Endogenous Glucagon-like Peptide-1 Suppresses High-Fat Food Intake by Reducing Synaptic Drive onto Mesolimbic Dopamine Neurons

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In Brief
Wang et al. elucidated that the centrally released endogenous glucagon-like peptide-1 from the nucleus tractus solitarius suppresses high-fat food intake by reducing synaptic drive onto mesoaccumbens dopaminergic neurons.

Highlights
- NTS GLP-1 neuron activation suppresses food intake
- Activation of GLP-1 terminals in the VTA suppresses high-fat diet intake
- GLP-1 specifically decreases excitatory synaptic input in mesolimbic DA neurons
Endogenous Glucagon-like Peptide-1 Suppresses High-Fat Food Intake by Reducing Synaptic Drive onto Mesolimbic Dopamine Neurons

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SUMMARY

Glucagon-like peptide-1 (GLP-1) and its analogs act as appetite suppressants and have been proven to be clinically efficacious in reducing body weight in obese individuals. Central GLP-1 is expressed in a small population of brainstem cells located in the nucleus tractus solitarius (NTS), which project to a wide range of brain areas. However, it remains unclear how endogenous GLP-1 released in the brain contributes to appetite regulation. Using chemogenetic tools, we discovered that central GLP-1 acts on the midbrain ventral tegmental area (VTA) and suppresses high-fat food intake. We used integrated pathway tracing and synaptic physiology to further demonstrate that activation of GLP-1 receptors specifically reduces the excitatory synaptic strength of dopamine (DA) neurons within the VTA that project to the nucleus accumbens (NAc) medial shell. These data suggest that GLP-1 released from NTS neurons can reduce highly palatable food intake by suppressing mesolimbic DA signaling.

INTRODUCTION

The central glucagon-like peptide-1 (GLP-1) system plays a crucial role in the control of food intake (Turton et al., 1996). GLP-1 signaling is among the most promising targets in the brain for treating overeating disorders (Alhadeff et al., 2012; Dossat et al., 2013; Drucker et al., 2008; Meenan et al., 1999; Secher et al., 2014; Sisley et al., 2014). GLP-1 analogs have been used to treat type 2 diabetes (for review, see Lovshin and Drucker, 2009), and a GLP-1 receptor (GLP-1R) agonist, Saxenda (liraglutide), has recently been approved to treat obesity (U.S. Food and Drug Administration, 2014). Central GLP-1 is mainly secreted by a small group of neurons located within the nucleus tractus solitarius (NTS) in the brainstem. GLP-1-expressing neurons project broadly to other brain regions including the hypothalamus, the ventral tegmental area (VTA), and the nucleus accumbens (NAc) (Gu et al., 2013). Accordingly, expression of GLP-1Rs has been detected in many brain areas, such as the VTA and NAc (Merchenthaler et al., 1999). Nevertheless, it is still not fully understood how release of central GLP-1 within the brain regulates food intake.

Regulatory mechanisms underlying the control of feeding may be divided into two categories: homeostatic (i.e., hunger-induced feeding to maintain energy balance) and reward-related (i.e., hedonic or pleasure-driven acquisition of highly palatable food). Feeding behavior is ultimately determined by a complex interaction between the two (Liu et al., 2015). Hedonic eating has become a key cause of weight gain and obesity. Therefore, there is a pressing need to further investigate the role of reward circuitry in the regulation of feeding behavior (Volkow et al., 2011). The neural circuits governing food intake intertwine with those mediating reward, and the midbrain dopamine (DA) system has been suggested to play a pivotal role in the regulation of reward-related behaviors, including eating (Liu et al., 2015; Volkow et al., 2011). Several studies report that pharmacologic manipulations of GLP-1 signaling, i.e., using GLP-1 analog Exendin-4 (Exn4) or GLP-1R blocker Exendin-9 (Exn9) infusions, in the VTA (Dickson et al., 2012; Mietlicki-Baase et al., 2013), NAc (Alhadeff et al., 2012; Dossat et al., 2011, 2013), NTS (Alhadeff and Grill, 2014), and hippocampus (Hsu et al., 2015), affect the appetitive and motivational aspects of feeding. Together, these findings suggest that GLP-1 signaling may affect hedonic food intake. Nevertheless, the neural basis of such effects remains enigmatic.

