IMPACT OF GHRELIN AND COCAINE ON INTRACRANIAL SELF-STIMULATION IN RATS

An Honors Fellows Thesis

by

TRACEY CHRISTINE KNIFFIN

Submitted to the Honors Programs Office
Texas A&M University
in partial fulfillment of the requirements for the designation as

HONORS UNDERGRADUATE RESEARCH FELLOW

April 2010

Major: Psychology and English

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Approved by:

Research Advisor:	Paul Wellman
Associate Director of the Honors Programs Office:	Dave A. Louis

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ABSTRACT

Impact of Ghrelin and Cocaine on Intracranial Self-Stimulation in Rats. (April 2010)

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Ghrelin (GHR) is a 28-amino acid peptide that is secreted in the peripheral and central nervous systems and correlates with hunger. GHR is the only gut hormone known to stimulate food intake. Psychostimulant drugs, such as cocaine (COC), induce locomotion and augment reward-seeking behavior. Recent studies have examined the effects of GHR on locomotion and report an augmenting effect when combined with COC. In the present study, we examine the effects of systemic injections of COC, GHR, and a pairing of both on rate-frequency curves, maximal response rate, and threshold (the frequency that produces 50% of the maximal response rate) obtained during intracranial self-stimulation (ICSS) of the medial forebrain bundle (MFB). In this task, rats press a lever to deliver electrical pulses to their own brain via an implanted electrode. Rats are run in 75 minute sessions, consisting of five 15-minute passes; during each pass, the intensity (in μamps) is kept constant, while the frequency of stimulation is lowered each minute from 141 Hz to 28 Hz (decreased in 0.05 log units). Each rat runs multiple trials on separate days and is injected with either vehicle (0.5 ml saline), COC (0, 2.5, and 5.0

mg/kg), GHR (5.0, 10.0, and 30.0 nMol/rat), or a combination of GHR and COC (10.0 nMol/rat GHR and 2.5 mg/kg COC). GHR produced a significant dose-dependent effect, where the rate-frequency curves shifted to the right, maximal response rate and 50% response rate decreased, and threshold increased. COC produced the opposite effect. A combination of GHR and COC shifted the rate-frequency curves to the right and caused a significant increase in threshold. Dopamine (DA) plays a critical role in reinforcement and is found in neurological reward pathways, such as the ventral tegmental area (VTA) and the nucleus accumbens (NA). Since GHR receptors exist in these two areas, some researchers suggest that there may be a common pathway shared by the rewarding aspects of food and psychostimulants leading to obesity and drug abuse, respectively. The present results suggest that in some circumstances, GHR may inhibit already active reinforcement systems.

ACKNOWLEDGMENTS

I would like to thank Dr. Paul Wellman for the time and effort he gave to this project. He went out of his way to help me on all aspects of this experiment throughout the year, from guidance in executing the experiment to trimming posters. His mentorship is truly appreciated.

I would also like to thank Shane Clifford for his help with surgeries, guidance during data analysis, and willingness to edit portions of this thesis. Additionally, the project could not have been completed without the help of Destri Schul and Samuel Hughes during data collection.

Finally, I need to thank my mother, father, and brother who were supportive of my efforts in this project, especially their tolerance of my constant talk of rats.

Portions of this project were supported by Texas A&M University Honors Programs to Tracey Christine Kniffin and NIDA 1R01DA013188B to Dr. Paul Wellman.

NOMENCLATURE

COC	Cocaine
DA	Dopamine
DAT	Dopamine Transporter
FR	Food Restriction
GHR	Ghrelin
ICSS	Intracranial Self-Stimulation
MFB	Medial Forebrain Bundle
NA	Nucleus Accumbens
SA	Self-Administration
VTA	Ventral Tegmental Area

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CHAPTER I

INTRODUCTION

Food restriction (FR) has been commonly used to facilitate the acquisition of cocaine (COC) or amphetamine self-administration (SA) and to increase the acquisition of COC-induced conditioned place preference (Wellman et al., 2005). Due to the effects of FR on drug-seeking behavior, it has commonly been used as a technique to facilitate shaping rats to SA psychostimulants (Bell et al., 1997; Carroll, 1985). Ghrelin (GHR) is a 28-amino acid peptide that is secreted in the peripheral and central nervous systems which has been observed to correlate with hunger, and it is possible that FR acts by increasing GHR levels in the body (Gardiner et al., 2008).

Several observations support the idea that GHR is directly related to FR such as the fact that GHR levels rise prior to and fall after meals. Additionally, GHR is the "only known gut hormone to stimulate food intake" (Gardiner et al., 2008). It should be noted that increased GHR is not the only effect of FR and represents only one of the possible pathways affected by a decrease in energy intake or availability. Therefore, the effects of GHR on COC do not necessarily lead to the conclusion that FR effects COC in the same way.

This thesis follows the style of Pharmacology, Biochemistry and Behavior.

Previous studies have shown that GHR does not only act to mimic the effects of FR and cause an increase in dietary consumption. GHR was also found to operate on the reward center of the brain that "communicates the hedonic and reinforcing aspects of natural rewards, e.g. food, as well as of addictive drugs" (Jerlhag et al., 2008). This may represent a common pathway in the brain shared by feeding and addiction. In other words, the feeding-relevant neural systems affected by FR may also "modulate the neural substrates by which cocaine and other psychostimulants alter locomotion and reinforcement" (Wellman et al., 2005).

It has been noted in recent research that "ghrelin may influence the activity of wide brainstem-hypothalamic pathways and networks" and its presence in reward regions such as the ventral tegmental area (VTA) suggests a link between GHR and feeding for reasons other than hunger or pure energy consumption (e.g. GHR may have a pharmacological effect independent of energy state). Specifically, GHR has been linked to the reward aspect of hunger, termed palatability (Olszewski et al., 2008). This suggests that GHR may influence the effects of COC when the two substances are presented together by acting on the reward center of the brain. Additionally, GHR has been found to increase the extracellular dopamine (DA) levels in the nucleus accumbens (NA) and also to augment COC-induced locomotion (Jerlhag et al., 2008; Quarta et al., 2009; Wellman et al., 2005). In a test of conditioned reward, Davis et al. (2007) reported that GHR facilitated the conditioned reinforcing effects of low COC doses (0.312 or 0.625 mg/kg) but repressed the reinforcing effect of a higher COC dose (1.25

mg/kg). Therefore, in some instances it appears that GHR administration can facilitate brain reinforcement associated with COC, but in other instances can inhibit it. There are currently no studies that report of the effects of GHR administration on the intravenous SA of COC.

