GLP-1 receptor signaling in the laterodorsal tegmental nucleus attenuates cocaine seeking by activating GABAergic circuits that project to the VTA

Nicole S. Hernandez[1,2], **Vanessa R. Weir**[2,3], **Kael Ragnini**[2,3], **Riley Merkel**[2,3], **Yafang Zhang**[2,3], **Kyla Mace**[4], **Matthew T. Rich**[5], **R. Christopher Pierce**[5], **Heath D. Schmidt**[2,3]

[1]Neuroscience Graduate Group, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

[2]Department of Psychiatry, Perelman School of Medicine, University of Pennsylvania, Philadelphia, PA 19104

[3]Department of Biobehavioral Health Sciences, School of Nursing, University of Pennsylvania, Philadelphia, PA 19104

[4]Pharmacology Graduate Group, Perelman School of Medicine, University of Pennsylvania, Phildelphia, PA 19104

[5]Brain Health Institute and Department of Psychiatry, Robert Wood Johnson Medical School, Rutgers University, Piscataway, NJ 08854

Abstract

An emerging preclinical literature suggests that targeting central glucagon-like peptide-1 receptors (GLP-1Rs) may represent a novel approach to treating cocaine use disorder. However, the exact neural circuits and cell types that mediate the suppressive effects of GLP-1R agonists on cocaine-seeking behavior are largely unknown. The laterodorsal tegmental nucleus (LDTg) expresses GLP-1Rs and functions as a neuroanatomical hub connecting the nucleus tractus solitarius (NTS), the primary source of central GLP-1, with midbrain and forebrain nuclei known to regulate cocaine-seeking behavior. The goal of this study was to characterize the role of LDTg GLP-1R-expressing neurons and their projections to the ventral tegmental area (VTA) in the reinstatement of cocaine-seeking behavior, an animal model of relapse. Here, we showed that administration of the GLP-1R agonist exendin-4 (Ex-4) directly into the LDTg significantly attenuated cocaine seeking at a dose that did not affect sucrose seeking, *ad libitum* food intake or body weight. Additionally, our studies revealed that selectively activating NTS-to-LDTg circuits attenuated cocaine seeking via a GLP-1R-dependent mechanism. We also demonstrated, for the

---

first time, that GLP-1Rs are expressed primarily on GABAergic neurons in the LDTg and that the efficacy of Ex-4 to reduce cocaine seeking depends, in part, on activation of LDTg-to-VTA GABAergic projections. Taken together, these studies identify a central mechanism by which Ex-4 attenuates cocaine seeking and highlight GABAergic GLP-1R-expressing circuits in the midbrain as important anti-craving pathways in regulating cocaine craving-induced relapse.

Keywords

relapse; addiction; drug self-administration; preproglucagon; exendin-4; cocaine

Introduction

Cocaine continues to be one of the most commonly abused illicit drugs in the United States[1]. Approximately 5.9 million Americans (2.2% of the population) 12 years of age or older reported having used cocaine in the past year[1]. Recent epidemiological data indicate that the prevalence of cocaine use is increasing[2]. For example, ~2% of all young adults (18–25 years of age) were classified as current users of cocaine in 2017, the highest rate reported for this age group in the past 10 years[1]. Despite decades of clinical and preclinical studies, there are still no FDA-approved pharmacotherapies to treat cocaine use disorder[3]. Therefore, an improved understanding of the neurobiological mechanisms that regulate cocaine taking and seeking is needed to inform conceptually new approaches to treating this disorder.

GLP-1 is a satiation hormone and neuropeptide that regulates the rewarding value of food and drugs of abuse via activation of GLP-1 receptors (GLP-1Rs) expressed in the mesolimbic reward system[4–11]. Our recently published findings indicate that systemic administration of the GLP-1R agonist exendin-4 (Ex-4) attenuates the reinstatement of cocaine-seeking behavior, an animal model of relapse, by activating central GLP-1Rs[8, 9]. Since GLP-1R agonists are FDA-approved for treating type II diabetes and obesity[12, 13], these pre-clinical findings suggest that these medications could be repurposed for treating cocaine use disorder[14]. However, the exact neurobiological mechanisms by which Ex-4 attenuates cocaine seeking are unclear. Therefore, to further support the clinical use of GLP-1R agonists in treating cocaine use disorder, it is imperative to investigate the central mechanisms that underly the efficacy of Ex-4 on cocaine seeking and expand our understanding of the GLP-1R-expressing circuits and cell types that regulate cocaine-mediated behaviors.

The laterodorsal tegmental nucleus (LDTg) of the mesopontine tegmentum receives input from the hindbrain and forebrain and sends direct projections to the ventral tegmental area (VTA)[15–17]. Based on this anatomy, the LDTg is uniquely positioned to influence the mesolimbic reward system and drug-mediated behaviors. The LDTg regulates burst firing of midbrain dopamine neurons and subsequent dopamine release in the nucleus accumbens (NAc) through its direct connections to the VTA[17–21]. Studies quantifying cell phenotypes in the LDTg show that ~22% are cholinergic, ~31–38% are glutamatergic and ~40–46% are GABAergic with negligible co-expression of these neurotransmitters[22, 23]. Although inhibitory GABAergic neurons are the major cell type in the LDTg, studies of the LDTg

in motivated behaviors have focused primarily on excitatory cholinergic and glutamatergic neurons. Activation of excitatory LDTg projections to the VTA elicits conditioned place preference (CPP) and reinforces operant responding in rodents[24–27]. With regard to cocaine-mediated behaviors, ablation or pharmacological inactivation of cholinergic neurons and decreased glutamate signaling in the LDTg increases the latency to self-administer cocaine, inhibits acquisition of cocaine CPP, and attenuates the reinstatement of cocaine-seeking behavior[28–30]. While these findings clearly indicate that excitatory LDTg projections in the midbrain drive rewarding and cocaine-mediated behaviors, the role of GABAergic LDTg circuits in these behaviors remains largely unknown. It is possible and likely that opposing LDTg circuits (inhibitory vs excitatory) differentially regulate drug seeking. Therefore, we hypothesized that activating LDTg GABAergic projections to the VTA would suppress cocaine seeking during abstinence.

GLP-1Rs are expressed in the LDTg[31–33] and the LDTg receives direct projections from GLP-1-producing neurons in the nucleus tractus solitarius (NTS) of the hindbrain[15, 33–35]. Studies show that cocaine increases neuronal activity in the NTS, including GLP-1-producing neurons[10, 36, 37]. Additionally, our previous studies show that cocaine taking and subsequent abstinence decrease expression of preproglucagon (PPG), the gene that encodes GLP-1, in the NTS[9]. Decreased PPG expression during abstinence may promote cocaine seeking via decreased GLP-1 tone in the brain and suggests that activating central GLP-1 circuits during abstinence may effectively prevent/reduce cocaine seeking. No studies to date, however, have examined the relevance of endogenous GLP-1 circuits in drug-seeking behavior. Thus, we hypothesized that activation of LDTg GLP-1Rs and NTS-to-LDTg circuit activation would significantly attenuate cocaine-seeking behavior during abstinence.

Overall, the present study aimed to: 1) determine if GLP-1R activation in the LDTg decreases cocaine seeking; 2) examine the role of GLP-1-expressing projections from the NTS to the LDTg in regulating cocaine seeking; and 3) investigate the circuits, cell types, and mechanisms by which Ex-4 acts in the LDTg to attenuate cocaine-seeking behavior. Using behavioral pharmacology, fluorescent *in-situ* hybridization, transgenic rat models, viral-mediated knockdown of GLP-1Rs and chemogenetic manipulations of hindbrain and midbrain circuits, our data establish an important role for LDTg GLP-1Rs in cocaine seeking. We show that infusion of Ex-4 into the LDTg attenuates cocaine seeking and that NTS-to-LDTg circuit activation is sufficient to decrease cocaine seeking through a GLP-1-dependent mechanism. We also discovered that GLP-1Rs are expressed primarily on GABA neurons in the LDTg and that Ex-4 activates GABAergic LDTg-to-VTA projections to reduce cocaine-seeking behavior. Together, these findings highlight a novel anti-craving pathway that could be targeted by GLP-1R agonists to reduce cocaine relapse in humans with cocaine use disorder.

Materials and Methods

Details regarding all drugs used, surgeries, behavioral experiments, immunohistochemistry, PCR, fluorescent *in situ* hybridization (FISH), and electrophysiology are available in the Supplement.

Animals and housing

Male Sprague-Dawley rats (*Rattus norvegicus*) weighing 220–250g were obtained from Taconic Laboratories (Rensselaer, NY). Transgenic rats expressing Cre recombinase under the GAD1 promoter (LE-Tg(Gad1-iCre)3Ottc) were purchased from the Rat Resource and Research Center (RRRC P40OD011062; University of Missouri, Columbia, MO). Rats were housed individually on a 12/12 hr light/dark cycle and maintained on *ad libitum* food and water. All experimental procedures were performed during the light cycle. The experimental protocols were consistent with the guidelines issued by the U.S. National Institutes of Health and were approved by the University of Pennsylvania's Institutional Animal Care and Use Committee.

Cocaine self-administration, extinction and reinstatement of cocaine-seeking behavior

Rats were allowed 7 days to recover from surgery before behavioral testing commenced. Initially, rats were placed in operant conditioning chambers and allowed to lever-press for intravenous infusions of cocaine (0.25 mg/kg/infusion, infused over a 5 s period) on a fixed-ratio 1 (FR1) schedule of reinforcement. Rats were allowed to self-administer a maximum of 30 injections per 120 min operant session. Once a rat achieved at least 20 infusions of cocaine in a single daily operant session under the FR1 schedule, the subject was switched to a fixed-ratio 5 (FR5) schedule of reinforcement. The maximum number of infusions was again limited to 30 per daily self-administration session under the FR5 schedule. Responses made on the inactive lever, which had no scheduled consequences, were also recorded during both the FR1 and FR5 training sessions. Rats self-administered cocaine for 21 days (total active lever responses on the last self-administration day: 141.1±4.3 (mean±SEM)). After cocaine self-administration, drug-taking behavior was extinguished by replacing the cocaine solution with 0.9% saline. Daily extinction sessions continued until responding on the active lever was <15% of the total active lever responses completed on the last day of cocaine self-administration (total active lever responses on the last extinction day: 23.2±1.4 (mean±SEM)). Typically, it took ~7 days for rats to meet this criterion. Once cocaine self-administration was extinguished, rats entered the reinstatement phase of the experiment. To reinstate cocaine-seeking behavior, rats received an acute priming injection of cocaine (10 mg/kg, i.p.) and then placed into the operant conditioning chambers. A two-hour reinstatement test session commenced immediately thereafter. During reinstatement test sessions, satisfaction of the response requirement (i.e., five presses on the active lever) resulted in an infusion of saline rather than cocaine. Using a between-sessions design, each reinstatement test session was followed by extinction sessions until responding was again <15% of the total active lever responses completed on the last day of cocaine self-administration. Generally, 1–2 days of extinction were necessary to reach extinction criterion between reinstatement test sessions. The sample size for each experiment was based on a combination of power analyses and previous publications from our lab and others. The assignment of animals to a particular experiment was random.

