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Activation of glucagon-like peptide-1 receptors in the nucleus accumbens attenuates cocaine seeking in rats

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Abstract

Recent evidence indicates that activation of glucagon-like peptide-1 (GLP-1) receptors reduces cocaine-mediated behaviors and cocaine-evoked dopamine release in the nucleus accumbens (NAc). However, no studies have examined the role of NAc GLP-1 receptors in the reinstatement of cocaine-seeking behavior, an animal model of relapse. Here, we show that systemic infusion of a behaviorally relevant dose of the GLP-1 receptor agonist exendin-4 penetrated the brain and localized with neurons and astrocytes in the NAc. Administration of exendin-4 directly into the NAc core and shell subregions significantly attenuated cocaine priming-induced reinstatement of drug-seeking behavior. These effects were not due to deficits in operant responding or suppression of locomotor activity as intra-accumbal exendin-4 administration had no effect on sucrose-seeking behavior. To determine the effects of GLP-1 receptor activation on neuronal excitability, exendin-4 was bath applied to *ex vivo* NAc slices from cocaine- and saline-experienced rats following extinction of cocaine-taking behavior. Exendin-4 increased the frequency of action potential firing of NAc core and shell medium spiny neurons (MSNs) in cocaine-experienced rats while no effect was observed in saline controls. In contrast, exendin-4 did not affect the frequency or amplitude of spontaneous excitatory post-synaptic currents (sEPSCs) or alter the paired-pulse ratios (PPRs) of

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Conflict of Interest

The authors declare no competing financial interests.

Authors Contributions

N.S.H and B.O'D contributed to the acquisition and analyses of the data as well as drafted the manuscript. H.D.S. was responsible for the study concept and design, supervised the acquisition of data and helped draft the manuscript. P.I.O. was also responsible for the study design and provided critical revision of the manuscript. All authors reviewed content and approved the final version for publication.

evoked EPSCs. These effects were not associated with altered expression of GLP-1 receptors in the NAc following cocaine self-administration. Taken together, these findings indicate that increased activation of GLP-1 receptors in the NAc during cocaine abstinence increases intrinsic, but not synaptic, excitability of MSNs and is sufficient to reduce cocaine-seeking behavior.

Introduction

Almost one million people aged 12 years or older initiated cocaine use in 2015, which represents a 61% increase compared to the total number of new users in 2013 (Hughes, 2016). Unfortunately, there are no FDA-approved medications to treat cocaine relapse, which continues to be a major public health concern (O'Brien, 1997; Pierce et al., 2012). Thus, novel molecular targets are needed to develop new medications for the treatment of cocaine addiction.

An emerging body of evidence indicates that metabolic regulators of hunger and satiety also modulate the rewarding and reinforcing properties of drugs of abuse (Engel and Jerlhag, 2014; Kenny, 2011). For example, glucagon-like peptide-1 (GLP-1) is a satiation hormone and neuropeptide that has been shown to regulate cocaine-, alcohol-, nicotine- and amphetamine-related behavioral responses in rodents (Egecioglu et al., 2013; Graham et al., 2013; Shirazi et al., 2013; Sorensen et al., 2015; Suchankova et al., 2015; Tuesta et al., 2017; Vallof et al., 2016). Since GLP-1 analogs are currently FDA-approved for treating type II diabetes and obesity (Lovshin and Drucker, 2009; Shukla et al., 2015), these pre-clinical findings suggest that GLP-1 receptor agonists could be repurposed for treating drug abuse in human addicts.

Studies have shown that systemic administration of GLP-1 receptor agonists attenuates cocaine-induced locomotion, cocaine conditioned place preference (CPP) and cocaine self-administration (Egecioglu et al., 2013; Graham et al., 2013; Sorensen et al., 2015). While systemic administration of GLP-1 receptor agonists attenuates cocaine-mediated behaviors, the role of central GLP-1 receptors in addiction-like behaviors is largely understudied (Hayes and Schmidt, 2016). GLP-1 receptors are expressed throughout the brain including the mesolimbic reward system (Merchenthaler et al., 1999). GLP-1 receptor activation in the nucleus accumbens (NAc) and ventral tegmental area (VTA) has been shown to decrease food intake by reducing the rewarding value of food (Alhadeff et al., 2012; Dickson et al., 2012; Dossat et al., 2013; Mietlicki-Baase et al., 2013). Since there is significant overlap between the neural mechanisms and circuits mediating the reinforcing effects of palatable food and drugs of abuse (Kenny, 2011; Narayanan et al., 2010; Volkow et al., 2013), it is likely that activation of central GLP-1 receptors also reduces voluntary cocaine taking- and seeking-behaviors. Indeed, we recently showed that activation of GLP-1 receptors in the VTA reduces the reinforcing efficacy of cocaine in rats self-administering cocaine (Schmidt et al., 2016). While these results clearly indicate a novel role for GLP-1 receptors in voluntary cocaine taking, no studies to date have investigated central GLP-1 receptors in the reinstatement of cocaine-seeking behavior, an animal model of relapse.

It is well established that the NAc core and shell subregions play critical roles in the reinstatement of cocaine-seeking behavior (Schmidt et al., 2005; Schmidt and Pierce, 2010).