Utilizing chemogenetic tools, we demonstrated that endogenously released GLP-1 from the NTS is sufficient to suppress high-fat (HF) food intake. More specifically, we found that activation of NTS-originating GLP-1 nerve terminals in the VTA is sufficient to suppress HF food intake. Furthermore, we uncovered that GLP-1R activation directly impedes excitatory synaptic drive onto VTA-to-NAc medial shell projecting DA neurons. Thus, GLP-1 released from NTS neurons may reduce highly palatable food intake through suppression of mesolimbic DA signaling.

RESULTS

Chemogenetic Activation of GLP-1-Expressing Neurons Suppresses Food Intake

To precisely target GLP-1-expressing neurons in the NTS, we took advantage of paired-like homeobox 2b (Phox2b)-Cre
bacterial artificial chromosome (BAC) transgenic mice, which express Cre recombinase in GLP-1-containing neurons within the NTS (Scott et al., 2011). Adeno-associated virus (AAV) expressing Cre-activated yellow fluorescent protein (YFP) was injected into the NTS of Phox2b-Cre animals to specifically visualize GLP-1 neurons (Figures 1A, 1B, and S1). We employed chemogenetics to address whether the activation of NTS GLP-1 neurons affects food intake by expressing designer receptors exclusively activated by designer drugs (DREADDs). Specifically, we expressed Cre-activated hM3Dq or hM4Di DREADDs in the NTS of Phox2b-Cre mice through local stereotactic injections of AAVs. Upon binding to clozapine-N-oxide (CNO), a synthetic agonist of DREADDs, Gq-coupled hM3Dq activates neuronal burst firing while Gi-coupled hM4Di inhibits neuronal firing (Sternson and Roth, 2014). By genetically encoding these designer receptors into GLP-1 neurons in the NTS (Figures S1B–S1D), we were able to control the activity of these neurons in a temporal and spatial manner and evaluate the subsequent impact on feeding behavior.

Immunostaining showed that neurons in the NTS expressing DREADDs or YFP were exclusively activated by designer drugs (DREADDs). Specifically, we expressed Cre-activated hM3Dq or hM4Di DREADDs in the NTS of Phox2b-Cre mice through local stereotactic injections of AAVs. Upon binding to clozapine-N-oxide (CNO), a synthetic agonist of DREADDs, Gq-coupled hM3Dq activates neuronal burst firing while Gi-coupled hM4Di inhibits neuronal firing (Sternson and Roth, 2014). By genetically encoding these designer receptors into GLP-1 neurons in the NTS (Figures S1B–S1D), we were able to control the activity of these neurons in a temporal and spatial manner and evaluate the subsequent impact on feeding behavior.

Immunostaining showed that neurons in the NTS expressing these DREADDs or YFP also express GLP-1 (Figure S1). To assay food-intake behavior, animals were given ad libitum access to both standard rodent chow and a highly palatable HF diet (Van Heek et al., 1997). Daily food intake was measured for 5 days before performing chemogenetic experiments, in order to establish a stable baseline of food consumption, and intraperitoneal (i.p.) CNO injections were then performed for 3 consecutive days as experimental trials (Figures 1C and 1D).

Activation of NTS GLP-1 neurons by CNO in Gq-coupled hM3Dq-expressing animals led to significant decreases in HF food intake compared to control mice expressing mCherry alone (Figures 1D and 1E). Interestingly, the same procedure showed little effect on standard chow intake (Figures S2A–S2C). This is in contrast to the behavior induced by pharmacological activation of GLP-1 receptors by Exn4 (Alhadeff et al., 2012). The activation of NTS GLP-1 neurons had little impact on body weight within 24 hr after application of CNO (Figure S2D), which is consistent with results from deletion of neuronal GLP-1Rs (Sisley et al., 2014). Conversely, hM4Di-expressing mice showed increased HF food intake (Figures 1E, S2, and S3), suggesting that inhibition of NTS GLP-1 neurons can facilitate food intake.

