NEUROSCIENCE

Rapid binge-like eating and body weight gain driven by zona incerta GABA neuron activation

Xiaobing Zhang and Anthony N. van den Pol*

The neuronal substrate for binge eating, which can at times lead to obesity, is not clear. We find that optogenetic stimulation of mouse zona incerta (ZI) γ-aminobutyric acid (GABA) neurons or their axonal projections to paraventricular thalamus (PVT) excitatory neurons immediately (in 2 to 3 seconds) evoked binge-like eating. Minimal intermittent stimulation led to body weight gain; ZI GABA neuron ablation reduced weight. ZI stimulation generated 35% of normal 24-hour food intake in just 10 minutes. The ZI cells were excited by food deprivation and the gut hunger signal ghrelin. In contrast, stimulation of excitatory axons from the parasubthalamic nucleus to PVT or direct stimulation of PVT glutamate neurons reduced food intake. These data suggest an unexpected robust orexigenic potential for the ZI GABA neurons.

Patients receiving deep brain stimulation of the subthalamus, including the zona incerta (ZI), for the treatment of movement disorders can exhibit characteristics of binge eating (*1*–*3*), a common eating disorder characterized by recurrent episodes of consuming large quantities of food, particularly highly palatable food (*4*, *5*). It is not clear why stimulation in the subthalamus would evoke eating, although sheep may release γ-aminobutyric acid (GABA) from the ZI in response to the sight or ingestion of food (*6*, *7*).

The ZI is one of the least-studied regions of the brain, despite its robust projections throughout the brain (*8*, *9*). To determine the role of the ZI in feeding and body weight regulation, we injected Cre recombinase–inducible adeno-associated viruses (AAV) expressing the optogenetic channelrhodopsin-like ChIEF fused with a tdTomato reporter [AAVdj-CAG-DIO-ChIEF-tdTomato (driven by the CAG promoter) (*10*, *11*)] bilaterally into the rostral ZI of vesicular GABA transporter (VGAT)–Cre mice that express Cre recombinase in GABA neurons (Fig. 1A). ChIEF-tdTomato was selectively expressed in ZI GABA neurons but not in lateral hypothalamic neurons (fig. S1). Laser stimulation (1 to 20 Hz) evoked depolarizing currents in ZI ChIEF-tdTomato–expressing VGAT neurons tested with whole-cell recording in brain slices, displaying a high-fidelity correspondence with stimulation frequency (Fig. 1B). In VGAT-Cre mice with ChIEF expression, bilateral laser stimulation (20 Hz) in the ZI increased food intake, with mice rapidly consuming 35.4% of their 24-hour ad libitum high-fat food intake in just 10 min (Fig. 1, C to E, and movie S1). In control mice with tdTomato expression, consumption was only 4% of their 24-hour intake during the same period (Fig. 1E). When stimulation of 10 min ON followed by 30 min OFF was repeated four times, ZI-VGAT-ChIEF mice consumed 74% of their normal 24-hour food intake, whereas control mice consumed only 22% (Fig. 1E). Food deprivation lasting 24 hours increased ZI GABA neuron activity and excitatory neurotransmission to these neurons (Fig. 1, F to J). Ghrelin, a hormone that signals a reduced gut energy state (*12*), excited ZI GABA neurons and increased excitatory synaptic input onto these neurons (Fig. 1, K to M, and fig. S2).

Anterograde AAV-ChIEF-tdTomato labeling of ZI GABA cells in VGAT-Cre mice showed strong axonal projections into the paraventricular thalamus (PVT) (Fig. 2A), consistent with previous observations that some ZI cells project to the PVT (*13*, *14*), a brain area that may contribute to energy homeostasis (*15*). Cre recombinase–dependent rabies virus–mediated monosynaptic retrograde pathway tracing in vGluT2–Cre recombinase mice confirmed that PVT glutamate neurons receive strong and direct innervation from ZI neurons (Fig. 2, B and C, and fig. S3). Food deprivation lasting 24 hours increased inhibitory synaptic neurotransmission to PVT glutamate neurons (fig. S4). We asked whether the PVT may be a critical target for ZI regulation of food intake. We crossed VGAT-Cre mice with vGlut2-GFP mice in which neurons expressing vesicular glutamate transporter (vGlut2) were labeled with green fluorescent protein (GFP) to study whether ZI GABA neurons release synaptic GABA to inhibit PVT glutamate neurons (*16*, *17*). One month after AAV-ChIEF-tdTomato was injected into the ZI of these mice (Fig. 2A), photostimulation of ZI VGAT-ChIEF-tdTomato terminals in the PVT evoked GABA-mediated inhibitory currents in PVT vGlut2-GFP neurons (Fig. 2D). In vivo stimulation (20 Hz) of axon terminals from ZI GABA neurons to PVT glutamate neurons ($VGAT^{ZI\text{-}PVT}$) evoked food-foraging behavior (movie S2). Continuous stimulation (20 Hz) for 10 min increased the intake of high-fat, sweet, and regular foods (Fig. 2E) in mice with ZI-VGAT-ChIEF-tdTomato expression. No effect of laser stimulation on high-fat food intake was detected in control mice with AAV-tdTomato in the ZI (Fig. 2F). The total feeding time for ZI VGAT-ChIEF-tdTomato mice was 7.1 ± 0.5 min compared with 0.3 ± 0.1 min for controls (Fig. 2G). Photostimulation of ZI-PVT inhibitory axons evoked gnawing, but not eating, of nonnutritional wood sticks (fig. S5, A and B); photostimulation leading to food intake eliminated subsequent evoked stick gnawing. A priori wood gnawing had no effect on photostimulation-evoked food intake (fig. S5, C and D). We then measured food intake of the same mice during three successive trials of 10-min laser stimulation with a 5-min interval without photostimulation between the trials. The food intake for the first trial was 4.95 ± 0.80 kcal. The amount for the second trial was reduced substantially to 0.72 ± 0.29 kcal and 0.49 ± 0.25 kcal, respectively (Fig. 2H). Satiety feedback signals can thus attenuate ZI-induced feeding.

