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. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** Temple 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):** Germaine A Calicat, MA

4. **Grant Contact Person's Telephone Number:** 215.204.7655

5. **Grant SAP Number:** 4100050909

6. **Project Number and Title of Research Project:** Project 7 - *Role of Dorsostriatal Glutamatergic Signaling in the Regulation of Cocaine-induced Synaptic and Behavioral Plasticity*

7. **Start and End Date of Research Project:** 01/01/11 – 12/31/12

8. **Name of Principal Investigator for the Research Project:** Vinay Parikh, Ph.D.

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:

   $ 75,978.92

   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	Position Title	% of Effort on Project	Cost
Tracy	Research Tech	10% yr. 1, 20% yr. 2; ca)	$9,257(direct)
Naughton	Undergrad Res Asst	25% summer (yr .1)	$3,125 (direct)

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	Position Title	% of Effort on Project
Parikh	Assistant Professor	15% (yrs 1 & 2)
Unterwald	Professor	10% (yrs 1 & 2)
Rawls	Associate Professor	10% (yrs 1 & 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

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:
	☐NIH ☐ Other federal (specify:_____ _____) ☐ Nonfederal source (specify: _____)		$	$
	☐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:

The data derived from this project will serve as the basis for a major (R01) grant application to NIH. We are planning to submit that later this year. We would be very competitive for such an application with the preliminary data generated by this PADOH-funded project.

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

    We plan to extend our studies to determine the contribution of glutamatergic signaling in the dorsomedial striatum in cocaine-induced deficits in executive processes.

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				
Unknown				
Total	**1**			

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic	1			
Unknown				
Total	**1**			

	Undergraduate	Masters	Pre-doc	Post-doc
White	1			
Black				
Asian				
Other				
Unknown				
Total	**1**			

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.

This project definitely enhanced the quality of our research by bringing investigators (Parikh, Unterwald and Rawls) from different disciplines (Liberal Arts College and the School of Medicine) together. These investigators brought a diverse set of expertise and technical skills together to gain an integrative perspective in order to answer major questions concerning the neurobiological basis of compulsive cocaine-seeking, and in general, drug addiction. The enormous data generated from this research, that takes into account the molecular, systems-level and functional (behavioral) viewpoint, provides important information concerning the mechanistic basis of addiction that may not be possible to attain by any individual investigator alone.

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.

Project Title

Role of Dorsostriatal Glutamatergic Signaling in the Regulation of Cocaine-induced Synaptic and Behavioral Plasticity

Project Duration:
1/1/2011 – 12/31/2012

Specific Aims

Aim 1: To test the hypothesis that chronic cocaine administration increases baseline and depolarization-evoked phasic glutamatergic activity in dorsal striatum of rats. As extrasynaptic glutamate is primarily regulated by astrocytic glutamate transporter 1 (GLT1)

activity, administration of GLT1 activator would attenuate reduced clearance kinetics of striatal glutamatergic signals in cocaine-treated animals.
Progress made on aim 1: **All experiments were successfully conducted and goals were fully achieved.**

Aim 2: To test the hypothesis that removal of dopaminergic inputs to dorsal striatum attenuates chronic cocaine-induced changes in resting glutamate levels and depolarization-dependent glutamate release.
Progress made on aim 2: **A major goal of the experiments proposed in Aim 2 was to test the hypothesis that dopamine, a neurotransmitter involved in reward, motivation and motor functions, regulates glutamate release in the dorsal striatum and this regulation is impaired in cocaine addiction. We decided to produce depletion of dopamine in the striatum of rats by using a neurotoxin, and then study how cocaine effects glutamate transmission in these animals. Removal of dopamine inputs from the striatum produced severe impairments in the locomotor activity of the awake animals and it was almost impossible to conduct electrochemical recordings in these animals. Therefore, we conducted studies looking into dopamine-glutamate interactions in anesthetized animals (refer Expt. 4 below). As brain-derived neurotrophic factor (BDNF) protein is implicated in addiction, and this trophic biomolecule also regulates glutamate transmission, we utilized transgenic mice that express reduced BDNF protein for these studies. We were able to conduct these experiments and generated important data on dopamine regulation of striatal glutamate in normal and BDNF mutant animals. However, we could not conduct experiments in awake rats treated with chronic cocaine, and therefore, our goals were only partially achieved.**