An alternative to the SA method is intracranial self-stimulation (ICSS), a procedure in which a rat is surgically implanted with an electrode in the medial forebrain bundle (MFB). The MFB is part of the mesolimbic pathway (with projections from the VTA to the NA), a well-known reinforcement system in the brain, and electrical stimulation of this area has been found to be reinforcing (Wise & Rompre, 1989). In this method, the rat is allowed to press a lever to stimulate its own brain; presumably this stimulation is pleasurable. Moreover, a change in the amount of effort a rat is willing to exert to obtain this stimulation is related to the degree of pleasure gained by the stimulation. Reductions in brain reward result in less effort while increases in brain reward result in greater effort. To date no published study has examined the impact of GHR on brain stimulation reward nor examined the interaction of COC and GHR on ICSS.

By researching the effect of COC, GHR, and a combination of both on ICSS, we will be able to better understand the processes that modify the reinforcing effects of COC and other psychostimulants. The gut peptide effects of GHR on COC make it one of only a few factors known to change the locomotion effects and reinforcing actions of COC. Other factors that modify the effects of COC are FR, stress, and ovarian hormones

(Wellman et al., 2005). With further research in this area, it may be possible to determine how to inhibit or augment the reinforcing effects of COC. Specifically, by examining the effects of COC and GHR on the threshold current required to support ICSS, we can better understand the effects GHR has on reward (Wellman et al., 2008).

The current study examined the effects of GHR, COC, and a combination of both on rodent ICSS and reward-seeking behavior. COC is known to increase ICSS at an injection of 1.25 mg/kg and to so profoundly increase ICSS responding with an injection of 5.0 mg/kg that animals continue lever-pressing until the end of trials without reaching a breakpoint (Wellman et al., 2008). The effects of GHR alone and GHR combined with COC on ICSS are unknown. The following hypotheses were the expected results of this research.

Hypothesis 1: An injection of systemic GHR will decrease rodent ICSS, indicating a decrease in reward-seeking behavior; however because the existing literature on GHR and reinforcement is conflicting, it is possible that GHR will facilitate rodent ICSS.

Hypothesis 2: An injection of COC will increase rodent ICSS, indicating an increase in reward-seeking behavior.

Hypothesis 3: An injection of systemic GHR followed by an injection of COC will decrease rodent ICSS or produce a smaller increase in ICSS than that produced by COC

administration alone. Alternatively, if GHR alone augments ICSS, then the combination may result in more activation than that induced by COC alone.

CHAPTER II

METHODS

Animals

The animals used were 9 adult male Sprague-Dawley albino rats (Harlan Industries, Houston, TX) weighing between 300-450 grams at the beginning of the study. The animals were housed individually in standard plastic rodent cages in a holding room and were allowed a 1-week adaptation period prior to the onset of behavioral testing in order to acclimatize them to daily handling and colony maintenance procedures. The animal holding room was maintained at 23+1.0°C, with a 12:12 L:D lighting schedule (lights off at 10:00 h). The rats were provided with continuous access to tap water and rodent chow pellets (Teklad). The procedures of the study were approved by the Texas A&M University Institutional Animal Care and Use Committee.

Drugs

COC solutions were prepared by dissolving COC hydrochloride (a gift provided by Dr. Kevin Gromley of the Basic Research Division of NIDA) into 0.9% saline at concentrations of 2.5 and 5.0 mg/ml. COC solutions were calculated as the salt. GHR solutions were prepared by dissolving rat GHR (No. B2709002; Piproteomics, Huntsville, AL) into a vehicle of 0.5 ml saline at concentrations of 5.0, 10.0, and 30.0 nMol.

Surgical procedures

Prior to surgery, each rat was injected (i.p.) with 0.4 mg/kg atropine sulfate (to minimize bronchial secretions), and then anesthetized with an injection (i.p.) of ketamine (Ketaset: 60 mg/kg) and xylazine (20 mg/kg). Each rat was mounted in a stereotaxic frame and the scalp incised using a sterile technique. A 2% lidocaine jelly was applied to the incised edges of the scalp. The periosteum was mechanically retracted and skull bleeding was terminated using a styptic gel (Kwik-Stop; Gimborn Pet, Atlanta, GA). A bipolar stimulating electrode with 0.125-mm wire diameter (No. 303/3; Plastics One, Roanoke, VA) was implanted into the MFB at the level of the lateral hypothalamus. The incisor bar was set at -2.7 mm, and coordinates were 3.2 mm posterior to bregma, 1.7 mm lateral to the sagittal suture, and 8.3 mm ventral to the skull surface. Electrodes were affixed to the skull with four skull screws and dental acrylic (Lang Dental; Wheeling, IL). The lateral edges of the scalp incision were coated with a 0.1% gentamicin sulfate ointment (E. Fougera; Melville, NY) and the ends of the incision were closed using cyanoacrylate. Following surgery, each rat was injected (i.m.) with penicillin (300,000 units each). A 7-day recovery period followed surgery, during which the rats were handled and weighed daily and had continuous access to water and food pellets.

Apparatus

The test chamber (Cambden Instruments) was constructed of Plexiglass and stainless steel with dimensions of 28x22x22 cm (Fig. 1). Two levers were mounted on opposite

sides of one wall 7 mm above the floor. Depression of the right lever was without consequence while depression of the left lever resulted in the delivery of a 500-ms