To unequivocally define the specific involvement of GLP-1 signaling in the suppression of HF food intake, we pre-administered GLP-1R antagonist Exn9 before the application of CNO in a subset of hM3Dq-expressing animals. Remarkably, we found that the food intake suppression induced by CNO activation of hM3Dq receptors was blocked, indicating that appetite suppression was specifically achieved by activating GLP-1 release from hM3Dq-expressing NTS neurons (Figure 1F). The terminals of NTS GLP-1 neurons projecting to VTA express vGluT2 (Zheng et al., 2014), but not vGluT1 (Figure S4A); thus, they may also release glutamate. However, our data provide strong evidence that endogenous GLP-1 released from NTS neurons projecting to the VTA suppresses food intake.

**Figure 1. Activation of NTS GLP-1 Neurons Releases GLP-1 and Reduces High-Fat Diet Intake**

(A and B) A representative sagittal mouse brain section showing viral infection of NTS neurons with Cre-dependent AAV-DIO-channelrhodopsin-YFP in Phox2b-Cre mice. (C) Illustration depicts Phox2b-Cre animals injected with AAV DIO-DREADDs (hM3Dq) and timeline of experiments (D–F). (D) Normalized high-fat (HF) diet intake measured 5 hr after i.p. injection of either PBS (control/recovery) or CNO. There was an overall time effect (p < 0.05) and time × group effect (p < 0.001); post hoc t tests were used for comparison of individual time points.

(E) Normalized HF diet intake at 5 hr during the CNO i.p.-injection period only (mCherry n = 7; hM3Dq n = 7; Exn9 n = 6). Absolute HF diet intake over 3 days of CNO application was normalized to the averages of the baseline (5 days). Absolute food intake amount in different groups at different times is shown in Figure S2. Student’s t tests were used.

(F) HF diet intake suppression induced by hM3Dq activation was blocked by the specific GLP-1 receptor antagonist Exendin-9 (Exn9) (mCherry n = 7; hM3Dq n = 7; Exn9 n = 6). Exn9 was applied 15 min before the application of CNO. There was an overall time effect (p < 0.001), group effect (p < 0.001), and time × group effect (p < 0.001); ANOVA 2 hr p < 0.001; post hoc Bonferroni: mCherry versus hM3Dq p < 0.001; mCherry versus hM3Dq + Exn9 p = 0.051; hM3Dq versus hM3Dq + Exn9 p = 0.002. ANOVA 5 hr: p < 0.001; post hoc Bonferroni: mCherry versus hM3Dq p < 0.001, mCherry versus hM3Dq + Exn9: p = 0.227, hM3Dq versus hM3Dq + Exn9: p = 0.002.

All values represent mean ± SEM. *p < 0.05, **p < 0.01, ***p < 0.001.
GLP-1 Release in the VTA Regulates HF Food Intake

Next, we asked whether GLP-1 signaling within the mesolimbic system is involved in regulating HF food intake. Consistent with previous reports (Dickson et al., 2012; Mietlicki-Baase et al., 2013), we demonstrated that intra-VTA infusion of Exn4 produced profound HF food intake suppression relative to control animals undergoing saline injections (Figures 2A and 2B). Therefore, we speculated that the VTA is a critical component of the neural circuitry through which GLP-1 controls reward-driven food intake. Next, we sought to use DREADDs to induce the release of GLP-1 from the nerve terminals. The hM3Dq is a Gq-coupled DREADD, and its activation stimulates the production of inositol triphosphate and diacylglycerol (Pei et al., 2008), both of which are known to increase synaptic vesicle release at the nerve terminal (Nakamura et al., 1999; Rhee et al., 2002). We injected CNO directly into the VTA of hM3Dq-mCherry-, hM4Di-mCherry-, and mCherry-expressing mice (Figure S4B), and we observed whether CNO altered food intake behavior. Interestingly, consistent with results from microinjections of Exn4 into the VTA (Figure 2B), local chemogenetic activation of GLP-1 nerve terminals in the VTA in hM3Dq-expressing mice significantly suppressed HF food intake (Figures 2C–2F). We cannot exclude the possibility that action potentials generated by CNO at the nerve terminals might back propagate to the cell body and then possibly release GLP-1 in other brain regions. However, our data suggest that suppression of food intake by hM3Dq and CNO is at least partially mediated by locally released GLP-1. Infusion of CNO into the VTA did not produce altered feeding behavior in hM3Di-expressing mice (Figures S4C and S4D), suggesting that dampening GLP-1 signaling in the VTA alone may not be sufficient to induce the acute overeating response observed after broader suppression of all GLP-1 neurons by i.p. injections of CNO (Figure 1E).