Here, we initially determined if systemic administration of the GLP-1 receptor agonist exendin-4 entered the brain, localized in the NAc and attenuated cocaine-seeking behavior. We then assessed the effects of direct activation of GLP-1 receptors in the NAc core and shell on cocaine-seeking behavior. Previous studies have shown that GLP-1 receptor agonists modulate neuronal activity in the brain by both pre-synaptic effects on neurotransmitter release and regulation of post-synaptic membrane excitability (Acuna-Goycolea and van den Pol, 2004; Korol et al., 2015; Mietlicki-Baase et al., 2014). Therefore, we aimed to identify a potential neurophysiological mechanism underlying the effects of intra-NAc exendin-4 on cocaine seeking by examining *ex vivo* electrophysiological measures of neuronal activity and GLP-1 receptor expression in the NAc of cocaine-experienced rats. Our results indicate that GLP-1 receptor activation in the NAc following extinction of voluntary cocaine taking increases the frequency of action potential firing in medium spiny neurons (MSNs) and attenuates the reinstatement of cocaine-seeking behavior.

Materials and Methods

Details regarding all drugs used, surgeries, cocaine and sucrose self-administration/extinction/reinstatement, verification of cannula placements, and electrophysiology are available in the Supplement.

Animals and housing

Male Sprague-Dawley rats (*Rattus norvegicus*) weighing 225–250 g were obtained from Taconic Laboratories. Rats were individually housed with food and water available *ad libitum* in their home cages. A 12/12 hr light/dark cycle was used with the lights on at 7:00 a.m. 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 and the University of South Carolina School of Medicine's Institutional Animal Care and Use Committee.

Cocaine reinstatement

To determine if systemic administration of a GLP-1 receptor agonist reduces cocaine seeking and penetrates the brain, initial studies utilized the GLP-1 receptor agonist exendin-4 tagged with fluorescein (fluoro-Ex-4). In addition to binding GLP-1 receptors *in vitro* and *in vivo*, fluoro-Ex-4 produces behavioral responses identical to unlabeled Ex-4 (Rajan et al., 2015; Reiner et al., 2016). Once cocaine-taking behavior was extinguished, rats were pretreated with vehicle, 0.1 and 0.2 µg/kg fluoro-Ex-4 (i.p.) one hour prior to a 10 mg/kg priming injection of cocaine and subsequent reinstatement test sessions. Using a within-subjects design, each rat served as its own control and fluoro-Ex-4 doses were counterbalanced to avoid rank order effects of drug treatment. To determine if the suppressive effects of exendin-4 on cocaine seeking are mediated, in part, by activation of central GLP-1 receptors, rats were deeply anesthetized and transcardially perfused with 0.1 M PBS, pH 7.4, followed with 4% formalin in 0.1 M PBS immediately following their cocaine priming-induced reinstatement test session (i.e., three hours post systemic fluoro-

Ex-4 administration). Once brains were removed and post-fixed overnight in 4% formalin in 0.1 M PBS and then cryoprotected in 20% sucrose in 0.1 M PBS at 4°C for three days. Coronal sections (30 µm) were then taken at the level of the striatum using a cryostat (Leica 3050S; Leica Corp., Deerfield, IL). Brain sections were stored in 0.1 M PBS at 4°C until processed using immunohistochemistry.

The effects of intra-accumbens core and shell infusions of exendin-4 on cocaine priming-induced reinstatement of drug-seeking behavior were studied in separate cohorts of rats. Obturators were removed from the guide cannulae and 33 gauge stainless steel microinjectors (16 mm; Plastics One) were inserted. Exendin-4 (0.005 and 0.05 µg/500 nl) and vehicle were microinjected into the core and shell 10 min prior to a priming injection of cocaine (10 mg/kg, i.p.). Microinjectors were left in place for an additional one min following infusions in order to allow for diffusion of the drug solution away from the tips of the microinjectors. Using a within-subjects design, each rat served as its own control. To control for potential rank order effects of drug and vehicle administrations, all treatments were counterbalanced across reinstatement test sessions.

Immunohistochemistry

Immunohistochemistry was performed on free-floating coronal sections containing the nucleus accumbens according to modified procedures from previously published studies (Reiner et al., 2016; Schmidt et al., 2016). Briefly, sections were washed with 1% sodium borohydride followed by 0.1 M PBS. Sections were then blocked in 0.1 M PBS containing 5% normal donkey serum and 0.2% Triton-X for 1 hr at room temperature. Sections were incubated in primary antibodies overnight, and then, following a PBS rinse, they were incubated in secondary antibodies for 2 hrs. The primary antibodies used were rabbit anti-NeuN (1:1000; ab177487, Abcam), and goat anti-GFAP (1:1000; ab53554, Abcam). Secondary antibodies were donkey anti-goat Alexa Fluor 594 (1:500), and donkey anti-rabbit Alexa Fluor 647 (1:500) from Jackson ImmunoResearch. Sections were then washed and mounted onto glass slides and coverslipped using Vectashield with DAPI (Vector Laboratories; Burlingame, CA). Sections were visualized with a Leica SP5 × confocal microscope using the 20× and 63× oil-immersion objectives along with 405, 488, 594 and 633 nm laser lines. Image z-stacks with the 63× oil-immersion were collected with a step size of 1 µm, while 2–3× optical zoom z-stack images using the same objective were collected with a step size of 0.5 µm.

