Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2010 - 12/31/2013

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814 935 1081

5. **Grant SAP Number:** 4100050904

6. **Project Number and Title of Research Project:** 44. Determining the Neurochemical Profile of Addiction in Near Real Time

7. **Start and End Date of Research Project:** 7/20/2012 - 12/31/2013

8. **Name of Principal Investigator for the Research Project:** Patricia Sue Grigson, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $ 76,500

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project	Cost
Freet, Christopher S.	Postdoctoral Fellow	40% 8/12-10/13	$20,200
Freet, Christopher S.	Postdoctoral Fellow	100% 11/13, 12/13	$7,288
Horvath, Nelli	Postdoctoral Fellow	50% 11/13	$1,523.5

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name, First Name	Position Title	% of Effort on Project
Grigson, Sue	PI	< 2 %

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
None		

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No___X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No____X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the application was submitted (column C), and the amount of funds requested (column D). If

you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____X____ No_____

If yes, please describe your plans:

I will apply for an R01 to NIDA in June 2014.

12. Future of Research Project. What are the future plans for this research project?

Having acquired this technique, we are now in a position to measure up to 17 neurochemicals at the same time from a given brain region during complex motivated behavior. This is very exciting and provides all manner of opportunities for future research. The first goal will be to obtain an R01 from NIDA to study the neurochemical mechanisms mediating drug-induced devaluation of an otherwise acceptable natural reward. When combined with a lesion assessment, this will allow for a full circuit analysis of the phenomenon and will, essentially, reinstate this line of research. We will know how these neurochemicals change when anticipating drug and when taking drug and we will know how they differ from one individual to the next. Thus, we will be able to determine the neurochemical profile in the nucleus accumbens and/or prefrontal cortex, for example, in those who are vulnerable to drug addiction and in those who are resistant. We will be able to see how it changes when exposed to conditions that promote drug-taking (e.g., sleep deprivation) or conditions that enhance resilience (e.g., exposure to environmental enrichment). Indeed, the sky is the limit. This is fascinating work that could not have been accomplished without the CURE funds.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes_____X____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				1
Female				1
Unknown				
Total				**2**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				2
Unknown				
Total				**2**

	Undergraduate	Masters	Pre-doc	Post-doc
White				2
Black				
Asian				
Other				
Unknown				
Total				**2**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes____X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

My having had funds to learn this new technique already has had a positive impact on the work of two colleagues, as well as myself. Thus, the laboratories of Drs. T. Subramanian and A. Hajnal also have been working with the Core Facility to collect such neurochemical data in the study of obesity and Parkinson's Disease. This, too, will give them a great edge in this competitive funding arena.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No____X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient

to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

We view addiction as a two-part problem. First, while natural rewards can protect against drug addiction under some circumstances, a prominent symptom of the disease is the devaluation of very important natural rewards. Second, addiction is a disease of chronic relapse where the drug, stress, or drug-associated cues can repeatedly initiate drug-seeking and drug-taking, even following very prolonged periods of abstinence**.** Here, we describe a paradigm that models both aspects of the disease and, as such, can be used to begin to identify the associated underlying neural substrates. Specifically, in our paradigm, rats avoid intake of a taste cue that predicts the availability of a drug such as morphine or cocaine. We have found that avoidance of the taste cue is associated with an elevation in levels of the stress hormone, corticosterone, a blunting of the reward transmitter, dopamine, in the nucleus accumbens, and the onset of frank aversive taste reactivity behaviors (i.e., gapes) when the taste cue is infused directly into the oral cavity. Cue-induced anticipation of drug availability (i.e., waiting for drug), then, is highly aversive. Importantly, greater aversiveness on these measures is associated with a shorter latency to take drug, greater load up on drug at the start of the session, and faster acquisition of the drug self-administration behavior.

Specific Aim 1 of this project will test whether a non-gustatory cue (i.e., an audiovisual cue) also can elicit the onset of the conditioned aversive state and whether the magnitude of this effect will predict drug-seeking and drug-taking. Until now, this effect has only been demonstrated with a gustatory cue and (with the exception of alcohol) audiovisual cues play a greater role than gustatory cues in relapse to drug-seeking in addicted humans. Therefore, it is important to demonstrate that the conditioned aversive state occurs in anticipation of drug delivery, regardless of the nature of the drug-paired cue. *Specific Aim 2* will test whether onset of the conditioned aversive state, and subsequent cocaine-seeking behavior, can be blocked by the infusion of a 5-HT1A agonist, 8-OH-DPAT, into the pariacqueductal gray (PAG). Finally, *Specific Aim 3* will adopt a new technique that will allow for minute by minute assessment of all major

neurotransmitters (e.g., dopamine, norepinephrine, serotonin, GABA, glutamate, acetylcholine) in the nucleus accumbens while the subject avoids the taste cue and then seeks and takes drug. This is a very powerful, cutting edge technique that will revolutionize our understanding of the neurochemical basis of cue-induced craving, withdrawal, and relapse.

Specific Aim 1 tests whether a non-gustatory cue (i.e., an audiovisual cue) also can elicit the onset of the conditioned aversive state and whether the magnitude of this effect will predict drug-seeking and drug-taking. *Hypothesis.* We predict that the audiovisual cue will elicit the onset of the learned aversive state and greater aversiveness will predict greater drug-seeking and taking.

Given the planned use of a non-gustatory cue, we proposed to use the measurement of ultrasonic vocalizations (USVs) to test whether the non-gustatory cue, like the gustatory cue, will elicit the onset of an aversive state when presented prior to drug access. To this end, we first needed to set up the system to measure USVs and then to verify that we could, in fact, measure both negative (i.e., 20kHz) and positive (i.e., 50kHz) calls. Figure 1 shows a number of positive calls (i.e., 50KHz) emitted by a rat during a session of play (tickling) in our laboratory with a familiar experimenter. Figure 2 shows aversive (i.e., approximately 20KHz) calls emitted while the rat was isolated in the chamber in our laboratory. These data demonstrate that our system has been appropriately designed to measure appetitive and aversive calls. This was, in fact, not simple and required a meeting of Dr. Grigson and her student Elizabeth Colechio with an expert, Dr. Jeffrey Burgdorf, at Northwestern, many follow up emails and phone calls, and also an in-house consultation with representatives from Med Associates, the company that supplies the hardware and software for USV detection in rodents.

Having established this methodology, we conducted a first experiment to (a) perfect our technique and (b) determine whether the USVs would change in a predictable manner during standard taste-drug pairings. Specifically, in two replications, we examined how USVs change with consumption of a palatable saccharin cue that predicts experimenter delivered morphine, cocaine, or LiCl. In this study, 80 (n = 32 for Replication 1 and n = 48 for Replication 2) naive water restricted (5 min a.m., 20 mls overnight) rats were placed in operant chambers and given 5 min to lick a palatable 0.15% saccharin solution. After a 5 min interstimulus interval, the rats were injected intraperitoneally (IP) or subcutaneously (SC) with saline or drug (see below). The rats were then placed back into the chambers for an additional 5 minutes before returning to their home cage. They received 20 ml overnight access to water. There was one such taste-drug pairing/trial for a total of 6 daily trials, and each occurred at 48 h intervals. On trial 1 and 2 rats were injected IP or SC with saline (n=16), SC with 10 mg/kg cocaine (n=24), IP with 15 mg/kg morphine (n=24), or IP with .009M LiCl (n=16). On trial 3 and 4 the LiCl dose was increased to 0.075 M LiCl. On trial 5 and 6, drug doses were increased to 20 mg/kg cocaine, 30 mg/kg morphine, or 0.15 M LiCl. USVs were summed across the 5 min saccharin access period, the 5 min wait before injection, and the 5 min period after injection. Here we show the behavioral data from both Replication 1 and 2, but the USV data from only Replication 1 (The data from Replication 2 are still being scored). *Hypothesis.* Because rats emit aversive orofacial responses (i.e., gapes) to a gustatory cue when paired with a drug of abuse, such as cocaine, we hypothesized that aversive USVs also would be emitted following presentation of the cocaine- and morphine-paired saccharin cue.