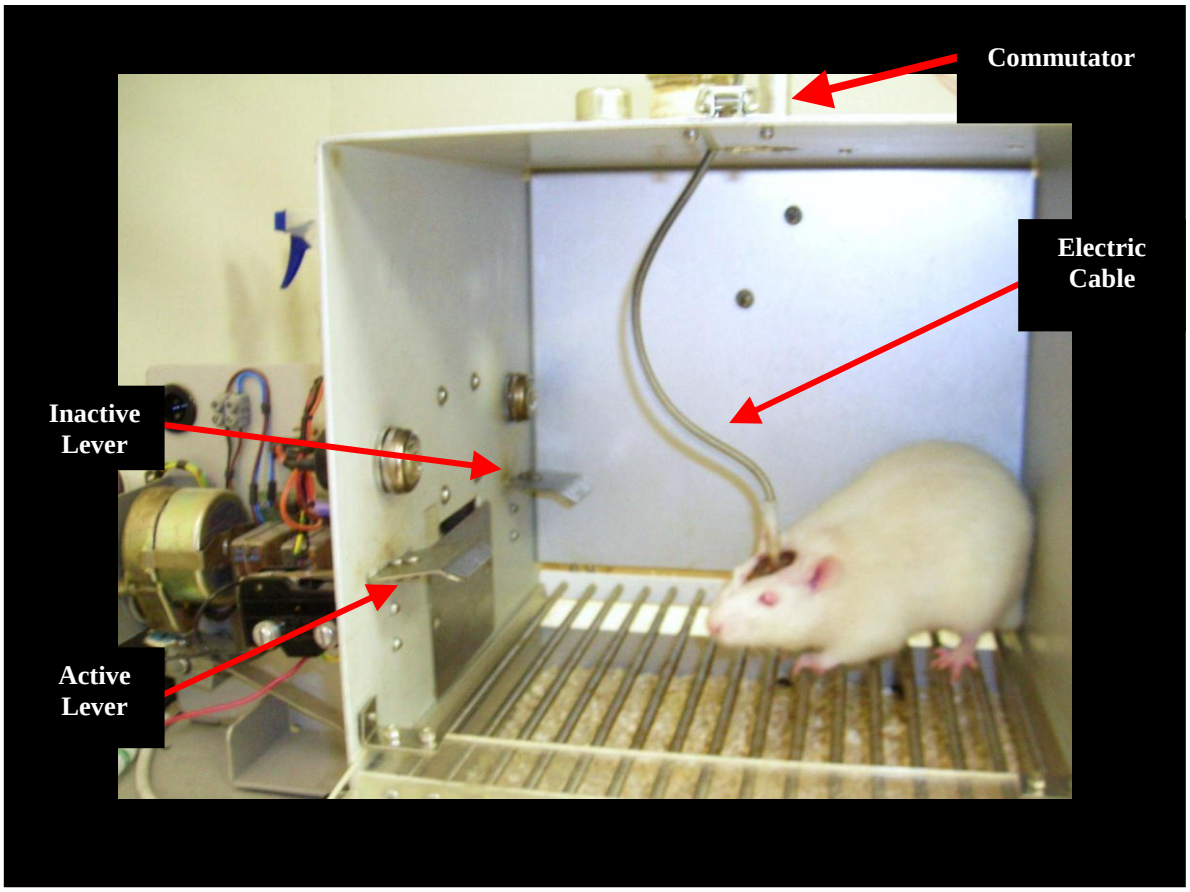

Fig. 1. Testing Apparatus. Lateral view of the ICSS test chamber. The lever on the left delivers brain stimulation via a cable attached to the top of the rat's skull.

train of monophasic rectangular pulses with 1-ms pulse duration delivered from a Grass S88 stimulator (Grass Instruments, Quincy, MA) and a constant current simulator (Model DS3; Digitimer, Hertfordshire, England) to the brain via a commutator and a flexible cable (Plastics One). All stimulation parameters were monitored on an oscilloscope (Model 645280; Jameco Electronics, Belmont, CA).

Procedure

After the recovery period, each rat was shaped to lever-press for rewarding brain stimulation on a fixed ratio-1 schedule. During shaping, current intensity was systematically increased until a minimum rewarding current (a current sufficient to elicit lever responding) was reached (typically between 50-200 μamps). Once the lever-pressing behavior was acquired, animals were run through 75 minute baseline trials consisting of five separate 15-minute passes. During each 15-minute pass the intensity was kept constant while the frequency of stimulation was lowered each minute from 141 Hz to 28 Hz (decreased in 0.05 log units). During testing, each rat ran multiple trials on separate days and was injected (i.p.) with either vehicle (0.5 ml saline), COC (0, 2.5, and 5.0 mg/kg), GHR (5.0, 10.0, and 30.0 nMol/rat), or a combination of GHR and COC (10.0 nMol/rat GHR and 2.5 mg/kg COC). GHR was injected one hour before the beginning of each trial and vehicle and COC were both injected 30 minutes before the beginning of each trial. Two days of vehicle trials were interposed between each drug trial. The number of lever-presses per minute was recorded for each rat throughout each 75 minute trial.

Histological analyses

At the conclusion of the experiment, each rat was overdosed with sodium pentobarbital (100 mg/kg, i.p.), and perfused through the heart with 0.9% formalin. Further fixation in 10% formalin/30% sucrose proceeded for at least 72 hours prior to sectioning each brain. Alternate 80 micron frozen sections were stained with cresyl violet and cover-slipped for permanent storage. Coronal scans were compared to standard atlas plates to verify electrode placements (Paxinos & Watson, 2005).

Data analyses

For each rat and session, the total number of responses, rate-frequency curve, maximal response rate (100% response rate), 50% response rate, and threshold (frequency which produced 50% response rate) were calculated for post-injection tests and compared to values obtained during baseline trials. Maximal response rate, 50% response rate, and threshold were assessed using one-way repeated measures analyses of variance and Holm-Sidak contrast tests. Differences less than 0.05 were deemed statistically significant.

CHAPTER III

RESULTS

Histological analysis

After testing, electrode placements were verified via histological analysis and all animals used in the present study showed correct electrode placement within the MFB at the level of the lateral hypothalamus (Fig. 2).

