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Supplementary Materials for

A lateral hypothalamus to basal forebrain neurocircuit promotes feeding by suppressing responses to anxiogenic environmental cues

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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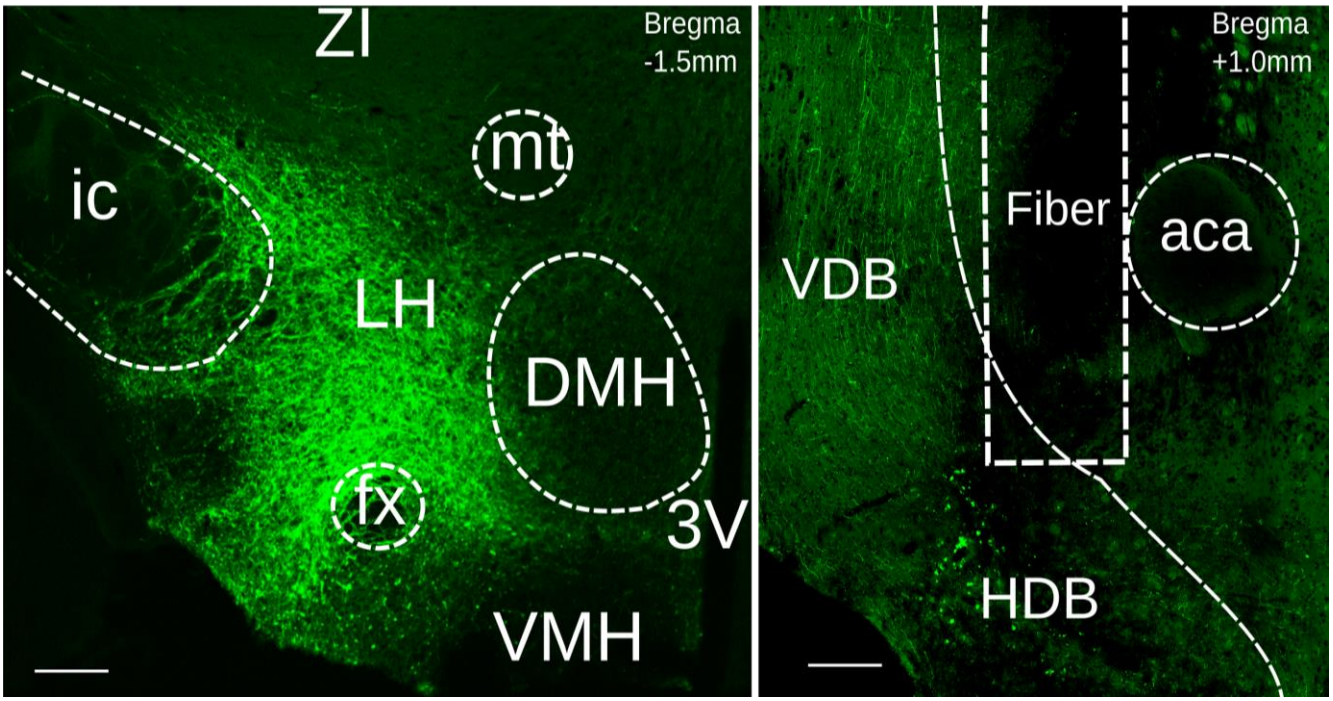

Fig. S1. Optogenetic targeting projections from LH neurons to neurons in the DBB. Coronal brain slices of Pdx1-cre mouse with LH injection of FLEX-ChR2-EYFP (left) and placement of optic fiber over the DBB (right). Scale bar- 250µM. 3V = third ventricle, aca = anterior commissure anterior portion, DMH = dorsomedial hypothalamic nucleus, fx = fornix, HDB = horizontal limb of DBB, ic = internal capsule, mt = mammilo-thalamic tract, VDB = ventral limb of DBB, VMH = ventromedial hypothalamic nucleus, ZI = zona incerta