Results and Discussion. The results shown in Figure 3 indicate that our hypothesis was not correct. Aversive USVs were emitted in response to the LiCl-paired saccharin cue, but not to the morphine- or cocaine-paired saccharin cue. Figure 3 shows data for rats in the saccharin-saline, saccharin-morphine, saccharin-cocaine, and saccharin-LiCl conditions on the first and last trial: Trial 1 and Trial 6. *Top panel.* The results show that while rats in the saccharin-saline condition increased intake of the saccharin cue from Trial 1 to Trial 6, other rats suppressed intake of the saccharin cue when paired with morphine, cocaine, or LiCl, ps < .05. *Second panel.* In addition to drinking less of the saccharin cue, rats in the cocaine and LiCl conditions also exhibited a longer latency to lick the drug-paired saccharin cue on Trial 6 ps < .05. As shown in *Panel 3*, ingestion of the saccharin cue was associated with emission of positive 50 kHz vocalizations and the number of appetitive calls increased from Trial 1 – Trial 6 for rats in the saccharin-morphine condition, p < .05. There was a tendency for an increase in aversive calls when saccharin was paired with the aversive illness-inducing agent, LiCl, but this trend did not attain statistical significance, p > .05 *(see Bottom Panel).* Interestingly, there were few if any aversive calls emitted to the saccharin cue when paired with morphine or cocaine. The failure to find aversive calls when exposed to the cocaine- or morphine-paired cue may have been due to the fact that we used a shorter access period to the saccharin cue (i.e., 5 min rather than 30 min). As discussed in Specific Aim 2, rats readily emit aversive orofacial responses (i.e., gapes) when a cocaine-paired cue is intraorally infused once/min over a 30 min period. Alternatively, the data may suggest that USVs provide information that differs from that obtained via the measurement of taste reactivity. Although additional study is required to make this determination, the overall effort for Specific Aim 1 has been highly successful as we now can reliably detect both appetitive and aversive calls. We have determined that rats appear to make aversive calls to a gustatory cue that has been paired with the illness-inducing agent, LiCl, but not to the same gustatory cue if paired with a drug of abuse such as morphine or cocaine. Once the analysis is complete, the data will be written up for publication. Finally, we now have a reliable measure (USVs) that will allow us to propose the use of audio/visual cues in our future NIH application.

Specific Aim 2 will test whether onset of the conditioned aversive state, and subsequent cocaine-seeking behavior, can be blocked by the infusion of a 5-HT1A agonist, 8-OH-DPAT, into the pariacqueductal gray (PAG). *Hypothesis.* We predict that pretreatment with 8-OH-DPAT will block the onset of the conditioned aversive response and subsequent seeking of drug.

First, for proof of principle, we sought to test whether pretreatment with 8-OH-DPAT could reduce aversive taste reactivity behavior (i.e., gapes) to the cocaine-paired saccharin cue if the 8-OH-DPAT were administered peripherally. Twelve Sprague-Dawley rats were anesthetized with a mixture of ketamine/Xylazine and implanted with a jugular catheter and two intraoral cannulae. Once recovered (about 20 days), the rats were placed on a water deprivation schedule as described above. During testing, they were intraorally infused with a grape Kool-Aid flavored saccharin solution once/min for 30 min. Immediately thereafter, the rats were given 2 h to lick an empty spout operant on a Fixed Ratio 10 to self-administer cocaine (0.33 mg/infusion) or saline. There was one such pairing a day for 16 trials (data not shown). The 8-OH-DPAT phase of the experiment was conducted from self-administration (SA) trials 17 – 27. Using a within subjects design, rats were pretreated with increasing doses of 8-OH-DPAT SC (.01, .06, .1, .25, and .5 mg/kg) or saline (.9%, 1 ml/kg) on alternating days. Injections were given 30' before rats were placed into the test chambers.

Results and Discussion. Figure 4 shows the mean number of gapes emitted by rats in the saccharin-saline condition and by Low and High Drug-Takers in the saccharin-cocaine condition pretreated with vehicle (VEH) or increasing doses of 8-OH-DPAT. As is evident, High Drug-Takers, but not Low Drug-Takers or saline treated rats, emitted aversive taste reactivity behavior (i.e., gapes) during the 30 min intraoral infusions of the cocaine-paired saccharin cue. Moreover, as hypothesized, this aversive taste reactivity behavior was nearly fully prevented in these High Drug-Takers following pretreatment with the serotonin 5HT 1a agonist, 8-OH-DPAT. Although pretreatment with 8-OH-DPAT blocked the aversive taste reactivity behavior to the drug-paired saccharin cue, particularly for the High Drug-Takers, it only tended to reduce subsequent cocaine self-administration behavior in these subjects. Thus, as shown in Figure 5, pretreatment with increasing doses of 8-OH-DPAT served to reduce, but not to eliminate, cocaine self-administration behavior in the High Drug-Takers. The downward trend, while not significant, may suggest the need for additional trials with a steady dose of 8-OH-DPAT pretreatment or, importantly, direct administration into the PAG. Follow up study is required to test the merits of these hypotheses.

Specific Aim 3 adopts a new technique that will allow for a minute by minute simultaneous assessment of all major neurotransmitters (e.g., dopamine, norepinephrine, serotonin, GABA, glutamate, acetylcholine) in the nucleus accumbens while the subject avoids the taste cue and then seeks and takes drug. *Hypothesis.* We predict that a key role for dopamine, GABA, glutamate, and Ach will be revealed.

At the start of this Bridge grant, Dr. Grigson, along with then student Jennifer Nyland and postdoctoral fellow, Dr. Christopher Freet, flew to the University of Michigan to visit Dr. Bob Kennedy. Dr. Kennedy is the scientist who developed the microdialysis/Mass Spec technique that allows for the measurement of multiple neurotransmitters in a single sample of perfusate from brain. This technique offers a great advantage over standard microdialysis because it allows for the measurement of not only one (as is common), but many neurotransmitters at a given time. In this way, it is possible to begin to examine a full functional circuit. We then brought this information back to our Core Facility. After some troubleshooting and correspondence between our faculty and the Kennedy laboratory, the Core Facility has now demonstrated that they can measure these analytes at concentrations that are within physiological range. See Figure 6 for a composite of the chromatograms from the standards of 17 analytes.

While it is the case that this technique can be used to assess changes in brain chemicals from minute to minute, and this ultimately will be highly advantageous, we chose to here begin with a more standard 15 min sample period to increase the volume of our perfusate and, thus, our chances for successful measurement using this new and sensitive technique. Here, we examined the profile of the neurochemical response in the nucleus accumbens to a saline and a drug-paired gustatory cue. In order to reduce the impact of between subject variability, we used a within subjects design where one flavor of saccharin (referred to as the CS-) predicted the opportunity to self-administer saline on one day and another flavor of saccharin (referred to as the CS+) predicted the opportunity to self-administer cocaine. During the test, perfusate was sampled at baseline, during the intraoral infusion of the CS-, during the intraoral infusion of the CS+, and for several minutes thereafter (referred to as Post). As such, it was possible for the first time to link the full pattern of the neurochemical profile to drug-induced devaluation of the Kool-Aid

flavored saccharin cue and cue-induced craving and/or anticipation of drug availability in sensitive and resilient rats.

Subjects. The subjects were 8 male Sprague-Dawley rats obtained from Charles River. Rats weighed between 293-339 g on the day of surgery. They were housed individually in standard, metal cages in a temperature-controlled (21 °C) animal care facility with a 12:12 hour light:dark cycle (lights on at 7:00 am). All experimental manipulations were conducted during the light phase of the cycle. The rats were maintained with free access to dry rodent chow (Harlan 2018) at the beginning of the experiment.

Surgery. Microdialysis Guide Cannulae. Implants were performed under ketamine/xyalzine anesthesia/analgesia. Each rat was implanted with a guide cannula (CMA/Microdialysis AB, Stockholm, REF 8309025) aimed at the right nucleus accumbens. Craniotomies were made at the following coordinates (relative to bregma) in a level-skull preparation: 1.2 mm lateral, 1.2 mm rostral. The guide cannula was inserted to a depth of 5 mm ventral to the skull surface and secured in a dental acrylic headcap for the duration of the experiment. *Self-administration catheter*. The self-administration catheter was prepared and implanted as described previously by Twining et al. (2009), as were the intraoral cannulae (see Colechio, Imperio, and Grigson, 2014). Each rat received 300000 units of GPenicillin (sq) at the beginning of the procedure, and 10 mL saline (sq) when it was complete. Rats recovered for 13-14 days before habituation began.