ZI-stimulated mice showed a preference for high-fat and sweet foods over normal food (Fig. 2I). Although mice prefer sweet and high-fat foods when stimulation is off, laser stimulation increased the relative preference for high-fat food (Fig. 2I). When normal, sweet, and high-fat foods were all available, mice consistently chose high-fat food during laser stimulation of ZI axons in the PVT (movie S3). ZI GABA neurons project to multiple brain regions, including the hypothalamus and midline thalamus (fig. S6). We therefore measured the relative contribution of stimulation of ZI somata with selective stimulation of ZI axons targeting the PVT. Stimulation of ZI VGAT cell bodies or $VGAT^{ZI\text{-}PVT}$ terminals in the PVT evoked similar levels of feeding (Fig. 2J). To further confirm the importance of the $VGAT^{ZI\text{-}PVT}$ projection in mediating ZI GABA neuron control of food intake, the type A GABA ($GABA_A$) receptor antagonist bicuculline (Bic) was microinjected into the PVT 10 min before photostimulation of $VGAT^{ZI\text{-}PVT}$ axon terminals. Bic attenuated photostimulation-evoked feeding (Fig. 2K). That Bic did not completely block photostimulation-evoked food intake could be a diffusion limitation of Bic after application, or ZI VGAT-Cre neurons may coexpress other neurotransmitters responsible for the remaining action. These results are consistent with an early report that lesions in the area of the ZI can alter food intake (*18*).

Stimulation of anorexigenic proopiomelanocortin (POMC) cells in the hypothalamic arcuate nucleus leads to a reduction in feeding slowly over the succeeding 24 hours, whereas stimulation of orexigenic hypothalamic neurons expressing agouti-related peptide (AgRP) leads to what has previously been considered to be a rapid increase in feeding with mean latency to eat of 6.1 min (range: 1.9 to 13.8 min) (*19*). To test the time course and efficiency of optogenetic activation of $VGAT^{ZI\text{-}PVT}$ inhibitory inputs to evoke feeding, we used a laser stimulation protocol of 10 s ON (20 Hz) followed by 30 s OFF for more than 20 min to study ZI axon stimulation in PVT brain slices and feeding behavior. Stimulation of ZI axons with this protocol hyperpolarized and inhibited PVT glutamatergic neurons each time the light was activated (Fig. 3A). Mice immediately started feeding for each of the

Department of Neurosurgery, Yale University School of Medicine, New Haven, CT 06520, USA.
*Corresponding author. Email: anthony.vandenpol@yale.edu

30 successive trials of ZI axon laser stimulation (Fig. 3B and movie S4). The mean latency to initiate feeding was 2.4 ± 0.6 s when we used laser stimulation of 20 Hz (Fig. 3C). This is almost 100 times faster than that reported for optogenetic stimulation of the AgRP neuron soma and 500 times faster than stimulation of AgRP-PVT axon terminals (*19*, *20*). As soon as the laser was turned off, the mice stopped eating. To test further whether photostimulation of $VGAT^{ZI\text{-}PVT}$ terminals evokes compulsive eating, food intake was measured when food was put in a brightly illuminated chamber in a two-chamber light-or-dark conflict test. Mice spent only 20% of their time in the brightly lit chamber with high-fat food when the laser was off, suggesting an aversion to the light (Fig. 3D). In spite of the light aversion, photostimulation of $VGAT^{ZI\text{-}PVT}$ terminals significantly increased the time mice spent on the illuminated side to 61% when high-fat food was available (Fig. 3D). Photostimulation increased high-fat food intake in bright light (Fig. 3E).

Binge eating has been linked to a reward-system disorder (*21*, *22*). To test the hypothesis that the $VGAT^{ZI\text{-}PVT}$ pathway is involved in a reward state, we explored the motivational valence of $VGAT^{ZI\text{-}PVT}$ in mice by using a two-chamber place preference test. In the absence of available food, optogenetic activation of the $VGAT^{ZI\text{-}PVT}$ pathway evoked a significant preference for the chamber associated with laser stimulation compared with the control chamber (Fig. 3, F and G).

To test whether activation of the $VGAT^{ZI\text{-}PVT}$ inhibitory pathway leads to body weight gain, we selectively photostimulated this pathway for only 5 min every 3 hours over a period of 2 weeks. Photostimulation increased food intake and body weight of mice with ChIEF-tdTomato expression in ZI GABA neurons (Fig. 3, H and I). After the days of photostimulation were completed, mice showed a significantly reduced food intake compared with that of controls (Fig. 3H). The body weight of mice that showed an increase with ZI GABA neuron photostimulation gradually returned to the prestimulation body weight level of controls (Fig. 3I), consistent with the perspective that the mice return to a normal body weight set point (*23*) in the absence of continuing ZI activation. To test whether ZI GABA neurons exert long-term effects on energy homeostasis, we microinjected AAV-flex-taCasp3-TEVp, which expresses caspase-3 (*24*), into the ZI of VGAT-Cre mice to selectively ablate ZI GABA neurons (fig. S7). Ablation of ZI GABA neurons decreased long-term food intake and reduced body weight gain by 53% over 8 weeks (Fig. 3, J and K).

