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**Supplemental Information**

# Chemogenetic Synaptic Silencing of Neural Circuits Localizes a Hypothalamus→Midbrain Pathway for Feeding Behavior

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Inventory of Supplemental Information

*Supplemental Data*

**Figure S1, Related to Figure 1 and Figure 2. hM4D/CNO neuronal and synaptic inhibition are reversible and repeatable**

Shows evidence that the effects of hM4D/CNO on both somatic hyperpolarization and synaptic silencing are reversible, and that repeated application of CNO can silence synaptic transmission.

**Figure S2, Related to Figure 3. $ARC^{AGRP}$→PVH circuits influence feeding**

Shows a schematic overview of the central hunger circuit examined in Figure 3 and shows data from brain slice physiology to verify that hM4D silences synaptic transmission from AGRP neurons.

**Figure S3, Related to Figure 5: PVH axon projection silencing with $hM4D^{nrxn}$**

Shows PVH descending projection field anatomy, ventricular injection control experiments, and repeatability of *in vivo* silencing.

*Supplemental Experimental Procedures*

*Supplemental Data*

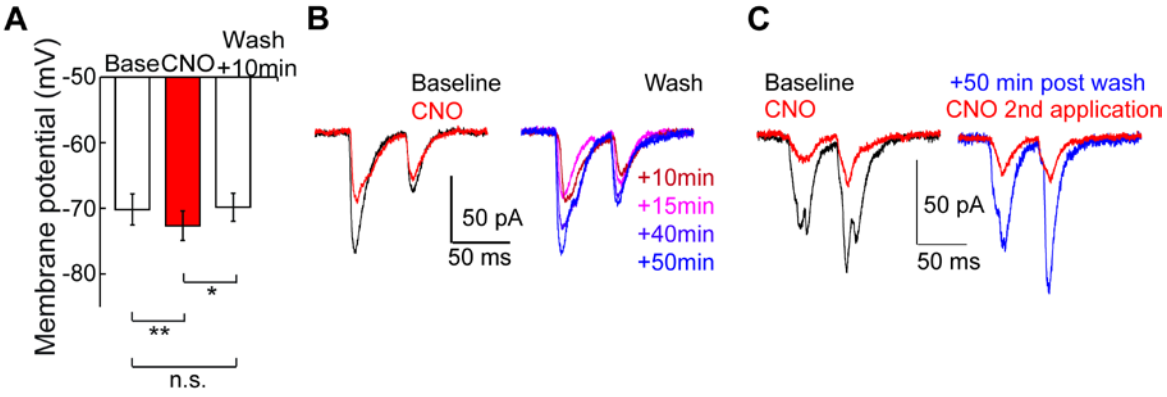


**Figure S1, Related to Figure 1 and Figure 2. hM4D/CNO neuronal and synaptic inhibition are reversible and repeatable**

A) In hM4D-expressing L2/3 neurons (n = 8), hyperpolarization is induced by 30 nM CNO and reverses rapidly, recovering to baseline in less than 10 min

B) Example of recovery of synaptic currents for L2/3→L5 connections after 100 nM CNO washout. Synaptic inhibition induced by CNO reverses more slowly, recovering in about an hour.

C) Synaptic inhibition by hM4D/CNO is repeatable. After recovery, a second application of CNO suppresses synaptic transmission.