Apparatus. Each rat was trained in one of 4 identical operant chambers (MED Associates, St. Albans, VT) described previously (see Colechio et al., 2014). Gustatory stimuli were delivered into the oral cavity using a computer controlled syringe pump system and orofacial responses were recorded using video monitoring (these data have not yet been scored). Operant responses for the IV infusion of saline or drug were made by contacting, via licks or nosepokes, an empty spout operant and completing a fixed ratio requirement. Thereafter, a computer controlled syringe pump delivered IV drug or saline. Events in the chamber and collection of data were controlled on-line with a Pentium computer that used programs written in the Medstate notation language (MED Associates).

Procedure. Once having recovered from surgery, rats were handled, fluid deprived (5 min a.m./20 ml access to fluid p.m.), and then habituated to the operant chambers for 5 min/day for 9 days. Water was available during this time. Using a within subjects design, the fluid deprived rats then received one intraoral (IO) infusion per minute for 30 min of one of two Kool-Aid flavored saccharin solutions. On day 1, the IO infusion of the CS+ flavor (e.g., orange) was followed by 2 h to self-administer cocaine (0.33 mg/infusion). On day 2, the IO infusion of the CS- flavor (e.g., grape) was followed by 2 h to self-administer saline. There were 5 such CS+ and 4 such CS- trials, occurring on alternating days, followed by one microdialysis test day.

Microdialysis Test Day. On the day of testing, each rat was briefly anesthetized with .1 mL ketamine (i/m). The guide cannula was removed and replaced with a probe (CMA/Microdialysis AB, Stockholm, PAES membrane, 2 mm membrane length, .5 mm membrane OD, 20 kDa cut-off, 14 mm shaft length, REF 8010432). Each rat equilibrated for 3h at 1 μl/min. Three 15 min baseline samples (samples 1-3) were then collected. One 15 min sample each was collected during the CS- (sample 4), ISI (sample 5, “inter”), and CS+ (sample 6) periods, followed by 5

post-injection samples (7-11). Ten μl from each sample were immediately pipetted from the collection tube into a vial and mixed with 5 μl each borate buffer and derivitizing agent before being placed onto dry ice. Samples were stored at -80 C and then analyzed using HPLC and Mass Spec. Rats were later sacrificed and the location of the guide cannulae in the nucleus accumbens verified.

Results and Discussion. Figure 7 shows data comparing neurochemical levels in the nucleus accumbens of one small drug-taker and one large drug-taker during the 15 min CS- period and averaged across the 15 min post periods (referred to as Post) following exposure to the CS+. The Post period was used as the best indication of the impact of CS+ delivery because, of all of the rats tested, and all the data points collected, the single CS+ sample was accidently lost for the rat with the history of the most drug-taking (and the highest gaping behavior to the CS+). We believe that the Post period is a reasonable indicator of the response to the IO delivery of the CS+. First and foremost, the data shown in Figure 7 reveal that, while Dopamine levels are low for both the low and the high drug-taker following the IO infusion of the CS-, infusion of the CS+ leads to a marked increase in dopamine, but only in the rat with a history of high drug-taking. This elevation in training (i.e., following just 5 pairings) was surprising. Later in testing, using very similar methodology, we know that the direction of this response is reversed, at least when assessed using voltammetry (i.e., dopamine levels are low following the IO infusion of the CS+ in the highest of drug-takers). It will, then, be critical to determine how and when the transition from reward (high dopamine) to aversion (low dopamine) occurs – as least as indicated by brain neurochemistry. Also noteworthy is the finding that GABA levels are increased in the high drug-taking rat, while acetylcholine levels and aspartate levels are reduced. Figure 8 shows the data for dopamine, again, in comparison to glycine, serine, taurine, histamine, and the serotonin precursor, 5-HIAA. During the Post session, the high drug-taker has higher levels of histamine, taurine, and 5-HIAA than does the rat with a history of low drug-taking. Finally, Figure 9 shows dopamine, again, and its metabolites. Observation reveals that the increase in dopamine during the Post period (i.e., following the IO infusion of the CS+) for the rat with a history of high drug-taking may be accompanied by an increase in both 3-MT and HVA. Although a full assessment is obviously required across a number of subjects, these data are very exciting and they are the first of their kind. With this expertise, we will be highly competitive in the current NIH climate and, importantly, we will be in position to uncover novel information about the functioning of the brain during complex motivated behavior.

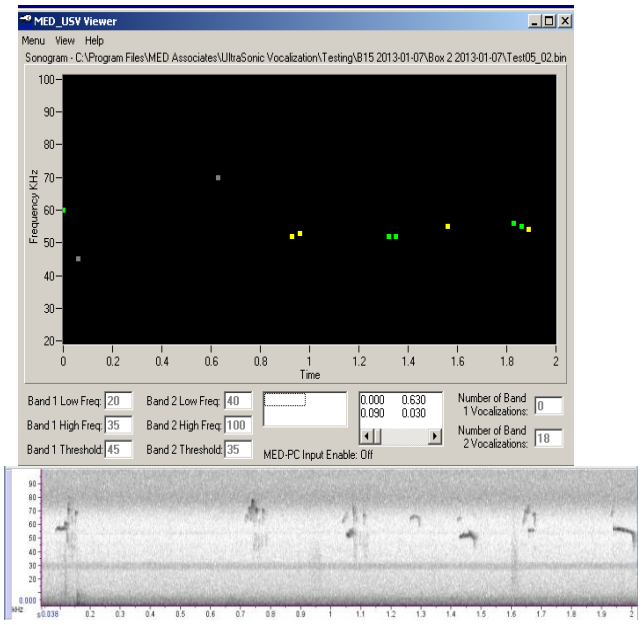


Figure 1. Call frequency (KHz) over time (sec). Shown here are a number of positive calls (i.e., those in the 50KHz range) emitted by a rat during a session of play (tickling) with the experimenter.

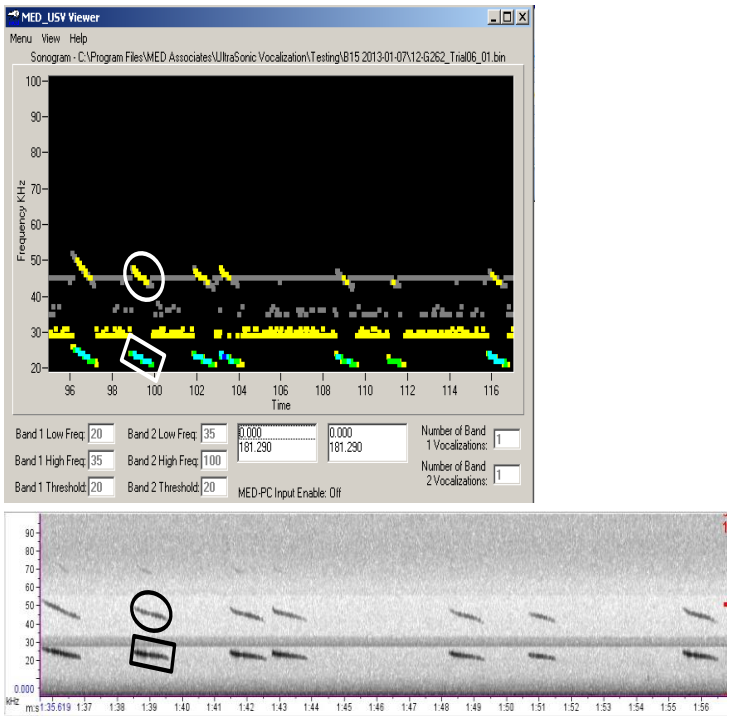


Figure 2. Call frequency (KHz) over time (sec). Aversive calls in the 20KHz range are indicated by the white rectangle. Yellow calls are more quiet and green calls more loud. As shown, this rat made a number of aversive low frequency calls when isolated from the experimenter. The white circle demarks a set of higher frequency harmonics. With a different program these harmonics of the aversive call might be mistakenly identified as an appetitive call. This no longer is the case.