Experiment 1: Determine the efficacy of intra-LDTg Ex-4 on cocaine- and sucrose-seeking behaviors

Using a within-subjects, counterbalanced design, rats were infused bilaterally with Ex-4 (0, 0.005 or 0.025 μg/100 nl) directly into the LDTg. Ten minutes later rats received a priming injection of cocaine immediately prior to a reinstatement test session. Further details and the timeline of this experiment are located in the Supplement and Figure1B.

Experiment 2: Determine the effects of intra-LDTg Ex-4 on the reinstatement of sucrose seeking

Sucrose seeking was assessed in a separate cohort of rats. Using a within-subjects, counterbalanced design, Ex-4 (0, 0.005 or 0.025 μg/100 nl) was infused into the LDTg 10 min prior to sucrose reinstatement test sessions. Further details and the timeline of this experiment are located in the Supplement and Figure 1B.

Experiment 3: Characterize the effects of chemogenetic activation of NTS-to-LDTg projections on cocaine reinstatement

Rats underwent catheter surgery and received bilateral infusions of the retrogradely infecting canine adenovirus-2 expressing Cre recombinase (CAV2-Cre) directly into the LDTg. During the same surgical session, an AAV expressing the neural activating Gq DREADD (AAV2-hSyn-DIO-hM3D(Gq)-mCherry) or control virus (AAV2-hSyn-DIO-mCherry) was infused bilaterally into the caudal NTS. This dual virus approach allowed for DREADD manipulation of NTS neurons that project specifically to the LDTg. Prior to reinstatement test sessions, CNO (0, 0.1 or 1.0 mg/kg, i.p.) was administered to activate endogenous NTS-to-LDTg projections. Further details and the timeline of this experiment are located in the Supplement and Figure 2A.

To determine if the effects of activating NTS-to-LDTg projections on cocaine seeking were due to increased GLP-1R activation in the LDTg, a subset of rats were implanted with LDTg guide cannulae following viral infusions. Using a within-subjects, counterbalanced design, rats were pretreated with intra-LDTg Ex-9 (10 μg/100nl) 10 min before a systemic injection of CNO (1.0 mg/kg, i.p.). Thirty minutes later rats received a priming injection of cocaine immediately prior to a reinstatement test session. Further details and the timeline of this experiment are located in the Supplement and Figure 2H.

Experiment 4: Phenotype GLP-1R-expressing neurons in the LDTg

Fluoresecent in-situ hybridization (FISH) was conducted using standard RNAscope methods. Pre-treated tissue sections were processed using probes designed by Advanced Cell Diagnostics (ACD) to detect GLP-1R mRNA (Rn-Glp1r; 315221), GAD1 mRNA (Rn-Gad1-C2; 316401-C2), vGlut2 mRNA (Rn-Slc17a6-C3; 317011-C3), and ChAT mRNA (Rn-Chat-C2; 430111-C2). See Supplement and Figure 3 for more details.

Experiment 5: Determine the effects of LDTg GABA neuron-specific GLP-1R knockdown on cocaine taking and seeking

In collaboration with the Viral Vector Core at the University of Pennsylvania, we created a virus that expresses a Cre-dependent short hairpin RNA (shRNA) sequence that selectively targets GLP-1R transcripts[10, 38] (AAV-FLEX-shRNA-GLP-1R). A GFP-expressing virus was used as a control (AAV-FLEX-GFP). For more details on these viruses, please see the Supplement.

For cell type-specific knockdown of GLP-1R expression in GABA neurons, GAD1-Cre rats received bilateral infusions of AAV-FLEX-shRNA-GLP-1R or AAV-FLEX-GFP directly into the LDTg. The effects of GLP-1R knockdown in LDTg GABA neurons on motivation to self-administer cocaine were tested using a progressive-ratio (PR) schedule of reinforcement. The ability of Ex-4 to reduce cocaine seeking was also assessed. Using a within-subjects, counterbalanced design, rats were pretreated with intra-LDTg vehicle or 0.005 μg Ex-4 10 min prior to a priming injection of cocaine. Separate rats received vehicle or 0.2 μg/kg Ex-4 (i.p.) one hour prior to a priming injection of cocaine. Further details and the timeline of this experiment are located in the Supplement and Figure 4A.

Experiment 6: Characterize the effects of chemogenetic inhibition and activation of GABAergic LDTg-to-VTA projections on cocaine seeking

GAD1-Cre rats received bilateral infusions of an AAV expressing the Cre recombinase-dependent inhibitory Gi DREADD (AAV2-hSyn-DIO-hM4D(Gi)-mCherry) or control virus (AAV2-hSyn-DIO-mCherry) into the LDTg. Once cocaine taking was extinguished, bilateral microinjections of CNO (0, 1 mM; 100nl) were administered into the VTA 10 min prior to a systemic injection of Ex-4 (0, 0.2 μg/kg, i.p). Cocaine priming-induced reinstatement was then assessed one hour post Ex-4 infusions to determine if inhibiting LDTg GABA terminals in the VTA prevents the ability of Ex-4 to attenuate drug seeking. Further details and the timeline of this experiment are located in the Supplement and Figure 5B.

Separate GAD1-Cre rats received intra-LDTg infusions of an AAV expressing a Cre-dependent activating Gq DREADD (AAV2-hSyn-DIO-hM3D(Gq)-mCherry) or a control virus (AAV2-hSyn-DIO-mCherry). Once cocaine taking was extinguished, bilateral microinjections of CNO (0, 1 mM; 100nl) were administered into the VTA 10 min prior to a cocaine priming-induced reinstatement test session. Further details and the timeline of this experiment are located in the Supplement and Figure 5F.

Statistics

For all cocaine and sucrose reinstatement experiments, the total mean active lever responses, inactive lever responses, and *ad libitum* food intake studies were analyzed with two-way repeated measures (RM) ANOVAs. Pairwise analyses were made using Bonferroni *post-hoc* tests (p<0.05). All other experiments were analyzed using unpaired *t*-tests or one-way ANOVAs. Quantification of FISH images was done by at least one experimenter blind to treatment group.

Results

Activation of GLP-1Rs in the LDTg decreases drug seeking and does not affect food intake or body weight in cocaine-experienced rats

Systemic administration of the GLP-1R agonist fluoro-Ex-4 (0.2 μg/kg) crosses the blood brain barrier and selectively decreases cocaine seeking without producing adverse feeding or malaise-like effects[9]. To define the central GLP-1 circuits regulating cocaine seeking, rats were pretreated with fluoro-Ex-4 and IHC analysis of the midbrain revealed fluoro-Ex-4 associated with both astrocytes and neurons in the LDTg (Figure 1A).

To determine if activation of LDTg GLP-1Rs is sufficient to reduce cocaine seeking, rats were pretreated with Ex-4 directly into the LDTg prior to reinstatement test sessions (Figure 1B&C). Intra-LDTg Ex-4 (0.005 or 0.025 μg) significantly attenuated drug seeking elicited by a priming injection of cocaine (Figure 1D). Intra-LDTg doses of Ex-4 as low as 0.025 μg are known to decrease body weight and food intake in drug-naïve rats[33]. Therefore, we screened for these adverse effects during the reinstatement phase. No differences in body weight were observed following intra-LDTg Ex-4 (Figure 1E). Additionally, there were no significant effects of 0.005 μg Ex-4 on *ad libitum* food intake (Figure 1F). To determine if the effects of intra-LDTg Ex-4 on cocaine seeking were due to potential motor suppressing effects or operant learning deficits, parallel studies of sucrose seeking were performed in a separate group of rats (Figure 1B&G). Only the high dose of intra-LDTg Ex-4 (0.025 μg) attenuated sucrose seeking (Figure 1G). Moreover, there were no effects of intra-LDTg Ex-4 on body weight in rats that underwent sucrose reinstatement (Figure 1H). Microinjection sites corresponding to these experiments are shown in Supplemental Figures 1A&B. Taken together, these findings indicate that intra-LDTg Ex-4 attenuates cocaine seeking at a dose (0.005 μg) subthreshold for effects on *ad libitum* food intake, body weight and sucrose-seeking behavior.

Cocaine self-administration and extinction do not affect GLP-1R expression in the LDTg

Next, we investigated whether cocaine exposure alters GLP-1R expression in the LDTg. There were no changes in GLP-1R mRNA expression in the LDTg of cocaine-experienced rats following one (Ext1) and seven (Ext7) days of extinction when compared to yoked saline controls (Supplemental Figures 1C&D) indicating that cocaine self-administration and subsequent extinction do not alter GLP-1R mRNA expression in the LDTg.

Activation of NTS-to-LDTg circuits attenuates drug seeking and does not affect food intake or body weight in cocaine-experienced rats

To date, no studies have investigated the role of endogenous NTS GLP-1 circuits in drug-seeking behavior. In order to selectively activate NTS-to-LDTg projections, a retrogradely infecting virus expressing Cre recombinase (CAV2-Cre) was infused into the LDTg and a Cre-dependent virus expressing a neural activating DREADD (AAV-DIO-hM3D(Gq)-mCherry) was infused into the NTS (Figures 2A&B). To validate that the DREADD agonist clozapine-n-oxide (CNO) activates hM3D(Gq)-expressing NTS neurons, IHC was performed to label cFos after CNO injection in both hM3D(Gq)- and mCherry control-expressing rats (Figure 2C). Quantification revealed that CNO significantly increased

cFos expression in NTS hM3D(Gq)-expressing neurons compared to controls (Figure 2C). Consistent with these findings, CNO increased neuronal firing in hM3D(Gq)-expressing NTS neurons (Supplemental Figures 2A&B). Systemic CNO also attenuated cocaine reinstatement in hM3D(Gq)-expressing rats (Figure 2D) indicating that activation of NTS-to-LDTg circuits is sufficient to reduce cocaine seeking and suggests that these projections may function to regulate craving-induced relapse.

Although CNO is often used as the inert ligand for DREADDs, recent studies show minimal, yet significant, reverse metabolism to clozapine, which may cause off-target effects on behavior[39]. To control for this possibility, a separate group of rats was infused with the control virus AAV-DIO-mCherry into the NTS and the same experimental procedures were performed as described above. There was no effect of CNO on lever responding in control rats (Figure 2E) indicating no impairments on operant responding or general motor activity. These findings indicate that CNO itself does not affect cocaine seeking and that the results in Figure 2D are due to activating NTS-to-LDTg projections and not off-target effects of CNO. Additionally, previous studies demonstrate that chemogenetic activation of NTS GLP-1-producing neurons transiently reduces food intake in rodents[33, 40–43]. Therefore, we determined if activation of NTS-to-LDTg projections produced adverse feeding effects during cocaine abstinence. No effects of CNO on food intake were observed in hM3D(Gq)-expressing or AAV-DIO-mCherry-expressing rats (Figure 2F). Consistent with these results, CNO did not alter body weight in DREADD-expressing or control rats (Supplemental Figures 2C&D).