Cocaine self-administration and yoked saline controls for electrophysiology experiments

Rats underwent catheterization as described above and were allowed to recover for 7 days before cocaine self-administration commenced. Each rat trained to respond for contingent cocaine infusions was paired with a yoked subject that received infusions of saline. Lever pressing for the saline-yoked rats had no scheduled consequences, but these animals received the same number and temporal pattern of infusions as self-administered by the paired cocaine-experimental rat. Cocaine-experimental rats were allowed to self-administer cocaine for 21 days. On day 22, cocaine was replaced with saline for the extinction phase, which lasted until total active lever responding decreased to <15% of the maximum number

of responses completed on the last day of cocaine self-administration. **Typically, it took ~7 days for rats to meet this criterion.**

Electrophysiology

NAc core and shell MSNs were identified by their morphology and low resting membrane potential (−70 to −85 mV) in current clamp. A current step protocol (from −500 to +500 pA; 20 pA increments; 500 ms step duration) was run to determine action potential frequency versus current (*f-I*) relationship. To evaluate spontaneous excitatory postsynaptic currents (sEPSCs) and paired-pulse ratio (PPR) of evoked EPSCs (eEPSCs), the cells were voltage-clamped at −70 mV. For PPR, evoked responses were triggered by 100 μ s constant-current pulses with an inter-stimulus interval of 100 ms generated by an A310 Accupulser (World Precision Instruments) and delivered at 0.2 Hz via a bipolar tungsten stimulation electrode positioned within 100 μ m of the recorded cell. Exendin-4 (1 μ M) was applied via the Y-tube perfusion system modified for optimal solution exchange in brain slices. Neurons from a total of four animals per group were analyzed. All exendin-4 data were collected after a minimum of 4 min of exendin-4 exposure. Currents were low-pass filtered at 2 kHz and digitized at 20 kHz using a Digidata 1440A acquisition board (Molecular Devices) and pClamp10 software (Molecular Devices). Access resistance (10–30 M Ω) was monitored during recordings by injection of 10 mV hyperpolarizing pulses; data were discarded if access resistance changed >25% over the course of data collection.

Cocaine self-administration and yoked saline controls for quantitative real-time PCR

A separate group of cocaine-experimental and yoked saline control rats was used to study the effects of voluntary cocaine taking and subsequent abstinence on GLP-1 receptor expression in the NAc. Cocaine-experimental rats were allowed to lever press for intravenous cocaine infusions as described above for a total of 21 days. Rats were then sacrificed either immediately after the first extinction session (Ext1) or following 7 consecutive days of extinction (Ext7), **a time point associated with robust cocaine-seeking behavior**. Brains were collected and flash frozen in −20°C isopentane and stored at −80°C. Brains were subsequently mounted on a cryostat (Leica 3050S; Leica Corp., Deerfield, IL) and coronal sections at the level of the NAc were taken. Bilateral 1 mm³ micropunches of the NAc core and shell were collected for quantitative real-time PCR analyses of GLP-1 receptor expression. mRNA expression was quantified using Taqman gene expression kits (GLP-1R: Rn00562406_m1; GAPDH: Rn01775763_g1; ThermoFisher Scientific, Waltham, MA). qPCR was conducted using an Eppendorf Mastercycler ep realplex2 and the comparative threshold cycle method was used to quantify relative mRNA expression. Relative fold-expression of NAc GLP-1 receptor transcripts at Ext1 and Ext7 were normalized to yoked saline controls and GAPDH levels.

Statistics

For all cocaine and sucrose reinstatement experiments, the total mean active lever responses, inactive lever responses, and infusions were analyzed with two-way repeated measures ANOVAs. Pairwise analyses were made using Bonferroni *post-hoc* tests ($p < 0.05$). For electrophysiological studies, all analyses were completed using Clampfit 10 (Molecular

Devices). **Differences in action potential frequency were analyzed with two-way repeated measures ANOVAs with current injection step and drug treatment as repeated measures and action potential frequency as a dependent variable. Bonferroni post-hoc tests were used for pair-wise comparisons ($p < 0.05$).** Action potential amplitude was measured from threshold to peak. Action potential duration was measured as duration at 50% of peak amplitude (i.e. half-width). Mean sEPSC frequencies were analyzed from 20 s trace segments. Mean sEPSC amplitude and duration were quantified from an average trace of at least 50 individual events in each cell. sEPSC duration was calculated as the rate of a mono-exponential fitted to the decay phase of the mean sEPSC trace. PPRs were calculated by averaging 5–10 responses and dividing the peak amplitude of the second evoked EPSC by the peak amplitude of the first evoked EPSC. Statistical comparisons of PPR and sEPSC data were done using two-tailed paired Student's *t* tests. Statistical significance thresholds were set at $p < 0.05$.