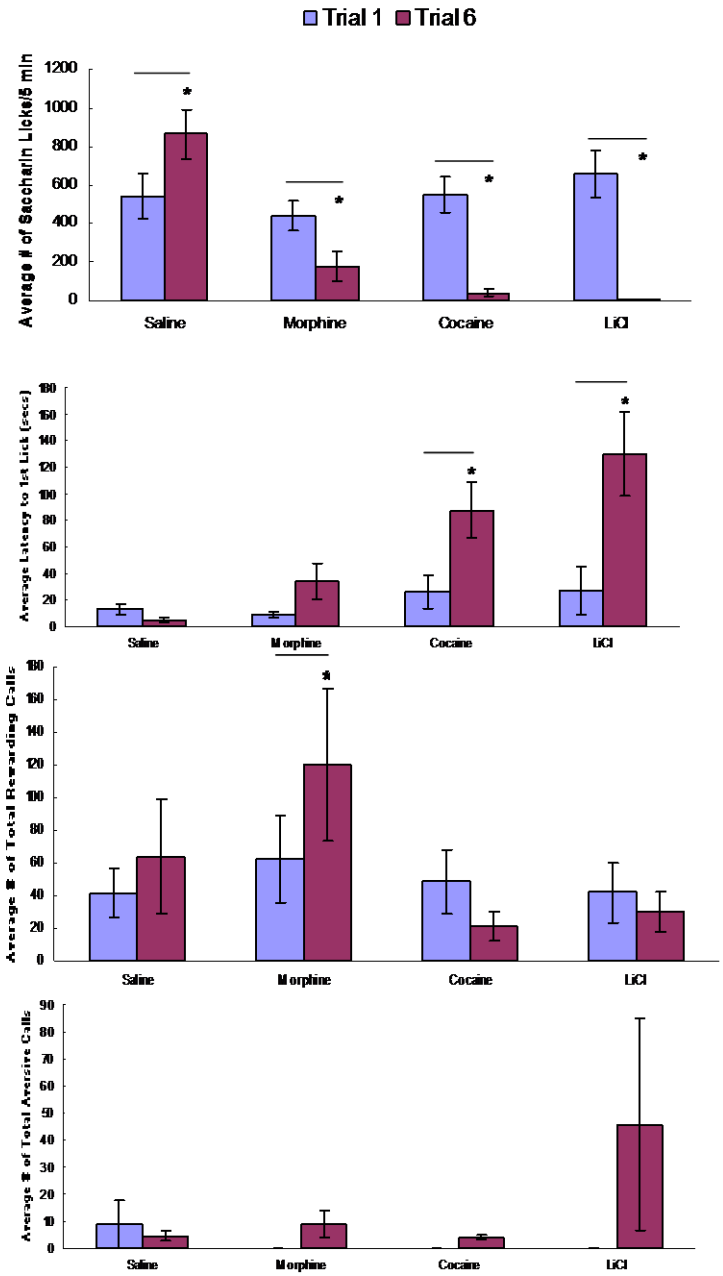


Figure 3. Top panel. Mean number of licks of 0.15% saccharin/5 min on Trial 1 and Trial 6 for rats in the saccharin-saline, saccharin-morphine, saccharin-cocaine, and saccharin-LiCl condition. Panel 2. Mean latency (s) to lick the saccharin cue on Trial 1 and Trial 6 for rats in the saccharin-saline, saccharin-morphine, saccharin-cocaine, and saccharin-LiCl condition. Panel 3. Total number of appetitive calls (50kHz) on Trial 1 and Trial 6 for rats in the saccharin-saline, saccharin-morphine, saccharin-cocaine, and saccharin-LiCl condition. Bottom panel. Total number of aversive calls (20kHz) on Trial 1 and Trial 6 for rats in the saccharin-saline, saccharin-morphine, saccharin-cocaine, and saccharin-LiCl condition. Appetitive and aversive calls were summed across the 5 min access period, the 5 min interval, and the 5 min period following injection of saline or drug.

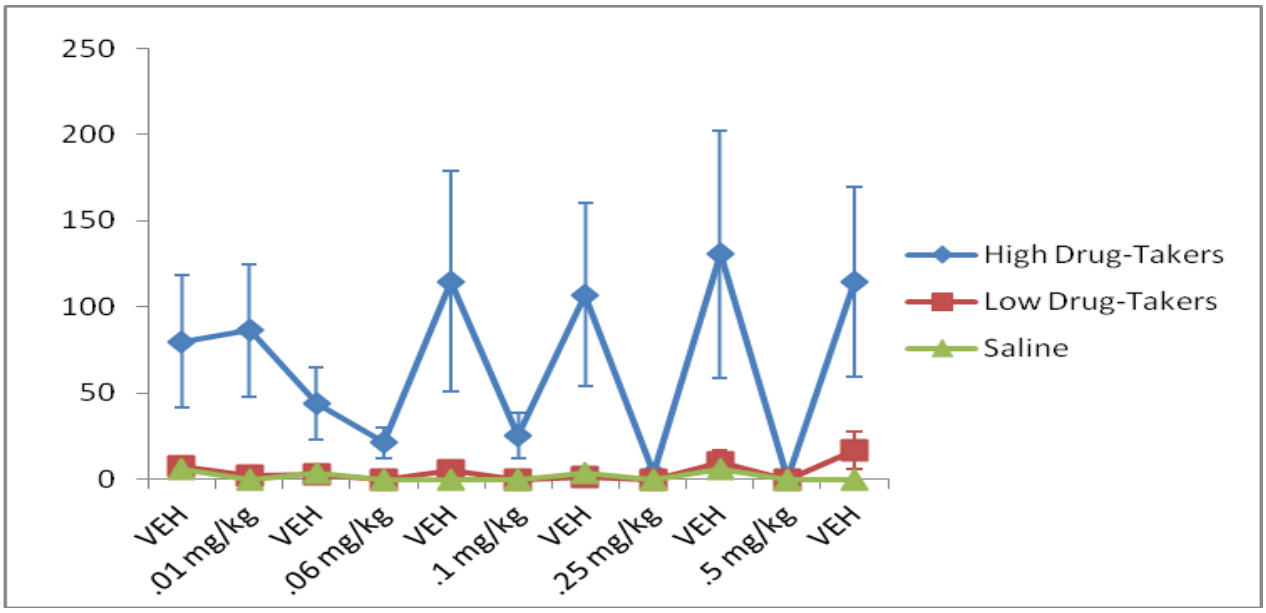


Figure 4. Mean number of gapes/5 min emitted by saccharin-saline rats, saccharin-cocaine Low Drug-Takers, and saccharin-cocaine High Drug-Takers as a function of pretreatment with vehicle (VEH) or increasing doses of 8-OH-DPAT across trials. Pretreatment with the serotonin 5HT 1a agonist, 8-OH-DPAT, nearly eliminated the gaping behavior that typically followed intraoral infusion of the drug-paired taste cue in High Drug-Takers. Rats in the saccharin-saline condition and Low Drug-Takers did not gape, with or without 8-OH-DPAT pretreatment.