Our data therefore demonstrate that local activation of NTS projections in the VTA is likely sufficient to decrease HF food intake, supporting the hypothesis that central GLP-1 release can regulate feeding behavior via signaling pathways within the reward centers of the brain.

GLP-1R Activation Suppresses Excitatory Synaptic Inputs onto the VTA-to-NAc-Projecting DA Neurons

Given that synaptic transmission and plasticity govern information flow in the brain and control behavioral outcomes, we hypothesized that GLP-1 signaling modulates synaptic transmission in the mesolimbic system. We focused on the modulatory effects of GLP-1 in VTA-to-NAc medial-shell-projecting neurons, which compose a well-established reward pathway (Lammel et al., 2011, 2012). To identify these neurons, we injected retrograde fluorescent microbeads into the NAc medial shell (Figure 3A). The majority of VTA-to-NAc-projecting neurons were DA, as shown by detection of tyrosine hydroxylase (TH) by immunohistochemistry (IHC) (Figure 3A). In order to study morphologic and electrophysiologic properties of these neurons, we performed whole-cell patch-clamp recordings. We included fluorescent dye (either Alexa 594-dextran or Alexa 488-biocytin) for filling the recorded cells. Neuronal subtypes were identified by post hoc IHC for TH (Figures 3B and 3C). We found that TH+ DA VTA neurons have more dendritic branch order than TH+ DA VTA neurons (n = 7). Student’s t tests were used. All values represent mean ± SEM. *p < 0.05, **p < 0.001. Post hoc t tests were used.
and TH \(^+\)/C0 VTA-to-NAc-projecting neurons. The sizes of \(I_\text{h}\) have been used for electrophysiologic identification of the VTA DA neurons (Chieng et al., 2011). However, these data suggest that categorizing VTA neurons as DA or non-DA by measuring \(I_\text{h}\) current alone may not be adequate, as described previously (Ungless and Grace, 2012).

To test whether GLP-1R activation regulates synaptic plasticity within the VTA, we recorded EPSCs in VTA-to-NAc medial-shell-projecting neurons in the absence or presence of GLP-1R agonist Exn4 (Figures 3, 4, and 5). Neuronal subtypes were identified by post hoc IHC for TH. In TH\(^+\) VTA-to-NAc projecting neurons, application of Exn4 consistently suppressed AMPA-receptor-mediated EPSCs (Figures 3D and 3E). However, no significant change was found in NMDA EPSCs before and after Exn4 application (Figure 3F). These data suggest that postsynaptic modifications reduce the excitatory synaptic strength, most likely through the specific removal of AMPA receptors from the postsynaptic membrane (otherwise, NMDA-receptor-mediated EPSCs would additionally show a reduction after Exn4 application). We then measured the AMPA/NMDA EPSC ratio, which suggested modifications in synaptic strength by the insertion or removal of AMPA receptors during long-term synaptic plasticity. We found a consistent reduction in the AMPA/NMDA EPSC ratio after the application of Exn4 (Figure 3G), further suggesting a postsynaptic modification.

In order to further delineate the pre- and post-synaptic mechanisms, we first calculated the coefficient of variation of evoked AMPA-receptor-mediated EPSCs, which showed no significant change with application of Exn4, suggesting no obvious alteration in presynaptic release probability. Moreover, we also recorded miniature EPSCs (mEPSCs) in the presence of tetrodotoxin (TTX) and found that the amplitude in DA neurons significantly decreased following application of Exn4 (Figures 4A–4C). Additionally, the paired-pulse ratio of evoked EPSCs showed no obvious change before or after Exn4 application (Figures S5I and S5J), again suggesting unaltered presynaptic vesicle release probability.