To explore the neuronal pathway postsynaptic to the $VGAT^{ZI\text{-}PVT}$ axon terminals, we injected Cre-inducible AAV-ChIEF–tdTomato selectively into the PVT of vGlut2-Cre mice (Fig. 4A and fig. S8A). In brain slices, laser stimulation excited PVT ChIEF-tdTomato–expressing glutamatergic neurons (Fig. 4C). Laser stimulation (20 Hz) above the PVT of ChIEF-tdTomato mice significantly inhibited normal, sweet, and high-fat food intake during 1-hour tests (Fig. 4D and fig. S8B). The mean latency for mice to stop eating was 6.1 ± 2.0 s after the laser (20 Hz) was turned on (Fig. 4E). After mice were partially fasted with only 60% of the normal food available during the preceding night, laser stimulation (20 Hz, 10 min ON followed by 10 min OFF, two times) of ChIEF-expressing PVT vGluT2 neurons reduced food intake (Fig. 4, F to H).

A chemo-genetic designer receptor exclusively activated by designer drugs (DREADD) was used

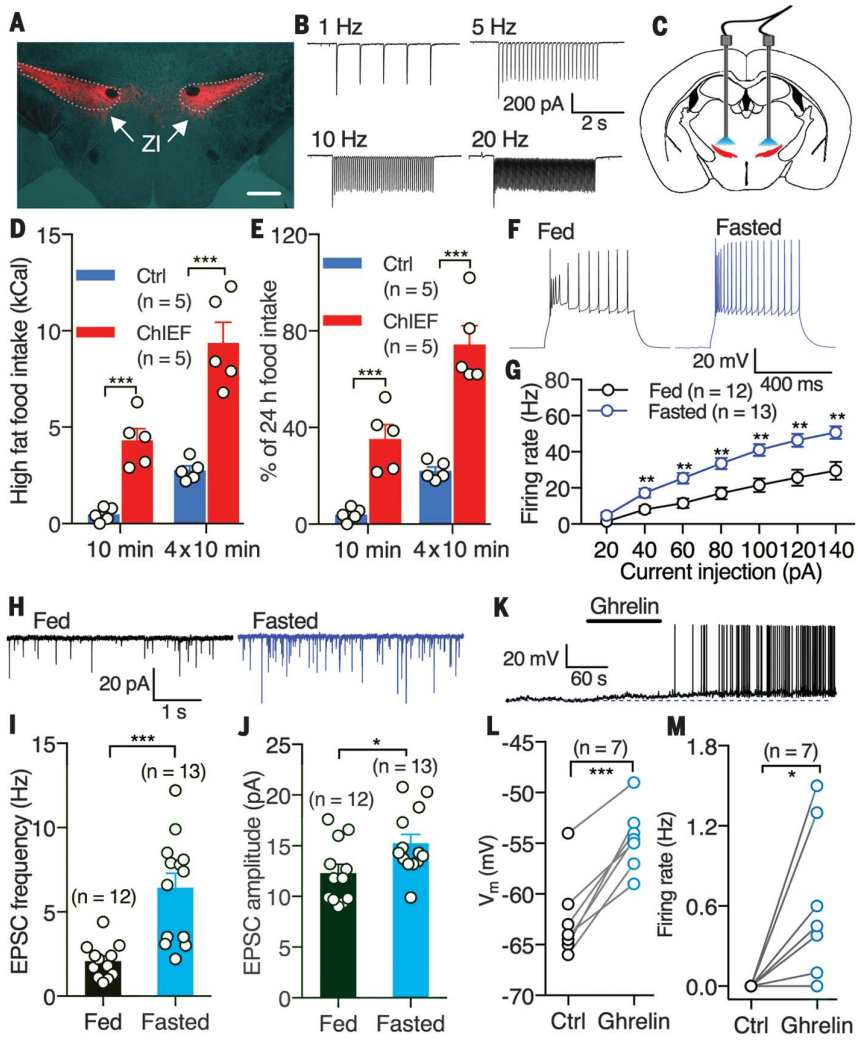

Fig. 1. Optogenetic activation of ZI GABA neurons rapidly evokes binge-like eating. (**A**) Red fluorescent image shows restricted expression of ChIEF-tdTomato in the ZI after AAV-ChIEF-tdTomato was bilaterally injected into the ZI of VGAT-Cre mice. Scale bar, 500 μm. (**B**) Optogenetic activation with varying frequency of a ZI GABA neuron in a brain slice. (**C**) Schematic illustration showing the location of optical fiber tips implanted above the ZI on both sides of the brain. (**D**) High-fat food intake during 10 min and four times 10 min from control mice with tdTomato and mice with ChIEF-tdTomato, both with ZI expression. For the 10-min trial, continuous light stimulation (10 ms, 20 Hz) was supplied to the ZI. For the 4 ×10-min trial, 10-min light stimulation (10 ms, 20 Hz) was followed by 30 min without stimulation, repeated four times. (**E**) High-fat food intake over 10 min and four times 10-min trial as a percentage of unstimulated 24-hour intake (100%). (**F**) Action potentials evoked by 100-pA current injection in ZI GABA neurons in brain slices of mice fed or fasted for 24 hours. (**G**) Firing rate at different levels of current injection from ZI GABA neurons in brain slices of mice fed or fasted for 24 hours. (**H**) Excitatory postsynaptic currents (EPSCs) in ZI GABA neurons of mice fed or fasted for 24 hours. (**I**) EPSC frequency from ZI GABA neurons of mice fed (n = 12 cells from each of four mice) or mice fasted for 24 hours (n = 13 cells from four mice). (**J**) EPSC amplitude from ZI GABA neurons in mice fed (n = 12 cells from four mice) or mice fasted for 24 hours (n = 13 cells from four mice). (**K**) Ghrelin (100 nM) excites a ZI GABA neuron. (**L**) Ghrelin depolarizes ZI GABA neurons. (**M**) Ghrelin increases the firing rate of ZI GABA neurons. Statistical analysis for comparison between two groups: Two-way analysis of variance (ANOVA) with Bonferroni post hoc comparison for (D) and (E); unpaired t test for (G), (I), (L) and (M). *P < 0.05; **P < 0.01; ***P < 0.001.

