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## Supplemental Information

# Depression of Accumbal to Lateral Hypothalamic Synapses Gates Overeating

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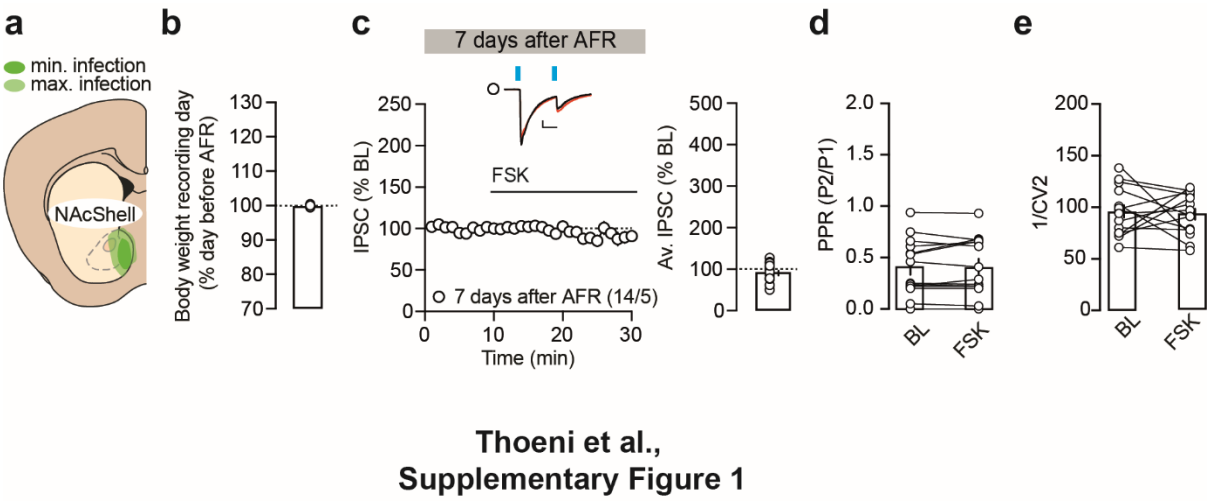



**Figure S1. Related to Figure 1.FSK induced i-LTP is absent 7 days after an acute food restriction episode.**

(**a**) Schematic representing the maximum and minimum infection with the AAV-DIO-ChR2 from animal that were kept in the study.
(**b**) Body weight from mice tested 7 days after a single 24h food restriction episode. Mice returned to their baseline weight.
(**c**) IPSC expressed as a percent of baseline (BL). Bath application of forskolin (FSK) was unable to potentiate the inhibitory transmission.
(**d**) PPR ratio was not changed by FSK.
(**e**) The coefficient of variation was not affected by FSK

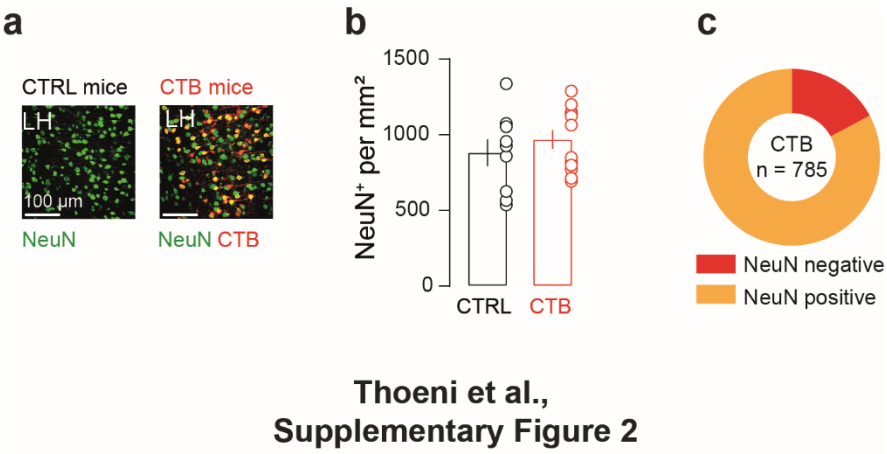



**Figure S2. Related to Figure 2. Quantification of NeuN expression in mice injected with CTB in the LH.**

(**a**) To control for CTB-induced lesion in the LH as a limitation of our strategy, we quantified the expression of NeuN in control or CTB-injected mice.
(**b**) NeuN expression density was similar in the LH of control and CTB-injected mice: $t(19) = 0.8260$, $p=0.4190$.
(**c**) 82.8% of CTB positive colocalized with NeuN, further demonstrating that CTB did induce a lesion in 10 days after the injection.
Plots show mean ± SEM.