Results

Peripheral administration of fluoro-exendin-4 penetrates the brain, localizes in the NAc and attenuates cocaine seeking in rats

Total active and inactive lever responses (mean $\pm$ SEM) in rats pretreated with systemic fluoro-Ex-4 (vehicle, 0.1 and 0.2 $\mu\text{g/kg}$, i.p.) prior to a cocaine priming-induced reinstatement test session are shown in Figure 1A. These data were analyzed with a two-way repeated measures ANOVA, which revealed significant main effects of treatment [$F(2,24)=7.681$, $p < 0.01$] and lever [$F(1,12)=10.21$, $p < 0.01$] as well as a significant interaction between lever and treatment [$F(2,24)=5.509$, $p < 0.05$]. Subsequent pairwise analyses indicated that active lever responses were significantly different between vehicle and 0.1 and 0.2 $\mu\text{g/kg}$ fluoro-Ex-4 (Bonferroni, $p < 0.05$). No significant differences were found on inactive lever responding between treatments.

Immediately following the reinstatement test session, rats pretreated with systemic fluoro-Ex-4 were sacrificed and their brains were collected to determine if fluoro-Ex-4 localized in the nucleus accumbens. Coronal sections of the striatum were processed using immunohistochemistry to label neurons and astrocytes. Confocal microscopy revealed colocalization of fluoro-Ex-4 with both GFAP-positive astrocytes and NeuN-positive neurons in both the accumbens core (Figure 1B) and shell (Figure 1C). Overall, these data revealed that systemic administration of fluoro-Ex-4 prior to a cocaine reinstatement test session penetrates the brain and may activate GLP-1 receptors in the nucleus accumbens to reduce drug-seeking behavior.

Administration of exendin-4 directly into the accumbens core and shell dose-dependently attenuates cocaine seeking in rats

To determine if activation of nucleus accumbens GLP-1 receptors alone is sufficient to attenuate cocaine reinstatement, exendin-4 was infused directly into the core and shell subregions of the accumbens 10 minutes prior to a cocaine priming-induced reinstatement test session. Total active and inactive lever responses (mean $\pm$ SEM) in rats pretreated with intra-accumbens core exendin-4 (vehicle, 0.005 or 0.05 $\mu\text{g}/500\text{nl}$) are shown in Figure 2A.

These data were analyzed using a two-way repeated measures ANOVA, which revealed significant main effects of treatment [$F(2,32)=5.004$, $p<0.05$] and lever [$F(1,16)=46.04$, $p<0.0001$] as well as a significant treatment $\times$ lever interaction [$F(2,32)=4.248$, $p<0.05$]. Subsequent pairwise analyses indicated that active lever responses were significantly different between vehicle and 0.005 and 0.05 μg exendin-4 (Bonferroni, $p<0.05$). No significant differences were found on inactive lever responding between treatments.

Total active and inactive lever responses (mean $\pm$ SEM) in rats pretreated with intra-accumbens shell exendin-4 (vehicle, 0.005 or 0.05 $\mu\text{g}/500\text{nl}$) are shown in Figure 2B. These data were analyzed using a two-way repeated measures ANOVA, which revealed significant main effects of treatment [$F(2,28)=5.401$, $p<0.01$] and lever [$F(1,14)=100.3$, $p<0.0001$] as well as a significant treatment $\times$ lever interaction [$F(2,28)=5.435$, $p<0.05$]. Subsequent pairwise analyses indicated that active lever responses were significantly different between vehicle and 0.05 μg exendin-4 (Bonferroni, $p<0.05$). No significant differences were found on inactive lever responding between treatments. Microinjections sites in the accumbens core and shell are illustrated in Figure 2C.

Administration of exendin-4 directly into the accumbens core and shell had no effect on sucrose seeking in rats

While intra-accumbens administration of exendin-4 had no significant effect on inactive lever responding, one could argue that responses were too low to assess the potential rate-suppressing effects of intra-accumbens exendin-4 treatment. Total active and inactive lever responses (mean $\pm$ SEM) are shown in Figures 3A and 3B for rats pretreated with intra-accumbens core and shell exendin-4, respectively, prior to a sucrose reinstatement test session. There were no effects of intra-accumbens core or shell exendin-4 on sucrose seeking. Taken together with the cocaine reinstatement studies in Figure 2, these data indicate that activation of GLP-1 receptors in the core and shell reduces cocaine seeking and that these effects are not due to general motor impairments.

Exendin-4 increases action potential frequency in nucleus accumbens MSNs of cocaine-experienced rats

Using whole-cell current clamp, we evaluated the effects of exendin-4 on action potential frequency of MSNs in the NAc of cocaine-experienced rats (Figure 4). Application of 1 μM exendin-4 increased the frequency of action potential firing in the NAc core (Figures 4A and 4B) and shell (Figures 4C and 4D) of cocaine-experienced rats. These data were analyzed with two-way repeated measures ANOVAs, which indicated no significant main effect of drug treatment [$F(1,6)=1.185$, $p=0.32$, partial $\eta^2=0.165$], a significant main effect of current injection [$F(10,60)=38.94$, $p<0.01$, partial $\eta^2=0.866$], and a significant drug by current interaction [$F(10,60)=3.18$, $p<0.01$, partial $\eta^2=0.346$] in the NAc core. Subsequent pairwise comparisons revealed significant differences between aCSF and exendin-4 at 100, 120, 140, 160, 180, 200, and 220 pA current steps (Bonferroni, $p<0.05$). In the NAc shell, two-way repeated measures ANOVAs revealed significant main effects of drug treatment [$F(1,5)=11.8$, $p<0.05$, partial $\eta^2=0.702$] and current injection [$F(10,50)=80.87$, $p<0.01$, partial $\eta^2=0.942$], and no drug by current interaction [$F(10,50)=0.864$, $p=0.57$, partial $\eta^2=0.147$]. Subsequent pairwise comparisons revealed significant differences between aCSF