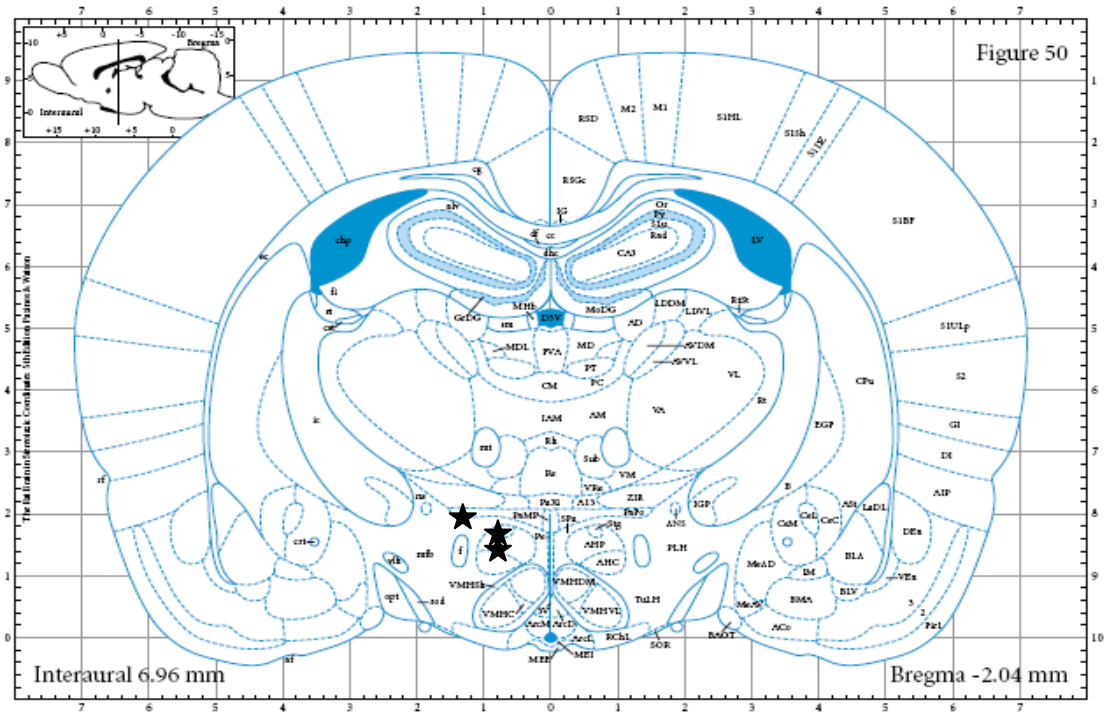

Fig. 2. Histological Analysis. A representative sample of electrode placement within the MFB at the level of the lateral hypothalamus. Fig. is derived from The Rat Brain (Paxinos & Watson, 2005).

GHR group analysis

Figure 3 depicts the rate-frequency curves for GHR trials with 0, 5.0, 10.0, and 30.0 nMol/rat GHR. After systemic injections of GHR, the rate-frequency curves dose-dependently shifted to the right and the maximal response rates decreased as GHR dose

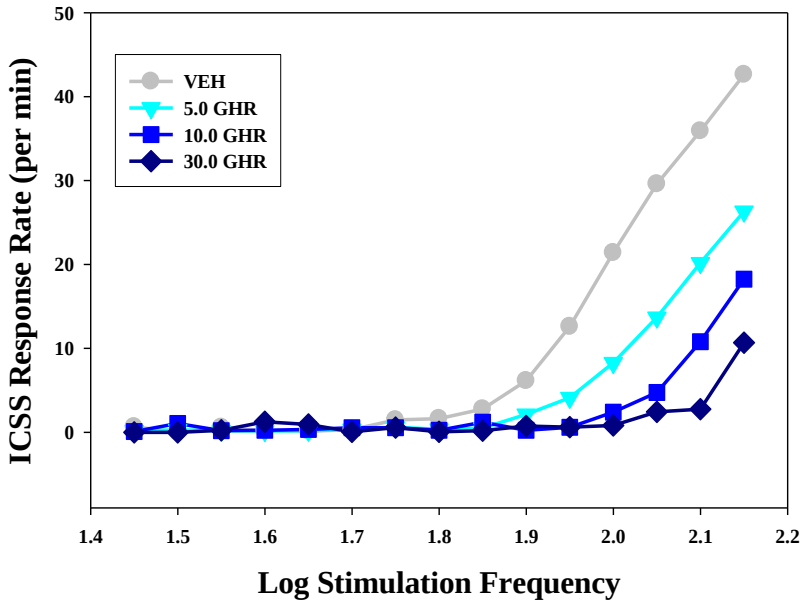

Fig. 3. Rate-frequency Curve for GHR Trials. Mean group response rates as a function of stimulation frequency for rats. Rats treated one hour prior to each test session with either 0.5 ml saline (VEH), 5.0, 10.0 or 30.0 nMol/rat GHR. Group sizes are n=5.

increased. Figure 4A-C depicts the mean group changes in ICSS responding after GHR administration. Panel A depicts the mean maximal response rate, panel B depicts the mean 50% response rate, and panel C depicts the mean threshold (frequency necessary to produce the 50% response rate) for the GHR testing condition. A one-way analysis of variance test, computed using Sigma Stat 3.0 (Systat Corporation; San Jose, CA), confirmed these results were significant between groups for the maximal response rate (100% response rate) ($F(3,12) = 10.371$, $p<0.001$). Additional analysis with the Holm-Sidak method compared the GHR dose values to the control condition and indicated a significant reduction in 100% response rate between each GHR dose (5.0, 10.0, and 30.0 nMol/rat GHR) and the control dose (0.5 ml saline) (p<at least 0.05). Similarly, a one-way analysis of variance test of the 50% response rate values showed that the results were significant between groups in GHR treatment ($F(3,12) = 12.455$, $p<0.001$). Analysis with the Holm-Sidak method also indicated a significant difference in 50% responses between each GHR dose and the control dose (p<at least 0.05). Finally, analysis of the threshold using a one-way analysis of variance test confirmed that the results were significant between groups ($F(3,12) = 10.999$, $p<0.001$). However, analysis with the Holm-Sidak method indicated a significant difference between the 10.0 and 30.0 nMol/rat GHR doses and the control dose (0.05 ml saline), but did not indicate a significant difference between the 5.0 nMol/rat GHR dose and the control dose (p<at least 0.05).