Activation of NTS-to-LDTg circuits attenuates cocaine seeking through a GLP-1-dependent mechanism

Since the NTS consists of a heterogeneous population of neurons[34, 37, 44], our next goal was to determine if the effects of non-cell type-specific NTS-to-LDTg circuit activation on cocaine seeking were due to increased GLP-1R signaling. IHC analysis showed colocalization of GLP-1 and hM3D(Gq) in NTS neurons that project to the LDTg (Figure 2G). To determine if reduced cocaine seeking following activation of NTS-to-LDTg circuits was mediated by GLP-1, rats were infused with CAV2-Cre into the LDTg, AAV-DIO-hM3D(Gq)-mCherry into the NTS, and implanted with guide cannula in the LDTg (Figure 2H). Prior to reinstatement test sessions, the GLP-1R antagonist Ex-9 was infused directly into the LDTg before CNO administration (Figures 2H&I). Pharmacological inhibition of LDTg GLP-1Rs blocked the ability of NTS-to-LDTg circuit activation to decrease cocaine seeking (Figure 2J). Corresponding microinjection sites are shown in Supplemental Figure 2E. These findings indicate that activation of NTS-to-LDTg projections attenuates cocaine seeking through a GLP-1-dependent manner.

LDTg GABA neurons express GLP-1Rs and are activated by Ex-4

Thus far, we showed that both exogenous and endogenous activation of GLP-1Rs in the LDTg decreased cocaine-seeking behavior. To further investigate the mechanisms by which LDTg GLP-1R activation attenuates cocaine seeking, we first characterized the neuronal cell types that express GLP-1Rs. Colocalization of GLP-1R transcripts with GAD1, vGLUT2 or ChAT mRNA are shown in Figures 3A–C. LDTg GLP-1R-expressing neurons were found

to co-express GAD1 and vGLUT2. In contrast, GLP-1R transcripts were not expressed in ChAT-labeled neurons. Quantification revealed that of the GLP-1R-expressing cells in the LDTg, 70% were GABAergic, 25% were glutamatergic, and 0% were cholinergic (Figure 3D). Since the majority of LDTg GLP-1Rs are expressed on GABA neurons, we hypothesized that activating GLP-1Rs on this cell population would attenuate cocaine seeking. First, we found that fluoro-Ex-4 bound putative GLP-1Rs on LDTg GABA neurons (Supplemental Figure 3A). Consistent with these effects, systemic Ex-4 increased cFos expression in LDTg GABA neurons when compared to saline-treated controls (Figures 3E&F). Collectively, these findings highlight a novel cell population that may regulate drug seeking and support the hypothesis that Ex-4 attenuates cocaine reinstatement, in part, by activating GABA neurons in the LDTg.

GLP-1Rs on LDTg GABA neurons are physiologically relevant for cocaine taking

To directly test the functional relevance of LDTg GABA GLP-1Rs in cocaine taking, AAV-FLEX-shRNA-GLP-1R was infused directly into the LDTg of GAD1-Cre rats to knockdown (KD) GLP-1R expression exclusively in GABA neurons (Figures 4A&B). Selective GLP-1R KD was validated using FISH (Figures 4C&D). After viral infusion, rats were allowed to self-administer cocaine. When challenged on a PR schedule, rats with reduced LDTg GABA GLP-1R expression self-administered more cocaine than control rats (Figure 4E–G). These findings indicate that endogenous GLP-1R activation on LDTg GABA neurons acts a 'brake' to reduce motivation to consume cocaine.

GLP-1Rs on LDTg GABA neurons mediate the efficacy of Ex-4 on cocaine seeking

After cocaine self-administration, rats underwent extinction and reinstatement tests to determine if Ex-4 acts on LDTg GABA GLP-1Rs to reduce cocaine seeking. Intra-LDTg Ex-4 did not alter cocaine seeking in rats with reduced GLP-1R expression in LDTg GABA neurons (Figure 4H). These findings indicate that GLP-1Rs on LDTg GABA neurons are necessary for the suppressive effects of intra-LDTg Ex-4 on cocaine seeking. Corresponding microinjection placements are shown in Supplemental Figure 3B. We also investigated the mechanisms by which systemic Ex-4 decreases cocaine seeking and hypothesized that these effects are mediated, in part, by activation of GLP-1Rs on LDTg GABA neurons. Systemic Ex-4 significantly reduced cocaine seeking in control rats but had no effect in GLP-1R KD rats (Figure 4I). Together, these studies reveal a cell type-specific mechanism by which Ex-4 decreases cocaine seeking and show that GLP-1Rs on LDTg GABA neurons function to reduce drug-seeking behavior.

Systemic Ex-4 decreases cocaine seeking by activating midbrain anti-craving circuits

Based on findings from the above cell type-specific studies, we hypothesized that Ex-4 reduces cocaine seeking by activating GLP-1Rs on inhibitory LDTg GABA neurons that project to the VTA. To determine if GLP-1Rs are expressed on LDTg-to-VTA GABAergic circuits, we infused the retrograde tracer fluorogold into the VTA. Using FISH, we showed that LDTg neurons that project to the VTA express GAD1 and GLP-1R transcripts (Figure 5A). Specifically, we found that ~50% of LDTg neurons that express GAD1 and GLP-1Rs project to the VTA (Supplemental Figure 4A). Next, we infused a virus expressing a Cre-dependent inhibitory DREADD (AAV-DIO-hM4D(Gi)-mCherry) into the LDTg of

GAD1-Cre rats prior to cocaine self-administration (Figures 5B&C). GABAergic terminals expressing hM4D(Gi) were visualized in proximity to dopamine neurons in the VTA (Figure 5C). CNO decreased firing of hM4D(Gi)-expressing LDTg neurons (Supplemental Figure 4B). Consistent with these effects, chemogenetic inhibition of LDTg GABA terminals in the VTA via local infusions of CNO prevented the ability of Ex-4 to reduce cocaine seeking (Figure 5D). Intra-VTA CNO itself did not affect Ex-4 efficacy in control rats (Figure 5E). Corresponding microinjection sites for these experiments are shown in Supplemental Figures 4C&D. These findings indicate that Ex-4 attenuated cocaine seeking, in part, by activating LDTg-to-VTA GABAergic circuits.

To further validate that activation of LDTg-to-VTA GABAergic projections is sufficient to reduce cocaine seeking, a virus expressing a Cre-dependent neural activating DREADD (AAV-DIO-hM3D(Gq)-mCherry) was infused into the LDTg of GAD1-Cre rats (Figure 5F–H). Intra-VTA administration of CNO significantly reduced cocaine seeking in rats expressing hM3D(Gq) in LDTg-to-VTA GABA neurons (Figure 5I). There were no effects of intra-VTA CNO on lever responding in control rats (Figure 5J). Microinjection sites corresponding to these experiments are shown in Supplemental Figures 4E&F. These studies reveal a novel GABAergic circuit in the midbrain that mediates the efficacy of Ex-4 on cocaine seeking and identify inhibitory LDTg-to-VTA projections that reduce drug-seeking behavior.

Discussion

The present study expands an emerging preclinical literature demonstrating an important role of GLP-1Rs in cocaine-mediated behaviors[4–10]. Specifically, we identified anti-craving circuits in the brain engaged by GLP-1R agonists to reduce cocaine-seeking behavior. First, we found that activation of LDTg GLP-1Rs attenuated cocaine seeking at a behaviorally-selective dose. We then showed that activation of NTS-to-LDTg projections attenuated cocaine seeking via increased GLP-1 signaling in the LDTg. Next, we discovered that GLP-1Rs are expressed primarily on LDTg GABA neurons and that Ex-4 activates this cell population. Using GAD1-Cre rats, we selectively reduced GLP-1R expression on LDTg GABA neurons to test the cell type-specific functional roles of LDTg GLP-1Rs in cocaine taking and seeking. Reduced GLP-1R expression on LDTg GABA neurons augmented cocaine self-administration and prevented the ability of Ex-4 to decrease cocaine seeking. Finally, we found that the efficacy of Ex-4 to reduce cocaine seeking is mediated, in part, by activation of LDTg-to-VTA GABAergic projections and that activating this inhibitory circuit alone is sufficient to decrease cocaine seeking. Overall, these findings highlight a novel, phenotypically distinct GABAergic population of neurons in the LDTg that expresses GLP-1Rs and projects to the VTA to inhibit cocaine-seeking behavior.

GLP-1R activation in the LDTg decreases cocaine-seeking behavior

The LDTg receives inputs from the prefrontal cortex and sends direct projections to the VTA that are essential for the burst firing of dopamine neurons[15, 17–19, 30, 45, 46]. Pharmacological blockade of excitatory signaling in the LDTg attenuates cocaine-mediated behaviors including cocaine reinstatement[28, 30, 47]. However, the role of other cell types

and mechanisms in the LDTg that act to regulate cocaine seeking are largely unexplored. Here, we showed that activation of LDTg GLP-1Rs attenuated cocaine seeking. Since Ex-4 reduces food intake and can cause nausea/malaise[48, 49], it is important to control for these adverse effects in order to support translating preclinical findings into clinical trials of GLP-1R agonists in humans with cocaine use disorder. Previous studies showed that doses of intra-LDTg Ex-4 as low as 0.025 μg decreased food intake and body weight in drug-naïve rats without producing malaise-like effects[33]. In contrast to these studies, we identified a dose of intra-LDTg Ex-4 (0.005 μg) that selectively attenuated cocaine seeking and did not affect food intake, body weight or sucrose-seeking behavior. These findings add to a growing literature that highlights doses of Ex-4 that selectively reduce drug seeking without producing notable feeding and malaise-like effects commonly associated with GLP-1R agonists in humans and rodents[8, 9, 50]. Together, the current literature and the present study suggest that motivated behaviors are differentially modulated by GLP-1R activation in cocaine-experienced versus drug-naïve rats. Support for this hypothesis comes from our previous studies that identified differential effects of Ex-4 on action potential firing of NAc medium spiny neurons in cocaine-experienced vs drug-naive rats[8, 51]. Future studies should continue to investigate the unique effects of GLP-1R activation on neural activity in drug-dependent rats. Overall, the translational implications of these findings are profound in that they support GLP-1R-focused therapeutic approaches for the treatment of cocaine craving and relapse. No studies to date, however, have examined potential sex differences in the efficacy of GLP-1R agonists to reduce cocaine-mediated behaviors. Recent evidence suggests that females may be more sensitive to the effects of GLP-1R agonists on sucrose self-administration[52]. Thus, future studies are also needed to determine if the potency of GLP-1R agonists differs between sexes in preclinical models of addiction.