and exendin-4 at 160, 180, and 200 pA current steps (Bonferroni, $p < 0.05$). When the core and shell data were combined, a two-way repeated measures ANOVA indicated a significant main effect of drug treatment [$F(1,12)=5.24$, $p < 0.05$, partial $\eta^2=0.304$], a significant main effect of current [$F(10,120)=105.7$, $p < 0.0001$, partial $\eta^2=0.898$], and a significant drug by current interaction [$F(10,120)=2.372$, $p < 0.05$, partial $\eta^2=0.165$]. Subsequent pairwise comparisons indicated significant differences between aCSF and exendin-4 at 120, 140, 160, 180, 200 and 220 pA current steps (Bonferroni, $p < 0.05$). Taken together, these results indicate that exendin-4 elicits broad changes in excitability of MSNs in the NAc core and shell of cocaine-experienced rats. Notably, the significant drug by current interaction in the core and combined core/shell datasets indicate that the effect of exendin-4 on intrinsic excitability may be sensitive to the depolarization state of the neuronal membrane (see Discussion).

Although we focused on electrophysiological correlates of behavioral effects in cocaine-experienced rats, we also collected data from MSNs in the NAc of yoked saline rats. We did not discriminate between NAc subregions in these experiments and our analyses of MSNs from these rats revealed no main effect of drug treatment [$F(1,6)=0.004$, $p=0.95$, partial $\eta^2=0.001$]; a significant main effect of current injection [$F(10,60)=37.44$, $p < 0.01$, partial $\eta^2=0.862$] and no drug by current interaction [$F(10,60)=1.253$, $p=0.27$, partial $\eta^2=0.173$] (Figures 4E and 4F, Table 1). These results indicate that, in contrast to our findings in the core/shell of cocaine-experienced rats, exendin-4 application has no effect on MSNs in the NAc of yoked saline rats. No other measures of membrane excitability were altered by exendin-4 application in cocaine-experienced or yoked saline control rats (Table 1).

Previously, we reported presynaptic effects of GLP-1 receptor activation in the VTA and NAc core of drug-naïve rats (Mietlicki-Baase et al., 2014; Mietlicki-Baase et al., 2013). To explore the role of presynaptic GLP-1 receptor activation, we analyzed sEPSCs and PPR of eEPSCs of NAc core and shell MSNs in cocaine-experienced rats and yoked saline controls. In contrast to our previous findings, exendin-4 application onto slices from cocaine-experienced rats had no effect on sEPSC frequency or PPR of eEPSCs (Figure 5). Exendin-4 also had no effect on sEPSC amplitude or duration in MSNs from cocaine-experienced rats (Table 1). Moreover, no effects of exendin-4 on PPR of eEPSCs or on sEPSC frequency, amplitude, and duration in MSNs from cocaine-naïve rats were observed (data not shown). Collectively, these findings indicate that stimulation of GLP-1 receptors is associated with increased intrinsic, but not synaptic, excitability of MSNs in the NAc core and shell.

Abstinence following cocaine self-administration has no effect on GLP-1 receptor expression in the NAc

Using quantitative real-time PCR, we assessed the effects of cocaine self-administration and subsequent extinction sessions on expression of GLP-1 receptors in the NAc core and shell to determine the potential effects of voluntary cocaine taking and subsequent abstinence on endogenous NAc GLP-1 signaling (Figure 6A). There were no changes in GLP-1 receptor mRNA expression in the NAc core (Figure 6B) and shell (Figure 6C) of cocaine-experienced rats following one (Ext1) and seven (Ext7) days of extinction when compared to yoked saline controls.

Discussion

The present study represents the first investigation of the role of GLP-1 receptors in the NAc core and shell in the reinstatement cocaine-seeking behavior. Here, we demonstrate for the first time that (1) systemically administered fluoro-exendin-4 localizes in the NAc and attenuates cocaine seeking; (2) direct activation of GLP-1 receptors in the NAc core and shell is sufficient to reduce cocaine, but not sucrose, seeking; (3) activation of GLP-1 receptors increases frequency of MSN action potential firing in the NAc core and shell following extinction of voluntary cocaine taking; and (4) abstinence following cocaine self-administration does not alter endogenous GLP-1 receptor expression in the NAc. Taken together, these results suggest that increased excitability of NAc MSNs may contribute to the suppressive effects of a GLP-1 receptor agonist on cocaine priming-induced reinstatement of drug-seeking behavior.