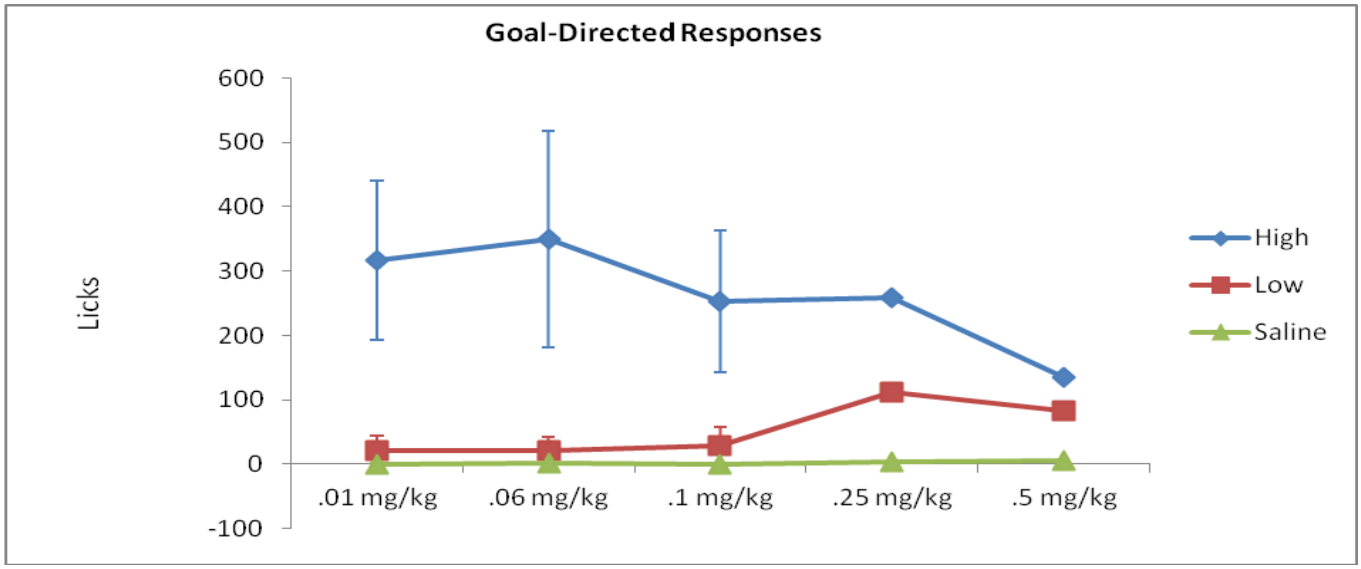
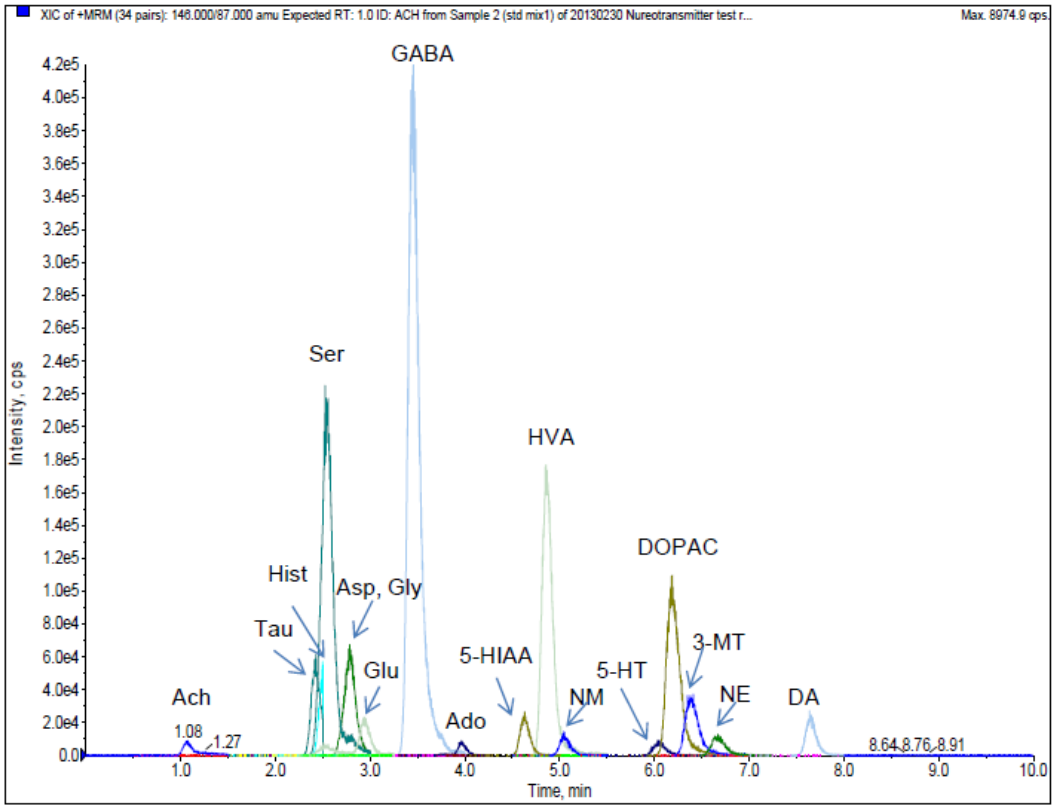


Figure 5. Goal directed responses (licks) for the active operant spout for cocaine/2 h. On the FR10 schedule of reinforcement, every 10 licks lead to an iv infusion of drug. Pretreatment with increasing doses of 8-OH-DPAT tended to reduce responding for cocaine by the High Drug-Takers over trials. This finding may suggest that responding for drug may be reduced in these subjects, but that additional treatment with 8-OH-DPAT is necessary.

Ion chromatogram for all 17 analytes.



1000 nM for Gly, Ser and Tau;
100 nM for Asp, Glu, GABA, Hist, Ado, HVA, 5-HIAA and DOPAC;
10 nM for Ach, 5-HT, NE, 3-MT, NM and DA.

Figure 6. Chromatograms from the standards shown in a composite figure, including 17 analytes at varying concentrations, and all within physiological range. These data verify that our Core Facility now has the expertise to measure all of these neurochemicals from a single sample.

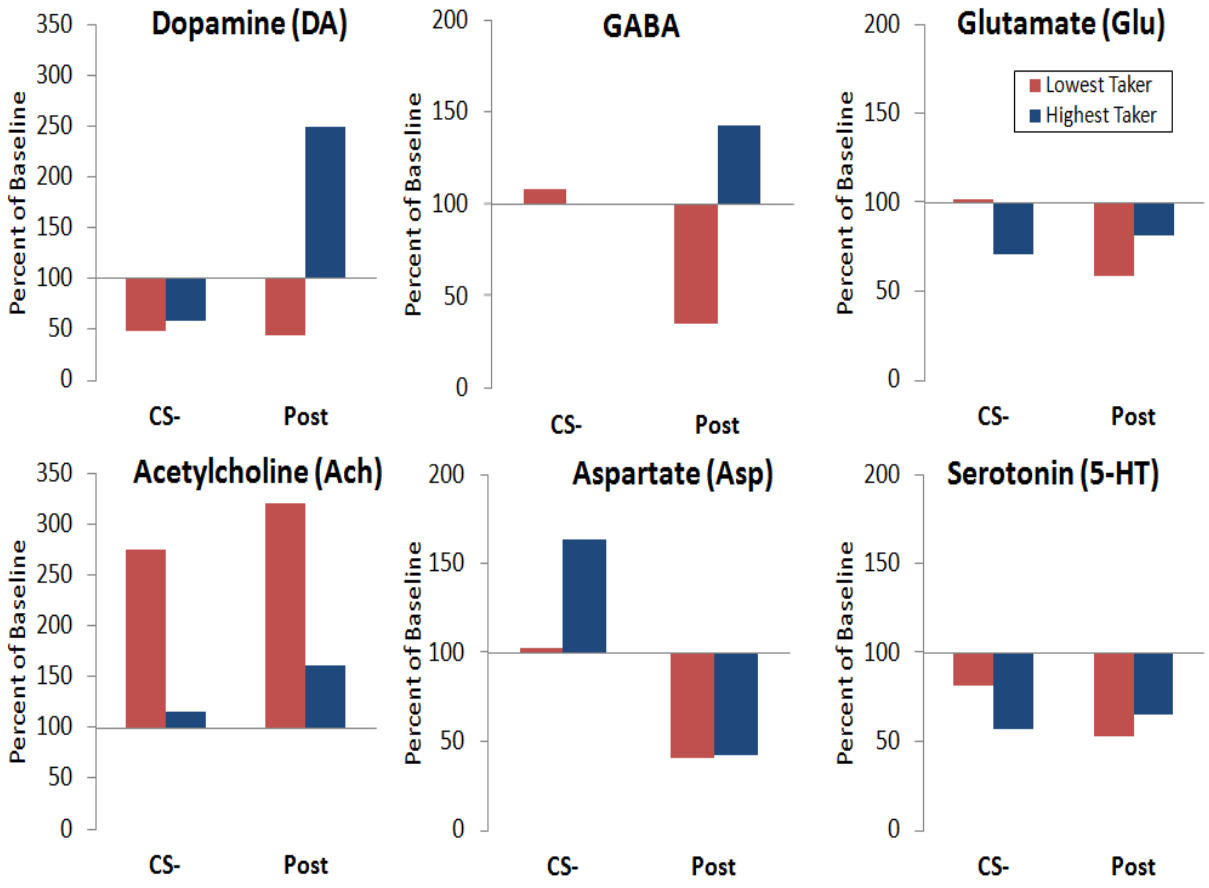


Figure 7. Percent change from Baseline for DA, GABA, Glutamate, Aspartate, Acetylcholine, and Serotonin for the lowest (red) and the highest drug-taker (blue) during infusion of the saline-paired CS- and during the period following the IO infusion of the cocaine-paired CS+ (referred to as Post).