Endogenous GLP-1-expressing circuits regulate cocaine-seeking behavior

While the current study revealed no effects of cocaine on LDTg GLP-1R expression, our previously published findings showed that cocaine taking and subsequent abstinence dynamically regulate expression of preproglucagon (PPG), the gene that encodes GLP-1, in the NTS. Specifically, cocaine self-administration increased PPG expression in the NTS[9]. These findings support the hypothesis that increased central GLP-1 signaling may serve as a 'brake' to reduce further cocaine consumption[10]. In contrast, PPG expression in the NTS was significantly decreased following seven days of abstinence[9], a time point that coincides with robust drug-seeking behavior[8, 53]. These results suggest that decreased PPG expression in the NTS may facilitate cocaine seeking during abstinence via reduced GLP-1 signaling in target nuclei. Here, we showed that chemogenetic activation of NTS-to-LDTg circuits decreased cocaine seeking via a GLP-1R-dependent mechanism. These findings indicate that endogenous GLP-1 signaling in the LDTg functions to reduce cocaine seeking and are the first to show that NTS circuits regulate drug-seeking behavior. It is important to note that NTS GLP-1-producing neurons are also known to co-release glutamate[44]. However, the suppressive effects of NTS-to-LDTg circuit activation on cocaine seeking are likely not due to increased glutamate release as blocking glutamate transmission in the LDTg attenuates cocaine-seeking behavior[30]. Our studies also revealed that not all hM3D(Gq)-expressing NTS-to-LDTg neurons were GLP-1 positive. In addition to GLP-1-producing neurons, cocaine activates noradrenergic neurons in the NTS[10, 37]. It is possible and likely that NTS

noradrenergic neurons play an important role in cocaine seeking especially when induced by stress as the noradrenergic system plays an important role in stress-induced drug seeking and extinction training enhances the ability of cocaine to activate NTS neuradrenergic neurons[37]. Moreover, the NTS sends projections to other nuclei involved in drug-mediated behaviors including the VTA and NAc[34, 54] and these circuits may also play a role in cocaine-seeking behavior. Future studies should continue to examine and characterize the role of NTS circuits in addiction-like behaviors.

The present study also demonstrated that activating NTS-to-LDTg circuits during abstinence had no effect on food intake or body weight in cocaine-experienced rats. These data are consistent with previous studies that chemogenetically activated GLP-1-producing neurons in the NTS of transgenic mice. These studies showed that both non-circuit-specific activation of NTS GLP-1-producing neurons and selective activation of NTS projections to the paraventricular hypothalamus reduced food intake only for 2–4 hrs with no effects at longer time points[40–43]. Taken together, these findings indicate that the suppressive effects of endogenous NTS circuit activation on food intake are transient and highlight the behavioral selectivity of NTS-to-LDTg circuit activation in reducing cocaine seeking.

Activation of GLP-1Rs on LDTg GABA neurons attenuates cocaine seeking

The LDTg contains distinct populations of cholinergic, glutamatergic and GABAergic neurons[22, 23]. Despite this heterogeneity, studies of LDTg circuits in drug taking and seeking have focused largely on excitatory cholinergic projections and their ability to promote reward-related behaviors[16–19, 26, 55–57]. The role of other cell types in the LDTg to regulate addiction-like behaviors is largely understudied. We phenotyped LDTg GLP-1R-expressing cells and found that 70% were GABAergic neurons, 25% were glutamatergic neurons and 0% were cholinergic neurons. The remaining 5% of GLP-1R-expressing cell types in the LDTg are most likely glial cells as astrocytes express GLP-1Rs[58] and bind fluoro-Ex-4 in the LDTg (present study). We also found that Ex-4 activated LDTg GABA neurons and that selective knockdown of GLP-1Rs in this cell population was sufficient to block the efficacy of both intra-LDTg and systemic Ex-4 to decrease cocaine seeking. Although 25% of LDTg GLP-1Rs are expressed on glutamate neurons, it is unlikely that activation of GLP-1Rs on this cell type decreased cocaine seeking. This is supported by previous studies showing that activation of LDTg-to-VTA glutamatergic neurons is rewarding and by a well-established literature demonstrating that glutamate relase in the mesolimbic reward system promotes drug-seeking behavior[24, 25, 30, 59]. To our knowledge, our study is the first to characterize GLP-1R-expressing neurons in the LDTg and provides evidence that GABAergic signaling is a key mechanism that underlies the effects of LDTg GLP-1R activation on motivated behaviors. Additionally, we found that reduced GLP-1R expression in LDTg GABA neurons augmented cocaine self-administration, indicating the endogenous relevance of GLP-1 signaling on this cell population in cocaine reinforcement. These data are consistent with our previously published findings showing non-cell type-specific knockdown of VTA GLP-1Rs increased cocaine self-administration[10]. With regard to drug seeking, reduced GLP-1R expression in LDTg GABA neurons did not augment cocaine reinstatement, possibly due to ceiling effects associated with the priming dose of cocaine. It is also possible that endogenous central GLP-signaling has differential roles in cocaine

taking and seeking[9]. Together, these findings reveal a critical role for LDTg GABA neurons in voluntary drug taking and seeking and, more specifically, that activation of GLP-1Rs on LDTg GABA neurons is sufficient to reduce cocaine-mediated behaviors.

Ex-4 attenuates cocaine seeking by activating GABAergic LDTg-to-VTA projections

To expand our understanding of LDTg GABA neurons in drug seeking, we investigated the role of GLP-1Rs on LDTg-to-VTA GABA circuits in regulating cocaine reinstatement. We found that GLP-1Rs are expressed on LDTg GABA neurons that project to the VTA and selectively inhibiting these projections reversed the suppressive effects of Ex-4 on cocaine seeking. These findings identify a novel neural mechanism by which Ex-4 attenuates cocaine seeking and highlight the relevance of LDTg-to-VTA GABA projections in drug-seeking behavior. Although the majority of neurons in the LDTg are GABAergic[22, 23], very little is known about the role of GABAergic LDTg circuits in motivated behaviors. LDTg GABA neurons synapse on VTA neurons[17] and non-cell type-specific stimulation of LDTg neurons in rats results in a ~30% inhibition of neuronal activity in the VTA including dopaminergic and GABAergic neurons[60]. Here, we showed that GABAergic LDTg fibers are localized in proximity to dopamine neurons in the VTA and that chemogenetic activation of LDTg-to-VTA GABA terminals attenuated cocaine seeking. These findings suggest that activation of GLP-1Rs in the LDTg decreases cocaine seeking by inhibiting VTA dopamine neurons. Support for this hypothesis comes from previous studies showing that systemic and ICV infusions of Ex-4 decreased cocaine-evoked dopamine release in the NAc[5, 6, 61]. However, future studies are needed to directly assess if LDTg GABA neurons make synaptic contacts onto VTA dopamine neurons and reduce dopamine cell firing. In addition to the VTA, LDTg GABA neurons project to other nuclei that regulate reward-related behaviors. For example, activation of LDTg GABAergic projections to the NAc decreased motivational drive[62], further supporting our hypothesis that LDTg GABAergic circuits function to decrease addiction-like behaviors. Overall, our study identified LDTg GABA neurons as important targets of Ex-4 action and is the first to establish the functional relevance of LDTg-to-VTA GABAergic circuits in cocaine-seeking behavior.

Conclusion

Here, we showed that central GLP-1Rs are expressed on specific cell types and circuits that reduce cocaine-seeking behavior. Specifically, we found that activation of GLP-1Rs in the LDTg and endogenous GLP-1-producing NTS circuits attenuated cocaine seeking. We phenotyped GLP-1R-expressing cell types and found that GLP-1Rs are expressed primarily on GABA neurons in the LDTg. Indeed, this cell population was found to be critical in controlling motivation to consume cocaine as well as the efficacy of Ex-4 to reduce cocaine seeking. Finally, we discovered a role for LDTg-to-VTA GABAergic projections in drug-mediated behaviors and showed that activating this cell type-specific circuit suppresses cocaine seeking. Taken together, our findings highlight GLP-1R-expressing anti-craving circuits in the brain that could serve as potential targets to reduce cocaine craving-induced relapse.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

References

1. NSDUH. Results from the 2017 National Survey on Drug Use and Health. vol. NSDUH Series H-53 Substance Abuse and Mental Health Services Administration2018.

2. Hughes A, Williams MR, Lipari RN, and Van Horn S. State estimates of past year cocaine use among young adults: 2014 and 2015. In: Report TC (ed). Center for Behavioral Health Statistics and Quality, Substance Abuse and Mental Health Services Administration: Rockville, MD, 2016.

3. Pierce RC, O'Brien CP, Kenny PJ, Vanderschuren LJ. Rational development of addiction pharmacotherapies: successes, failures, and prospects. Cold Spring Harb Perspect Med 2012; 2(6): a012880. [PubMed: 22675669]

4. Graham DL, Erreger K, Galli A, Stanwood GD. GLP-1 analog attenuates cocaine reward. Mol Psychiatry 2013; 18(9): 961–962. [PubMed: 23089631]

5. Sorensen G, Reddy IA, Weikop P, Graham DL, Stanwood GD, Wortwein G et al. The glucagon-like peptide 1 (GLP-1) receptor agonist exendin-4 reduces cocaine self-administration in mice. Physiol Behav 2015; 149: 262–268. [PubMed: 26072178]

6. Egecioglu E, Engel JA, Jerlhag E. The glucagon-like peptide 1 analogue, exendin-4, attenuates the rewarding properties of psychostimulant drugs in mice. PLoS One 2013; 8(7): e69010. [PubMed: 23874851]

7. Harasta AE, Power JM, von Jonquieres G, Karl T, Drucker DJ, Housley GD et al. Septal Glucagon-Like Peptide 1 Receptor Expression Determines Suppression of Cocaine-Induced Behavior. Neuropsychopharmacology 2015; 40(8): 1969–1978. [PubMed: 25669605]

8. Hernandez NS, O'Donovan B, Ortinski PI, Schmidt HD. Activation of glucagon-like peptide-1 receptors in the nucleus accumbens attenuates cocaine seeking in rats. Addict Biol 2019; 24(2): 170–181. [PubMed: 29226617]

9. Hernandez NS, Ige KY, Mietlicki-Baase EG, Molina-Castro GC, Turner CA, Hayes MR et al. Glucagon-like peptide-1 receptor activation in the ventral tegmental area attenuates cocaine seeking in rats. Neuropsychopharmacology 2018; 43(10): 2000–2008. [PubMed: 29497166]

10. Schmidt HD, Mietlicki-Baase EG, Ige KY, Maurer JJ, Reiner DJ, Zimmer DJ et al. Glucagon-Like Peptide-1 Receptor Activation in the Ventral Tegmental Area Decreases the Reinforcing Efficacy of Cocaine. Neuropsychopharmacology 2016; 41(7): 1917–1928. [PubMed: 26675243]

11. Reddy IA, Pino JA, Weikop P, Osses N, Sorensen G, Bering T et al. Glucagon-like peptide 1 receptor activation regulates cocaine actions and dopamine homeostasis in the lateral septum by decreasing arachidonic acid levels. Transl Psychiatry 2016; 6: e809. [PubMed: 27187231]

12. Lovshin JA, Drucker DJ. Incretin-based therapies for type 2 diabetes mellitus. Nat Rev Endocrinol 2009; 5(5): 262–269. [PubMed: 19444259]