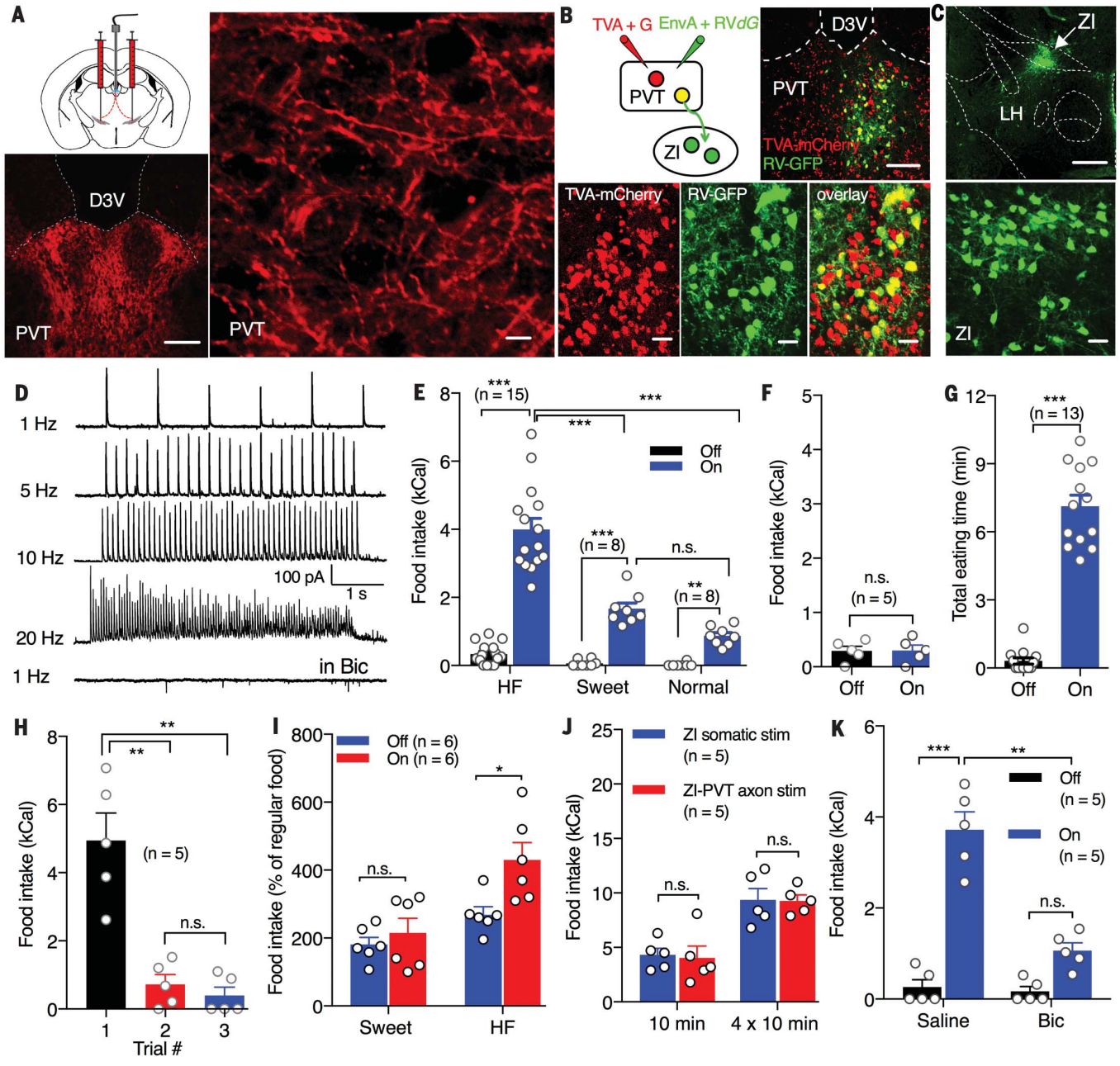

Fig. 2. Optogenetic activation of ZI GABA axon terminals in PVT rapidly evokes eating with preference for high-fat food. (**A**) Anterograde mapping of ZI GABA neuron projections to PVT. (Top left) Schematic shows bilateral injection of AAV-ChIEF-tdTomato into ZI and placement of fiber optic tip above PVT. (Bottom left) Red fluorescent image shows strong projection to PVT from ZI-VGAT-ChIEF-tdTomato neurons. D3V, dorsal third ventricle. Scale bar, 100 μm. (Right) ZI axons in PVT. Scale bar, 5 μm. (**B**) Retrograde mapping of presynaptic neurons to PVT glutamate neurons. (Top left) Schematic shows the strategy for tracing presynaptic ZI projections to PVT glutamate neurons. TVA, the avian tumor virus receptor A; RV*dg*, glycoprotein–deleted rabies virus (RV). (Top right) Selective expression of RV-GFP (green) and TVA-mCherry (red) in PVT. Scale bar, 300 μm. (Bottom) PVT neurons detected with TVA-mCherry (left), RV-GFP (middle) and merged image (right) shows originating cells (yellow, expressing both GFP and mCherry). Scale bar, 20 μm. (**C**) RV-labeled presynaptic neurons in ZI. LH, lateral hypothalamus. Scale bars: top, 300 μm; bottom, 20 μm. (**D**) Optogenetically evoked inhibitory postsynaptic currents (IPSCs) of PVT vGlut2 neurons at 1, 5, 10, and 20 Hz (membrane potential clamped at −40 mV). In bicuculline (Bic, 30 μM), 1-Hz pulses evoked no obvious current. (**E**) Photostimulation (10 ms, 20 Hz) of ZI-VGAT-ChIEF neuron terminals in PVT increases food intake during 10-min trial. (**F**) Photostimulation of PVT has no effect on food intake of VGAT-Cre mice after control AAV-tdTomato injection into ZI. (**G**) Cumulative time during eating by VGAT-ChIEF mice during 10-min photostimulation (10 ms, 20 Hz). (**H**) Food intake induced by photostimulation is greater in first 10-min trial, and reduced in second and third trial, with a 5-min interval between photostimulations. (**I**) $VGAT^{ZI\text{-}PVT}$ photostimulation increased preference for high-fat food. (**J**) Food intake for 24 hours with photostimulation of ZI VGAT neurons or $VGAT^{ZI\text{-}PVT}$ terminals. For 4 ×10-min trial, 10-min light stimulation (10 ms, 20 Hz) followed by 30-min no stimulation, repeated four times. (**K**) Bic attenuates optogenetic stimulation of food intake in PVT. Statistical analysis for comparison between two groups: two-way ANOVA with Bonferroni post hoc comparison for (E) and (J); unpaired *t* test for (G); one-way ANOVA repeated measure with Bonferroni post hoc comparison for (H); two-way ANOVA repeated measure with Bonferroni post hoc comparison for (I) and (K). n.s., not significant; $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$.