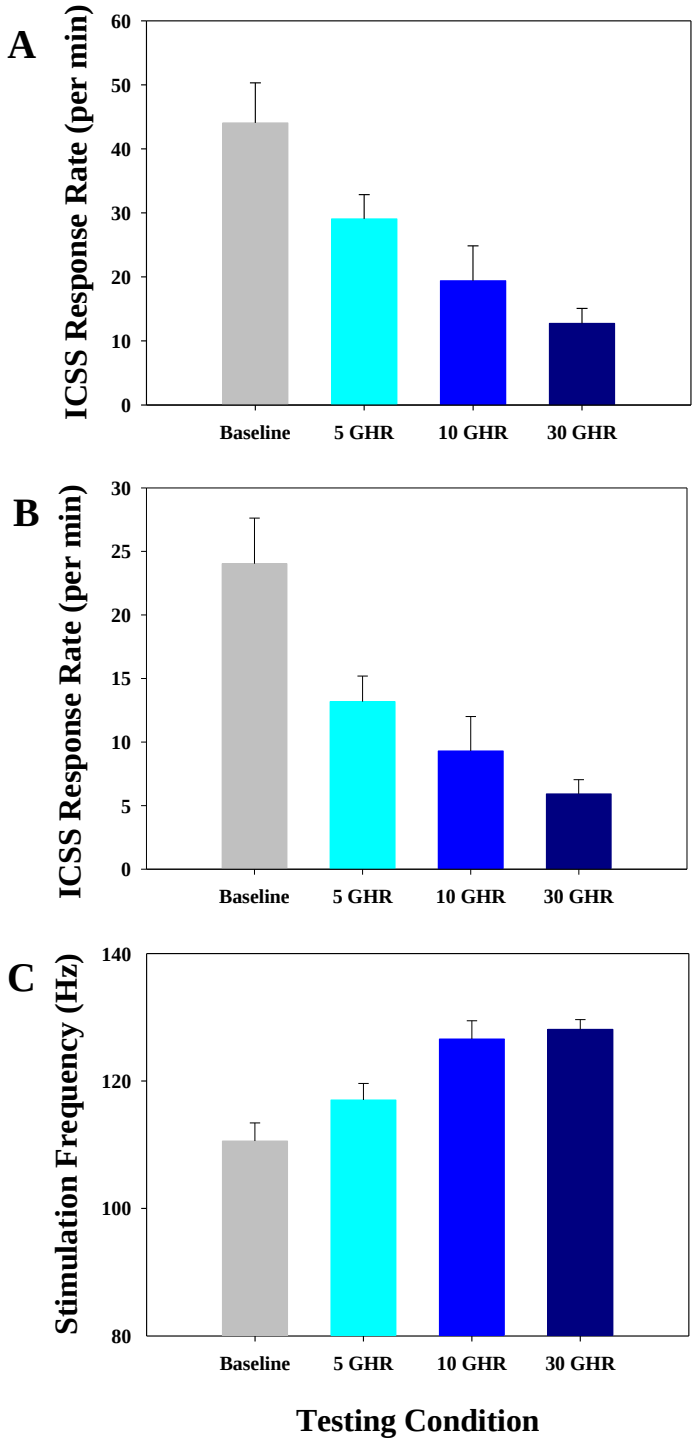

Fig. 4. Mean Group Changes in ICSS Responses after 0, 5.0, 10.0, or 30.0 nMol/rat GHR. Panel A: Maximal response rate for ICSS; Panel B: 50% response rate for ICSS; Panel C: Threshold for ICSS.

COC group analysis

Figure 5 depicts the rate-frequency curves for COC trials with 0, 2.5, and 5.0 mg/kg COC. After systemic injections of COC, the rate-frequency curves dose-dependently shifted to the left and the maximal response rates increased as COC dose increased.

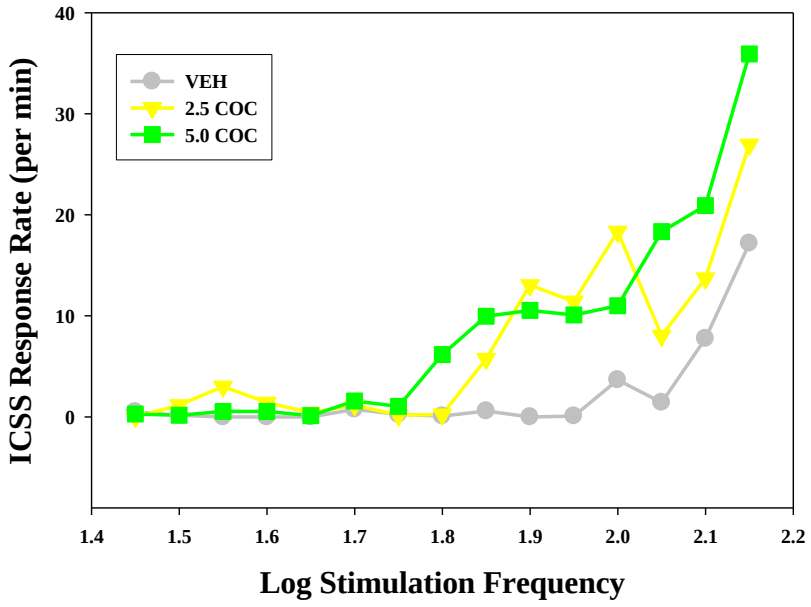

Fig. 5. Rate-frequency Curve for COC Trials. Mean group response rates as a function of stimulation frequency for rats. Rats treated 30 min prior to each test session with either 0.5 ml saline (VEH), 2.5, or 5.0 mg/kg COC. Group sizes are n=3.

Figure 6A-C depicts the mean group changes in ICSS responding after COC administration. Panel A depicts the mean maximal response rate, panel B depicts the mean 50% response rate, and panel C depicts the mean threshold for the COC testing condition. A one-way analysis of variance test confirmed these results were significant between groups for the maximal response rate (100% response rate) ($F(2,4) = 7.009$, $p<0.049$). Additional analysis with the Holm-Sidak method compared each COC dose value to the control condition and indicated a significant difference between the 5.0 mg/kg dose of COC and the control dose (0.5 ml saline), but no significant difference between the 2.5 mg/kg dose of COC and the control dose ($p<$at least 0.05). One-way analysis of variance test of the 50% response rate did not show a significant difference between groups in COC treatment with 0, 2.5, and 5.0 mg/kg doses ($F(2,4) = 3.295$, $p<0.143$). Finally, analysis of the threshold using a one-way analysis of variance test did not show a significant difference between groups ($F(2,4) = 0.356$, $p<0.721$).