Aim 3: To test the hypothesis that pharmacological blockade of striatal ionotropic glutamate receptors (iGluRs) in dorsolateral striatum will impair cocaine-induced locomotor sensitization.
Progress made on aim 3: **All experiments were successfully conducted and goals were fully achieved.**

Background information and goals of the study

Deficits in cognitive control play an important role in the representation of compulsive drug-taking and seeking behaviors. Recent evidence suggests that diminished ability to control cocaine seeking arise from perturbations in glutamate homeostasis that produces impairments in prefrontal regulation of striatal circuitry. Corticostriatal circuits involving discrete regions of the dorsal striatum contribute directly to the manifestation of executive functions through the integration of sensorimotor, cognitive and motivational information. Specifically, components of medial and lateral dorsostriatal structures are essential for learned associations to become habitual, and abnormal activity within these loops is implicated in compulsive cocaine seeking. However, mechanisms underlying the drug-induced neuroadaptations in glutamate homeostasis in dorsal striatal structures and their role in enduring drug use and relapse, and executive dysfunction are not known.

The primary goal of this proposal was to determine how cocaine produces neuroadaptive changes in striatal glutamatergic system and whether these changes are linked to cocaine-induced behavioral sensitization. We studied the effects of acute and chronic cocaine administration on spatiotemporal dynamics of glutamatergic transmission in the dorsal striatum of rats. The focus of these studies was kept primarily on the dorsolateral striatum as this region is implicated in habit learning and locomotor sensitization effects of psychostimulants. We utilized enzyme-based biosensors and amperometry to measure basal glutamate levels and the release of this neurotransmitter following terminal depolarization in the dorsolateral striatum of *awake* freely-moving animals. The effect of ceftriaxone (CTX), a beta-lactam antibiotic known to upregulate the expression and functional activity of glial glutamate transporter (GLT1), was also be examined on locomotor sensitization and striatal glutamate homeostasis in chronic cocaine-treated animals. As striatal dopamine mediates the reinforcing effects of drugs and also regulates glutamatergic activity, we also elucidated the contribution of striatal dopaminergic input system in the maintenance of glutamate homeostasis. Finally, we evaluated the effects of pharmacological manipulations that disrupt signaling via ionotropic glutamate receptors on the acquisition and expression of cocaine-induced locomotor sensitization.

Major studies and main results

Subjects: Adult male Wistar rats (2-3 months; 250-300g) were procured from Charles River Laboratories (Malvern, PA). The animals were housed individually in a temperature-/humidity-controlled environment with a 12-hour dark/light cycle and had free access to food and water. Male C57BL/6J mice (PND 42-56) were purchased from Jackson Laboratories (Bar Harbor, ME). All procedures were conducted in adherence with protocols approved by the Temple University's Institutional Animal Care and Use Committee (IACUC) and Institutional Biosafety Committee (IBC).

Statistics: Data are M±SEM from 5-8 animals/group. Statistical analyses were performed using SPSS/PC+ V17.0 (IBMSPSS, Armonk, NY, USA). *Unpaired t* comparisons were conducted to analyze group comparisons for signal amplitudes and clearance time. Brain-derived neurotrophic factor (BDNF) dose effect was analyzed using one-way analysis of variance (ANOVA) followed by Fisher's *LSD* test. Behavioral and immunoblotting data were analyzed using a two-way ANOVA followed by a Bonferroni test to identify group differences. Cumulative activity data was analyzed by one-way ANOVA followed by a Newman-Keul test for multiple comparisons.