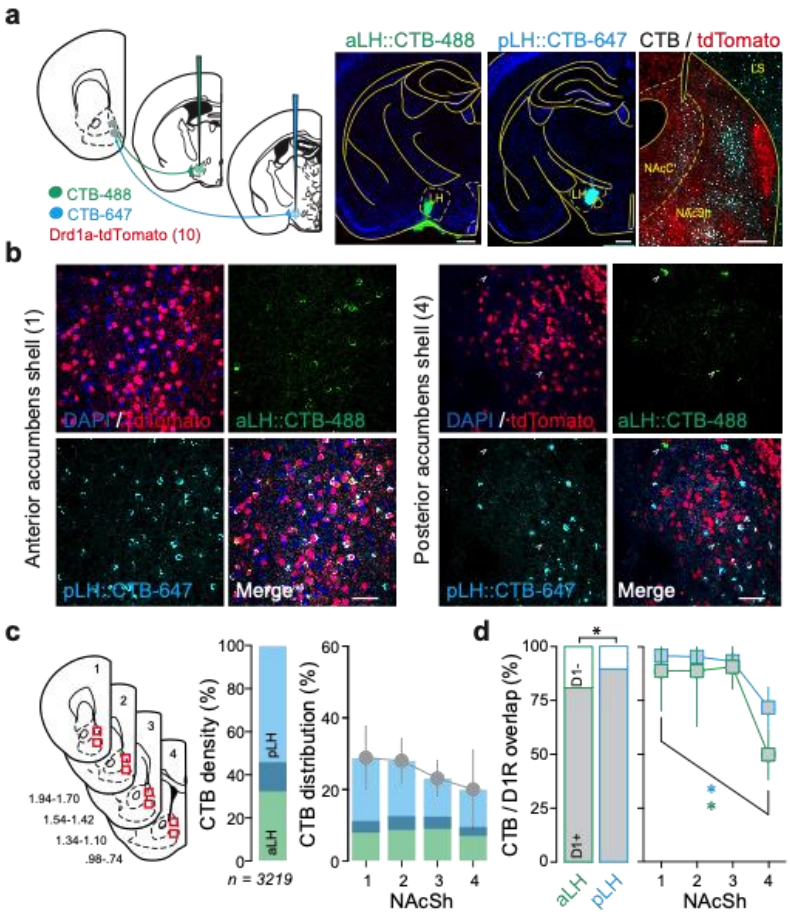



**Figure S3. Related to Figure 2. Dual retrograde tracing from anterior and posterior lateral hypothalamus in Drd1a-tdTomato mice**