to test the hypothesis that silencing the cells postsynaptic to ZI GABA axons, the PVT glutamate neurons, would enhance food intake. We injected Cre-inducible AAV5-hSyn-HA-hM4D(Gi)-IRES-mCherry coding for the clozapine-*N*-oxide (CNO) receptor into the PVT of vGlut2-Cre mice (*25*, *26*) (fig. S9, A and B). CNO inhibited PVT neurons with hM3D(Gi)-mCherry receptor expression (fig. S9C). Intraperitoneal CNO produced an increase in food intake during a 3-hour trial (fig. S9D). To test whether PVT neuronal activity affects body weight gain, we microinjected AAV-flex-taCasp3-TEVp into the PVT of vGlut2-Cre mice to induce Cre-dependent caspase expression and selectively ablate PVT glutamatergic

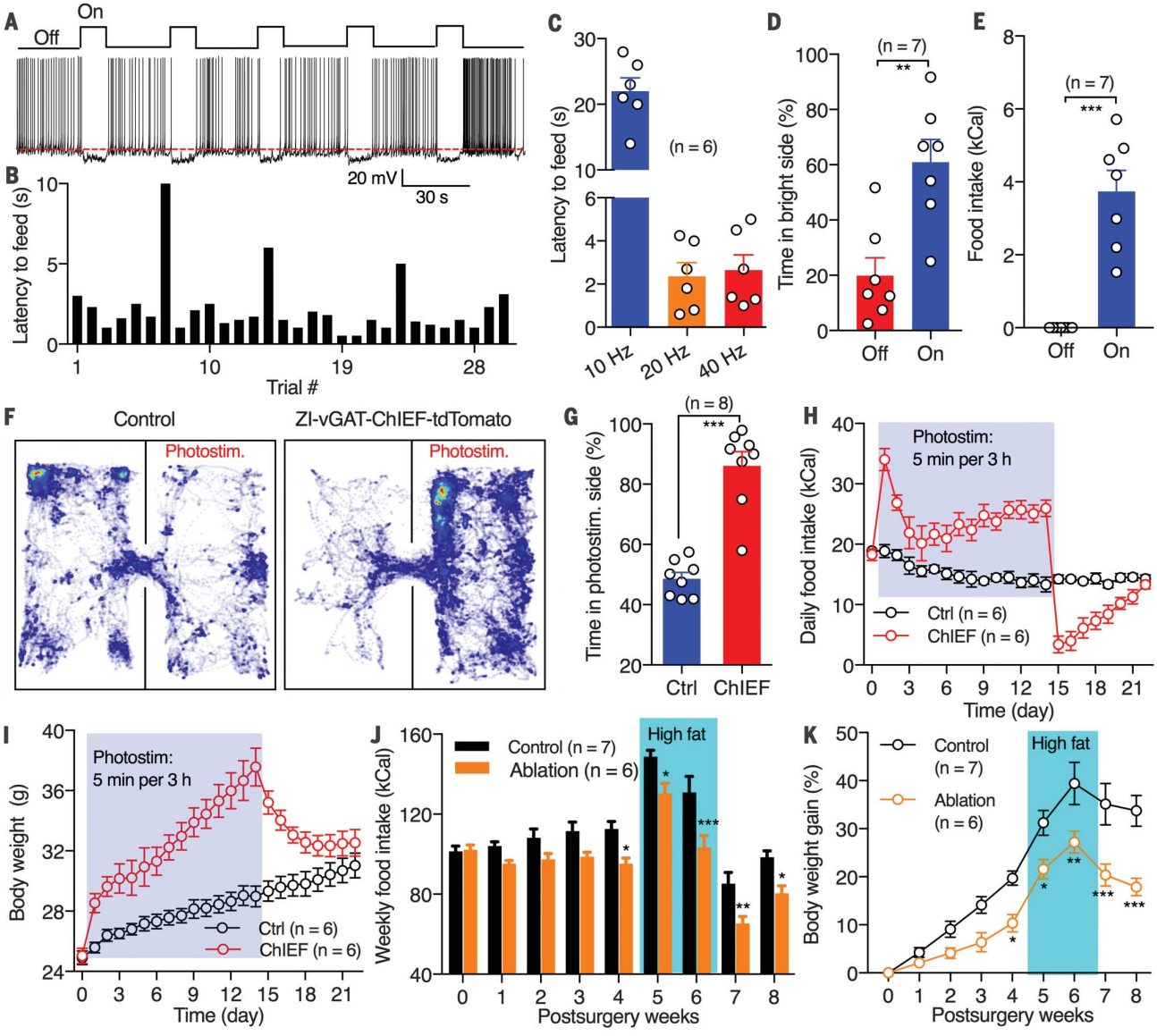

Fig. 3. Binge-like eating evoked by optogenetic activation of ZI GABA axon terminals produces rapid increase in body weight and shows positive hedonic association. (**A**) Photostimulation of ChIEF-expressing ZI axons reduces activity of PVT glutamate neuron in brain slice during repeated 10-s photostimulations (10 ms, 20 Hz) with 30-s rest interval. (**B**) Latency for representative mouse to rapidly initiate feeding in response to photostimulation over 30 consecutive trials. Photostimulation protocol same as in (A). (**C**) Latency of feeding initiation using 10-s photostimulation (10-ms pulses) at 10, 20, and 40 Hz. (**D**) Photostimulation increases time in lit chamber during light-dark conflict test. (**E**) Light-dark conflict test shows photostimulation increases high-fat food intake in brightly lit chamber. (**F**) Real-time place-preference data show tracks of control (left) and ZI VGAT-ChIEF mouse (right) in photostimulation-paired (photostim.) and nonpaired chambers. (**G**) Time (%) that control and ChIEF-tdTomato mice stay in photostimulation-paired chamber during a 20-min trial. (**H**) Daily food intake of control tdTomato and ChIEF-tdTomato mice with photostimulation of $VGAT^{ZI\text{-}PVT}$ terminals for 5 min (20 Hz) every 3 hours repeated over 14 days (shaded box) then continued without photostimulation (unshaded). $F_{1,230} = 343.9$, $P < 0.0001$, two-way ANOVA. (**I**) Body weight of tdTomato control and ChIEF-tdTomato mice with a 5-min (20-Hz) photostimulation of $VGAT^{ZI\text{-}PVT}$ terminals every 3 hours repeated over 14 days (shaded box), then continued without photostimulation (unshaded). Data were from the same mice tested in (D). $F_{1,230} = 73.45$, $P < 0.0001$, two-way ANOVA. (**J**) Weekly food intake from control and ZI VGAT neuron ablation. Ablation versus control: $F_{1,99} = 55.84$, $P < 0.0001$. (**K**) Body weight gain of mice from control group and ZI VGAT neuron ablation. Ablation versus control: $F_{1,99} = 60.12$, $P < 0.0001$. Statistical analysis for comparison between two groups: Paired *t* test for (D) and (E); unpaired *t* test for (G); two-way ANOVA with Bonferroni post hoc comparison for (J) and (K). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