Our findings contribute to and expand upon previous studies demonstrating a role for GLP-1 receptors in addiction-like behaviors. An emerging literature indicates that systemic administration of GLP-1 receptor agonists reduces the rewarding and reinforcing effects of cocaine (Egecioglu et al., 2013; Graham et al., 2013; Sorensen et al., 2015). However, the mechanisms underlying these effects are not clear. GLP-1 receptors are expressed throughout the brain in nuclei known to mediate cocaine reinforcement and drug-seeking behavior, including the VTA and NAc (Merchenthaler et al., 1999; Schmidt et al., 2005; Schmidt and Pierce, 2010). We have shown previously that activation of GLP-1 receptors in the VTA attenuates the reinforcing efficacy of cocaine (Schmidt et al., 2016). Here, we show that systemic fluoro-exendin-4 crosses the blood brain barrier, localizes in the NAc and attenuates cocaine priming-induced reinstatement of drug-seeking behavior. Fluoro-exendin-4 was identified in proximity to both neurons and astrocytes in the NAc core and shell suggesting that GLP-1 receptor activation on these two cell populations mediates the effects of peripheral fluoro-exendin-4 on cocaine seeking. However, future studies are required to investigate the exact role of GLP-1 receptors on neurons and astrocytes in the NAc in cocaine seeking.

Infusions of exendin-4 directly into the NAc core and shell were sufficient to attenuate cocaine priming-induced reinstatement of drug-seeking behavior. These are the first data supporting a novel role for GLP-1 receptors in the NAc in drug-seeking behavior, generally, and cocaine reinstatement, specifically. Interestingly, the effects of GLP-1 receptor signaling on cocaine seeking differed between NAc subregions. Cocaine-seeking behavior was decreased in rats pretreated with 0.05 µg exendin-4 in both the NAc core and shell. In contrast, 0.005 µg exendin-4 significantly attenuated cocaine seeking when infused directly into the NAc core, but not shell. GLP-1 signaling in the NAc has also been shown to affect food intake (Dossat et al., 2013). The effects of GLP-1 receptor activation on intake of palatable food are more pronounced when exendin-4 is infused into the NAc core compared to the shell (Alhadeff et al., 2012). The lowest effective dose of exendin-4 used in this study was 0.025 µg, which produced a transient effect on food intake following activation of GLP-1 receptors in the NAc core and no effect on food intake when infused into the NAc shell (Alhadeff et al., 2012). Collectively, these findings suggest that GLP-1 signaling in the NAc core may play a more prominent role in regulating drug-seeking behavior and hedonic

feeding. Moreover, results from the present study also identify doses of a GLP-1 receptor agonist that selectively reduce cocaine seeking and do not affect *ad libitum* food intake (Alhadeff et al., 2012; Dickson et al., 2012; Miettlicki-Baase et al., 2014) or produce aversive malaise-like effects (Dickson et al., 2012; Hayes et al., 2011; Kanoski et al., 2012).

Administration of exendin-4 directly into the NAc dose-dependently reduces sucrose self-administration on a progressive ratio schedule of reinforcement (Dickson et al., 2012). Doses of exendin-4 lower than 0.1 µg when infused into the NAc do not affect operant responding for sucrose or chow intake (Dickson et al., 2012). Consistent with these effects, the behaviorally relevant dose of exendin-4 (0.05 µg) that attenuated cocaine seeking when infused into the NAc core and shell did not affect the reinstatement of sucrose-seeking behavior. Combined with our systemic administration study identifying doses of exendin-4 that reduce cocaine seeking and do not produce adverse feeding effects (Hayes et al., 2011; Kanoski et al., 2012), these findings further strengthen evidence supporting the selectivity of lower doses of GLP-1 receptor agonists in attenuating cocaine seeking versus non-drug motivated behaviors.

The neural mechanisms underlying the effects of GLP-1 receptor agonists on drug-mediated behaviors are not clear. GLP-1 receptor agonists have been shown to elicit increases in neuronal activity in various brain regions by both pre- and post-synaptic mechanisms. In hypocretin/orexin neurons of the lateral hypothalamus, exendin-4 increased action potential firing by post-synaptic sodium channel-dependent mechanisms and also increased pre-synaptic glutamate release (Acuna-Goycolea and van den Pol, 2004). In our study, the interaction between injected current level and exendin-4 suggests that exendin-4 may exert a preferential effect at specific subtypes of voltage-gated sodium channels or unidentified low voltage-gated conductances. Exendin-4 has also been shown to enhance GABA_A receptor-mediated synaptic and tonic currents in rat hippocampal pyramidal CA3 neurons (Korol et al., 2015). Finally, we have previously reported that exendin-4 increases miniature EPSC frequency and decreases PPR of AMPA receptor-mediated currents in the VTA and NAc core of drug-naïve rats, effects consistent with increased release of pre-synaptic glutamate (Miettlicki-Baase et al., 2014; Miettlicki-Baase et al., 2013). Interestingly, while cell-to-cell variability in the NAc of yoked saline animals (Figure 4F, inset) may indicate discrete effects in the core and the shell, it also raises the possibility that regulation of action potential firing may depend on relative efficacy of exendin-4 at excitatory vs. inhibitory inputs to NAc MSNs. Together, these observations paint a complex picture of the mechanisms by which GLP-1 receptor activation regulates neuronal output. Here, we show that post-synaptic action potential frequency was increased by exendin-4 application in both the NAc core and shell of cocaine-experienced rats. However, pre-synaptic glutamate release mechanisms were not affected by GLP-1 receptor activation in the NAc of cocaine-experienced or yoked saline rats. Our findings suggest that cocaine and/or operant chamber experience may facilitate a shift from a pre-synaptic to a post-synaptic site of exendin-4 action in the NAc. Notably, our qPCR results indicate that this dynamic switch is unlikely to result from an overall difference in GLP-1 receptor mRNA expression in the NAc of cocaine-experienced rats, although a change in the ratio of pre-synaptic versus post-synaptic GLP-1 receptor protein levels remains a possibility.

