Scale bar = 25 s.
(C-F) Ca^{2+} signal of water-excited neurons around the onset of drinking-related behaviors.
(C,D) n = 48 neurons, *** P < 0.001. (E,F) n = 64 neurons, *** P < 0.001, Mann-Whitney U test.
(G-J) Ca^{2+} signal of water-inhibited neurons around the onset of drinking-related behaviors.
(G,H) n = 11 neurons, ns P > 0.05, * P = 0.028. (I,J) n = 27 neurons, 2 mice. n = 22 neurons, ns P > 0.05, ** P = 0.0011, *** P < 0.001, Mann-Whitney U test.
(K-N) Food-responsive Nts^{LH} neurons.
(K,L) Food-excited neurons. (K) Ca^{2+} signal around entry (dashed line) into FL, local fit, Fe: n = 59, 2 mice. (L) Ca^{2+} signal before and after entry into FL, ** P = 0.0095, paired Wilcoxon signed-rank test.
(M,N) Food-inhibited neurons. (M) As in (K), n = 35 neurons, 2 mice. (N) As in (L), ns.
(C-J) Scale bar: x = 5 s, y = 0.5 SD. (K,M) Scale bar: x = 5 s, y = 0.2 SD.
Data shown are mean ± SEM. ns – not significant, * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure S7