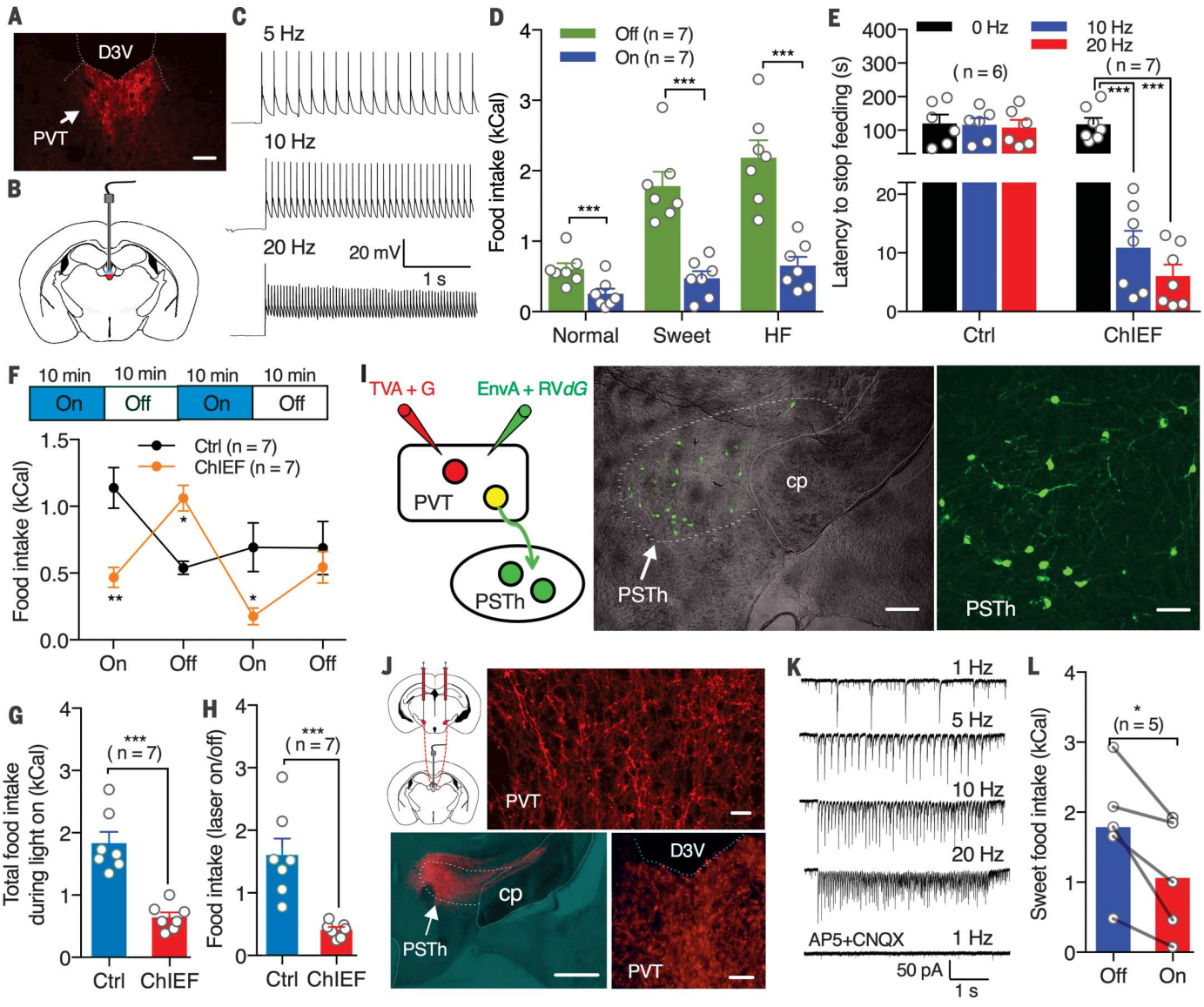

Fig. 4. Direct and indirect activation of glutamatergic PVT neurons postsynaptic to ZI axons inhibits food intake. (**A**) Fluorescent image shows restricted expression of ChIEF-tdTomato in PVT after AAV Cre-dependent ChIEF-tdTomato injected into PVT of vGlut2-Cre mice. Scale bar, 100 μm. (**B**) Implanted optical fiber tip above PVT. (**C**) Photostimulation (10-ms pulses) at 5, 10, and 20 Hz excites vGlut2-ChIEF neurons in PVT. (**D**) Photostimulation of PVT vGlut2-ChIEF neurons decreases intake of normal, sweet, and high-fat (HF) foods over 1 hour. (**E**) Latency in eating cessation in control and PVT vGlut2-ChIEF mice. (**F**) (Top) Four consecutive 10-min trial periods with first and third paired with stimulation (10 ms, 20 Hz). Food intake of control and vGlut2-ChIEF mice with partial food restriction during each period. (**G**) Total food intake is reduced by photostimulation in PVT of vGlut2-ChIEF mice with food restriction over 40 min compared with controls. (**H**) Food intake ratio of photostimulated to unstimulated periods in food-restricted control and vGlut2-ChIEF mice. (**I**) (Left) Schematic shows strategy for tracing presynaptic PSTh projections to PVT glutamate neurons. (Middle and right) Green images show fluorescent RV-labeled presynaptic PSTh neurons. cp, Cerebral peduncle. (**J**) Schematic shows bilateral AAV-ChIEF-tdTomato injections in parasubthalamic nucleus (PSTh) and placement of fiber optic tip above PVT. (Left bottom) Restricted expression of ChIEF-tdTomato in PSTh after AAV-ChIEF-tdTomato injection in vGlut2-Cre mice. Scale bar, 500 μm. (Right bottom) PSTh vGlut2 neurons project to PVT. Scale bar, 100 μm. (Top) Higher magnification shows PSTh vGlut2 neuron terminals in PVT. Scale bar, 5 μm. (**K**) EPSCs in PVT glutamate neurons evoked by photostimulation (10-ms pulses) at 1, 5, 10, and 20 Hz (membrane potential clamped at −70 mV). In 2-amino-5-phosphonopentanoic acid (AP5) (50 μM) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (10 μM), photostimulation of 1 Hz evoked no obvious current. (**L**) Photostimulation (10 ms, 20 Hz) of excitatory vGlut2 neuron terminals in PVT projecting from the PSTh decreased food intake. Statistical analysis for comparison between two groups: paired *t* test for (D) and (L); one-way ANOVA with Bonferroni post hoc comparison for (E) and (F); unpaired *t* test for (G) and (H). **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