Experiment 1: Effects of acute and chronic cocaine treatment on glutamate dynamics in the dorsal striatum.

<u>Rationale:</u> Dorsolateral striatum receives glutamatergic inputs primarily from sensorimotor cortex. This brain region plays a critical role in the acquisition of motor and associational stimulus-response (habit) learning. Repeated exposure with psychostimulant drugs like cocaine produces sensitization in motor behavior by recruiting sensorimotor circuits involving dorsolateral striatum. Although changes in glutamate homeostasis in ventral striatum are linked to rewarding effects of cocaine, the effect of chronic cocaine on the

dynamics of synaptic/extrasynaptic glutamate release in the dorsal striatum and how it relates to behavioral sensitization in cocaine addicts have never been studied. The goal of this experiment was to investigate the effect of acute and chronic cocaine administration during withdrawal and cocaine challenge (expression of behavioral sensitization), on basal (tonic) and depolarization-dependent (phasic) glutamate release in the dorsolateral striatum of freely-moving rats.

Methods: *a)* Drug administration and experimental design: Prior to the start of drug treatment, animals were handled for a week and habituated to the recording box that consisted of a 45(w)x45(d)x60(h) cm plywood-constructed chamber, with a full-size front door and a 30x30 cm observation window. Animals were exposed to the drug inside the chamber. Rats received either saline or cocaine (20mg/Kg/day; i.p.) for 1 week followed by a 10 days of withdrawal period to produce sensitization effects. Every day during the withdrawal phase, the animals were kept in the testing chamber for 45-60 min. 3-4 days prior to the challenge day, the animals were prepared for microelectrode implantation surgeries. The animals were challenged with either saline (SAL/SAL; COC/SAL) or cocaine (SAL/COC; COC/COC). The experimental protocol for drug pretreatment, withdrawal and challenge is depicted in **Fig 1**.

b) Microelectrode preparation and calibration: Ceramic-based, multi-site microelectrodes featuring four 50x150 μm platinum- recording sites (Quanteon LLC, Nicholasville, KY) were used for amperometric recordings of glutamatergic activity in freely-moving animals. Microelectrodes were modified by soldering one end of four enamel-coated magnet wires (30 ga) to the terminals on the electrode panel and the other end to gold-pin connectors. Reference electrodes were constructed by soldering Ag/AgCl reference electrodes prepared from 0.008" silver wire (A-M Systems, Carlsberg, WA) to gold-pin connectors. The gold-pins were inserted into a miniature 9-pin plastic strip connector that was glued to the microelectrode using 5-min epoxy. The electrodes were then prepared for enzyme coatings. Briefly, glutamate oxidase (Glu-Ox) was cross-linked with the BSA-glutaraldehyde mixture and immobilized onto the bottom pair of recording sites. The remaining upper two recording sites were coated only with the BSA-glutaraldehyde solution and served to record background activity. Enzyme-coated microelectrodes were air-dried for 48-72 hrs and electroplated with m-PD at 0.5 V for 60 min prior to calibration. This procedure was required to eliminate non-specific current produced as a result of electroactive interfering analytes in vivo. Glu-Ox microelectrodes were calibrated using a FAST-16 electrochemical recording system (Quanteon) for glutamate sensitivity and selectivity prior to implantation in animals.

Microelectrodes were dipped in 0.05M PBS for 30 min prior to calibration. Calibrations were performed using fixed potential amperometry by applying a constant voltage of 0.7 V versus Ag/AgCl reference electrode in a beaker containing a stirred solution of 0.05M PBS maintained at 37ºC. Amperometric currents were digitized at a frequency of 5 Hz. After baseline currents stabilized, aliquots of stock solutions of ascorbic acid (AA; 20 mM), glutamate (20 mM), and dopamine (DA; 2 mM) were added to the calibration beaker such that the final concentrations were 250 μM AA, 20, 40, 60 and 80 μM glutamate and 2 μM DA (**Fig 2**). The slope (sensitivity), limit of detection (LOD) and linearity (R^2) for choline, as well as selectivity ratio for AA and DA, were calculated for each individual recording site and recordings from Glu-Ox-coated sites