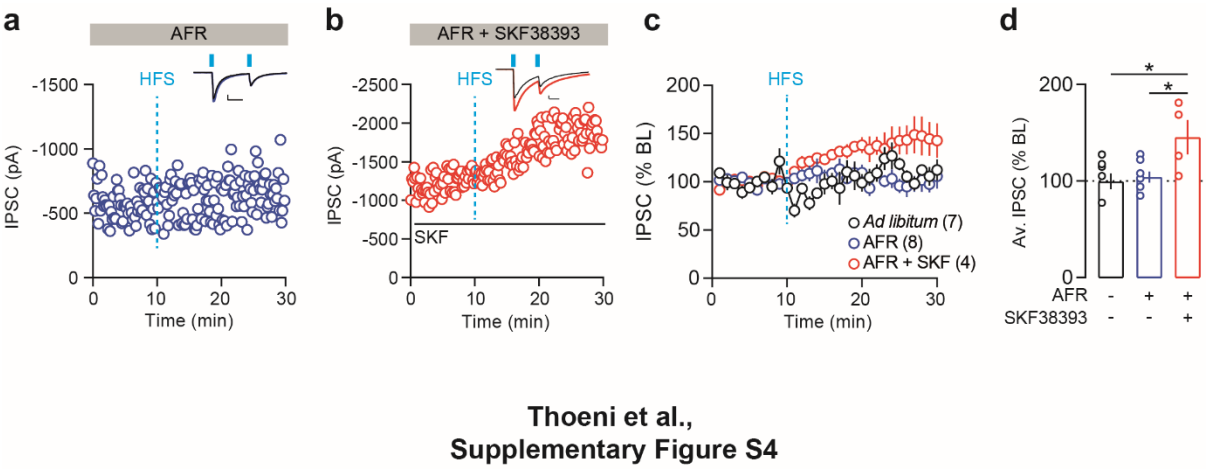
(**a**) Schematic of experiment (left), with example images of Cholera toxin subunit-B (CTB) injection sites in anterior and posterior lateral hypothalamus (aLH/pLH) and CTB-labeled cell bodies visible in the nucleus accumbens. NAcC: accumbens core, NAcSh: accumbens shell, LS: lateral septum. All images are from the same animal. Scale bars: left & center: 500μm; Right, 200μm.

(**b**) From the animal in (A), confocal images from anterior (left) and posterior (right) NAcSh showing colocalization of CTB with tdTomato (i.e. D1R-MSNs). Arrowheads indicate non-colocalizing neurons located in posterior NAcSh. Scale bars: 50μm.

(**c**) Schematic indicating imaging sites across anterior/posterior NAcSh (left), with proportion of all CTB cells identified in NAcSh (n=3219) as aLH (green), pLH (light blue) or dual projecting (dark blue; center) and density distribution of each CTB type across NAcSh (right; grey points show group mean total CTB densities ± SEM).

(**d**) Proportion of all aLH or pLH projecting MSNs that colocalize with tdTomato (i.e. D1R-MSNs; left). Significantly fewer MSNs projecting to aLH vs. pLH were D1R+ (80.9 ± 3.51% vs. 89.5 ± 0.7%; paired t-test, *p < 0.05). Distribution of CTB/tdTomato overlap across anterior/posterior NAcSh (right). Significantly fewer MSNs located in the most posterior quarter of NAcSh and projecting to either aLH or pLH were D1R+ (area 1 vs. 4, Bonferroni corrected t-test, *p < 0.016).

Plots shows means ± SEM.

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**Supplementary Figure S4**

**Figure S4. Related to Figure 6. Ex vivo 100Hz high frequency optogenetic stimulation at D1-MSN to LH synapse.**

(**a**) IPSC example recorded from a cell in the LH in food restricted mice. Stimulation occurred at 10 min.
(**b**) IPSC example recorded from a cell in the LH in food restricted mice. In this preparation, the D1 agonist SKF38393 was present in the bath during the entire recording.
(**c**) IPSC expressed as a percentage of baseline for all recorded cell from control, AFR and AFR + SKF38393 groups.
(**d**) Grouped IPSC. The IPSC was significantly increased in AFR mice when SKF38393 was bath applied. ANOVA: $F(2,16) = 4.073$, $p<0.05$. Post-hoc Bonferonni t-test: * $p<0.05$.

## SUPPLEMENTARY TABLES

| Experiment & Figure | Mouse line | Virus / Tracer | Injection site | n |
|---|---|---|---|---|
| CTB in LH and VP<br>Fig. 2 | Drd1a-tdTomato | CTB-Alexa488, CTB-Alexa567 | LH, VP | 5 |
| CTB in LH and VTA<br>Fig. 2 | Drd1a-tdTomato | CTB-Alexa488, CTB-Alexa567 | LH, VTA | 6 |
| Rabies tracing<br>Fig.3 | Drd1a-tdTomato X VGluT2Cre | AAV5-Flex-TVA-mCherry<br>AAV8-Flex-RG<br>SADΔG-EFG(EnvA) | LH | 2 |
| aLH and pLH<br>Fig. S3 | Drd1a-tdTomato | CTB-Alexa488, CTB-Alexa567 | aLH, pLH | 10 |