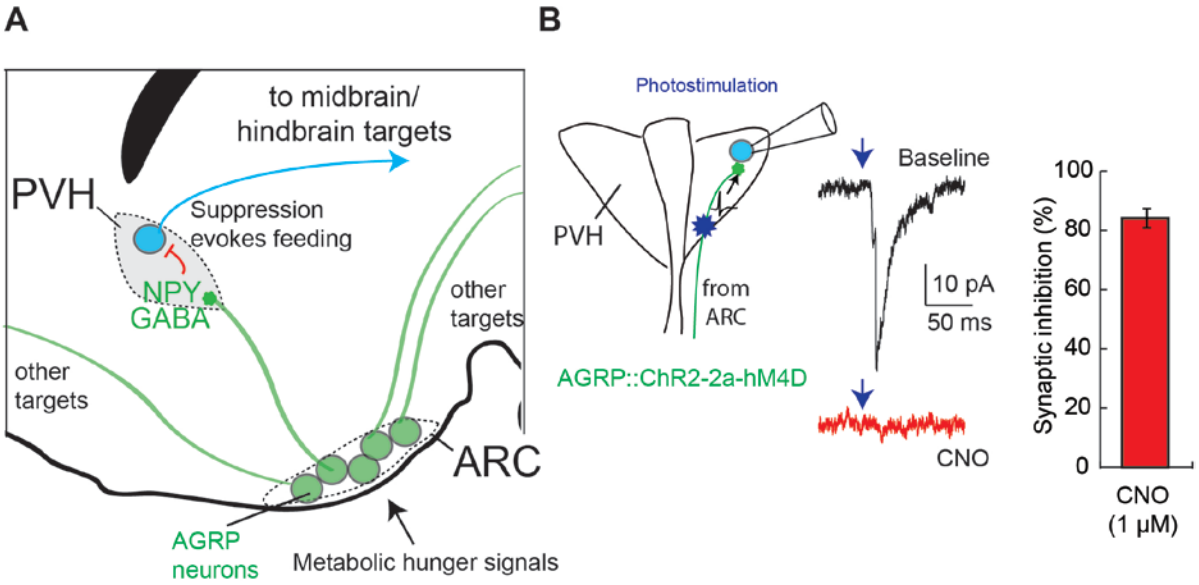


**Figure S2, Related to Figure 3. ARC$^{AGRP}$→PVH circuits influence feeding**

A) Schematic of AGRP neuron circuits that influence feeding and that are manipulated in this study to demonstrate *in vivo* efficacy of hM4D/CNO-mediated synaptic silencing. Circulating hormonal signals of energetic state influence electrical activity in AGRP neurons such that energy deficit results in increased AGRP neuron firing rates. Elevated AGRP neuron activity is sufficient to elicit feeding behavior. Selective activation of the ARC$^{AGRP}$→PVH circuit is also sufficient to evoke food intake and this is mediated by release of NPY and GABA. The ARC$^{AGRP}$→PVH circuit projection inhibits PVH neuron electrical activity. In turn, silencing PVH neuron activity also elicits feeding behavior.

B) Photostimulation of ChR2 and hM4D co-expressing AGRP axons (signified by blue starburst) in the PVH elicits GABA-mediated synaptic currents (20 μM CNQX) in PVH neurons *ex vivo*, which are blocked upon exposure to CNO (1 μM, n = 5). Values are means ± SEM.