13. Shukla AP, Buniak WI, Aronne LJ. Treatment of obesity in 2015. J Cardiopulm Rehabil Prev 2015; 35(2): 81–92. [PubMed: 25714749]

14. Hernandez NS, Schmidt HD. Central GLP-1 receptors: Novel molecular targets for cocaine use disorder. Physiol Behav 2019; 206: 93–105. [PubMed: 30930091]

15. Cornwall J, Cooper JD, Phillipson OT. Afferent and efferent connections of the laterodorsal tegmental nucleus in the rat. Brain Res Bull 1990; 25(2): 271–284. [PubMed: 1699638]

16. Oakman SA, Faris PL, Kerr PE, Cozzari C, Hartman BK. Distribution of pontomesencephalic cholinergic neurons projecting to substantia nigra differs significantly from those projecting to ventral tegmental area. J Neurosci 1995; 15(9): 5859–5869. [PubMed: 7666171]

17. Omelchenko N, Sesack SR. Laterodorsal tegmental projections to identified cell populations in the rat ventral tegmental area. J Comp Neurol 2005; 483(2): 217–235. [PubMed: 15678476]

18. Lodge DJ, Grace AA. The laterodorsal tegmentum is essential for burst firing of ventral tegmental area dopamine neurons. Proc Natl Acad Sci U S A 2006; 103(13): 5167–5172. [PubMed: 16549786]

19. Dautan D, Souza AS, Huerta-Ocampo I, Valencia M, Assous M, Witten IB et al. Segregated cholinergic transmission modulates dopamine neurons integrated in distinct functional circuits. Nat Neurosci 2016; 19(8): 1025–1033. [PubMed: 27348215]

20. Forster GL, Blaha CD. Laterodorsal tegmental stimulation elicits dopamine efflux in the rat nucleus accumbens by activation of acetylcholine and glutamate receptors in the ventral tegmental area. Eur J Neurosci 2000; 12(10): 3596–3604. [PubMed: 11029630]

21. Blaha CD, Allen LF, Das S, Inglis WL, Latimer MP, Vincent SR et al. Modulation of dopamine efflux in the nucleus accumbens after cholinergic stimulation of the ventral tegmental area in intact, pedunculopontine tegmental nucleus-lesioned, and laterodorsal tegmental nucleus-lesioned rats. J Neurosci 1996; 16(2): 714–722. [PubMed: 8551354]

22. Wang HL, Morales M. Pedunculopontine and laterodorsal tegmental nuclei contain distinct populations of cholinergic, glutamatergic and GABAergic neurons in the rat. Eur J Neurosci 2009; 29(2): 340–358. [PubMed: 19200238]

23. Luquin E, Huerta I, Aymerich MS, Mengual E. Stereological Estimates of Glutamatergic, GABAergic, and Cholinergic Neurons in the Pedunculopontine and Laterodorsal Tegmental Nuclei in the Rat. Front Neuroanat 2018; 12: 34. [PubMed: 29867374]

24. Lammel S, Lim BK, Ran C, Huang KW, Betley MJ, Tye KM et al. Input-specific control of reward and aversion in the ventral tegmental area. Nature 2012; 491(7423): 212–217. [PubMed: 23064228]

25. Steidl S, Wang H, Ordonez M, Zhang S, Morales M. Optogenetic excitation in the ventral tegmental area of glutamatergic or cholinergic inputs from the laterodorsal tegmental area drives reward. Eur J Neurosci 2017; 45(4): 559–571. [PubMed: 27740714]

26. Xiao C, Cho JR, Zhou C, Treweek JB, Chan K, McKinney SL et al. Cholinergic Mesopontine Signals Govern Locomotion and Reward through Dissociable Midbrain Pathways. Neuron 2016; 90(2): 333–347. [PubMed: 27100197]

27. Steidl S, Veverka K. Optogenetic excitation of LDTg axons in the VTA reinforces operant responding in rats. Brain Res 2015; 1614: 86–93. [PubMed: 25911581]

28. Shinohara F, Kihara Y, Ide S, Minami M, Kaneda K. Critical role of cholinergic transmission from the laterodorsal tegmental nucleus to the ventral tegmental area in cocaine-induced place preference. Neuropharmacology 2014; 79: 573–579. [PubMed: 24467849]

29. Steidl S, Cardiff KM, Wise RA. Increased latencies to initiate cocaine self-administration following laterodorsal tegmental nucleus lesions. Behav Brain Res 2015; 287: 82–88. [PubMed: 25746513]

30. Schmidt HD, Famous KR, Pierce RC. The limbic circuitry underlying cocaine seeking encompasses the PPTg/LDT. Eur J Neurosci 2009; 30(7): 1358–1369. [PubMed: 19788581]

31. Merchenthaler I, Lane M, Shughrue P. Distribution of pre-pro-glucagon and glucagon-like peptide-1 receptor messenger RNAs in the rat central nervous system. J Comp Neurol 1999; 403(2): 261–280. [PubMed: 9886047]

32. Goke R, Larsen PJ, Mikkelsen JD, Sheikh SP. Distribution of GLP-1 binding sites in the rat brain: evidence that exendin-4 is a ligand of brain GLP-1 binding sites. Eur J Neurosci 1995; 7(11): 2294–2300. [PubMed: 8563978]

33. Reiner DJ, Leon RM, McGrath LE, Koch-Laskowski K, Hahn JD, Kanoski SE et al. Glucagon-Like Peptide-1 Receptor Signaling in the Lateral Dorsal Tegmental Nucleus Regulates Energy Balance. Neuropsychopharmacology 2018; 43(3): 627–637. [PubMed: 28920591]

34. Rinaman L Ascending projections from the caudal visceral nucleus of the solitary tract to brain regions involved in food intake and energy expenditure. Brain Res 2010; 1350: 18–34. [PubMed: 20353764]

35. Grill HJ, Hayes MR. Hindbrain neurons as an essential hub in the neuroanatomically distributed control of energy balance. Cell Metab 2012; 16(3): 296–309. [PubMed: 22902836]

36. Zahm DS, Becker ML, Freiman AJ, Strauch S, Degarmo B, Geisler S et al. Fos after single and repeated self-administration of cocaine and saline in the rat: emphasis on the Basal forebrain and recalibration of expression. Neuropsychopharmacology 2010; 35(2): 445–463. [PubMed: 19794406]

37. Buffalari DM, Rinaman L. Cocaine self-administration and extinction alter medullary noradrenergic and limbic forebrain cFos responses to acute, noncontingent cocaine injections in adult rats. Neuroscience 2014; 281C: 241–250.

38. Alhadeff AL, Mergler BD, Zimmer DJ, Turner CA, Reiner DJ, Schmidt HD et al. Endogenous Glucagon-like Peptide-1 Receptor Signaling in the Nucleus Tractus Solitarius is Required for Food Intake Control. Neuropsychopharmacology 2017; 42(7): 1471–1479. [PubMed: 27782127]

39. Manvich DF, Webster KA, Foster SL, Farrell MS, Ritchie JC, Porter JH et al. The DREADD agonist clozapine N-oxide (CNO) is reverse-metabolized to clozapine and produces clozapine-like interoceptive stimulus effects in rats and mice. Sci Rep 2018; 8(1): 3840. [PubMed: 29497149]

40. Liu J, Conde K, Zhang P, Lilascharoen V, Xu Z, Lim BK et al. Enhanced AMPA Receptor Trafficking Mediates the Anorexigenic Effect of Endogenous Glucagon-like Peptide-1 in the Paraventricular Hypothalamus. Neuron 2017; 96(4): 897–909 e895. [PubMed: 29056294]

41. Gaykema RP, Newmyer BA, Ottolini M, Raje V, Warthen DM, Lambeth PS et al. Activation of murine pre-proglucagon-producing neurons reduces food intake and body weight. J Clin Invest 2017; 127(3): 1031–1045. [PubMed: 28218622]

42. Holt MK, Richards JE, Cook DR, Brierley DI, Williams DL, Reimann F et al. Preproglucagon Neurons in the Nucleus of the Solitary Tract Are the Main Source of Brain GLP-1, Mediate Stress-Induced Hypophagia, and Limit Unusually Large Intakes of Food. Diabetes 2019; 68(1): 21–33. [PubMed: 30279161]

43. Shi X, Chacko S, Li F, Li D, Burrin D, Chan L et al. Acute activation of GLP-1-expressing neurons promotes glucose homeostasis and insulin sensitivity. Mol Metab 2017; 6(11): 1350–1359. [PubMed: 29107283]

44. Zheng H, Stornetta RL, Agassandian K, Rinaman L. Glutamatergic phenotype of glucagon-like peptide 1 neurons in the caudal nucleus of the solitary tract in rats. Brain Struct Funct 2015; 220(5): 3011–3022. [PubMed: 25012114]

45. Satoh K, Fibiger HC. Cholinergic neurons of the laterodorsal tegmental nucleus: efferent and afferent connections. The Journal of comparative neurology 1986; 253(3): 277–302. [PubMed: 2432101]

46. Sesack SR, Deutch AY, Roth RH, Bunney BS. Topographical organization of the efferent projections of the medial prefrontal cortex in the rat: an anterograde tract-tracing study with Phaseolus vulgaris leucoagglutinin. J Comp Neurol 1989; 290(2): 213–242. [PubMed: 2592611]

47. Shabani S, Foster R, Gubner N, Phillips TJ, Mark GP. Muscarinic type 2 receptors in the lateral dorsal tegmental area modulate cocaine and food seeking behavior in rats. Neuroscience 2010; 170(2): 559–569. [PubMed: 20667466]

48. Hayes MR, Kanoski SE, Alhadeff AL, Grill HJ. Comparative effects of the long-acting GLP-1 receptor ligands, liraglutide and exendin-4, on food intake and body weight suppression in rats. Obesity (Silver Spring) 2011; 19(7): 1342–1349. [PubMed: 21415845]

49. Kanoski SE, Rupprecht LE, Fortin SM, De Jonghe BC, Hayes MR. The role of nausea in food intake and body weight suppression by peripheral GLP-1 receptor agonists, exendin-4 and liraglutide. Neuropharmacology 2012; 62(5–6): 1916–1927. [PubMed: 22227019]

50. Zhang Y, Kahng MW, Elkind JA, Weir VR, Hernandez NS, Stein LM et al. Activation of GLP-1 receptors attenuates oxycodone taking and seeking without compromising the antinociceptive effects of oxycodone in rats. Neuropsychopharmacology 2020; 45(3): 451–461. [PubMed: 31581176]

51. Mietlicki-Baase EG, Ortinski PI, Reiner DJ, Sinon CG, McCutcheon JE, Pierce RC et al. Glucagon-like peptide-1 receptor activation in the nucleus accumbens core suppresses feeding by increasing glutamatergic AMPA/kainate signaling. J Neurosci 2014; 34(20): 6985–6992. [PubMed: 24828651]

52. Richard JE, Anderberg RH, Lopez-Ferreras L, Olandersson K, Skibicka KP. Sex and estrogens alter the action of glucagon-like peptide-1 on reward. Biol Sex Differ 2016; 7: 6. [PubMed: 26779332]

53. Schmidt HD, McFarland KN, Darnell SB, Huizenga MN, Sangrey GR, Cha JH et al. ADAR2-dependent GluA2 editing regulates cocaine seeking. Mol Psychiatry 2015; 20(11): 1460–1466. [PubMed: 25349168]

54. Alhadeff AL, Rupprecht LE, Hayes MR. GLP-1 neurons in the nucleus of the solitary tract project directly to the ventral tegmental area and nucleus accumbens to control for food intake. Endocrinology 2012; 153(2): 647–658. [PubMed: 22128031]

55. Mena-Segovia J, Winn P, Bolam JP. Cholinergic modulation of midbrain dopaminergic systems. Brain Res Rev 2008; 58(2): 265–271. [PubMed: 18343506]

56. Holmstrand EC, Sesack SR. Projections from the rat pedunculopontine and laterodorsal tegmental nuclei to the anterior thalamus and ventral tegmental area arise from largely separate populations of neurons. Brain Struct Funct 2011; 216(4): 331–345. [PubMed: 21556793]

57. Kaneda K Neuroplasticity in cholinergic neurons of the laterodorsal tegmental nucleus contributes to the development of cocaine addiction. Eur J Neurosci 2018.