were self-referenced. To be implanted into the cortex, electrodes were required to meet the following characteristics: 1) sensitivity for detecting glutamate: >3 pA/µM, with a background current of <200 pA; 2) limit of detection (LOD): <300 nM glutamate; 3) selectivity for glutamate:AA: >80:1; 4) detection of increasing glutamate concentrations (20-80 µM): R^2: >0.98. After calibration, an infusion guide cannula (26 g; Plastics One Inc., Roanoke, VA) was attached to the microelectrode assembly so that the tip of the cannula was positioned between the two pairs of platinum recording sites, ~200 µm away from the ceramic-based microelectrode. The assembly was then implanted into the dorsal striatum.

c) Stereotaxic surgery and *in vivo* recordings in freely-moving rats: Surgeries were performed under aseptic conditions. Anesthesia was induced with 4-5% isoflurane using an anesthesia machine (Anesco/Surgivet, Waukesha, WI). Anesthetized rats with shaved heads were placed in stereotaxic frame (David Kopf, Model # 962, Tujunga, CA) and their body temperature was maintained at 37ºC using Deltaphase isothermal pad (Braintree Scientific, Braintree, MA). Anesthesia was maintained with 2% isoflurane along with oxygen at a flow rate of 1 mL/min throughout the surgical procedure. The scalp was cleansed with betadine scrub and incised along the midline for 10mm. Three stainless steel screws were threaded into the cranium to anchor microelectrode assembly with dental cement. The microelectrode assembly was slowly lowered into the dorsolateral striatum of the right hemisphere (AP: +1.2 mm; ML: -3.5 mm, measured from Bregma; DV: -4.0 mm, measured from dura) using a microdrive (MO-10; Narishige, International, East Meadow, NY). A stylet (32 ga) was placed into the infusion cannula to prevent obstruction. Topical antibiotic (bacitracin, polymixin and neomycin) was applied to the wound immediately after surgery. All animals received an antibiotic (amikacin; 100 mg/kg; s.c.), an analgesic (buprenorphine, 0.05 mg/kg; s.c.), and saline (1.0 mL; i.p.) daily for two days post-operatively while remaining in their home cages with food and water *ad libitum*. Thereafter, the animals were prepared for recording sessions in a shielded recording chamber. Animals were placed in the chamber and the microelectrode assembly was connected to the FAST-16 system through a shielded cable and a low-impedance commutator via a miniature potentiostat headstage (mk-II RAT HAT; Quanteon, Lexington, KY). Amperometric recordings were collected every 500 ms by applying a fixed potential of 0.7 V to the microelectrode using the FAST-16 recording system. Data were digitized using FAST-16 software. Amperometric recordings were conducted at 2Hz by applying a fixed potential of +0.7 V. The effects of intrastriatal infusions of KCL (70 mM; 1µL), (3*S*)-3-[[3-[[4-(Trifluoromethyl)ben zoyl]amino]phenyl]methoxy]-L-aspartic acid (TFB-TBOA 100 uM; 1µL), a glial glutamate transporter blocker, and tetrodotoxin (TTX 100 uM; 1µL), a sodium channel blocker were assessed on glutamate dynamics following the drug/saline challenge. Amperometric recordings were time-locked and synchronized by marking all events (drug injection/infusions) via TTL pulses. After completion of recording sessions, 10 and 20 nmoles of glutamate were infused through the guide cannula to determine the sensitivity of the microelectrode to glutamate. Self-referenced amperometric recordings were boxcar-averaged over 1-3 data points to minimize noise levels (determined by averaging the difference between two successive data points across the 10 s pre-cue baseline) to ≤200 nM. Signal amplitudes were calculated by determining the change in current following drug infusions as compared to pre-drug baseline and dividing it by the

calibration slopes. Clearance kinetics of glutamate signals were determined by calculating T80 values; time required to clear 80% of the signal from the peak amplitude.