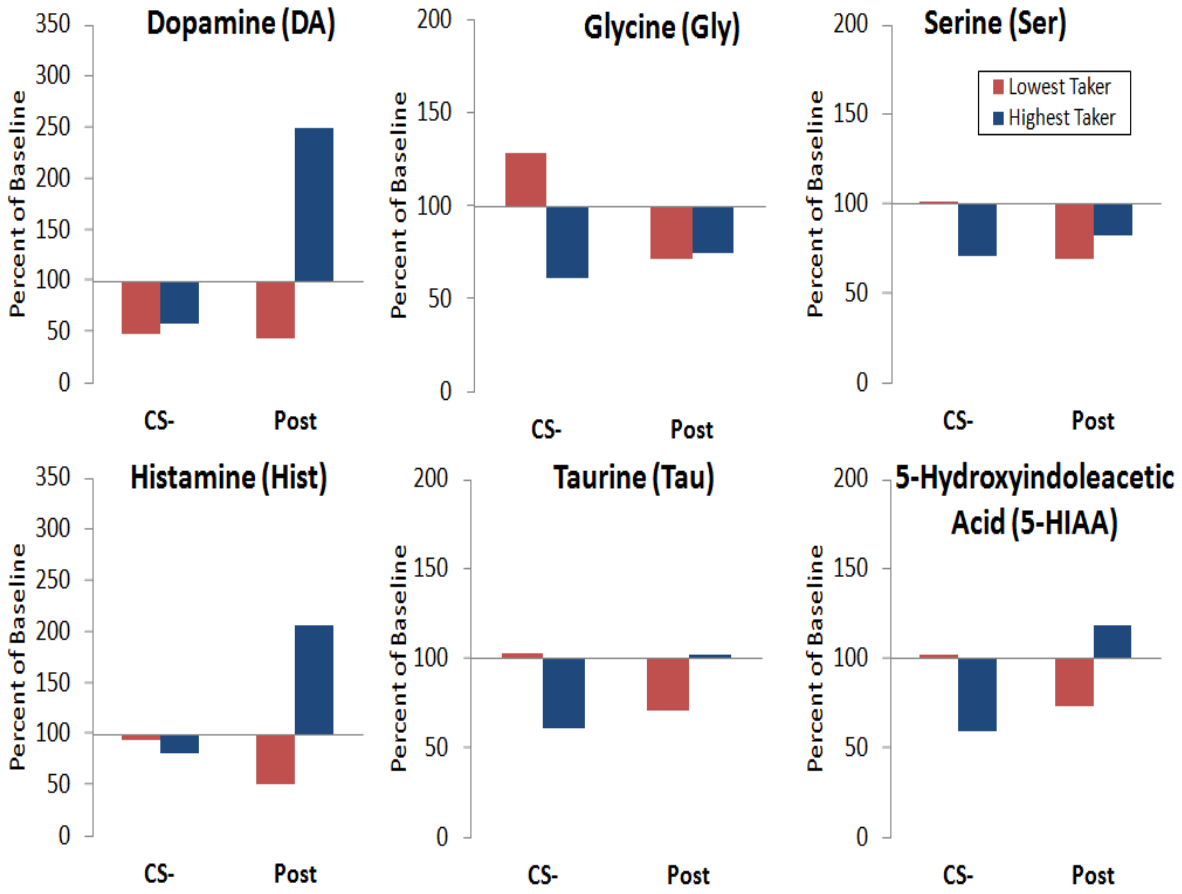


Figure 8. Percent change from Baseline for DA (same data as shown in Figure 5), Glycine, Serine, Histamine, Taurine, and 5-Hydroxyindoleacetic Acid for the lowest (red) and the highest drug-taker (Blue) during infusion of the saline-paired CS- and during the period following the IO infusion of the cocaine-paired CS+ (referred to as Post).

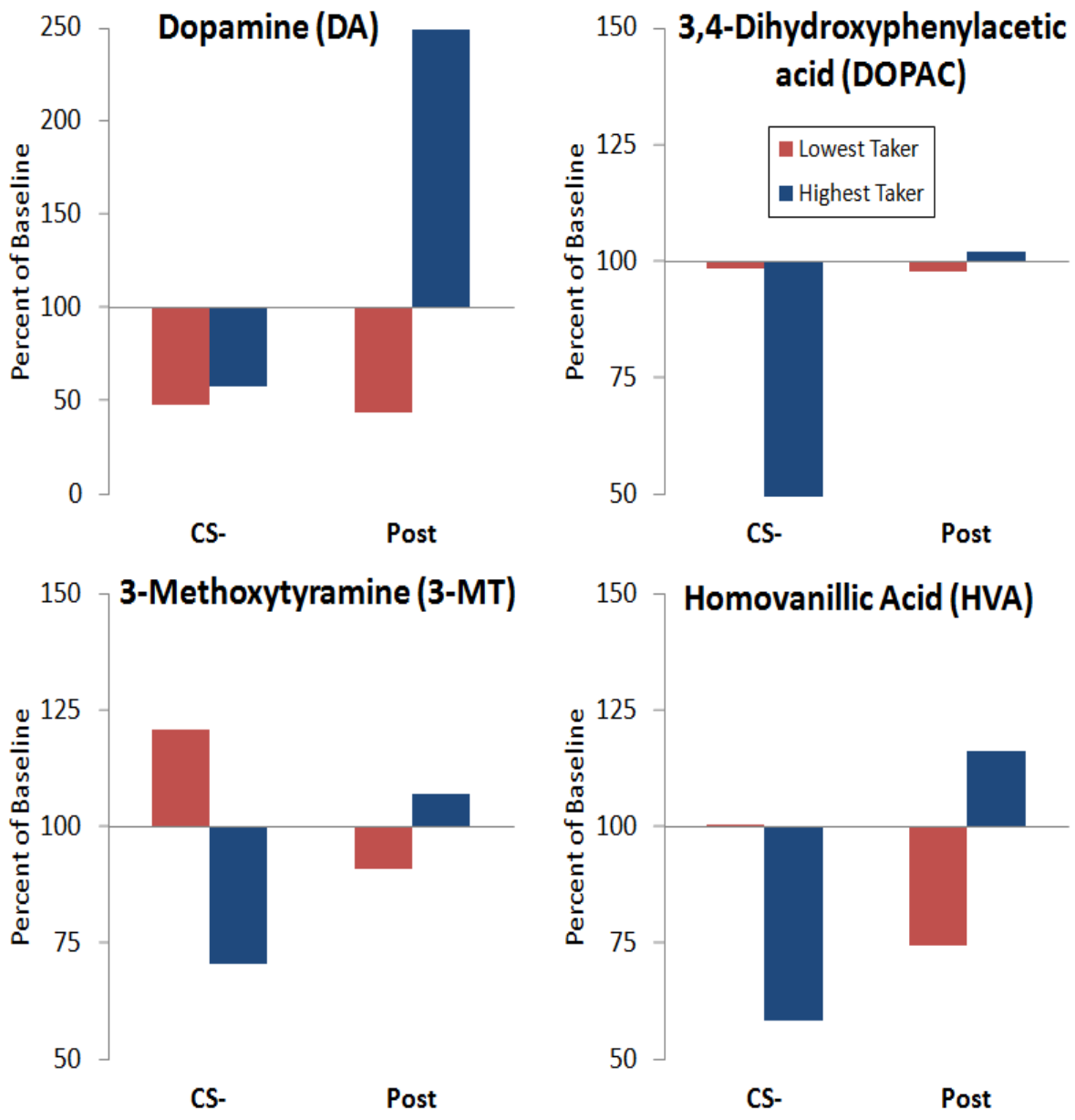


Figure 9. Percent change from Baseline for DA and its metabolites, DOPAC, 3-MT, and HVA, for the lowest (red) and the highest drug-taker (Blue) during infusion of the saline-paired CS- and during the period following the IO infusion of the cocaine-paired CS+ (referred to as Post).

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
___X__No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

_____Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

_____Number of subjects originally targeted to be included in the study
_____Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
_____Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
_____Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
___X_ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, and an abbreviated title of the publication. For example, if you submit two publications for Smith (PI for Project 01), one

publication for Zhang (PI for Project 03), and one publication for Bates (PI for Project 04), the filenames would be:

Project 01 – Smith – Three cases of isolated
Project 01 – Smith – Investigation of NEB1 deletions
Project 03 – Zhang – Molecular profiling of aromatase
Project 04 – Bates – Neonatal intensive care

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. None				☐Submitted ☐Accepted ☐Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes____x_____ No_____

If yes, please describe your plans:

The data resulting from the study completed and described for Specific Aim 1 will be written up and submitted for publication.