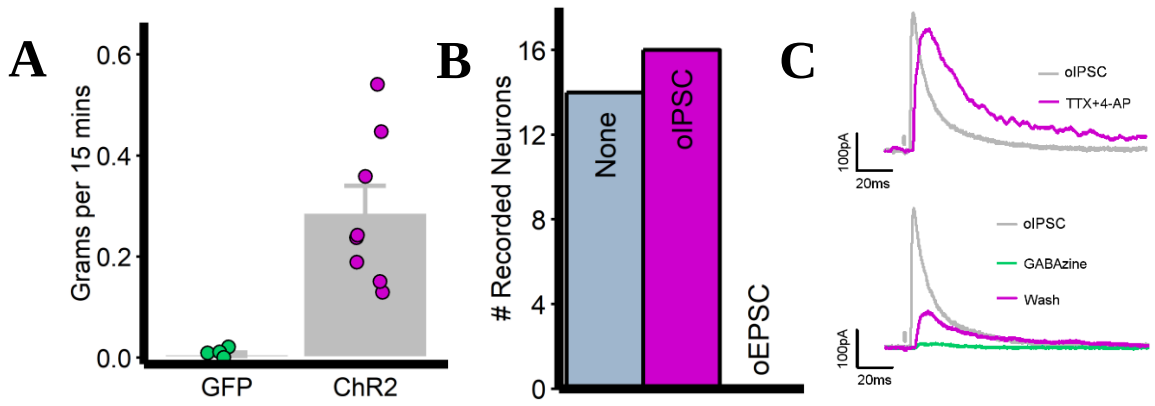

Fig. S2. Electrophysiological recordings on DDB neurons that receive monosynaptic inputs from LH neurons. (A) Activation of LH^{Pdx1}→DBB neurons induces feeding behavior. (B) Brain slice electrophysiology of downstream neurons in the DBB (n=4 animals) identifies 16 neurons with inhibitory post-synaptic current (oIPSC), 14 with no response, and 0 with excitatory post-synaptic current (oEPSC). (C) oIPSC is monosynaptic (not blocked by tetrodotoxin (TTX) and 4-aminopyridine (4-AP) and dependent on local GABA-A receptors (blocked by GABAzine) (right).