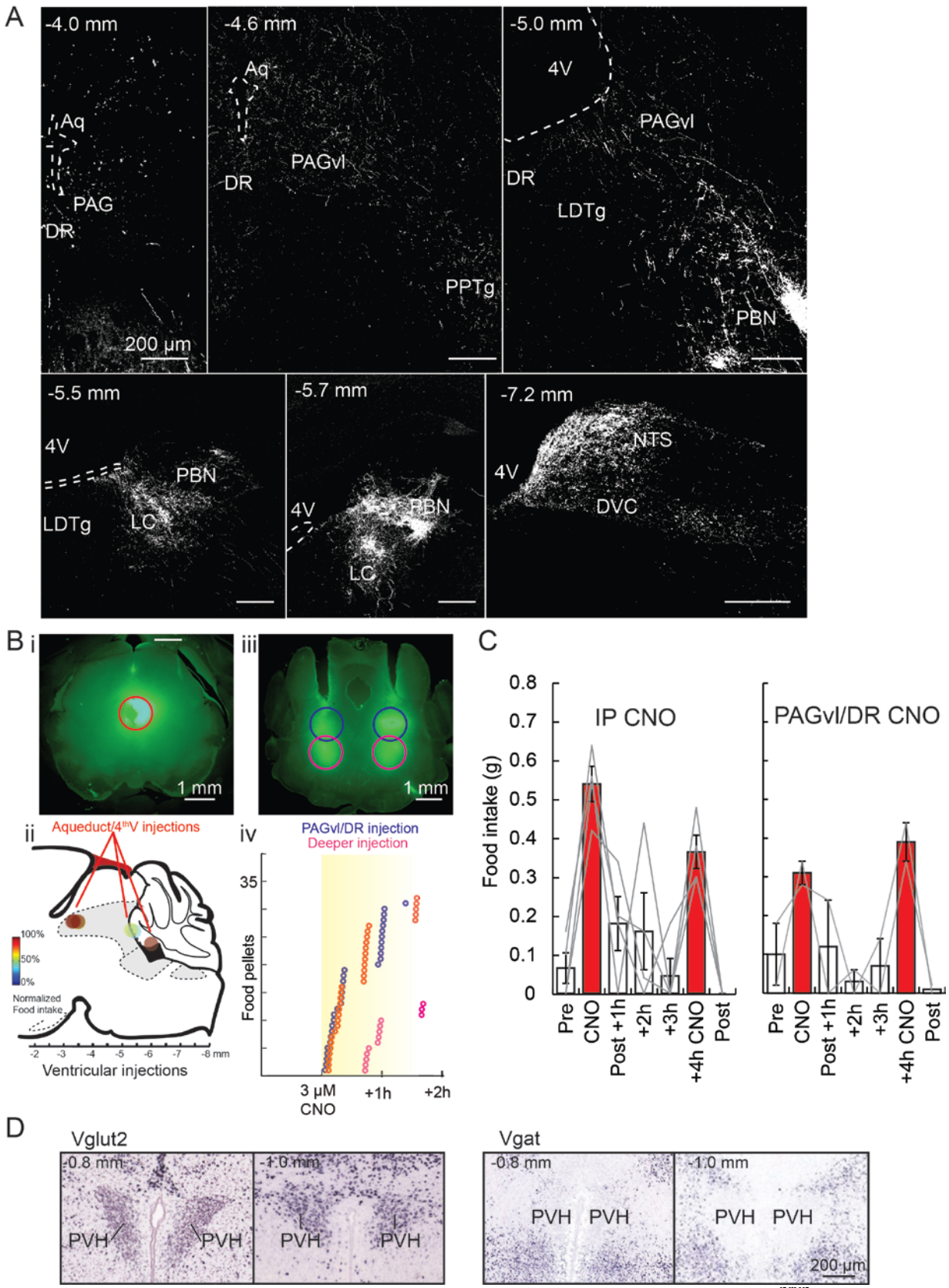


**Figure S3, Related to Figure 5: PVH axon projection silencing with hM4D$^{nrxn}$**

**Figure S3, Related to Figure 5: PVH axon projection silencing with hM4D$^{nrxn}$**

A) PVH$^{SIM1}$ axons transduced with mCherry-2a-hM4D$^{nrxn}$ are visible (mCherry immunofluorescence) within the anterior PAG (-4.0 mm) and DR, with more robust innervation of these structures caudally (PAGvl, -4.4 mm and -5.0 mm). Fibers innervate the PBN and LC (-5.5 mm and -5.7 mm), as well as the NTS (-7.2), with some fibers distributed sparsely in the DVC. DR: dorsal raphe nucleus; DVC: dorsal vagal complex; LC: locus coeruleus; LDTg: lateral dorsal tegmental area; NTS: nucleus of the solitary tract; PAG: periaqueductal grey; PAGvl: periaqueductal grey, ventrolateral portion; PBN: parabrachial nucleus; PPTg: pedunculopontine tegmental area; Aq: cerebral aqueduct; 4V: fourth ventricle.

B) For mice expressing hM4D$^{nrxn}$ in PVH$^{SIM1}$ neurons, food intake is elicited by microinjections of CNO (3 µM) targeted directly to (Bi) the cerebral aqueduct rostral to the PAGvl/DR (red circle) or to (Bii) the 4$^{th}$ ventricle caudal to PAGvl/DR. Fluorogold injected through the cannula prior to perfusion in order to mark the injection site.