Our results show that GLP-1 receptor activation in the NAc core and shell of cocaine-experienced rats facilitates MSN firing rates and attenuates cocaine-seeking behavior. Cocaine abstinence is associated with decreased NAc membrane excitability due to changes in conductance of sodium, calcium and potassium (Hu et al., 2004; Zhang et al., 2002; Zhang et al., 1998). Lower membrane excitability during cocaine abstinence may enhance action potential sensitivity to GLP-1 receptor activation that we report here. Cocaine exposure is likely a critical component in this scenario, given the previous findings of minimal effects of exendin-4 on NAc MSN firing in drug-naïve rats (Mietlicki-Baase et al., 2014). Indeed, a recent study showed that exendin-4 reduces cocaine-evoked, but not electrically stimulated, phasic release of dopamine in the NAc core (Fortin and Roitman, 2017). Moreover, systemic exendin-4 pretreatment reduces cocaine-evoked dopamine release in the NAc (Egecioglu et al., 2013; Sorensen et al., 2015) and lateral septum (Reddy et al., 2016). However, GLP-1 signaling has also been shown to regulate other neurotransmitter systems including glutamatergic (Mietlicki-Baase et al., 2014) and GABAergic (Korol et al., 2015) transmission in the brain. Understanding the mechanisms by which GLP-1 receptor activation in the NAc reduces cocaine seeking will benefit from a comprehensive analysis of how exendin-4 regulates integration of synaptic and intrinsic MSN excitability in the context of drug priming-induced reinstatement. Our results indicate that intrinsic GLP-1 receptor-coupled mechanisms may contribute to previous findings that a broad increase in NAc MSN action potential output is associated with attenuation of rewarding consummatory behaviors (Nicola et al., 2004; Wheeler and Carelli, 2009)

Previously, we showed that cocaine activates GLP-1-expressing neurons in the nucleus tractus solitarius (NTS) of the caudal brainstem (Schmidt et al., 2016). Since the NTS sends monosynaptic GLP-1-expressing efferent projections to the NAc (Alhadeff et al., 2012), these findings suggest that increased endogenous GLP-1 signaling in the brain may represent a homeostatic response to cocaine that functions to reduce on-going cocaine consumption (Schmidt et al., 2016). However, no studies to date have investigated the potential role of endogenous central GLP-1 signaling in cocaine seeking. Here, we show that GLP-1 receptor mRNA expression in the NAc core and shell was not altered following one and seven days of extinction. Exendin-4 binding has been shown to regulate trafficking and surface expression of GLP-1 receptors (Roed et al., 2014). It is possible that exendin-4 infusions into the NAc increase trafficking of GLP-1 receptors to the post-synaptic membrane resulting in enhanced firing of MSNs. Additionally, cocaine may affect endogenous GLP-1 release from the NTS, which could also result in altered GLP-1 receptor trafficking and surface expression in the NAc. Future studies are required to further delineate the role of endogenous central GLP-1 signaling in cocaine seeking, including whether cocaine affects GLP-1 receptor trafficking and/or the kinetics of ligand-mediated GLP-1 receptor trafficking.

A growing literature supports the efficacy of GLP-1 receptor agonists in reducing cocaine-mediated behaviors. Our findings expand this literature and are the first to indicate that exogenous activation of GLP-1 receptors in the NAc core and shell is sufficient to reduce cocaine-seeking behavior. Importantly, the doses of exendin-4 shown to attenuate cocaine seeking do not produce adverse feeding and malaise-like effects. While future studies are needed to further delineate the molecular and neurophysiological mechanisms by which

GLP-1 receptor activation in the NAc reduces cocaine seeking, our data show that the suppressive effects of intra-NAc exendin-4 on cocaine seeking are associated with increased action potential firing of MSNs. These findings provide strong rationale for pilot translational studies screening the efficacy of GLP-1 receptor agonists in human cocaine addicts.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. Systemic administration of the GLP-1 receptor agonist exendin-4 attenuates cocaine seeking and localizes in the nucleus accumbens

Rats were pretreated with intraperitoneal infusions of vehicle, 0.1 and 0.2 µg/kg fluoro-exendin-4 prior to a cocaine priming-induced reinstatement test session ($n=7$ per treatment).

(A) Total numbers of active lever responses (mean $\pm$ SEM) were significantly different between rats pretreated with vehicle and 0.1 and 0.2 µg/kg fluoro-exendin-4. (* $p<0.05$, Bonferroni). Immediately following the reinstatement test session, rats pretreated with systemic fluoro-exendin-4 were sacrificed and brains removed. Immunohistochemical

analyses identified fluoro-exendin-4 (green fluorescence = fluoro-exendin-4) in the nucleus accumbens core (**B**) and shell (**C**) where it localized with neurons (blue fluorescence = NeuN) and astrocytes (red fluorescence = GFAP).