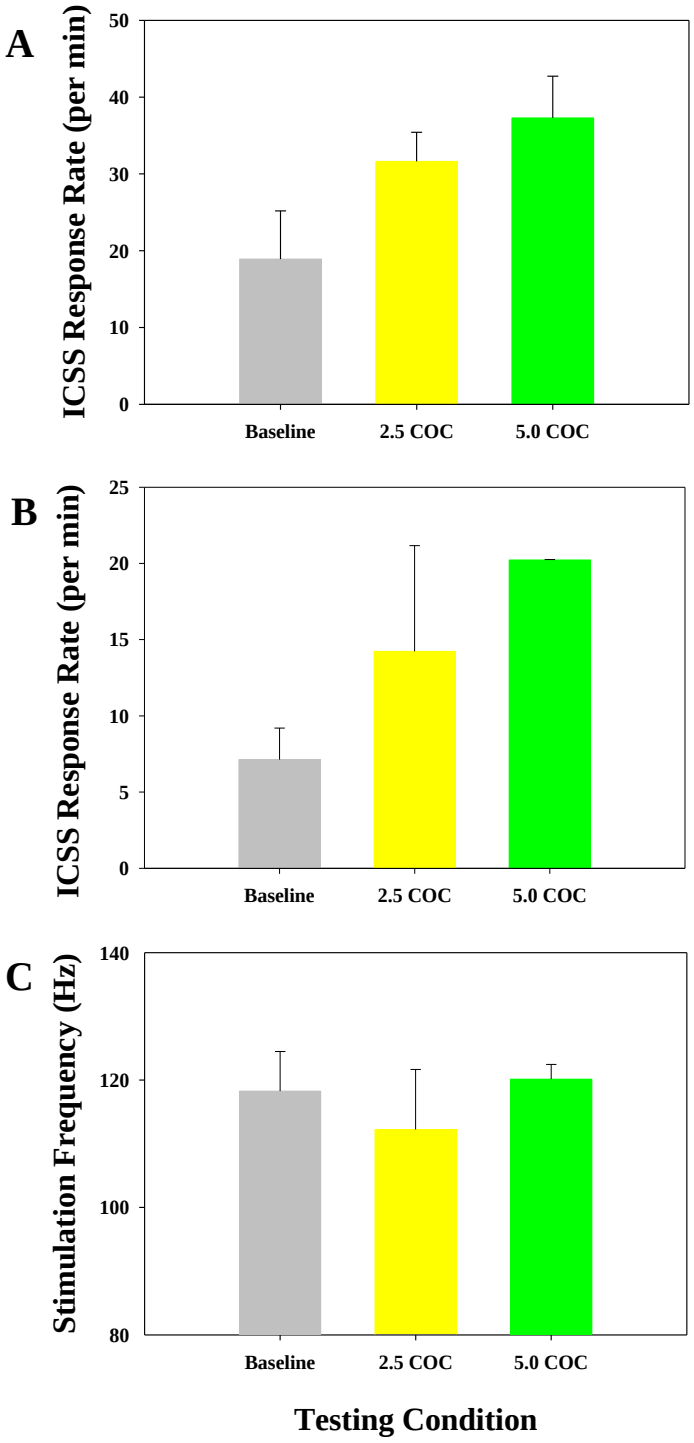

Fig. 6. Mean Group Changes in ICSS Responses after 0, 2.5, or 5.0 mg/kg COC. Panel A: Maximal response rate for ICSS; Panel B: 50% response rate for ICSS; Panel C: Threshold for ICSS.

Combination group analysis

Figure 7 depicts the rate-frequency curves for the combination GHR and COC trials with vehicle (0.5 ml saline), 10.0 nMol/rat GHR, and 2.5 mg/kg COC combined with 10.0 nMol/rat GHR. After systemic injections of the GHR and COC combination, the rate-

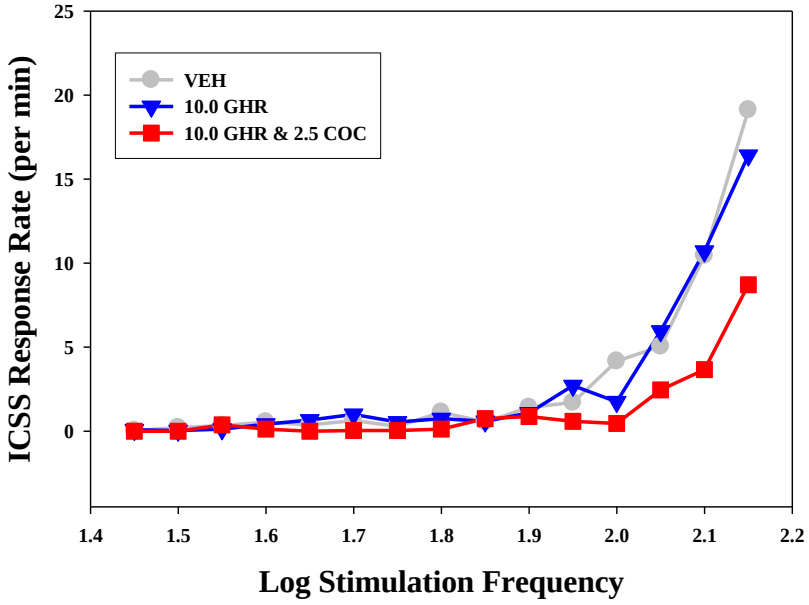

Fig. 7. Rate-frequency Curve for Combination Trials. Mean group response rates as a function of stimulation frequency for rats. Rats treated one hour prior to each test session with 10.0 nMol/rat GHR and 30 min prior to each test session with 2.5 mg/kg COC. Group sizes are n=3.

frequency curves shifted to the right and the maximal response rate decreased. Figure 8 A-C depicts the mean group changes in ICSS responding after administration of GHR and COC. Panel A depicts the mean maximal response rate, panel B depicts the mean 50% response rate, and panel C depicts the mean threshold for the combination testing condition. A one-way analysis of variance test confirmed these results were significant between groups for the threshold ($F(2,4) = 8.710$, $p<0.035$). Additional analysis with the Holm-Sidak method compared each dose value to the control condition and indicated a significant difference between the combination dose (2.5 mg/kg COC and 10.0 nMol/rat GHR) and the control dose (0.5 ml saline), but no significant difference between the 10.0 nMol/rat GHR dose and the control dose (p<at least 0.05). One-way analysis of variance test of the maximal response rate (100% response rate) did not show a significant difference between groups ($F(2,4) = 1.197$, $p<0.391$). Finally, analysis of the 50% response rate did not show a significant difference between groups ($F(2,4) = 0.670$, $p<0.561$).