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention, diagnosis and treatment that are attributable to the completed research project. If there were

no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None.

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No___X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____ X _____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages.

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Patricia Sue Grigson	Professor
eRA COMMONS USER NAME (credential, e.g., agency login)	
psgrigson	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Elizabethtown College, Elizabethtown, PA	B.A.	1984	Psychology
Rutgers University, New Brunswick, NJ	M.S.	1987	Psychology
Rutgers University, New Brunswick, NJ	Ph.D.	1990	Psychology
Penn State College of Medicine, Hershey, PA	Postdoc	1990-93	Behavioral Neuroscience

A. Personal Statement

One primary means by which cues elicit relapse is by inducing, through experience with drug, the onset of withdrawal, and evidence suggests withdrawal is accompanied by the onset of an aversive state. Rats, it turns out, make orofacial responses following the intraoral delivery of palatable and aversive sapid stimuli and these orofacial responses can be used to test whether a stimulus is palatable or aversive (i.e., positive or negative). Dr. Grigson reported on these faces in 1997. She was then prepared to discover, along with her former students and colleagues, that aversive faces (i.e., gapes) are elicited by the intraoral infusion of a drug-paired taste cue and that greater aversion predicts greater responding for drug. In this case, however, the taste reactivity behavior was measured at the end of training, following multiple taste-drug pairings. More recently, we examined the behavior early in training and found early indicators of later vulnerability for addiction. Current efforts seek to determine whether the affective state of the rat also can be detected by the measurement of ultrasonic vocalizations – which are much easier to score than taste reactivity behavior. Finally, we seek to identify the neurochemical milieu that accompanies avoidance of a drug-paired cue, cue-induced craving, and the associated individual vulnerabilities.

B. Positions and Honors

Positions and Employment

1990-1993 Postdoctoral Fellow - Penn State University, College of Medicine, Hershey, PA
1993-1995 Sr. Research Associate -Penn State University, College of Medicine, Hershey, PA
1995-2000 Assistant Professor - Penn State University, College of Medicine, Hershey, PA
2000-2007 Associate Professor - Penn State University, College of Medicine, Hershey, PA
2007-present Professor – Penn State University, College of Medicine, Hershey, PA

Honors and Awards

1990-1993 Postdoctoral National Research Service Award
May, 2000 Recipient of the Annual Hinkle Society Junior Investigator Award
July, 2004 Recipient of the Alan N. Epstein Research Award, Society for the Study of Ingestive Behavior

C. Selected peer-reviewed publications (out of 70)

Grigson, P.S. Conditioned taste aversions and drugs of abuse: A reinterpretation. (1997). Behavioral Neuroscience, 111, 129-136. **PMID: 9109631**

Grigson, P.S. & Twining, R.C. (2002). Cocaine-induced suppression of saccharin intake: A model of drug-induced devaluation of natural rewards. Behavioral Neuroscience, 116, 321-333. **PMID:11996317**

Grigson, P.S., & Hajnal, A. (2007). Once is too much: Conditioned changes in dopamine following a single saccharin-morphine pairing. Behavioral Neuroscience, 121: 1234-1242**.** **PMID: 18085877**

Wheeler, R.A., Twining, R.C., Jones, J.L., Slater, J.M., Grigson, P.S., and Carelli, R.M. (2008). Cue-induced negative affect: A behavioral and neural mechanism of cocaine seeking. Neuron 57(5): 774-785. **PMID: 18341996**

Kuntz, K.L., Twining, R.C., Baldwin, A.E., Vrana, K.E., & Grigson, P.S. (2008). Heroin self-administration: I. Incubation of goal-directed behavior in rats. Pharmacology Biochemistry & Behavior, 90(3): 344-348. **PMID: 18471868**

Kuntz, K. L., Patel, K. M., Grigson, P. S., Freeman, W. M., & Vrana, K. E. (2008). Heroin self-administration: II. CNS gene expression following withdrawal and cue-induced drug-seeking behavior. *Pharmacology Biochemistry & Behavior*, 90(3): 349-356. **PMID: 18466961**

Freet, C.S., Steffen, C., Nestler, E.J., & Grigson, P.S. (2009). Over expression of ΔFosB is associated with attenuated cocaine-induced suppression of saccharin intake in mice. Behavioral Neuroscience, 123(2): 397-407. **PMID: 19331462**

Twining, R.C., Bolan, M., & Grigson, P.S.(2009). Yoked delivery of cocaine is aversive and protects against motivation for drug in rats. Behav Neurosci. 123: 913-925. **PMID: 19634952**

Puhl, M. D., Fang, J., & Grigson, P. S. (2009). Acute sleep deprivation increase the rate and efficiency of cocaine self-administration, but not the perceived value of cocaine reward in rats. *Pharmacology Biochemistry and Behavior*, 94:262-270. **PMID: 19751755**

Puhl, M.D., Cason, A.M., Wojnicki, F.H.E., Corwin, R.L., & Grigson, P.S. (2011). A History of Bingeing on Fat Enhances Cocaine Seeking and Taking. Behavioral Neuroscience, 125: 930-942. **PMID: 21988520**

Puhl, M. D., Blum, J. S., Acosta-Torres, S., & Grigson, P. S. (2011). Environmental enrichment protects against the acquisition of cocaine taking and seeking in adult male rats, but does not attenuate avoidance of a drug-associated saccharin cue. Behavioural Pharmacology, Dec 8 [Epub ahead of print]. **PMID: 22157144**

Liang, N-C., Grigson, P.S., & Norgren, R. (2012). Pontine and Thalamic Influences on Fluid Rewards: II. Sucrose and Corn Oil Conditioned Aversions. Physiology & Behavior, 105: 589-5946 **PMID: 21699909.**

Liang, N-C., Norgren, R., & Grigson, P.S. (2012). Pontine and Thalamic Influences on Fluid Rewards: III. Anticipatory Contrast for Sucrose and Corn Oil. Physiology & Behavior, 105: 595-6066 **PMID: 21703289.**

Nyland, J., & Grigson, P.S. (2012). A drug-paired taste cue elicits withdrawal and predicts cocaine self-administration. Behav Brain Res **PMID: 23174208**

Puhl MD, Boisvert M, Guan Z, Fang J, Grigson PS. (2013). A novel model of chronic sleep restriction reveals an increase in the perceived incentive reward value of cocaine in high drug-taking rats. Pharmacol Biochem Behav. 109: 8-15. **PMCID: PMC3740787**

D.Research Support

Active

Sponsor: NIH/NIDA R37 DA09815-09 (Renewal)
Title: Drugs of Abuse and Learned Aversions: Solving a Paradox

Period: 5/1/11-4/30/16
Role: Grigson (PI) 40% effort

Sponsor: CURE Grant, PA Department of Health, SAP#4100055573
Title: A Multidisciplinary Research Paradigm for Assessing & Guiding Addiction Treatment.
Period: 6/01/11-5/31/15
Role: Grigson (PI) 25% effort

Sponsor: National Institute on Drug Abuse Fellowship Grant
Title: Substance abuse: Individual differences in behavior and epigentetics
Period: 08/01/2013-07/31/2016
Role: Grigson (Mentor)

Pending
Sponsor: National Institutes of Health
Title: Heroin Addiction: Predicting Vulnerability & Identification of a Novel Treatment
Project Period: 4/1/2014-03/31/2016
Role: Grigson (Co-I) 5% 0.6 calendar months

Sponsor: NIDA DA038775
Title: Random Nicotine Delivery: A Novel Intervention for Smokers
Period: 09/01/14 – 08/31/16
Role: Grigson (Co-I)

BIOGRAPHICAL SKETCH

Provide the following information for the Senior/key personnel and other significant contributors.
Follow this format for each person. **DO NOT EXCEED FOUR PAGES.**

NAME	POSITION TITLE
Freet, Christopher Samuel	Postdoctoral Scholar
eRA COMMONS USER NAME (credential, e.g., agency login)	
CFREET	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as nursing, include postdoctoral training and residency training if applicable.)*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	MM/YY	FIELD OF STUDY
Shippensburg University, Shippensburg, PA	B.A	05/1996	Psychology/Biology
The Penn State College of Medicine, Hershey, PA	M.S	05/2001	Anatomy
The Penn State College of Medicine, Hershey, PA	Ph.D.	05/2010	Neuroscience
The Penn State College of Medicine, Hershey, PA	Postdoc	2010 -	Neuroscience