Results: Raw traces of glutamate signals from a control (saline pretreated/saline challenged; SAL/SAL) and drug (cocaine pretreated/cocaine challenged; COC/COC) treated animal following local infusions of potassium (KCl 70mM; 1 µL) are shown (**Fig. 3A**). Cocaine pretreatment (COC/SAL) reduced the amplitudes of depolarization-evoked glutamate signals (t (8) = 2.97; p = 0.017 vs. SAL/SAL; **Fig 3B**). However, there was a trend for an increase in potassium-evoked signal following challenge (COC/COC; p = 0.1). Both cocaine pretreatment and challenge produced slower clearance of signals reflecting downregulation of transporter-mediated glutamate uptake (SAL/SAL: 21.0 ± 4.0 s; COC/SAL: 36.6 ± 2.4; COC/COC: 53.5 ± 3.5; **Fig. 3C**). TTX infusions profoundly reduced the resting levels of glutamate (**Fig. 3D**). Moreover, TTX-induced reduction in baseline glutamate was attenuated in cocaine pretreated and cocaine challenged animals (**Fig. 3E**; *, $p < 0.05$ vs. SAL/SAL). Finally, the effects of chronic cocaine were examined on increases in resting glutamate levels following blockade with TFB-TBOA, a non-specific glutamate transporter blocker. Cocaine pretreatment reduced the clearance of glutamate signals following local TFB-TBOA infusions (COC/COC: 65.6 ± 12.65 s; SAL/SAL: 19.5 ± 2.59 s; $p < 0.05$).

Experiment 2: Ceftriaxone (CTX) attenuates the effects of chronic cocaine on glutamate clearance kinetics

Rationale: Extracellular glutamate concentration is tightly regulated by a family of excitatory amino acid transporter proteins localized on neuronal and non-neuronal cells. One such protein, astrocytic glutamate transporter-1 (GLT1), is responsible for the removal of ~90% glutamate from extrasynaptic space. Downregulation of GLT1 by chronic cocaine and resultant increase in basal glutamate levels in nucleus accumbens is hypothesized to mediate cocaine relapse. If an increase in basal extracellular glutamate levels in the dorsolateral striatum of chronic cocaine-treated animals is a result of downregulation of GLT1 activity, then administration of GLT1 activator, CTX should restore the astrocytic but not neuronal component of basal glutamate levels. Furthermore, enhanced clearance of depolarization-evoked glutamate release in cocaine-sensitized animals will be normalized after CTX treatment.

Methods: The drug treatment regimen for this experiment was similar to experiment 1 except that animals received CTX (200 mg/Kg/day) along with cocaine (COC/CTX) or saline (SAL/CTX). Following withdrawal, rats were prepared for electrochemical recordings as described above.

Results: CTX reduced depolarization-evoked glutamate release in chronic cocaine pretreated and cocaine challenged animals (CTX/COC) as compared to COC/COC group (t (9) = 2.30, p = 0.04; **Fig. 4**). Furthermore, slower clearance of depolarization-evoked glutamate signals in cocaine-pretreated/challenged animals returned to normal levels following CTX treatment (T80; CTX/COC: 24.8 ± 3.44 s; COC/COC: 65.6 ± 12.65 s; **Fig. 4**). Although, CTX did not affect TFB-TBOA-induced glutamate signal amplitudes, it did reduce clearance time in cocaine pretreated/challenged animals (COC/COC: 20.6 ± 3.55 s; CTX/COC: 56.33 ± 10.07

s; $p < 0.05$; **Fig. 4**). CTX treatment per se did not affect potassium-evoked glutamate release (CTX/SAL: 6.54 ± 0.56 µM; SAL/SAL: 6.17 ± 0.38 µM) nor it affected the kinetics of signals (CTX/SAL: 26.75 ± 5.76 s; SAL/SAL: 19.2 ± 3.59 s; $p > 0.05$).