58. Reiner DJ, Mietlicki-Baase EG, McGrath LE, Zimmer DJ, Bence KK, Sousa GL et al. Astrocytes Regulate GLP-1 Receptor-Mediated Effects on Energy Balance. J Neurosci 2016; 36(12): 3531–3540. [PubMed: 27013681]

59. Schmidt HD, Pierce RC. Cocaine-induced neuroadaptations in glutamate transmission: potential therapeutic targets for craving and addiction. Ann N Y Acad Sci 2010; 1187: 35–75. [PubMed: 20201846]

60. Coimbra B, Soares-Cunha C, Borges S, Vasconcelos NA, Sousa N, Rodrigues AJ. Impairments in laterodorsal tegmentum to VTA projections underlie glucocorticoid-triggered reward deficits. Elife 2017; 6.

61. Fortin SM, Roitman MF. Central GLP-1 receptor activation modulates cocaine-evoked phasic dopamine signaling in the nucleus accumbens core. Physiol Behav 2017; 176: 17–25. [PubMed: 28315693]

62. Coimbra B, Soares-Cunha C, Vasconcelos NAP, Domingues AV, Borges S, Sousa N et al. Role of laterodorsal tegmentum projections to nucleus accumbens in reward-related behaviors. Nat Commun 2019; 10(1): 4138. [PubMed: 31515512]
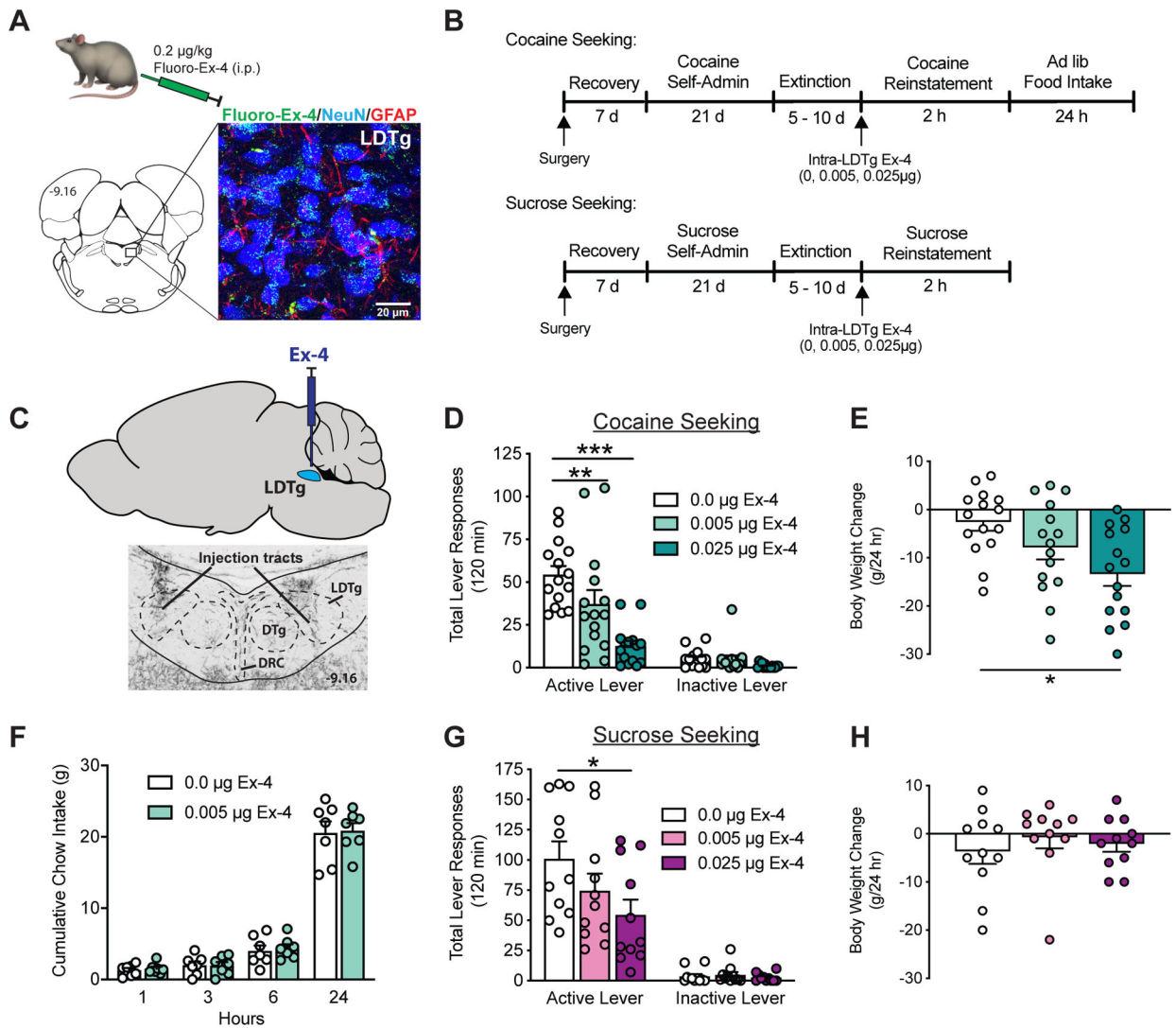
Figure 1. Intra-LDTg Ex-4 attenuates cocaine seeking at a dose that does not affect sucrose seeking, food intake or body weight.

(A) 0.2 μg/kg fluoro-Ex-4 (green) colocalized with NeuN-positive neurons (blue) and GFAP-positive astrocytes (red) in the LDTg. (B) Schematic of the behavioral paradigms and experiments. (C) Representative histological section verifying infusions of Ex-4 directly into the LDTg. (D) Total active and inactive lever responses in rats pretreated with Ex-4 (0, 0.005 or 0.025 μg/100nl, n=15) prior to cocaine priming-induced reinstatement test sessions. Two-way RM ANOVA revealed significant main effects of treatment [F(2,28)=15.05, p<0.001], lever [F(1,14)=76.23, p<0.001], and treatment × lever interaction [F(2,28)=15.89, p<0.001]. *Post-hoc* analyses revealed significant differences between vehicle and both Ex-4 treatments (Bonferroni, p<0.01). No significant differences were found on inactive lever responding. (E) 24 hr body weight in rats pretreated with Ex-4 (0, 0.005 or 0.025 μg/100nl, n=15) prior to subsequent cocaine reinstatement test sessions. One-way ANOVA revealed a significant main effect of treatment [F(2,28)=6.10, p<0.01]. Significant differences between vehicle and 0.025 μg Ex-4 treatments were observed (Bonferroni, p<0.05). No differences were observed between vehicle and 0.005 μg Ex-4. (F) There were no significant effects of 0.005 μg Ex-4