A. Personal Statement

My first experience with planning and conducting research occurred during the senior year of my undergraduate studies. I was able to conduct an undergraduate research project with Dr. Robert Hale, a professor in the Psychology Department at Shippensburg University. After receiving a small grant from the Shippensburg University Foundation to help fund the project, we conducted an experiment to evaluate the effects of an aspirin pretreatment on ethanol withdrawal severity in DBA/2 mice. This project provided me my first practical experience with experimental design and data analysis and exposed me to animal care and handling procedures common in behavioral studies. Following a stint as a research technician, I entered the Masters program in Anatomy at the Penn State University College of Medicine and was able to not only gain knowledge through graduate level classes, but also to broaden my behavioral experimental skills to include molecular techniques. Switching from behavioral paradigms (such as using modular chambers to monitor various responses in rats) to molecular studies (such as determining mRNA levels in the brain using subcloning, in situ hybridization, and immunohistochemistry) allowed me to study the distribution of the insulin regulated aminopeptidase (IRAP) and the glucose transporters GLUT4 and GLUT8 in embryonic and postnatal rat brain.

Following completion of my Masters degree, I continued to develop my research experience in the Neuroscience doctoral program at the Penn State University College of Medicine. As a student in the laboratory of Dr. Sue Grigson, I received training in a variety of behavioral, pharmacological, and cellular/molecular techniques necessary to study the role of deltaFosB in responding to natural rewards, drugs of abuse, and drug-induced devaluation of natural rewards. Shortly before entering the program, however, my oldest son was diagnosed with autism and I have had to reorganize my strategies for pursuing an academic career. I am grateful, however, for two things that have allowed me to reorganize instead of abandon that goal. First, a network of autism support in the area and a strong school system has allowed my son to flourish. Second, the excellent collaborative atmosphere at the Penn State University College of Medicine has allowed me to gain the experience I need from those within the university as well as from institutions across the country. As such, although I suffered some delay and am now tied to the area, I was able to collaborate with Dr. Eric Nestler, at the

University of Texas SW, and was able to complete my dissertation with Dr. Grigson to evaluate sensitivity to absolute and relative properties of natural rewards and drugs of abuse as a function of strain in rats and mice.

Following completion of my dissertation, I accepted a postdoctoral position with Dr. Xiaorui Tang at Penn State which allowed me to gain experience in more physiology-oriented experiments as well as further develop my writing skills. During this time, I worked with Dr. Tang on the development of a novel treatment for hypertension utilizing tetanic stimulation of the carotid baroreflex. The project required that I develop my knowledge of the cardiovascular system and led to a first-authorship review on the effects of hypoxic exposure on the baroreflex and chemoreflex systems. Unfortunately, Dr. Tang failed to obtain tenure and soon left the institution. However, I quickly shifted back to the study of substance abuse and addiction with Dr. Grigson as a postdoctoral scholar. During this time I increased focus on data publication, and in addition to my hypoxia review, I have published two first-authorship articles with Dr. Grigson this year including an evaluation of the C57BL/6J and DBA/2J mice in drug-induced suppression and the effects of a kappa-opioid agonist on drug-induced suppression in Lewis and Fischer rats. In addition, I have a manuscript evaluating drug-induced suppression in the humanized OPRM1 A118G mice ready for submission. I have also gained additional teaching experience during this time. In the 2013 spring semester, I served as an adjunct professor at Elizabethtown College teaching the undergraduate level *Introduction to Neuroscience* course and lectured two graduate classes, *Techniques of Animal Experimentation* and *Biological Basis of Human Health & Disease*, in the spring of 2014. Currently, I am fully responsible for the establishment and growth of the mouse research program in Dr. Grigson's lab designed to compliment and advance the models currently developed in rats. To this end, I have been working to develop and improve upon the self-administration technique in mice. The goal now is to advance this research program and to establish my independence. Space has been identified and both Dr. Grigson and Dr. Barnstable, the Department Chair, are in full support.

B. Positions and Honors

Positions and Employment

1996-1999	Research Lab Technician, Department of Behavioral Science, Penn State College of Medicine
2000	Teaching Assistant, Department of Neuroscience and Anatomy, Penn State College of Medicine, Hershey, PA
2001-2002	Senior Research Lab Technician, Department of Behavioral Science, Penn State College of Medicine
2002-2003	Research Support Associate, Department of Behavioral Science, Penn State College of Medicine
2003-2010	Graduate Assistant, Department of Neural and Behavioral Sciences, Penn State College of Medicine, Hershey, PA.
2010-	Postdoctoral Scholar, Department of Neural and Behavioral Sciences, Penn State College of Medicine
2013	Adjunct Professor, Department of Psychology, Elizabethtown College, Elizabethtown, PA

Other Experience and Professional Memberships

2003-	Member, Society for Neuroscience (SFN)

C. Peer-reviewed Publications

Most relevant to the current application

1. Grigson PS & **Freet CS (2000)**. The Suppressive Effects of Sucrose and Cocaine, but Not Lithium Chloride, Are Greater in Lewis Than in Fischer Rats: Evidence for the Reward Comparison Hypothesis. *Behavioral Neuroscience*, **114(2)**: 353-363. **PMID: 10832796**.
2. **Freet CS**, Tesche JD, Tompers DM, Riegel KE, Grigson PS **(2006)**. Lewis rats are more sensitive than Fischer rats to successive negative contrast, but less sensitive to the anxiolytic and appetite stimulating effects of chlordiazepoxide. *Pharmacology, Biochemistry, and Behavior*, **85(2)**: 378-384. Epub 2006 Oct 16. **PMID: 17049372**.
3. Grigson PS, Twining RC, **Freet CS**, Wheeler RA, Geddes RI **(2009)** Drug-induced suppression of CS intake: Reward, aversion, and addiction. In: *Conditioned Taste Aversion: Behavioral and Neural Processes, S. Reilly & T. Schachtman, (Eds).* pp 74-91. New York: Oxford University Press.
4. **Freet CS**, Steffen C, Nestler EJ, Grigson PS **(2009)**. Over expression of DeltaFosB is associated with attenuated cocaine-induced suppression of saccharin intake in mice. *Behavioral Neuroscience*, **123(2)**: 397-407. **PMCID:PMC2819926**
5. **Freet CS**, Arndt A, Grigson PS **(2013)**. Compared with DBA/2J Mice, C57BL/6J Mice Demonstrate Greater Preference for Saccharin and Less Avoidance of a Cocaine-Paired Saccharin Cue. *Behavioral Neuroscience,* **127(3)**: 474-484. Epub 2013 Apr 1. **PMID:23544599[PubMed - indexed for MEDLINE]**
6. **Freet CS**, Wheeler RA, Leuenberger E, Mosblech, NAS, Grigson PS **(2013)**. Fischer Rats Are More Sensitive Than Lewis Rats to the Suppressive Effects of Morphine and the Aversive Kappa-Opioid Agonist Spiradoline. *Behavioral Neuroscience*, **127(5)**: 763-770. **PMID:24128363[PubMed - in process]**

Additional recent publications of importance to the field

1. Bruno KJ, **Freet CS**, Twining RC, Egami K., Grigson PS, Hess EJ **(2007)**. Abnormal latent inhibition and impulsivity in coloboma mice, a model of ADHD, *Neurobiology of Disease*, **25(1)**: 206-216. Epub 2006 Oct 24. **PMID: 17064920**.
2. Liang NC, **Freet CS**, Grigson PS, Norgren R **(2012)**. Pontine and thalamic influences on fluid rewards: I. Operant responding for sucrose and corn oil. *Physiology & Behavior,* **105(2)**: 576-588. Epub 2011 Jun 16. **PMCID:PMC3207032**
3. **Freet CS**, Stoner JF, Tang X **(2013)**. Baroreflex and chemoreflex controls of sympathetic activity following intermittent hypoxia. *Autonomic Neuroscience: Basic and Clinical*, **174(1-2)**: 8-14. Epub 2013 Jan 8. **PMID:23305890[PubMed - indexed for MEDLINE]**

D. Research Support

Active

None