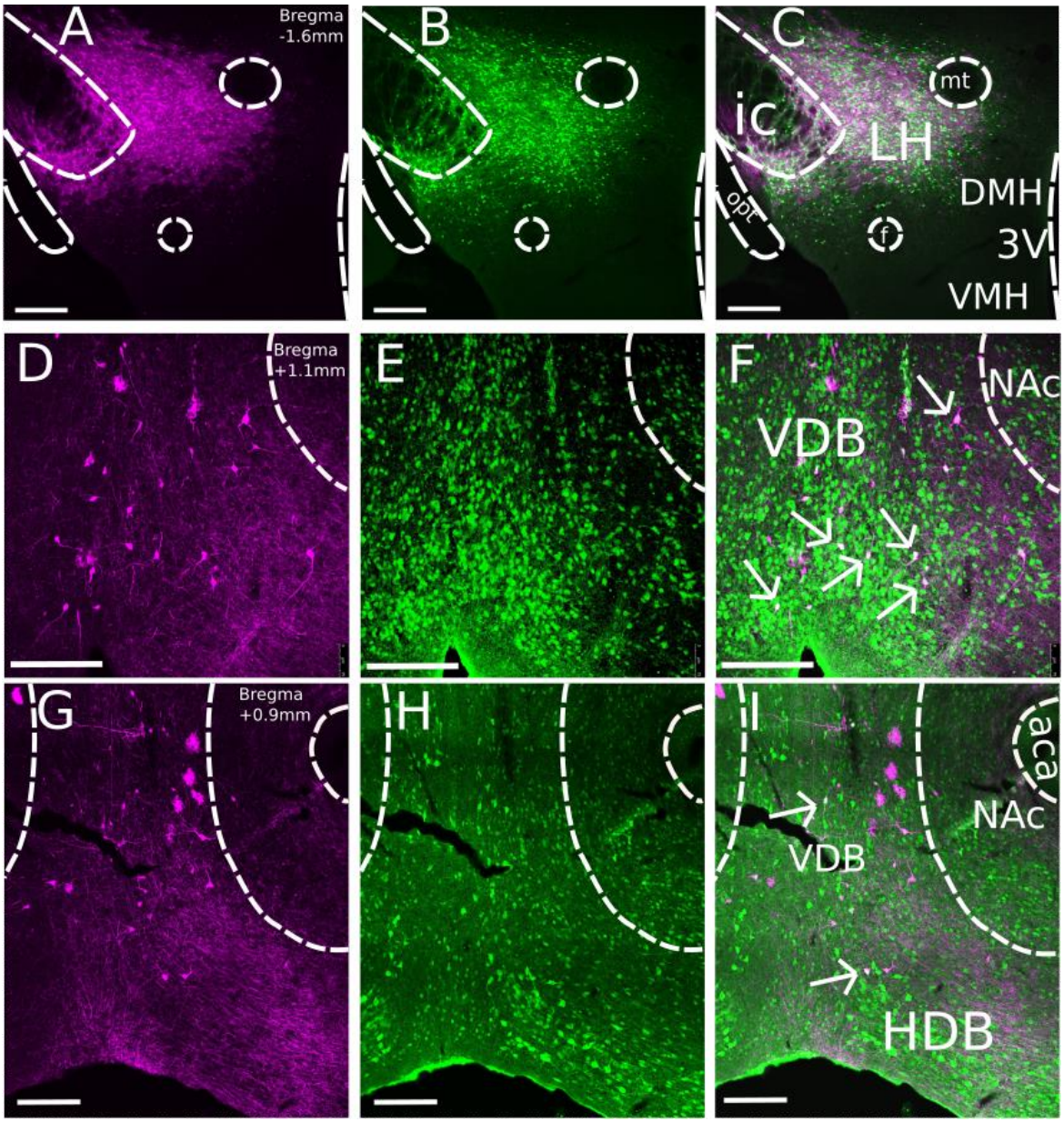

Fig. S3. Tracing DDB neurons that receive direct inputs from LH. (A-I) Coronal slices of Ai9+ (Cre-dependent mCherry) mouse brain after unilateral injection of anterograde tracer AAV1-Cre-GFP into the LH, and the Cre activity was reported in the DBB. Antibody staining against choline acetyltransferase (ChAT) was shown (green). Magenta = mCherry, Green = ChAT. Scale bar- 250μM 3V = third ventricle, aca = anterior commissure anterior portion, DMH = dorsomedial hypothalamic nucleus, f = fornix, HDB = horizontal limb of DBB, ic = internal capsule, mt = mammilo-thalamic tract, Nac=nucleus accumbens, VDB = ventral limb of DBB, VMH = ventromedial hypothalamic nucleus.