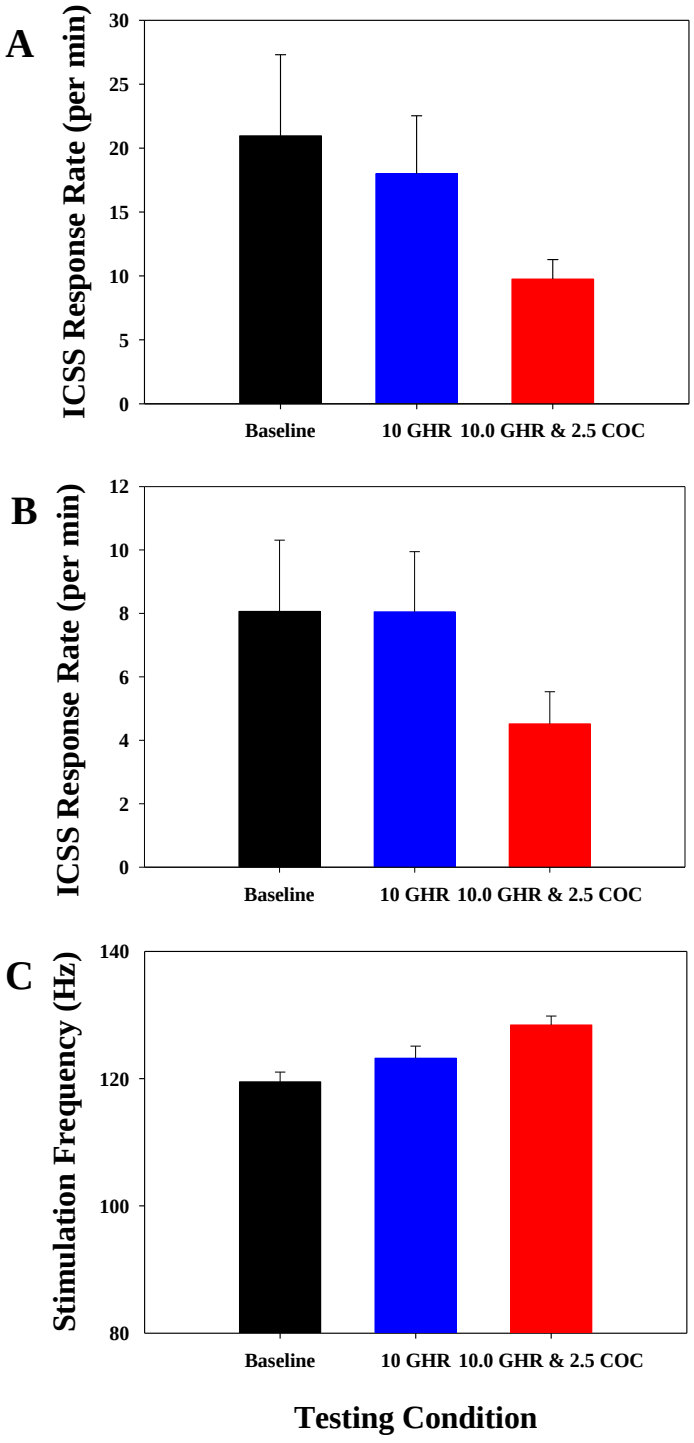

Fig. 8. Mean Group Changes in ICSS Responses after 0, 10.0 nMol/rat GHR, or 10.0 nMol/rat GHR and 2.5 mg/kg COC. Panel A: Maximal response rate for ICSS; Panel B: 50% response rate for ICSS; Panel C: Threshold for ICSS.

Across trials analysis

Figure 9 depicts the rate-frequency curves for the animal that was run in every trial of the current experiment (GHR, COC, and combination trials). After systemic injections of 10.0 nMol/rat GHR, the rate-frequency curves shifted to the right and the maximal response rates decreased. After systemic injections of the 2.5 mg/kg COC, the rate-frequency curves shifted to the left and the maximal response rates increased. After systemic injections of a combination of 2.5 mg/kg COC and 10.0 nMol/rat GHR, the rate-frequency curves shifted to the right and the maximal response rate decreased (both effects were greater than those seen from administration of GHR alone).

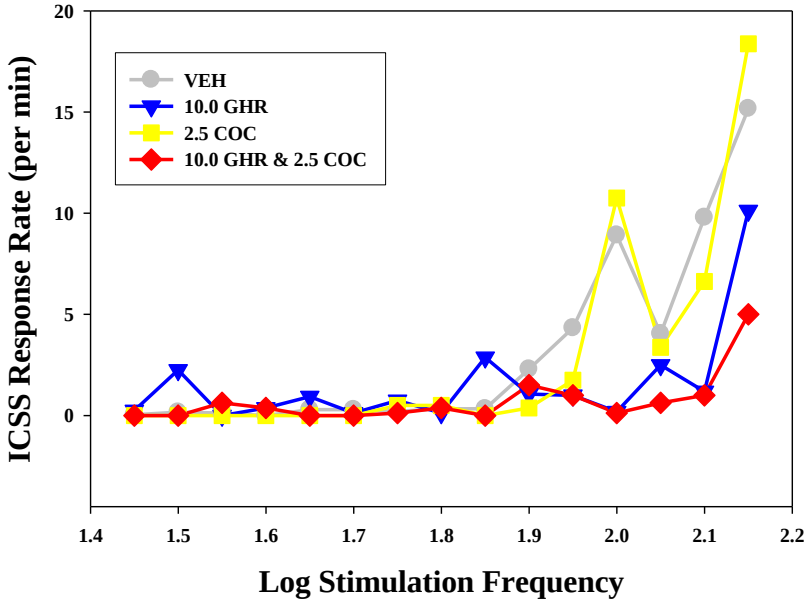

Fig. 9. Rate-frequency Curve for Animal Run on All Test Conditions. Mean response rates as a function of stimulation frequency for one rat. Rat was treated one hour prior to each test session with 10.0 nMol/rat GHR during GHR trials, 30 min prior to each test session with 2.5 mg/kg COC during COC trials, and one hour prior with 10.0 nMol/rat GHR and 30 min prior with 2.5 mg/kg COC prior to each test session during combination trials. Group size are n=1.

CHAPTER IV

SUMMARY AND CONCLUSIONS

Summary

The major findings of the present experiment are that systemic administration of 5.0, 10.0, and 30.0 nMol/rat GHR causes a significant, dose-dependent reduction in maximal response rate and 50% response rate, and an increase in threshold for ICSS. In contrast, administration of 2.5 and 5.0 mg/kg COC caused a significant increase in maximal response rate, and the trend in COC data for 50% response rate and threshold mimicked that of other experiments (increase in 50% response rate and reduction of threshold). Finally, the interaction of GHR and COC (10.0 nMol/rat GHR and 2.5 mg/kg COC) caused a significant increase in threshold. In other words, administration of GHR alone caused a decrease in reward-seeking behavior as measured through ICSS response, while COC alone caused an increase in reward-seeking behavior. When GHR was administered together with COC it seemed to inhibit the effects of COC on ICSS; in other words, there was a significantly smaller increase in reward-seeking behavior than that normally produced by the administration of COC alone. These findings suggest that GHR decreases reward-seeking behavior when administered alone but may also inhibit the effects of COC on reward-seeking behavior by affecting the reinforcement pathway in the brain.

These results support the hypotheses that GHR alone would inhibit ICSS responding, COC alone would augment ICSS responding, and that the combination of the two drugs would cause a smaller increase in ICSS responding than that produced by COC alone.

Conclusions

Previous experiments on the effects of GHR on reward-related behavior have found similar results to the present study. Davis et al. (2007) reported that administration of GHR facilitated the effects of COC on conditioned place preference for low COC doses (0.312 and 0.625 mg/kg) but not for higher COC doses (1.25 mg/kg). The COC doses used in the Davis et al. study were not sufficient to activate the VTA, and therefore to cause more DA in the NA; however, when paired with a relatively small dose of GHR (5.0 nMol/rat), Davis et al. reported a significant conditioned place-preference effect. On the other hand, the combination of 5.0 nMol/rat GHR with a slightly larger dose of COC (1.25 mg/kg) did not facilitate conditioned place-preference. Therefore, we can assume that low doses of COC may not fully activate the reward system in the brain.