**Table S1. Related to Figure 2 and Figure 3. Details of mice used in tracing experiments.**

| Experiment & Figure | Mouse line | Virus / Tracer | Injection site | n |
|---|---|---|---|---|
| HFS, FSK and Control cells<br>Fig. 1 | D1Cre | AAV5-EF1A-DIO-ChR2(H134R)-eYFP | NAcSh | 22 |
| FSK in the VP and Control cells<br>Fig. 1 | D1Cre | AAV5-EF1A-DIO-ChR2(H134R)-eYFP | NAcSh | 5 |
| FSK and Control cells<br>Fig. 3 | VGATCre | AAV5-hsyn-ChR2(H134R)-eYFP<br>AAV5-DIO-EF1A-mCherry | NAc (Chr2)<br>LH (mCherry) | 6 |
| LH VGlut2 connectivity<br>Fig. 3 | VGluT2Cre | AAV5-hsyn-ChR2(H134R)-eYFP<br>AAV5-DIO-EF1A-tdTomato | NAcSh (ChR2)<br>LH (TdTomato) | 5 |
| FSK and Control cells<br>Fig. 3 | VGluT2Cre | AAV5-hsyn-ChR2(H134R)-eYFP<br>AAV5-DIO-EF1A-mCherry | NAc (Chr2)<br>LH (mCherry) | 6 |
| Acute food restriction and FSK<br>Fig. 1 | D1Cre | AAV5-EF1A-DIO-ChR2(H134R)-eYFP | NAcSh | 12 |
| 7 days after acute food restriction<br>Fig. S1 | D1Cre | AAV5-EF1A-DIO-ChR2(H134R)-eYFP | NAcSh | 14 |
| Acute food restriction and FSK in LH GABA cells<br>Fig. 3 | VGATCre | AAV5-hsyn-ChR2(H134R)-eYFP<br>AAV5-DIO-EF1A-mCherry | NAc (Chr2)<br>LH (mCherry) | 12 |
| Acute food restriction and FSK in LH glutamate cells<br>Fig. 3 | VGluT2Cre | AAV5-hsyn-ChR2(H134R)-eYFP<br>AAV5-DIO-EF1A-tdTomato | NAcSh (ChR2)<br>LH (TdTomato) | 10 |
| WIN55212-2 and Control cells in ad libitum fed mice<br>Fig. 4 | D1Cre | AAV5-EF1A-DIO-ChR2(H134R)-eYFP | NAcSh | 16 |
| Acute food restriction and CB1R antagonist on slice<br>Fig. 4 | D1cre | AAV5-EF1A-DIO-ChR2(H134R)-eYFP | NAcSh | 15 |

| | | | | |
|---|---|---|---|---|
| Acute food restriction and CB1R antagonist<br>Fig. 5 | D1Cre | AAV5-EF1A-DIO-ChR2(H134R)-eYFP | NAcSh | 32 |
| High fat exposure<br>Fig. 1 and 5 | D1Cre | AAV5-EF1A-DIO-ChR2(H134R)-eYFP | NAcSh | 16 |

**Table S2. Related to Figure 1, Figure 3, Figure 4 and Figure S1. Details of mice used for whole-cell patch clamp recordings.**

| **Experiment & Figure** | **Mouse line** | **n** |
|---|---|---|
| Acute food restriction and systemic CB1R antagonist<br>Fig. 5 | C57BL/6J | 14 |
| High fat exposure and CB1R antagonist<br>Fig. 5 | C57BL/6J | 15 |
| Acute food restriction and HFS in vivo<br>Fig. 6 | D1cre | 7 |
| Local infusion of CB1R agonists and antagonist<br>Fig. 5 | C57BL/6J | 12 |

**Table S3. Related to Figure 5 and Figure 6. Details for the mice used for behavioural experiments.**