

Vglut2-IRES-cre mice after injection of AAV-DIO-hGlyR bilaterally or controls after injection of AAV-DIO-ChR in the DHA. (A) Mice expressing hGlyR received treatment of IVM (red) or vehicle (blue) 24h before LPS (20µg/Kg) injections, controls were treated with IVM 24h before LPS (black). Mice with inhibition of the DHA<sup>Vglut2</sup> neurons had less stress fever due to the injection itself ( $0.3 \pm 0.15^{\circ}$  C after IVM vs. 0.8 ± 0.25° C before IVM vs. 1.0 ± 0.25°C control) ( $F_{(2,8)}$ =12.75 one-way ANOVA, followed by Bonferroni's post-hoc test p<0.003) during the first 45 min after injection and a lower increase in body temperature during first peak of fever (30-60 min) after LPS injection (1.1 ± 0.03° C after IVM vs. 1.8 ± 0.05°C before IVM vs. 2.0 ± 0.05°C control) ( $F_{(2,10)}=19.87$  one-way ANOVA, followed by Bonferroni's post-hoc test p<0.0003), n=9 hGlyR and n=5 controls. (B) Neurons in the dorsal hypothalamic area (DHA) showed an increase in cFOS (red) immunoreactivity 4h after LPS (i.p.20µg/Kg) injection but not after saline (E). Panels C and F show Vglut2 neurons (green, due to GFP expression in these L10 reporter mice) and panels D and G merge the same two fields in these experiments. Nearly all of the neurons in the DHA that showed cFOS after LPS treatment also expressed Vglut2. Abbreviation: 3V, third ventricle.

Figure S1. Inhibition of DHA<sup>vglut2+</sup> neurons reduces the peak of fever induced by LPS, Related to Figure 2. Time course of the changes in temperature after I.P. injection of LPS in

(60,610)=0.9209, two-way ANOVA, followed by Bonferroni's post hoc test p>0.05.



Figure S2. Activation of DHA<sup>vglut2+</sup> neurons does not further increase hyperthermia or hyperactivity during psychological stress, Related to Figure 3. (A) A schematic illustration of stress protocol. (B) Effect on core temperature in Vglut2-IRES-cre mice that were bilaterally injected with AAV-DIO-hM3Dq-mcherry in the DHA. The elevation of Tc during cage exchange stress and fall after return of mice to their home cage were not statistically different between mice treated I.P. with saline, n=6) vs. CNO (0.3mg/kg). (C) Similar results were seen in LMA in the same mice. (M3 CNO, n=6)  $F_{Tc (180,1088)}$ =1.056 and  $F_{LMA (60,610)}$ =0.9209, two-way ANOVA, followed by Bonferroni's post hoc test p>0.05.  $F_{Tc (180,1088)}$ =0.9279 and  $F_{LMA}$ 



Figure S3. Activation of DHA<sup>Vglut2+</sup> neurons with hM3Dq induced an increase in cFOS (black nuclei) expression in the DLPO and RPa, Related to Figure 3. Injection of CNO in Vglut2-IRES-cre mice after bilateral transduction of the AAV8-hSyn-DIO-hM3D(Gq)-mCherry (brown) (A) induced a statistically significant increase in cFos activation of neurons in the DLPO (B.D) but despite more than doubling did not reach statistical significance in the RPa (C,D). RPa p=0.09 t-test=2.016 df=5 and DLPO \*p = 0.0172 t-test=3.503 df=5.

![](_page_3_Figure_0.jpeg)

## Figure S4. Mapping inputs to DHA neurons using a traditional retrograde tracer Cholera Toxin subunit b (CTb), Related to Figure 5. Note that the retrograde tracing with CTb verifies that the inputs to DHA<sup>Vglut2</sup> --> RPa in the rabies experiment were retrogradely labeled from the DHA, rather than from intermediate sites of replication. (A) A schematic illustrations of experiments injecting the CTb (in red) in the dorsal hypothalamic area (DHA) of wild type mice, n=4. (B) Neurons labeled in the median preoptic area (MnPO), (C) parastrial nucleus (PS), (d) dorsolateral and ventrolateral preoptic area (DLPO and VLPO, respectively), (E) suprachiasmatic nucleus, (F) paraventricular hypothalamic nucleus anterior parvic (PVHap), (G) lateral hypothalamus (LH) and dorsomedial hypothalamus (DMH) and (H) lateral and MPB). Anatomic reference: 3V, third ventricle; ac, anterior commissure; oc, optic chiasm; and scp - superior cerebellar peduncle.