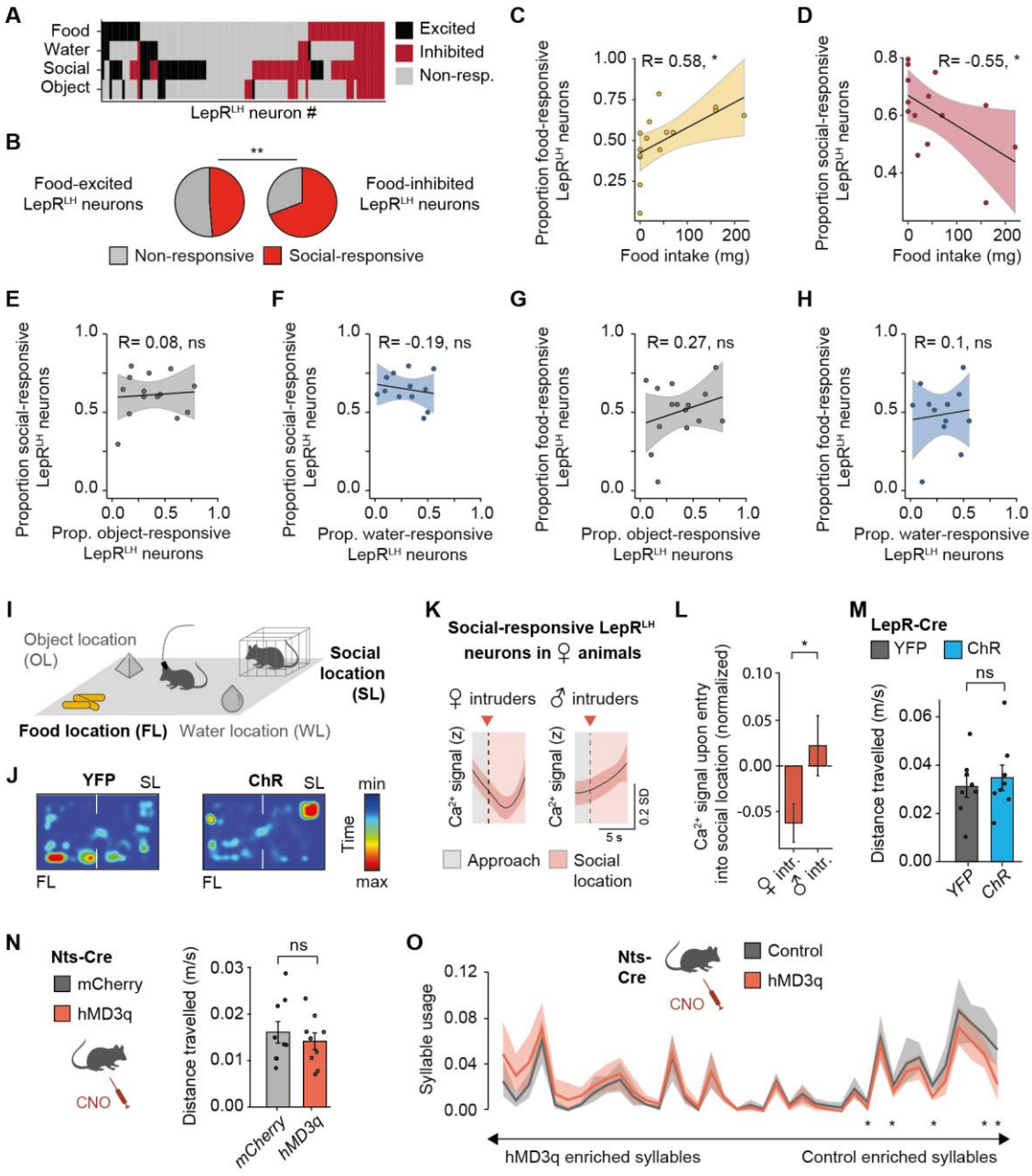
A
Food
Water
Social
Object
LepR^{LH} neuron #
Excited
Inhibited
Non-resp.
B
**
Food-excited LepR^{LH} neurons
Food-inhibited LepR^{LH} neurons
Non-responsive
Social-responsive
C
R= 0.58, *
Proportion food-responsive LepR^{LH} neurons
Food intake (mg)
D
R= -0.55, *
Proportion social-responsive LepR^{LH} neurons
Food intake (mg)
E
R= 0.08, ns
Proportion social-responsive LepR^{LH} neurons
Prop. object-responsive LepR^{LH} neurons
F
R= -0.19, ns
Proportion social-responsive LepR^{LH} neurons
Prop. water-responsive LepR^{LH} neurons
G
R= 0.27, ns
Proportion food-responsive LepR^{LH} neurons
Prop. object-responsive LepR^{LH} neurons
H
R= 0.1, ns
Proportion food-responsive LepR^{LH} neurons
Prop. water-responsive LepR^{LH} neurons
I
Object location (OL)
Social location (SL)
Food location (FL)
Water location (WL)
J
YFP
SL
ChR
SL
min
Time
max
FL
FL
K
Social-responsive LepR^{LH} neurons in ♀ animals
♀ intruders
♂ intruders
Ca^{2+} signal (z)
Ca^{2+} signal (z)
0.2 SD
5 s
Approach
Social location
L
*
Ca^{2+} signal upon entry into social location (normalized)
♀ intr.
♂ intr.
M
LepR-Cre
YFP
ChR
ns
Distance travelled (m/s)
YFP
ChR
N
Nts-Cre
mCherry
hMD3q
CNO
ns
Distance travelled (m/s)
mCherry
hMD3q
O
Nts-Cre
CNO
Control
hMD3q
Syllable usage
hMD3q enriched syllables
Control enriched syllables

Figure S7. Contribution of $LepR^{LH}$ and Nts^{LH} neurons to social behaviors. Related to Figs. 6 and 7.

(A) Stimulus selectivity of individual $LepR^{LH}$ neurons across three states (sated, acute food restriction, chronic food restriction), n = 357 neurons.

(B) Proportion of social-responsive neurons, ** P = 0.0017, Pearson's $\chi 2$ test.

(C,D) Food intake and proportion of (C) food-responsive neurons, * P = 0.023, or (D) social-responsive neurons, * P = 0.041, Pearson's correlation.

(E,F) Proportion of social-responsive and (E) object-responsive, ns, or (F) water-responsive neurons, ns, Pearson's correlation.