neurons. To confirm that PVT vGlut2 neurons were killed by the virus-generated caspase-3, we injected the Cre-dependent reporter construct AAV-tdTomato simultaneously with AAV-flex-taCasp3-TEVp to corroborate that reporter-expressing neurons were absent after selective caspase expression. With coinjection, little tdTomato expression was detected, whereas many cells were detected with injections of AAV-tdTomato by itself, consistent with the elimination of vGluT2 neurons in the PVT (fig. S10, A to D). Ablation of PVT vGluT2 neurons substantially increased both food intake and body weight gain for an extended period (16-week study) (fig. S10, G and H).

In our monosynaptic retrograde tracing with Cre-dependent rabies virus, although less robust than the projection from the ZI, we found a substantial projection to PVT glutamate neurons from the parasubthalamic nucleus (PSTh) (Fig. 4I and fig. S11) (*27*, *28*). That the PSTh may be involved in feeding is suggested by increased c-*fos* expression in the PSTh during anorexia induced by amino acid deficiency (*29*). To test whether the PSTh maintains an excitatory input to PVT glutamatergic neurons that could serve to antagonize binge-like eating evoked by $VGAT^{ZI\text{-}PVT}$ inhibitory pathway activation, we injected Cre-inducible AAV-ChIEF–tdTomato bilaterally into the PSTh of vGlut2-Cre mice (Fig. 4J). Restricted expression of ChIEF–tdTomato was observed in the PSTh cell bodies (Fig. 4J) and in large numbers of PSTh axon terminals in the PVT (Fig. 4J). Brain slice electrophysiology confirmed that optogenetic activation of PSTh glutamatergic neuron terminals in the PVT evoked strong glutamate-mediated postsynaptic excitatory currents in PVT vGlut2-GFP neurons, suggesting a functional role for PSTh glutamate neurons in the synaptic excitation of PVT glutamate neurons (Fig. 4K). Stimulation of PSTh glutamatergic neuron terminals in the PVT inhibited food intake (Fig. 4L). Furthermore, optogenetic activation of the $vGlut2^{PSTh\text{-}PVT}$ excitatory pathway in a two-chamber place-preference test generated a significant aversion associated with the laser stimulation–paired chamber (fig. S12).