Experiment 3: Effects of chronic ceftriaxone (CTX) treatment on, behavioral sensitization and GLT-1 expression

Rationale: Psychostimulants may produce changes in glutamate transporter subtype 1 (GLT-1) function that contribute to their addictive properties. Animal studies indicate that GLT-1 activation by the β-lactam antibiotic CTX attenuates acute and sensitized behavioral responses to amphetamine in rats, prevents relapse to cocaine seeking in rats, and inhibits the direct reinforcing properties of cocaine in mice maintained on a progressive-ratio schedule of reinforcement). However, the contribution of GLT-1 in the dorsal striatum to the development of cocaine-induced behavioral sensitization has not been elucidated.

Methods: a) Drug and dosing regimen: Rats were injected with CTX (200 mg/kg) or saline once daily for 10 days. Beginning on the fourth day, rats from both groups were injected with cocaine (20 mg/kg) or saline for 7 days. All rats were then withdrawn from drug exposure for 10 days and then challenged with either saline or cocaine (20 mg/kg) in the behavioral chamber (see below).

b) Behavioral measures: Locomotor activity was measured using a Digiscan DMicro System (Accusan, Columbus, OH, USA) that consists of clear 20 x 20 x 42 cm plastic cages lined with horizontal photo-beams and detectors that are interfaced with an output computer. Ambulatory activity and stereotypy activity were recorded as counts of consecutive and repetitive photobeam breaks respectively. Following measurement of baseline motor activity (ambulatory, stereotypical) for at least 60 min, rats were injected with cocaine (or saline) and activity was measured for 80 min post-injection.

c) GLT-1 Immunoblotting: Immediately following behavioral experimentation, rats were euthanized and decapitated following brief exposure to carbon dioxide. For comparative purposes, 3 brain regions – dorsal striatum, nucleus accumbens (NAcc) and prefrontal cortex (PFC) were rapidly dissected, frozen on dry ice and stored at -80° C. Tissues were sonicated in the lysis buffer consisting of 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 1 % Triton, 0.1% SDS in 50 mM (pH 7.4) Tris with a protease inhibitor cocktail added. Supernatants were collected following centrifugation at 13,000 rpm for 15min. at 4° C. Protein levels were determined by the Lowry method. Tissue samples were boiled for 5 min in a Laemmli buffer containing 100 mM dithiothreitol. Proteins were separated by SDS-polyacrylamide gel electrophoresis and then transferred to nitrocellulose membranes. GLT1-immunoreactive bands were detected using a Guinea Pig Anti-Glutamate Transporter antibody (Millipore). Visualization was done with the Pierce Supersignal enhanced chemiluminesent substrate (Pierce, Rockford, IL, USA), read on Fujifilm image gage hardware (Fujifilm, Chicago, IL, USA) and quantified with Science Lab 99 L-Process v1.96 software (Fujifilm). GLT1 bands were normalized using β-tubulin.

Results: CTX pretreatment blocked the development of ambulatory activity and stereotypy in cocaine pretreated animals (**Fig. 5 & 6**; *p < 0.05 compared to SAL-SAL). To further investigate whether attenuation of cocaine-induced behavioral sensitization by CTX involve alterations in GLT-1 expression, the effects of repeated CTX exposure on baseline and cocaine-induced changes in GLT-1 expression in the nucleus accumbens (NAc), prefrontal cortex (PFC), and dorsal striatum of rats were assessed. CTX pretreatment upregulated the expression of GLT-1 in the dorsal striatum of control animals (**Fig. 7**; * $P < 0.05$ compared to respective SAL - SAL group). However, GLT-1 expression levels remain unaffected in cocaine pretreated animals in the presence and absence of CTX. Furthermore, drug treatments did not alter GLT-1 levels in the PFC and ventral striatum (NAcc).