In contrast, in TH\(^-\) (non-DA) VTA-to-NAc-projecting neurons, the application of Exn4 produced minor changes in AMPA- and
NMDA-receptor-mediated evoked EPSCs, a moderate increase in the AMPA/NMDA ratio (in contrast to the decrease in TH+ neurons), and no change in the coefficient of variance of EPSCs (Figures 3C and 3I–3M). Moreover, we did not observe changes in mEPSCs in non-DA neurons (Figures 4D–4F). Together with the data from VTA-to-NAc-projecting DA neurons, these data suggest a cell-type-specific effect of GLP-1R activation on synaptic plasticity in the VTA.

Considering that neurons in the VTA also receive inhibitory synaptic inputs that are mediated by GABA (Johnson and North, 1992), we also studied the regulatory effects of GLP-1R activation on inhibitory synaptic inputs onto VTA-to-NAc medial-shell-projecting neurons. Activation of GLP-1R-s by Exn4 resulted in an increase in the amplitude of spontaneous inhibitory postsynaptic currents (sIPSCs) in DA VTA neurons (Figures 5A–5D). In contrast, GLP-1R activation had no obvious impact on sIPSCs in non-DA neurons (Figures 5E–5H). Furthermore, no significant differences were detected in miniature inhibitory postsynaptic currents (mIPSCs) in the presence of TTX (Figures 5I and 5J). Thus, Exn4 may facilitate inhibitory synaptic transmission mediated by spontaneous action potentials in the VTA-to-NAc-projecting DA neurons.

**DISCUSSION**

This study demonstrates that release of endogenous GLP-1 from NTS neurons projecting to the VTA is sufficient to reduce highly palatable food intake in mice. Moreover, we identified that excitatory synaptic drive onto VTA DA neurons projecting to the medial shell of the NAc is specifically suppressed upon GLP-1R activation, implying a downregulation of VTA-to-NAc DA signaling.

GLP-1 receptors are broadly expressed in the brain including the hypothalamus and reward centers such as the NAC and VTA (Merchenthaler et al., 1999). Food intake can be regulated by homeostatic (hunger-driven) signals and hedonic (reward-related) signals, and the mesolimbic DA system is a common neural integrator involved in hedonic food intake control (Kenny, 2011; Volkow et al., 2011). Exogenous application of GLP-1 analogs in the VTA suppresses food intake (Dickson et al., 2012; Mietlicki-Baase et al., 2013). Consistent with these observations, our data show that exogenous application of GLP-1R agonist Exn-4 in the VTA is sufficient to reduce consumption of HF food. We also demonstrate that endogenously released GLP-1 in the VTA is sufficient to reduce HF food intake (Figure 1). We thus propose that endogenous GLP-1 signaling within the mesolimbic system may be an effective target to manipulate eating behavior.

The mechanisms underlying GLP-1-mediated food-intake suppression are not fully understood. Limited reports on the role of GLP-1 in regulating synaptic function show that GLP-1R activation increases firing in hypothalamic orexin/hypocretin neurons by regulating intrinsic membrane properties (e.g., sodium and calcium channel conductance and spontaneous neurotransmitter release) (Acuna-Goycolea and van den Pol, 2004). In pancreatic-projecting neurons in the brainstem, GLP-1 has been shown to facilitate sEPSC and sIPSC release (Wan et al., 2007). Our data indicate that GLP-1 analog Exn4 regulates synaptic transmission in a cell-type-specific and synapse-specific manner. Among the VTA-to-NAc medial-shell-projecting neurons, DA neurons show a decrease in excitatory synaptic strength and a facilitation of inhibitory synaptic inputs upon administration of Exn4 (Figures 4D and 5F). However, non-DA neurons show increased excitatory synaptic strength (without perturbed inhibitory synaptic strength) upon GLP-1R activation (Figures 4 and 5).

Unequivocal identification of the specific pathways (e.g., VTA to NAC) and cell types (e.g., DA versus non-DA neurons) is particularly important for interpreting functional information in the VTA, because (1) previous identification of DA neurons within the VTA based on electrophysiological characteristics remains controversial (Ungless and Grace, 2012) and (2) VTA DA neurons projecting to the NAc or prefrontal cortical brain regions have differential responses toward reward and aversion (Lammel et al., 2011, 2012). Our data support this complexity, although they somewhat contradict results from a previous report.
showing facilitation of excitatory synaptic transmission by Exn4 in putative DA neurons identified by the sizes of $I_h$ current in the rat VTA (Mietlicki-Baase et al., 2013). The discrepancy could arise from differences in neuronal subtypes, given that as mentioned previously, electrophysiology may not be a reliable method for identifying DA VTA neurons and projection pathways were not defined. Of course, species differences may also exist.