Together, our data demonstrate a powerful inhibitory projection from the ZI to the PVT that can reliably generate rapid and substantial eating. That the ZI GABA cells may participate in energy homeostasis is suggested by electrophysiological data showing increased activity of these cells after food deprivation and in the presence of the empty gut–signaling peptide ghrelin. Based on retrograde rabies virus and anterograde AAV tracing, ZI axonal projections to the excitatory neurons of the PVT appear more robust than those from other known regions of the brain involved in food intake, suggesting the ZI is not a minor component; furthermore, optogenetic stimulation of the ZI generated a more robust feeding response than stimulation of the much-studied lateral hypothalamus, further suggesting that the ZI can play a substantive role in enhancing food consumption. Our study provides a potential explanation for why clinical deep brain stimulation in the ventral thalamus near the ZI can increase binge eating.

REFERENCES AND NOTES

1. L. B. Zahodne *et al*., *J. Neuropsychiatry Clin. Neurosci.* **23**, 56–62 (2011).
2. P. Amami *et al*., *J. Neurol. Neurosurg. Psychiatry* **86**, 562–564 (2015).
3. L. Novakova *et al*., *Neuroendocrinol. Lett.* **32**, 437–441 (2011).
4. J. I. Hudson, E. Hiripi, H. G. Pope Jr., R. C. Kessler, *Biol. Psychiatry* **61**, 348–358 (2007).
5. S. A. Swanson, S. J. Crow, D. Le Grange, J. Swendsen, K. R. Merikangas, *Arch. Gen. Psychiatry* **68**, 714–723 (2011).
6. D. L. Benson, P. J. Isackson, C. M. Gall, E. G. Jones, *Neuroscience* **46**, 825–849 (1992).
7. K. M. Kendrick, M. R. Hinton, B. A. Baldwin, *Brain Res.* **550**, 165–168 (1991).
8. N. Urbain, M. Deschênes, *Neuron* **56**, 714–725 (2007).
9. J. Chen, A. R. Kriegstein, *Science* **350**, 554–558 (2015).
10. J. Y. Lin, M. Z. Lin, P. Steinbach, R. Y. Tsien, *Biophys. J.* **96**, 1803–1814 (2009).
11. X. Zhang, A. N. van den Pol, *Nat. Neurosci.* **19**, 1341–1347 (2016).
12. M. Tschöp, D. L. Smiley, M. L. Heiman, *Nature* **407**, 908–913 (2000).
13. J. S. Lee, E. Y. Lee, H. S. Lee, *Brain Res.* **1598**, 97–113 (2015).
14. G. J. Kirouac, M. P. Parsons, S. Li, *J. Comp. Neurol.* **497**, 155–165 (2006).
15. T. R. Stratford, D. Wirtshafter, *Brain Res.* **1490**, 128–133 (2013).
16. E. E. Hur, L. Zaborszky, *J. Comp. Neurol.* **483**, 351–373 (2005).
17. H. Huang, P. Ghosh, A. N. van den Pol, *J. Neurophysiol.* **95**, 1656–1668 (2006).
18. B. A. Stamoutsos, R. G. Carpenter, L. Grossman, S. P. Grossman, *Physiol. Behav.* **23**, 771–776 (1979).
19. Y. Aponte, D. Atasoy, S. M. Sternson, *Nat. Neurosci.* **14**, 351–355 (2011).
20. J. N. Betley, Z. F. Cao, K. D. Ritola, S. M. Sternson, *Cell* **155**, 1337–1350 (2013).
21. E. M. Schulte, C. M. Grilo, A. N. Gearhardt, *Clin. Psychol. Rev.* **44**, 125–139 (2016).
22. R. M. Kessler, P. H. Hutson, B. K. Herman, M. N. Potenza, *Neurosci. Biobehav. Rev.* **63**, 223–238 (2016).
23. R. E. Keesey, T. L. Powley, *Annu. Rev. Psychol.* **37**, 109–133 (1986).
24. C. F. Yang *et al*., *Cell* **153**, 896–909 (2013).
25. B. N. Armbruster, X. Li, M. H. Pausch, S. Herlitze, B. L. Roth, *Proc. Natl. Acad. Sci. U.S.A.* **104**, 5163–5168 (2007).
26. G. M. Alexander *et al*., *Neuron* **63**, 27–39 (2009).
27. M. Goto, L. W. Swanson, *J. Comp. Neurol.* **469**, 581–607 (2004).
28. S. Li, G. J. Kirouac, *Brain Struct. Funct.* **217**, 257–273 (2012).
29. X. Zhu *et al*., *Am. J. Physiol. Endocrinol. Metab.* **303**, E1446–E1458 (2012).

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SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/356/6340/853/suppl/DC1
Materials and Methods
Figs. S1 to S12
References
Movies S1 to S4

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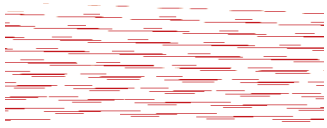

Rapid binge-like eating and body weight gain driven by zona incerta GABA neuron activation

Xiaobing Zhang and Anthony N. van den Pol (May 25, 2017)

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Editor's Summary

A neuronal circuit for overeating

Recurrent binge eating is a common eating disorder. Zhang and van den Pol investigated an understudied brain region known as the zona incerta and found that it projects inhibitory inputs to the paraventricular thalamus, a brain region involved in suppressing feeding behavior. In mice, acute stimulation of this inhibitory projection resulted within seconds in overeating, especially high-fat food. Chronic stimulation induced persistent overeating and weight gain.

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