Additionally, unpublished data on the effects of GHR and COC on ICSS measured using a progressive ratio method (fig. 10) showed identical results to those found in the present study (Wellman; personal communication, 2010). In the progressive ratio method of ICSS, rats are required to exert increasingly greater effort to earn a burst of ICSS stimulation; the breakpoint is defined as the point at which the rat stops responding. In this figure, data represent the percent change of breakpoint for post- relative to pre-test

conditions; thus, for example, 75% would indicate an increase in effort exerted to receive reinforcement whereas -75% would indicate a decrease in effort exerted. The data compiled in the progressive ratio study showed a dose-dependent increase following COC administration, a reduction following GHR, and a smaller increase after the combination of COC and GHR than when COC was administered alone.

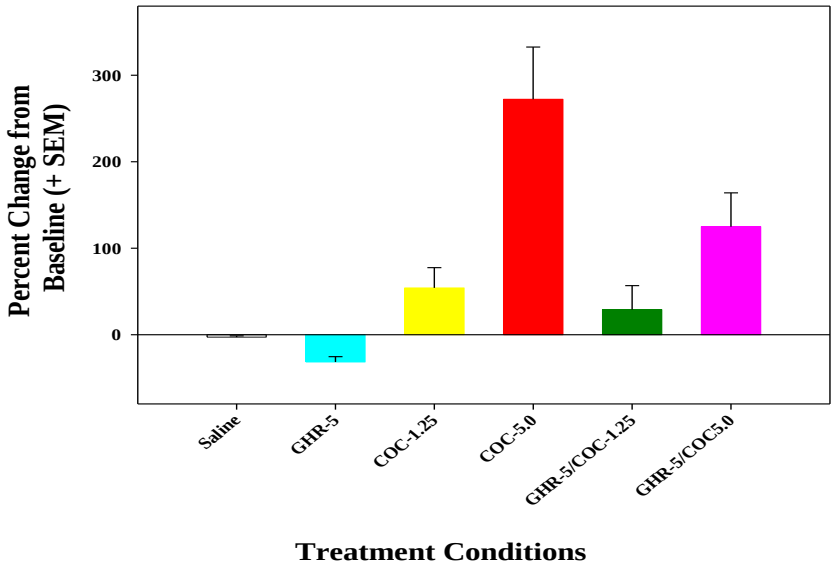

Fig. 10. Percent Change from Baseline (+SEM) for Progressive Ratio ICSS. Percent change was measured as the difference in breakpoint for pre- versus post-test conditions. Rats were treated with either vehicle (0.5 ml saline), GHR (5.0 nMol/rat), COC (1.25 or 5.0 mg/kg), or a combination of GHR and COC (5.0 nMol/rat GHR and 1.25 mg/kg COC or 5.0 nMol/rat GHR and 5.0 mg/kg COC).

GHR receptors and DA receptors are found in many common areas in the reward pathway in the brain such as the VTA and NA in the mesolimbic pathway (Olszewski et al., 2008; Naleid et al., 2005). Additionally, GHR receptors have been located on brain DA neurons (Davis et al., 2007). The connection between DA and GHR suggests that both may play a role separately, or may interact, to affect the reward pathway in the brain. One of the clearest connections between GHR and DA exists in the mesolimbic system between the VTA and the NA (when the VTA is activated, it causes a greater release of DA in the NA). One possible theory to explain the effects of GHR and COC on reward-seeking behavior suggests that administration of GHR partially activates the VTA in the brain. Therefore administration of COC doses (which on their own are insufficient to activate the VTA), when combined with GHR, become sufficient to activate this area and to allow the release of more DA in the NA which thus facilitates conditioned place preference. However, this does not fully explain the results of the present study or the progressive ratio ICSS study (e.g. why does administration of GHR alone cause a decrease in ICSS if it is partially activating the VTA).

Another theory to explain these results involves the dopamine transporter (DAT) which is responsible for the internalization of the neurotransmitter DA. High levels of DAT cause smaller levels of DA in the synapse, whereas low levels of DAT cause higher levels of DA in the synapse. COC cannot increase reinforcement without the presence of DAT because COC acts by blocking DAT (which increases DA levels). A larger amount of DAT in the brain means that there is more DAT for COC to block; and,

therefore, the COC will have a larger reinforcing effect. In other words, larger amounts of DAT lead to greater effects of COC on reinforcement. Unpublished data by Abizaid (2010) reports that mice with a GHR knockout gene (such mice retain the GHR receptor but lack plasma GHR) show an increase in DAT in the NA and lower levels of DA in the NA. This data suggest that administration of GHR alone may cause a decrease of DAT in the NA, which in turn causes an increase in the levels of synaptic DA in the NA (longer-lasting DA in the synapse). At present, we are unsure how this effect of GHR when administered alone causes a decreased in reward-seeking behavior in ICSS. It is possible that other neural systems (e.g. interaction of the medial prefrontal cortex with the VTA and NA) are also affected by the administration of GHR and are responsible for portions or all of the observed effect.

The interaction of GHR and COC is interesting because administration of GHR seemed to inhibit the reinforcing effects of COC. It is possible that GHR inhibits the reinforcing effects of COC by inhibiting DAT in the mesolimbic pathway (causing down-regulation of DAT in the NA) which decreases the number of DAT available to be blocked by COC which in turn causes a smaller reinforcing effect of COC (and also increases synaptic DA levels). Supporting this theory, Thomsen et al. (2009) reported that DAT knockout mice failed to show reliable COC-induced SA which suggests that DAT is critical in mediating the reinforcing effects of COC.

The manipulation that GHR demonstrates on the effects of COC on reinforcement indicates the possibility of its use in treatment of COC addicts. A preliminary study in our lab suggests that GHR reduces operant responding for COC. Therefore, similar to the use of naloxone or naltrexone to treat opioid addiction, the ability of GHR to inhibit the effects of COC could be utilized during COC addiction treatment by administration of a GHR agonist.

Future research

In future studies, we plan to examine the effects of a GHR antagonist on ICSS responding and other paradigms used to measure reward-seeking behavior. Additionally, we plan to examine the effects of GHR and COC administration in GHR receptor knockout rats in ICSS, SA, and locomotion studies. Results consistent with those found in previous literature would bolster the claim that GHR could be used in treatment of psychostimulant addiction. Additional future research could examine the differences between the effects of GHR administration on acute versus chronic psychostimulant administration (both acquisition of SA and sensitization to the drug).

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