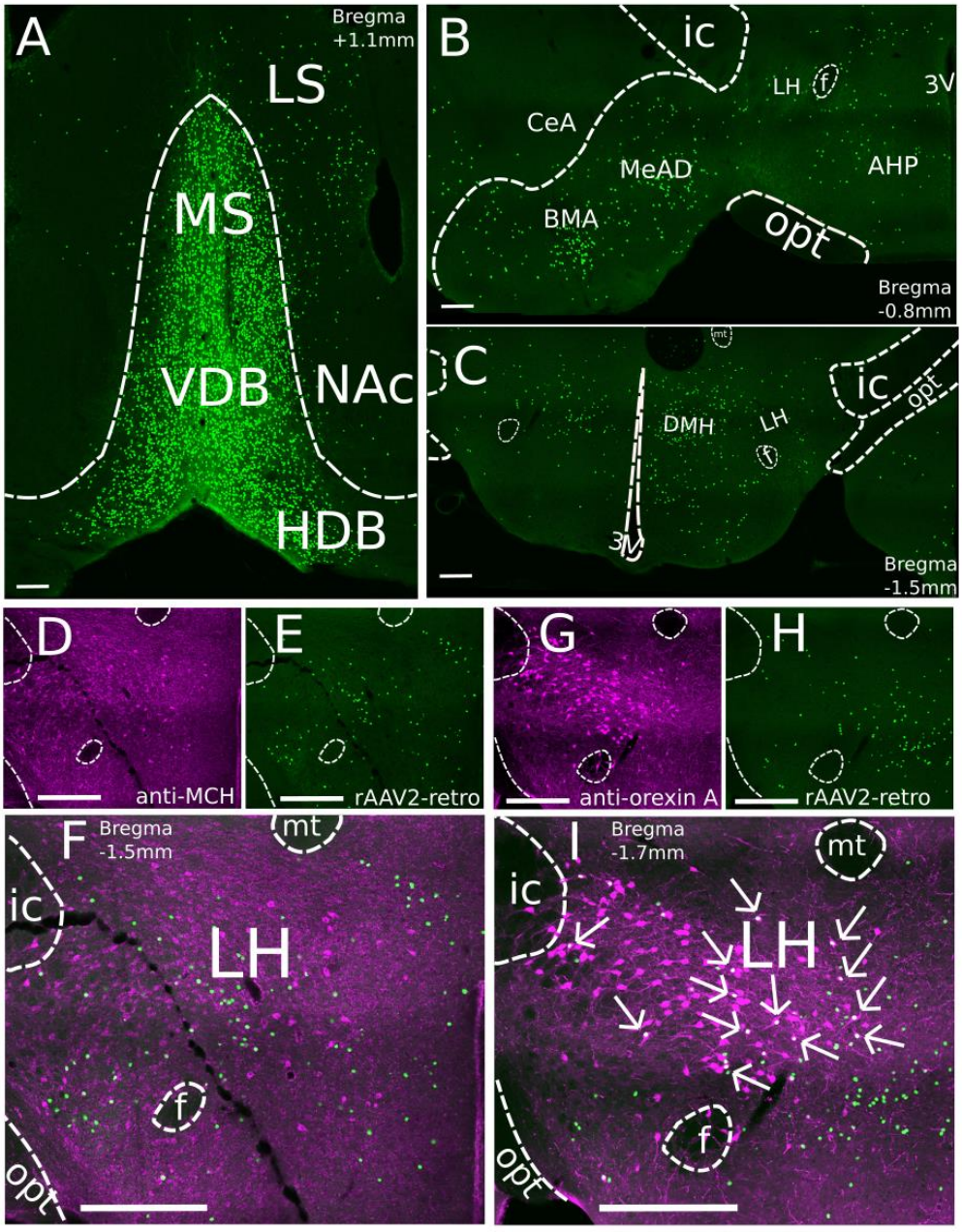

Fig. S4. Tracing LH neurons that send direct projections to DBB neurons. (A-C) Coronal slices of mouse brain after midline injection of retrograde tracer rAAV2-retro carrying a plasmid encoding Cre and H2B::Venus (green) into the DBB, demonstrating selective expression in the medial septum/DBB complex, amygdyla, and hypothalamus. (D-F) Antibody staining against orexin (purple) demonstrates minimal colocalization. (G-I) Antibody staining against melanin-concentrating hormone (MCH; purple) demonstrates no colocalization. Scale bar- 250µM 3V = third ventricle, aca = anterior commissure anterior portion, DMH = dorsomedial hypothalamic nucleus, f = fornix, HDB = horizontal limb of DBB, ic = internal capsule, mt = mammilo-

thalamic tract, Nac=nucleus accumbens, VDB = ventral limb of DBB, VMH = ventromedial hypothalamic nucleus.