(G,H) Proportion of food-responsive and (G) object-responsive, ns, or (H) water-responsive neurons, ns, Pearson's correlation.

(I) Enclosure (30 x 50 cm) with free access to a food (FL), water (WL), object (OL), and social location (SL).

(J) Representative heatmap of zone occupation in the free access enclosure during optogenetic activation of $LepR^{LH}$ neurons following acute food restriction.

(K) Ca^{2+} signal around entry (dashed line) into SL; female intruders' session: n = 21 neurons, 5 mice; male intruders' session: n = 23 neurons, 6 mice, GAM fit.

(L) Ca^{2+} signal upon entry into social location, normalized to approach phase. * P = 0.0357, Mann-Whitney U test.

(M) Optogenetic activation and locomotion. YFP: n = 8 mice; ChR: n = 8 mice. ns, Student's t-test.

(N,O) Nts^{LH} neurons. Chemogenetic activation, control: n = 9 mice; hM3Dq: n = 9 mice.

(N) Locomotion. ns, Student's t-test.

(O) Behavioral syllable usage in social zone sorted by group. Average syllable usage per animal calculated using bootstrap estimation. Bootstrap estimates (n = 1000): mean indicated with solid lines, 95% confidence interval indicated with fainter lines. * P < 0.05, z-test on bootstrapped syllable usage distributions (one test/syllable) with Hochberg correction.

Data shown are mean $\pm$ SEM. ns – not significant, * P < 0.05, ** P < 0.01, *** P < 0.001.

Figure S8

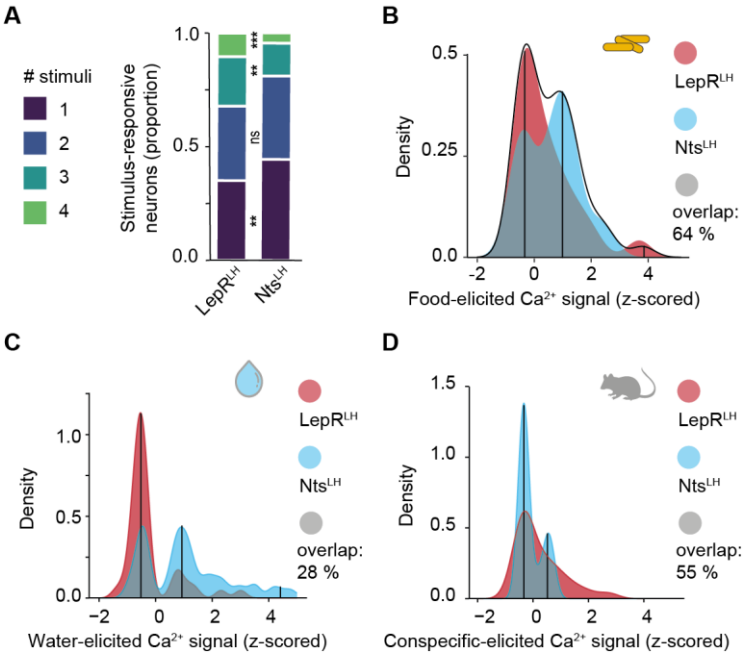

Figure S8. Multimodal responses of LepR^{LH} and Nts^{LH} neurons to nutritional and non-nutritional rewards. Related to Figs. 3, 4 and 7.

(A) Selectivity for four stimuli (food, water, conspecific, object) across three states (sated, acute food restriction, chronic food restriction). LepR^{LH}, n = 443; Nts^{LH}, n = 717 neurons.

(B) Population density of food-elicited responses from food-responsive neurons registered across sated and acute food restriction state, n = 96 neurons, P = 0.044, excess mass test with kernel density estimation.

(C) Population density of water-elicited responses from water-modulated neurons, n = 130 neurons, P < 0.001, as in (B).

(D) Population density of conspecific-elicited responses from social-responsive neurons, n = 161 neurons, P = 0.018, as in (B).