on *ad libitum* food intake in a subset of rats that underwent cocaine reinstatement (n=7) [F(1,6)=0.16, p=0.70]. (G) Total active and inactive lever responses in rats pretreated with Ex-4 (0, 0.005 or 0.025 μg/100nl, n=11) prior to sucrose reinstatement test sessions. Two-way RM ANOVA revealed significant main effects of treatment [F(2,56)=6.735, p<0.01], lever [F(1,56)=75.37, p<0.001], and treatment × lever interaction [F(2,56)=6.828, p<0.01]. Significant differences between vehicle and 0.025 μg Ex-4 treatments were observed (Bonferroni, p<0.05). In contrast, no significant differences were observed between vehicle and 0.005 μg Ex-4. No significant differences were found on inactive lever responding. (H) There was no effect of intra-LDTg Ex-4 on body weight in rats that underwent sucrose reinstatement [F(2,20)=0.35, p=0.70]. Data are mean ± SEM. *p<0.05, **p<0.01, ***p<0.001
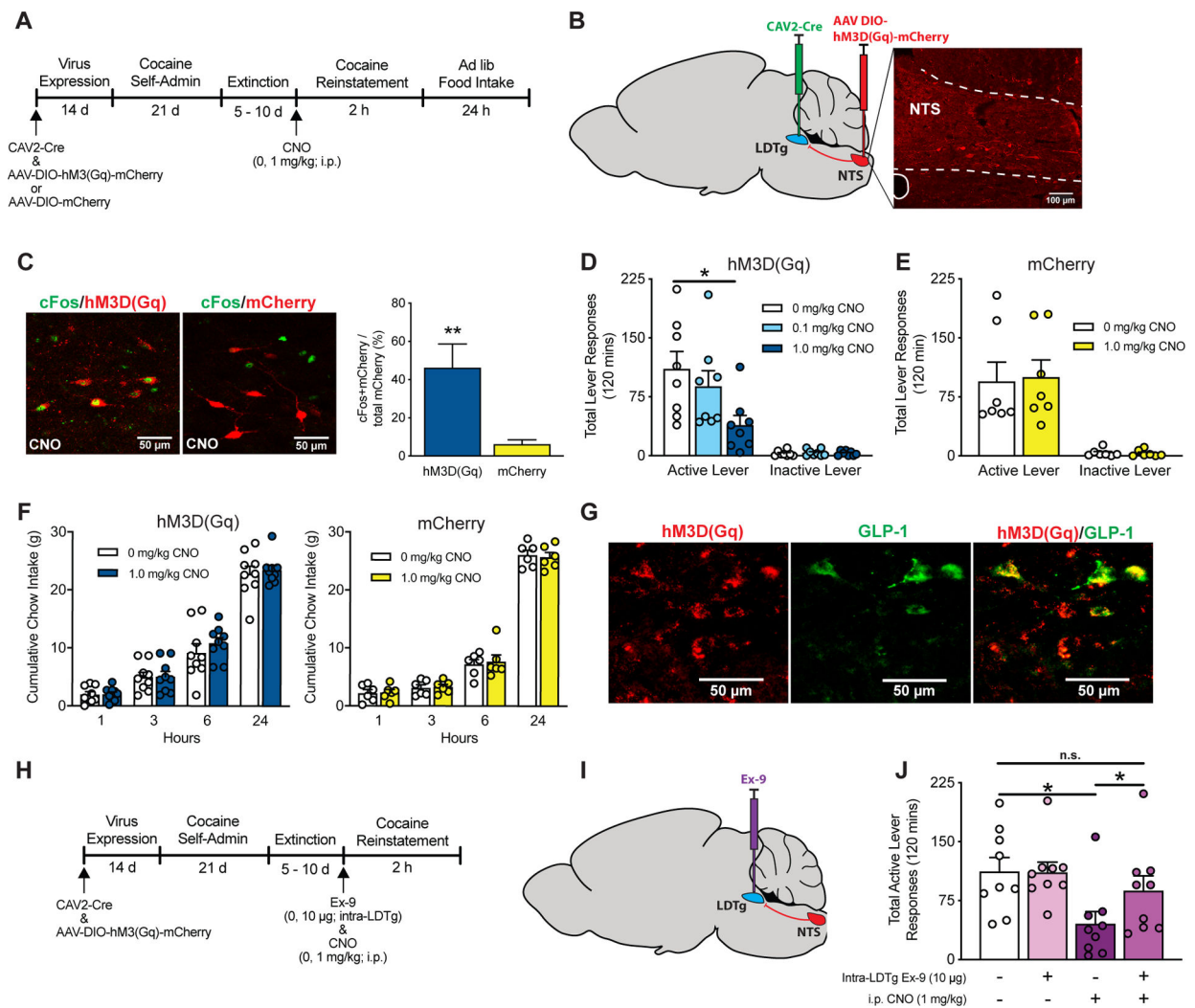
Figure 2. Activation of NTS-to-LDTg circuits is sufficient to attenuate cocaine seeking.
(A) Schematic of the behavioral paradigm and experiment. (B) CAV2-Cre and a Cre-
dependent AAV2 expressing the activating DREADD hM3D(Gq) (AAV-DIO-hM3D(Gq)-
mCherry) were administered directly into the LDTg and NTS, respectively. Representative
image shows hM3D(Gq) expression (red) in NTS neurons that project to the LDTg. (C)
Representative images and quantification showing CNO induced cFos expression (green)
in ~46% of NTS hM3D(Gq)-expressing neurons (red; n=5) compared to just ~6% of
mCherry-expressing neurons (red; n=6) [t(9)=3.50, p<0.01]. (D) Total active and inactive
lever responses in rats that were pretreated with CNO (0, 0.1, 1.0 mg/kg, i.p, n=8) prior
to cocaine reinstatement test sessions. Two-way RM ANOVA revealed significant main
effects of treatment [F(2,14)=11.93, p<0.01], lever [F(1,7)=21.21, p<0.01], and treatment ×
lever interaction [F(2,14)=11.87, p<0.01]. *Post-hoc* analyses revealed significant differences
between vehicle and 1.0 mg/kg CNO treatments (Bonferroni, p<0.05). (E) CNO had no
effect on cocaine seeking in rats infused with control virus (n=7) [F(1,6)=0.24, p=0.63].
(F) CNO had no effect on *ad libitum* chow intake in cocaine-experienced rats expressing
hM3D(Gq) (n=9) [F(1,8)=0.74, p=0.41]. CNO had no effect on *ad libitum* chow intake in

rats expressing the control virus (n=6) [F(1,5)=0.02, p=0.89]. (G) Representative images showing co-expression of hM3D(Gq) (red) and GLP-1 (green) in NTS neurons that project to the LDTg. (H & I) Prior to reinstatement test sessions, the GLP-1R antagonist Ex-9 (10 μg/100nl) or vehicle (Veh) was infused directly into the LDTg before administration of CNO (0, 1 mg/kg, i.p.). (J) Total active lever responses for all four treatment conditions (n=9). Two-way RM ANOVA revealed a significant systemic treatment × intra-LDTg treatment interaction [F(1,8)=7.69, p<0.05]. Responding in Veh/CNO-treated rats was significant different from all other treatments (Bonferroni, p<0.05). No effects on total inactive lever responding were observed (data not shown). Data are mean ± SEM. *p<0.05
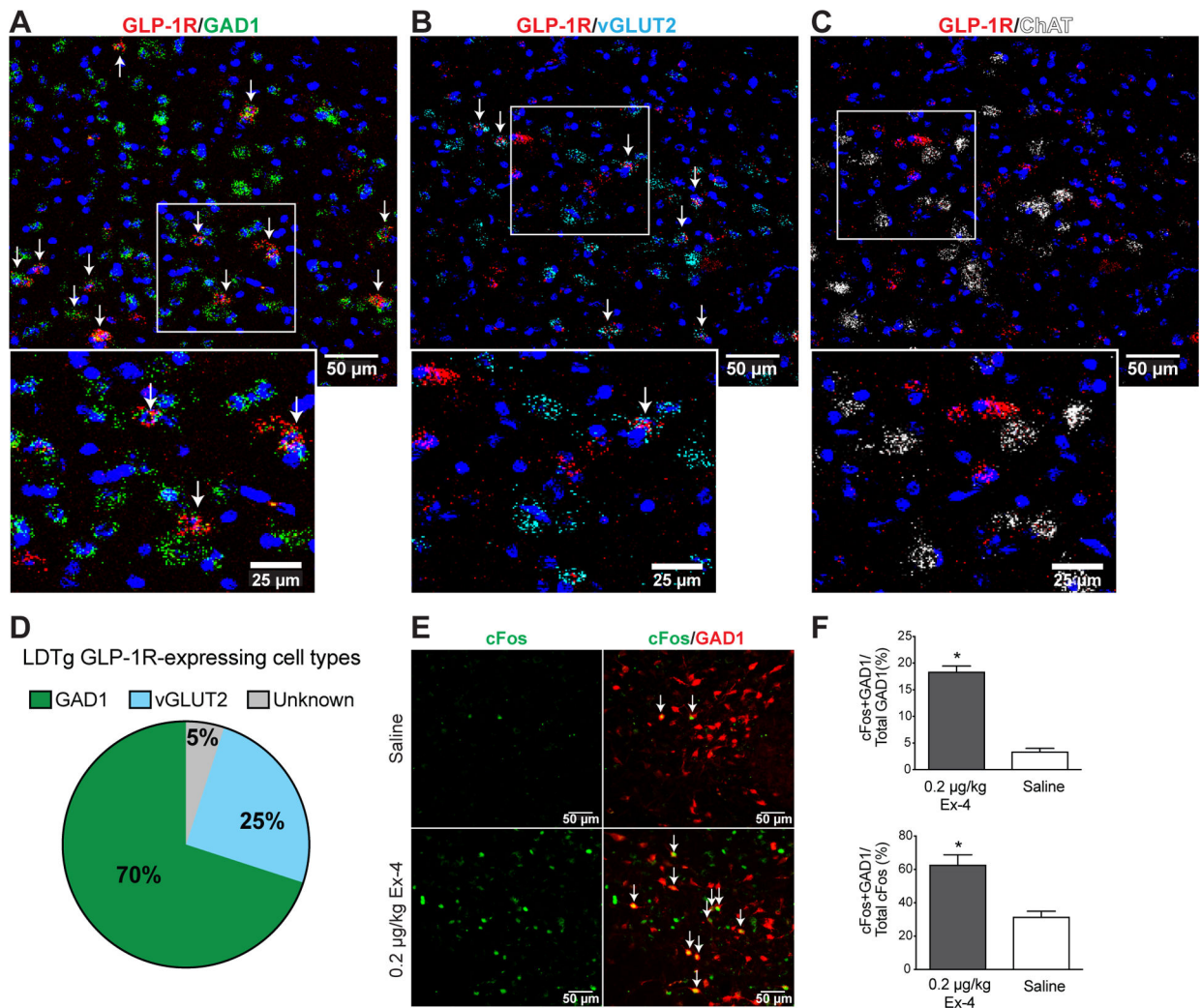
Figure 3. GLP-1Rs are expressed primarily on GABA neurons in the LDTg.
(A-C) Representative images of FISH in the LDTg with GLP1R (red) and DAPI (blue) with GAD1 (green), vGLUT2 (cyan), and ChAT (white). (D) Quantification of GLP-1R colocalization with different cell types. (E) Representative images show 0.2 μg/kg Ex-4 increases cFos (green) in LDTg GABA neurons (red) compared to saline (F) Quantification revealed ~18% of LDTg GAD1+ cells co-expressed cFos in Ex-4-treated rats while ~3% of LDTg GAD1+ cells co-expressed cFos in saline-treated controls [t(6)=12.49, p<0.001; n=4/ treatment]. In addition, ~62% of cFos-positive cells co-expressed GAD1 in Ex-4-treated rats while ~31% of cFos-positive cells co-expressed GAD1 in saline-treated controls [t(6)=4.57, p<0.01; n=4/treatment]. White arrows indicate colocalization. Data are mean ± SEM. *p<0.05
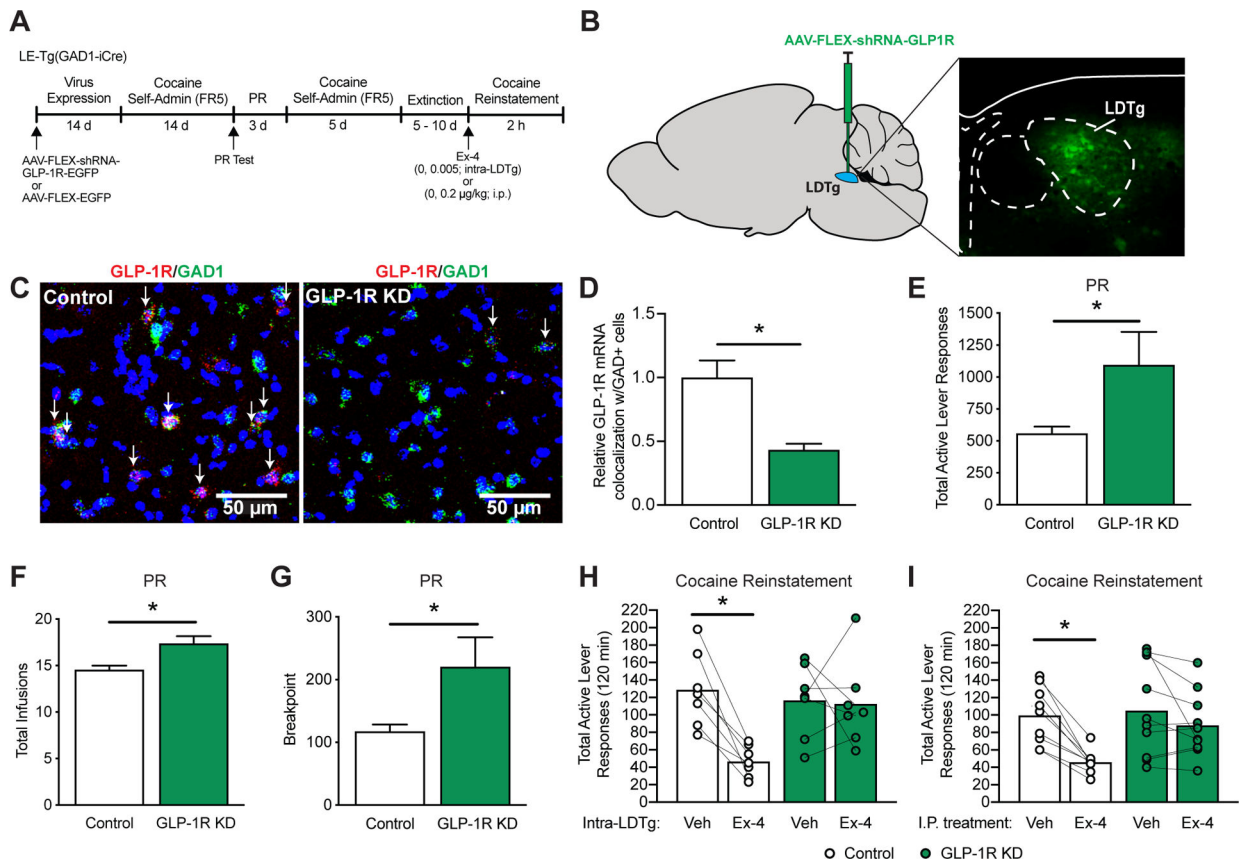
Figure 4. Reduced GLP-1R expression in LDTg GABA neurons augments cocaine taking and prevents the ability of Ex-4 to reduce cocaine seeking.