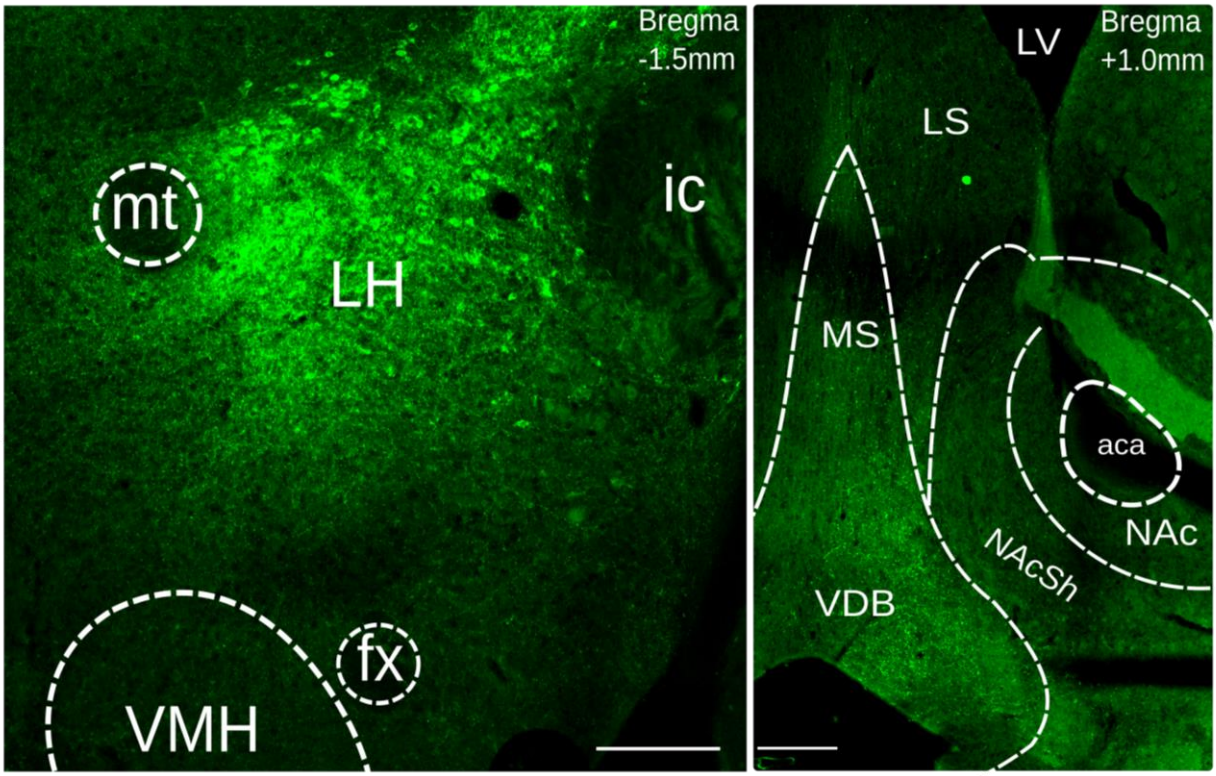

Fig. S5. LH^{GABA} neurons send direct projections to the DBB. Injection of LH^{Vgat} neurons with Flex-Synaptophysin::EGFP reveals strong synaptic projection to the medial septum (MS) and vertical and horizontal limbs of the diagonal band of Broca (VDB, HDB), with only sparse connection lateral septum (LS) or nucleus accumbens core (NAc) or shell (NAcSh). 3V = third ventricle, aca = anterior commissure anterior portion, DMH = dorsomedial hypothalamic nucleus, fx = fornix, HDB = horizontal limb of DBB, ic = internal capsule, LS = lateral septum, LV = lateral ventricle, mt = mammilo-thalamic tract, VDB = ventral limb of DBB, VMH = ventromedial hypothalamic nucleus. Scale bar- 250µM