Within the core of the NAc, GLP-1Rs seem to have no regulatory effect on DA nerve terminals, because application of Exn4 appears to not alter DA release in striatal brain slices lacking midbrain cell bodies (Mietlicki-Baase et al., 2014). These data simply indicate that GLP-1R signaling in the NAc core does not involve local regulation of DA release and are not at all contradictory to our hypothesis that DA released by VTA-to-NAc-projecting neurons functions to reduce appetite by decreasing pleasure and reward derived from palatable food. Support for this hypothesis was provided by a recent study in humans demonstrating that DA enhances the neural reward response to food, thereby influencing food-intake behavior (Medic et al., 2014). We thus hypothesize that endogenous release of central GLP-1 suppresses mesolimbic DA signaling and reduces appetite by decreasing the pleasure and reward derived from palatable food.

We show evidence that excitatory input to VTA-to-NAc medial-shell-projecting DA neurons is reduced, implying
decreased release of DA into the NAc. In exploring the relationships among food intake, GLP-1 signaling, and synaptic transmission within the reward neural circuitry, we provide an integrated perspective that likely has implications for other motivated behaviors, such as drug abuse and addiction (Egecioglu et al., 2013; Shirazi et al., 2013). Further studies examining other brain regions, circuits, and cell types, as well as the underlying molecular mechanisms, are needed to comprehensively understand the role of GLP-1 in regulating food intake and other reward-driven behaviors.

EXPERIMENTAL PROCEDURES

Animals

All procedures involved using mice were approved by the Rutgers Robert Wood Johnson Medical School Institutional Animal Care and Use Committee. The Phox2b-Cre mice (Scott et al., 2011) were obtained from The Jackson Laboratory (stock 016223). Wild-type mice (C57/B16) were purchased from The Jackson Laboratory.

AAV Injection of NTS Neurons

Under isoflurane inhalation anesthesia (VetEquip) and using a stereotaxic instrument (KOPF M1900), Phox2b-Cre mice were injected bilaterally (0.6–1 µl each side) in the NTS (bregma −3.15 mm; lateral ±0.6 mm; ventral 4.15 mm) with either AAV-hSyn-DIO-hM4Di-mCherry, AAV-hSyn-DIO-hM3Dq-mCherry, or AAV-hSyn-DIO-hmCherry, or AAV-DIO-Chr-YFP (UNC GTC Vector Core). The Chr-YFP is membrane-bound and allows for detailed morphological analyses of both the cell bodies and axons of Cre-expressing neurons. Mice were allowed a survival period of 14 days prior to experimental manipulation. Injections sites were confirmed in all animals.

IHC

Mice were anesthetized with euthasol and transcardially perfused with 4% paraformaldehyde (PFA) (PBS pH 7.4). Either coronal or sagittal brain slices (50 µm) were prepared, and a standard IHC protocol was followed. The primary antibodies used were TH (affinity-purified rabbit anti-tyrosine hydroxylase 1:1,000; Abcam), vGlut1 (mouse monoclonal 1:1,000; Abcam), vGlut2 (NeuroMab), and GLP-1 (affinity-purified chicken anti-GLP-1, peptide sequences HAEQFTSDVSSYC, or Peninsula Laboratories T-4363). Alexa Fluor secondary antibodies used to visualize the signal using a confocal microscope. For slice physiology post hoc immunostaining, brain slices were fixed in 4% PFA for 2 hr, then washed in PBS and processed with IHC.