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A. NAc core

B. NAc shell

C.

Figure 2. Administration of exendin-4 directly into the nucleus accumbens dose-dependently attenuates cocaine-seeking behavior in rats

Cocaine seeking was attenuated in rats pretreated with exendin-4 in the accumbens core and shell prior to a cocaine priming-induced reinstatement test session. **(A)** Total numbers of active lever responses (mean $\pm$ SEM) were significantly different between rats pretreated with vehicle and 0.005 and 0.05 μ g exendin-4 in the accumbens core ($n=9$ per treatment). **(B)** Total numbers of active lever responses were also significantly different between rats pretreated with vehicle and 0.05 μ g exendin-4 in the accumbens shell ($n=8$ per treatment). **(C)** Microinjection sites corresponding with infusions into the core (open circles) and shell (closed circles). (* $p<0.05$, Bonferroni)

Figure 3. Intra-accumbens administration of exendin-4 does not affect sucrose seeking in rats
 Exendin-4 (0.005 and 0.05 µg) administered directly into the accumbens core (A) and shell (B) did not alter sucrose-seeking behavior in rats. Microinjection sites corresponding with infusions into the core (open circles) and shell (closed circles) are shown in C. ($n=6$ per treatment in the core; $n=8$ per treatment in the shell)

Figure 4. Exendin-4 increases action potential frequency in NAc core and shell MSNs in cocaine-experienced rats

Representative membrane potential traces recorded before (aCSF) and after (Ex-4) application of exendin-4 (1 μ M) in response to a hyperpolarizing (−100 mV) and a depolarizing (+200 mV) current step in MSNs from cocaine-experienced and yoked saline rats (A, C, E). Corresponding plots of mean action potential frequency ($\pm$ SEM) across a range of injected current steps are shown in B, D and F. Insets illustrate cell-to-cell variability of spike frequency at the +180 pA step for all recorded neurons before and after Ex-4 application. Note that cells with identical aCSF or Ex-4 values are not distinguishable. Thick black lines in the insets represent spike frequency means. (* $p < 0.05$, for drug treatment by current interaction in Cocaine: NAc core and * $p < 0.05$, for drug treatment in Cocaine: NAc shell, two-way repeated measures ANOVAs). Cocaine: NAc core, $n = 7$ cells, $N = 4$ rats; Cocaine NAc shell, $n = 6$ cells, $N = 4$ rats; Yoked Saline: NAc core/shell, $n = 7$ cells, $N = 3$ rats.

Figure 5. Exendin-4 effects on action potential firing in NAc MSNs are not modulated by presynaptic mechanisms

Representative traces of sEPSCs from before (aCSF) and after (Ex-4) application of exendin-4 (1 μ M) in the NAc core and shell are shown in A. Exendin-4 had no effect on sEPSC frequency in either subregion of the accumbens (B). Representative traces showing paired pulse ratios (PPRs) before and after exendin-4 application are shown in C. Exendin-4 had no effect on PPR in the core and shell (D). ($n=8$ cells, $N=4$ rats for Cocaine: NAc core; $n=8$ cells, $N=4$ rats for Cocaine: NAc shell).

Figure 6. Cocaine self-administration and subsequent abstinence are not associated with altered expression of GLP-1 receptors in the nucleus accumbens

(A) Experimental design depicting the drug taking and extinction phases. Tissue was collected immediately after extinction sessions. GLP-1 receptor mRNA expression in the nucleus accumbens core (B) and shell (C) was not altered following one (Ext1; $n=7$) and seven (Ext7; $n=9$) days of abstinence compared to yoked saline controls. ($p>0.05$; un-paired t-tests)

Table 1

Effects of extendin-4 on neuronal activity in the nucleus accumbens

	Cocaine: NAc Core		Cocaine: NAc Shell		Yoked Saline: NAc Core/Shell	
	aCSF	extendin-4	aCSF	extendin-4	aCSF	extendin-4
Resting membrane potential	76.0 ± 1.3 mV	71.0 ± 1.9 mV	73.5 ± 1.4 mV	72.0 ± 2.1 mV	78.0 ± 2.4 mV	77.0 ± 2.3 mV
Rheobase	160 ± 24 pA	132 ± 37 pA	151 ± 16 pA	140 ± 16 pA	190 ± 27 pA	176 ± 25 pA
Action potential amplitude	88 ± 1.9 mV	87 ± 2.6 mV	83 ± 1.5 mV	83 ± 3.2 mV	86 ± 2.8 mV	85 ± 3.3 mV
Action potential duration	1.1 ± 0.04 ms	1.2 ± 0.07 ms	1.1 ± 0.03 ms	1.1 ± 0.07 ms	1.02 ± 0.04 ms	1.05 ± 0.05 ms
sEPSC amplitude	24.58 ± 1.93 pA	24.09 ± 2.02 pA	25.88 ± 2.39 pA	24.45 ± 1.27 pA	24.79 ± 4.0 pA	23.93 ± 2.43 pA
sEPSC duration	4.52 ± 0.42 ms	4.86 ± 0.19 ms	4.21 ± 0.23 ms	4.05 ± 0.42 ms	4.75 ± 0.42 ms	4.86 ± 0.19 ms