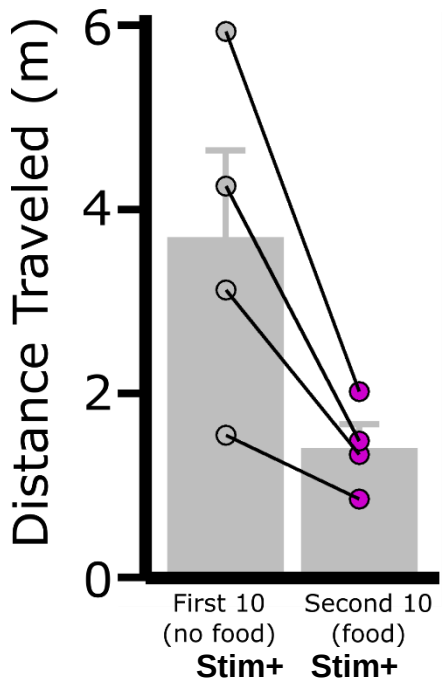

Fig. S6. Optogenetic stimulation of LH^{GABA} to DBB projections increased food-dependent exploratory behavior. In two sequential 10-minute testing periods while $LH^{Vgat\text{-}ChR2}$→DBB projections were photostimulated, fasted mice show more locomotion prior to food exposure, than afterwards, showing food-dependent exploratory behavior (n=4, p=0.044, paired t-test)