Experiment 4: Effects of striatal dopaminergic deafferentation and brain-derived neurotrophic factor (BDNF) on glutamatergic transmission.
Rationale: Dopamine is a critical player involved in reinforcement learning and hence, addiction. Extensive evidence suggests that the primary rewarding effects of addictive drugs are mediated by dopamine, and dopamine signaling in dorsal striatum facilitates the progression of compulsive cocaine seeking behavior. Dopamine maintains striatal glutamate tone by exerting an inhibitory effect on glutamatergic activity. Therefore, deafferentation of dopaminergic inputs to dorsal striatum may influence TTX-sensitive resting and depolarization-evoked glutamate levels. Brain-derived neurotrophic factor (BDNF) is a growth-promoting protein that also modulates synaptic transmission. As the rewarding effects of cocaine may involve alterations in BDNF and this protein facilitates the release of dopamine, we also investigated the effects of BDNF on basal and depolarization-evoked glutamate release in dopaminergic deafferented animals. 6-hydroxy dopamine (6-OHDA) is a neurotoxin that selectively destroys dopaminergic neurons and terminals. We infused 6-OHDA in the dorsal striatum to produce dopaminergic deafferentation.
Note: *Originally, these studies were proposed to be conducted in awake, freely-moving rats. We faced a lot of challenges in conducting electrochemical recordings from dopamine-depleted animals due to impairments in locomotor activity. Therefore, these studies were conducted in anesthetized animals. As we were interested in exploring the role of BDNF, and it is easier to experimentally genetically manipulate mice, these studies were conducted in mouse species. In future, we wish to expand this research in BDNF mutants, so mouse was an ideal model for this aspect of the study.*

Methods: a) Lesion surgeries: Mice were anesthetized with isoflurane and placed in the stereotaxic frame. The skull was open and holes were drilled at the following coordinates (given relative to bregma: A/P: +1.0, M/L: ±1.7, D/V: -3.0). Mice received bilateral infusions of either 6-OHDA; 4µL/hemisphere of a 2µg/µL in 0.2% ascorbic acid saline solution) or saline (4µL/hemisphere). The animals were allowed to recover and prepared for amperometric recordings two weeks later.
b) Amperometric recordings: Urethene-anesthetized mice were prepared for amperometric recordings of glutamatergic activity. Ceramic-based, multi-site microelectrodes featuring four rectangular (15x333 µm) platinum- recording sites arranged side-by-side in pairs were coated with glutamate oxidase (GluOx) and prepared for *in vivo* recordings of glutamate activity as described above. Single barrel glass capillaries (~15 µm diameter) were pulled using a micropipette puller and attached to the electrode with the tip positioned between all four recording sites and 70-100 µm from the surface of the electrode. The assembly was lowered via

microdrive into the dorsal striatum of the anesthetized animal. Drug solutions (KCl (70mM; 200 nL), TTX (100 µM; 1 µL), and BDNF (1-100 ng/µl) were pressure ejected from the micropipette via picospritzer and the volume of each ejection was monitored by stereomicroscope fitted with a reticule. Recordings were made every 500 ms using FAST-16 recording system (Quanteon). Following recording, animals were perfused with 4% paraformaldehyde and serial sections were stained either with cresyl violet to confirm probe placements or to confirm dopamine lesions.

c) Immunohistochemistry: 6-OHDA lesions were confirmed by visualizing dopaminergic terminals with tyrosine hydroxylase (TH) immunostaining using a monoclonal mouse primary antibody. HRP-conjugated secondary antibody was used to develop staining with diaminobenzidine. Sections were dehydrated, coverslipped with permount and observed under bright-field Leica Microscope.