(A) Schematic of behavioral paradigm and experiments. (B) A Cre-dependent AAV1 expressing a shRNA targeting GLP-1R transcripts was infused into the LDTg of GAD1-Cre rats. Representative image shows GLP-1R shRNA expression (green) in the LDTg. (C) Representative FISH images of GLP-1R and GAD colocalization in control vs GLP-1R knockdown rats. White arrows indicate neurons co-expressing both transcripts. (D) Infusions of AAV-FLEX-shRNA-GLP-1R into the LDTg resulted in a ~55% reduction of GLP-1R mRNA expression in GAD-positive cells [t(7)=3.59, p<0.01] (n=5 for control, n=4 for GLP-1R KD). (E-G) Total active lever responses [t(20)=2.42, p<0.05], total infusions [t(20)=3.44, p<0.01], and breakpoints [t(20)=2.53, p<0.05] during PR test sessions were significantly increased in rats with reduced GLP-1R expression in LDTg GABA neurons (n=9) compared control rats (n=13). (H) Total active lever responses for control rats (n=7) and GLP-1R KD rats (n=7) that were pretreated with intra-LDTg Ex-4 (0, 0.005 μg/100nl) prior to cocaine priming-induced reinstatement test sessions. Two-way RM ANOVA revealed a significant effect of treatment [F(1,12)=9.744, p<0.01] and treatment × group interaction [F(1,12)=7.96, p<0.05]. *Post-hoc* analyses revealed significant differences between vehicle and Ex-4 treatments in control rats (Bonferroni, p<0.05) but not rats with GLP-1R KD in LDTg GABA neurons. Additionally, Intra-LDTg Ex-4 treatment was significantly different in controls vs GLP-1R KD rats [t(12)=3.31, p<0.01]. There were no effects of GLP-1R KD on total inactive lever responses (data not shown). (I) Total active lever responses for control rats (n=9) and GLP-1R KD rats (n=10) that were pretreated with

systemic Ex-4 (0, 0.2 μg/kg, i.p.) prior to cocaine reinstatement tests. Two-way RM ANOVA revealed a significant effect of treatment [F(1,17)=24.33, p<0.001] and treatment × group interaction [F(1,17)=6.59, p<0.05]. Vehicle and Ex-4 treatments were significantly different in control rats (Bonferroni, p<0.05) but not rats with GLP-1R KD in LDTg GABA neurons. Additionally, systemic Ex-4 treatment was significantly different in controls vs GLP-1R KD rats [t(17)=3.24, p<0.01]. There were no effects of either treatment on total inactive lever responses (data not shown). Data are mean ± SEM. *p<0.05
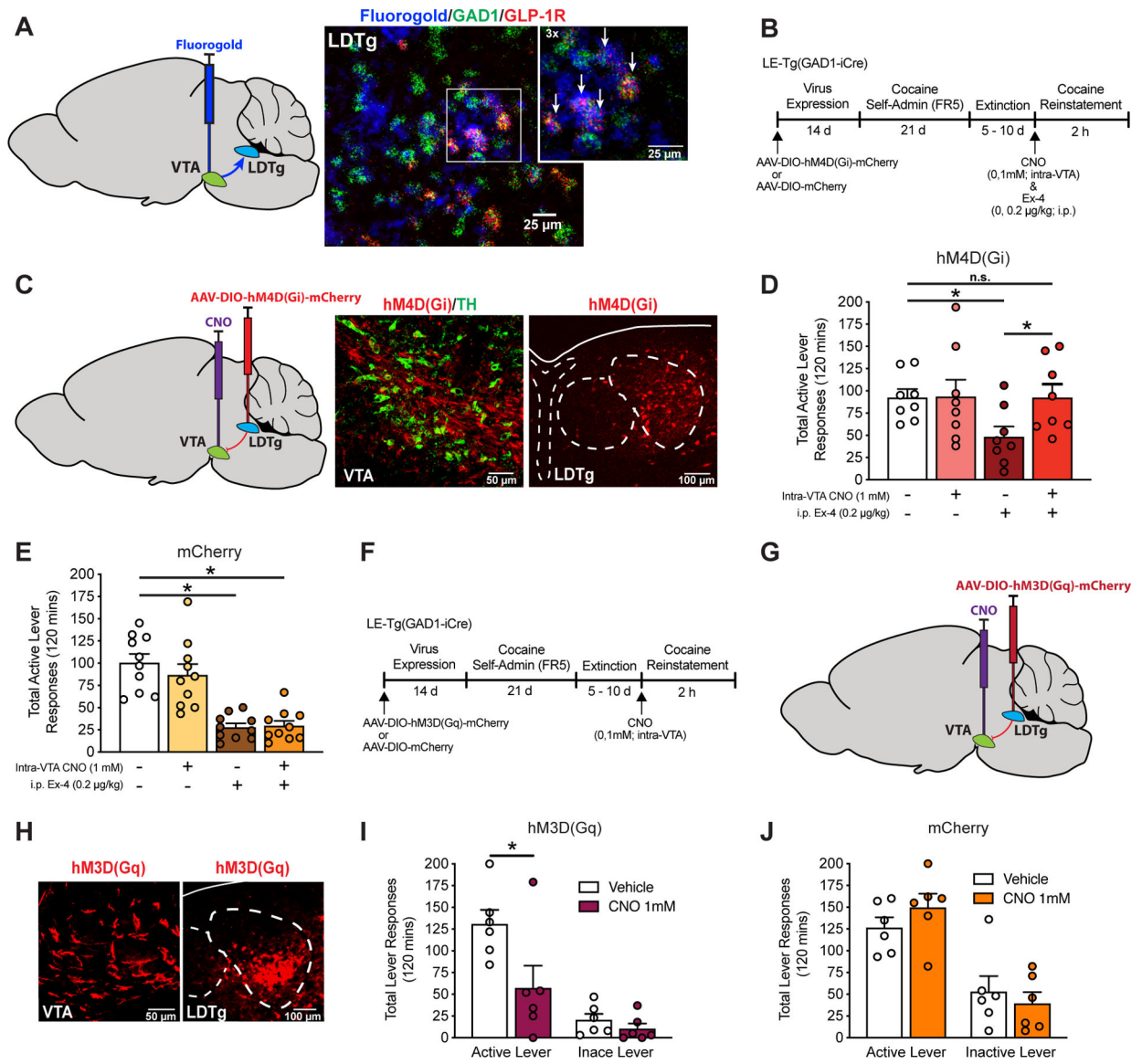
Figure 5. LDTg-to-VTA GABA circuits regulate cocaine seeking and mediate the efficacy of Ex-4 on drug-seeking behavior.

(A) The retrograde tracer fluorogold (blue) was infused into the VTA. LDTg GABA neurons that project to the VTA (blue) co-express GAD1 (green) and GLP-1R (red) transcripts (white arrows). (B) Schematic of the behavioral paradigm and experiments for data in D and E. (C) A Cre-dependent virus expressing the inhibitory DREADD hM4D(Gi) (AAV-DIO-hM4D(Gi)-mCherry) was infused into the LDTg of GAD1-Cre rats. Representative images show LDTg GABA hM4D(Gi)-expressing terminals (red) in proximity to dopamine neurons in the VTA and hM4D(Gi) expression (red) in the LDTg. (D) Prior to cocaine priming-induced reinstatement test sessions, CNO (0, 1mM, 100nl) was infused into the VTA to inhibit LDTg GABA terminals before a systemic injection of Ex-4 (0, 0.2μg/kg, i.p). Total active lever responses for all 4 treatment conditions (n=8) are shown. Two-way RM ANOVA revealed significant main effects of systemic [F(1,7)=8.93, p<0.05] and intra-VTA [F(1,7)=6.16, p<0.05] treatments and a systemic treatment × intra-VTA treatment interaction

[F(1,7)=6.82, p<0.05]. *Post-hoc* analyses revealed significant differences between Veh/Ex-4 and all other treatments (Bonferroni, p<0.05). No effects on total inactive lever responding were found (data not shown). (E) Intra-VTA CNO did not alter the effects of Ex-4 in control rats (n=10). There was a significant main effect of systemic treatment [F(1,9)=43, p<0.001] but no significant main effect of intra-VTA treatment [F(1,9)=0.73, p=0.41]. *Post-hoc* analyses revealed significant differences between i.p. vehicle treatments (Veh/Veh, CNO/Veh) and i.p. Ex-4 treatments (Veh/Ex-4, CNO/Ex-4) (Bonferroni, p<0.05). (F) Schematic of the behavioral paradigm and experiments for data in I and J. (G) A Cre-dependent virus expressing the excitatory DREADD hM3D(Gq) (AAV-DIO-hM3D(Gq)-mCherry) was infused into the LDTg of GAD1-Cre rats. (H) Representative images show hM3D(Gq)-expressing neurons (red) in the LDTg and terminals in the VTA. (I) Total active lever responses are shown for rats pretreated with intra-VTA CNO (n=6) prior to cocaine reinstatement test sessions. Two-way RM ANOVA revealed significant main effects of treatment [F(1,5)=34.44, p<0.01], lever [F(1,5)=16.95, p<0.01], and treatment × lever interaction [F(1,5)=12.13, p<0.05]. Significant differences between vehicle and CNO treatments were observed (Bonferroni, p<0.01). (J) There was no effect of intra-VTA CNO on cocaine seeking in rats infused with the control virus (n=6) [F(1,5)=0.12, p=0.73]. Data are mean ± SEM. *p<0.05