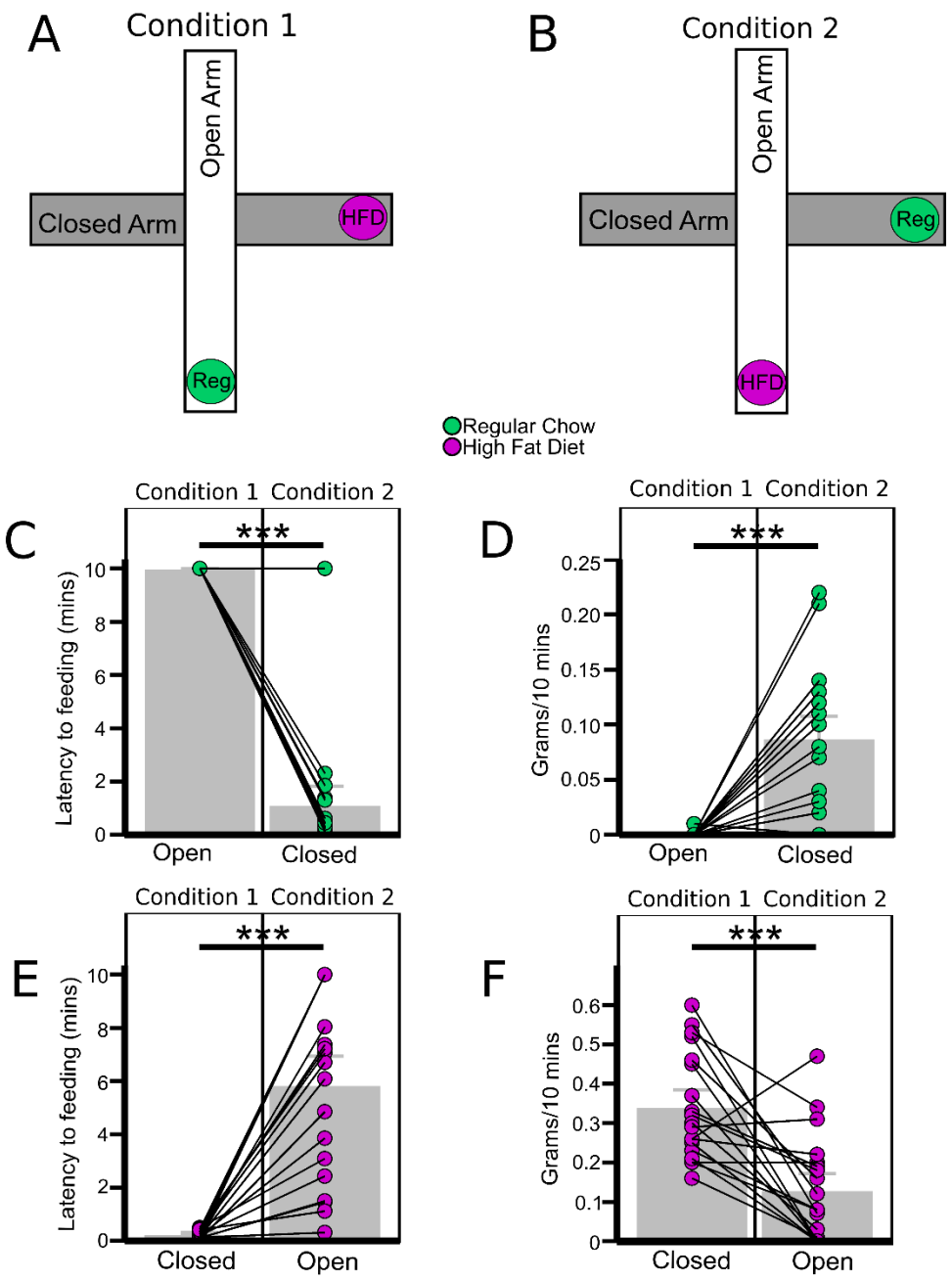

Fig. S7. Competition between anxiogenic conditions and feeding behavior. Mice (n=20) were fasted overnight prior to all experiments and habituated to the elevated plus maze (EPM) prior to food placement; lines connect same mouse across conditions. (A-B) Schematic representing two experimental conditions: condition 1, regular chow (reg) is placed on the open arm of the EPM and high fat diet (HFD) on the closed arm; condition 2, regular chow is placed on closed arm and HFD on the open arm (C) Latency to regular chow consumption between the two conditions

($p<0.001$) (D) Amount of regular chow consumed for 10 minutes after first bite ($p<0.001$) (E) Latency to HFD consumption between the two conditions ($p<0.001$) (F) Amount of HFD consumed for 10 minutes after first bite ($p<0.001$).