(Bii) Sagittal schematic illustrates feeding responses elicited after injection of CNO (3 µM) to the cerebral aqueduct or 4$^{th}$ ventricle and normalized to food intake after IP delivery of CNO.

(Biii) Microinjection sites at two dorsal/ventral positions (circles) without damage to the cerebral aqueduct. Fluorogold injected through the cannula prior to perfusion.

(Biv) Food pellet consumption for CNO (3 µM) microinjections from Bi and Biii shows that feeding can be evoked by intracranial injections that did not damage the cerebral aqueduct (blue), and this was of similar magnitude to CNO (3 µM) microinjections into the cerebral aqueduct (red). For the same animal from Biii, a deeper injection failed to evoke robust food pellet consumption (pink).

C) Evoked feeding with hM4D is repeatable. IP injection of CNO (1 mg/kg) evokes feeding, primarily during the first hour post-injection. Repeated CNO delivery 4 h later also evokes feeding (n = 4). Feeding induced by intracranial microinjection of CNO (3 µM) to the PAGvl/DR is repeatable 4 hours later (n = 2). Values are means ± SEM.

D) In situ hybridization in the PVH shows extensive signal for vesicular glutamate transporter 2 (Vglut2) and scant signal for vesicular GABA transporter (Vgat), indicating that most PVH neurons are glutamate releasing and likely excitatory. Approximate positions relative to Bregma are noted. Images: ©2012 Allen Institute for Brain Science. Allen Mouse Brain Atlas. Available from: http://mouse.brain-map.org/).

*Supplemental Experimental Procedures*

**Electrophysiology.** Coronal brain slices were prepared in cold cutting solution containing (in mM): 75 sucrose, 87 NaCl, 25 $NaHCO_3$, 25 D-glucose, 2.5 KCl, 7 $MgCl_2$, and 1.25 NaH2PO4, aerated with 95% $O_2$ / 5% $CO_2$. Slices were transferred to artificial cerebrospinal fluid (aCSF) containing (in mM): 128 NaCl, 26 $NaHCO_3$, 10 D-glucose, 3 KCl, 1.25 $MgCl_2$, 2 $CaCl_2$ and 1.25 $NaH_2PO_4$, aerated with 95% $O_2$ / 5% $CO_2$. To reduce spontaneous synaptic activity for cortical laser scanning photostimulation experiments, aCSF was as described above with the following changes (in mM): 123 NaCl, 4 $MgCl_2$, 4 $CaCl_2$. The intracellular solution for whole recordings contained (in mM): 125 K gluconate, 2 KCl, 10 HEPES, 10 phosphocreatine, 4 $MgCl_2$, 1 EGTA, 0.1 $CaCl_2$, 4 ATP, 0.4 GTP, pH 7.35, 300 mOsm. For whole cell recording of inhibitory currents in PVH, the extracellular solution contained 20 µM CNQX, and the intracellular solution contained (in mM): 130 CsCl, 10 HEPES, 10 phosphocreatine, 1 EGTA, 1 $MgCl_2$, 0.1 $CaCl_2$, 4 ATP, 0.4 GTP, 10 lidocaine N-ethyl bromide, pH 7.35, 300 mOsm. The holding potential for voltage clamp recordings was -70 mV unless otherwise indicated.

**Hippocampal neuronal culture and electroporation**. A mixed cell culture (neurons and glia) was prepared from Sprague-Dawley rat pups. Briefly, P0 pups were decapitated and the brains dissected into ice-cold neural dissection solution (HBSS + Hepes, pH 7.4). Hippocampi were removed, enzymatically digested with papain, washed with pre-warmed plating medium, mechanically digested by trituration, and electroporated. Cells were plated on coverslips coated with poly-D-lysine, and kept at 37 °C, 5% $CO_2$ in plating medium for 1 h, then in NbActiv4 medium (BrainBits) for 6-20 days in vitro. In some cases, mature neurons were transfected using Lipofectamine 2000 (Promega) after plating.