Behavioral Test

Mice (7–8 weeks old) were housed on a 12-hr light (06:00)/dark (18:00) cycle with ad libitum access to water, standard chow (fat 21.63% mouse diet 20, LabDiet), and HF diet (45 kcal% fat, Research Diet) in all behavioral experiments. Note that when mice were given a standard chow and HF diet, the animals would mainly consume the HF diet. Prior to experiments, animals were housed in individual cages for 3 days. All i.p. and stereotaxic injections with Exn4 (Tocris, 2.4 µg/kg, i.p. and 0.24 µg/kg for intracraniol) 0.9% saline or CNO (0.3 µg/kg, 100 µl, i.p. and 0.03 µg/kg, 50 µl each side, for intracranial) were performed at the beginning of the dark cycle (start time 18:00 hr). For assaying the involvement of GLP1-R in food intake suppression by NTG GLP1 neuronal activation using chemogenetics, Exn9 (8.4 µg/kg) was injected i.p. 15 min prior to i.p. injection of CNO. Exn4, Exn9, and CNO (Tocris) were stored in aliquots at −20°C and dissolved in vehicle (0.9% sodium chloride) before use. Food weight was measured at 18:00, 20:00, 23:00, and 06:00 to calculate food intake volume. Intake was adjusted for spillage.

Retrograde Labeling of VTA-to-NAc Medial-Shell-Projecting Neurons

Mice (6 weeks old) were anesthetized using isoflurane, placed into a stereotactic frame (KOPF M1900), and red or green RetroBeads (100 nl; LumaFluo) were injected bilaterally in the medial shell region of the NAc (bregma 2.2 mm; lateral ±0.5 mm; ventral 4.5 mm). To allow adequate time for retrograde transport of the RetroBeads to the somata of VTA neurons, mice were allowed to survive for 14 days prior to slice physiology. Injections sites were confirmed in all animals.

Electrophysiological Recordings from Adult Mouse Midbrain

Electrophysiology was performed as described previously (Pang et al., 2002) with modifications. Briefly, mice were deeply anesthetized with Euthasol. Coronal midbrain slices (300 µm) were prepared and whole-cell patch-clamp recordings were done at 30°C. Patch pipettes (3.8–4.4 MΩ) were pulled from borosilicate glass and filled with internal solution containing 40 mM CsCl, 10 mM HEPES, 0.05 mM EGTA, 1.8 mM NaCl, 3.5 mM KCl, 1.7 mM MgCl2, 2 mM Mg-ATP, 0.4 mM Na2-GTP, 10 mM phosphocreatine, and 5 mM QX-314. Alexa Fluor 594 (Life Technologies) or 0.2% Neurobiotine-488 (Life Technologies) was added to label recorded neurons (pH 7.2; 280-290 mOsm). Whole-cell patch-clamp recordings were done using Axon 700B amplifier. Data were filtered at 2 kHz, digitized at 10 kHz, and collected using Clampex 10.2 (Molecular Devices). To record EPSCs, picrotoxin (50 µM, Sigma-Aldrich) was added to block IPSCs mediated by GABA? receptors. TTX (1 µM) was added to block action potential for mEPSCs recording. To record inhibitory currents, the NMDAR antagonist D-APV (50 µM) and the AMPAR antagonist CNQX (20 µM) were added to block excitatory currents. A bipolar stimulating electrode was placed 100–300 µm lateral to the recording electrode and used to stimulate afferents at 0.05 Hz. Neurons were voltage-clamped at −70 mV to record AMPAR EPSCs or IPSCs and at +40 mV to record dual-component EPSCs containing NMDAR EPSCs. AMPAR/NMDAR ratios were also calculated by dividing the peak of the AMPAR EPSC at −70 mV by the value of the NMDAR EPSC after stimulation start time 50 ms at +40 mV.

Data Analysis

Repeated-measures ANOVA and multiple t test with Bonferroni were used. Student’s t test or paired t test were used to determine statistical differences. Two-way ANOVA analysis was used for multiple treatments with multiple groups. Statistical significance was set at p < 0.05. All data values are presented as means ± SEM.

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2015.06.062.

AUTHOR CONTRIBUTIONS

X.W. and J.J.L. conducted the electrophysiological, morphological, and behavioral experiments; J.X. conducted behavioral and morphological experiments; J. L. conducted Exendin-9 experiments and immunohistochemical analysis and performed statistical analysis of the data; V.M. contributed significantly to data interpretation and the writing of the manuscript; and Z.P.P. designed the study and wrote the manuscript. All authors contributed to the writing of the paper.

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