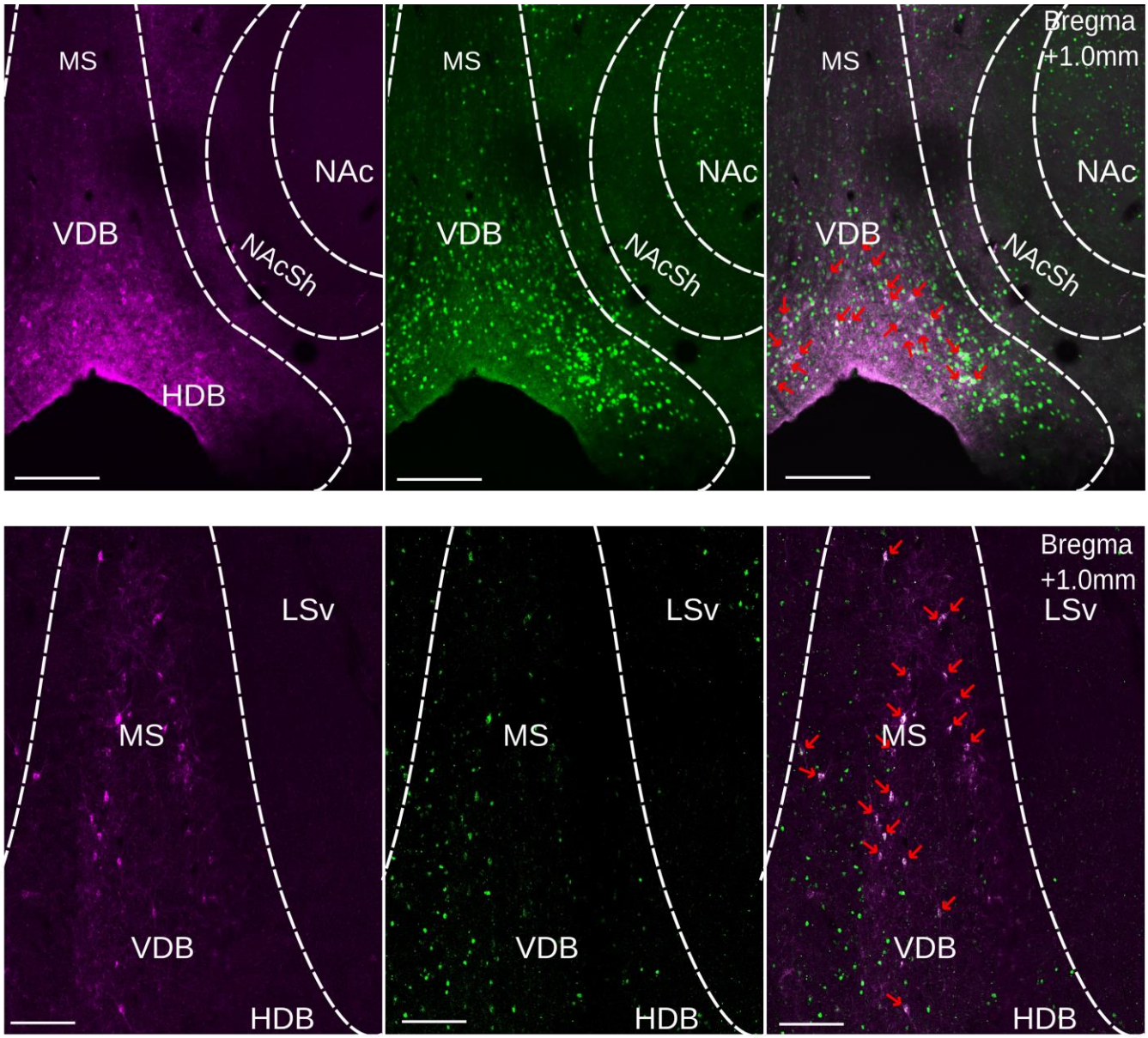

Fig. S8. Verification of synthetic excitatory receptor (hm3Dq) expression in DBB neurons. (Top Row; scale bar = 250μm) Brain slices showing expression DIO-hm3Dq in DBB Vgat neurons (magenta); c-fos immunohistochemical staining (green) in these neurons 3 hours after injection of clozapine-N-oxide (CNO). (Bottom Row; scale bar = 100μm) Brain slices showing expression of DIO-hm3Dq DBB Vglut2 neurons (magenta) and c-fos expression (green) 3 hours after injection of CNO. Arrows indicate co-localization.

Movie S1. Activation of LH^{Vgat}→DBB fibers (Laser ON) induces immediate increase in sniffing, licking, and exploratory behavior, followed by consumption of chow. Behaviors cease once laser is turned off.

Movie S2. Fiber photometry data of novel object interaction. DBB$^{Vgat\text{-}GCaMP6m}$ calcium-dependent fluorescence (magenta line) increases in activity immediately upon novel object interaction, while calcium-independent signal (green line) remains constant.

Movie S3. Fiber photometry data of food consumption after 24 hours fasting. DBB$^{Vgat\text{-}GCaMP6m}$ calcium-dependent fluorescence (magenta line; %dF/F0) increases in activity on food interaction (at 15s), then drops below baseline for the duration of feeding bout. Resumes normal levels of activity upon cessation of feeding (at 30s). The calcium-independent signal remains constant (green line).