Results: Local application of BDNF produced a dose-dependent increase in glutamate signal amplitudes (**Fig. 8A**; $F(3,15) = 12.17$, $p = 0.001$). Moreover, co-administration of TTX with BDNF reduced glutamate release confirming neuronal origin of the neurotransmitter (**Fig. 8B**). BDNF also increased depolarization-evoked glutamate release in the dorsal striatum (saline: 7.21 ± 0.51 µM; BDNF 10ng: 10.09 ± 0.82 µM; $p < 0.05$). Representative sections depicting TH immunoreactivity in the dorsal striatum of control and lesioned mice are shown (**Fig. 9A**). 6-OHDA produced ~80% loss of dopaminergic terminals. Neuronal component of resting glutamate levels reduced following TTX infusion in 6-OHDA lesions animals (saline: 1.43 ± 0.23 µM; 6-OHDA: 2.2 ± 0.39 µM; $p < 0.05$; **Fig. 9B**) confirming inhibitory dopamine tone over glutamatergic transmission. However, BDNF-induced enhancement of depolarization-evoked glutamatergic activity in lesioned animals remained identical to sham animals (**Fig. 9C**). These findings suggest that acute effects of BDNF are specific to glutamatergic synapse only and do not involve dopamine release.

Experiment 5: AMPA receptor antagonism in the dorsolateral striatum attenuates the acquisition of cocaine-induced behavioral sensitization.

Rationale: AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid) glutamate receptors are involved in cocaine-induced synaptic alterations in the nucleus accumbens. However, the role of these ionotropic glutamate receptors in the dorsal striatum has not been elucidated. If AMPA receptors in the dorsal striatum play a necessary role in neuroplasticity induced by cocaine, then administration of the AMPA receptor antagonist CNQX should prevent the development of cocaine-induced behavioral sensitization when administered prior to each cocaine injection.

Methods: Rats were bilaterally infused with either vehicle (0.9% saline) or the AMPA receptor antagonist CNQX (0.3µg /0.5µl/side) into the dorsolateral (DL)- striatum 15 mins prior to a cocaine (20 mg/kg, i.p.) injection for 7 days. After a 10-day drug-free period, all rats were challenged with cocaine (20 mg/kg, i.p.) and behavioral responses were measured for 60 min after the injection.

Results: In the absence of CNQX (**Fig. 10**; red bars), rats injected repeatedly with cocaine on Days 1-7 had a significantly greater ambulatory response to a cocaine challenge compared with control animals injected with saline on Days 1-7, indicating a sensitized behavioral response.

Bilateral intrastriatal infusion of CNQX (blue bars) blocked the sensitized behavioral response in rats injected with repeated cocaine. In the absence of CNQX (red bars), rats injected repeatedly with cocaine on Days 1-7 had a significantly greater stereotypy response to a cocaine challenge compared with control animals injected with saline on Days 1-7, indicating sensitization to stereotypy (**Fig. 10**). Bilateral intrastriatal infusion of CNQX (blue bars) blocked the sensitized behavioral response in rats injected with repeated cocaine.

Discussion

The present study shows that chronic cocaine treatment produced robust reduction in the clearance of depolarization-evoked glutamate signals. Furthermore, such slower decay in clearance rate was also seen in signals-evoked by glutamate transporter blocker, TFB-TBOA. These findings suggest that chronic cocaine downregulates glutamate transporter-mediated clearance mechanisms leading to an increase in resting glutamate levels. As cocaine itself increases dopamine by inhibiting the reuptake process, and this neurotransmitter inhibits glutamate release, reduced potassium-evoked signals in cocaine-sensitized but unchallenged animals may indicate increased dopamine tone in these animals. These findings are further strengthened by the dopamine lesions studies that show enhanced resting glutamate levels following the removal of dopaminergic afferents. TTX-infusions reduced baseline glutamate levels in the control group supporting the notion that a fraction of glutamate present in the extracellular space is impulse-dependent. Attenuation of TTX-dependent component of glutamate in cocaine pretreated and cocaine challenged animals is suggestive of the presence of compensatory mechanisms that normalizes extrasynaptic glutamate levels in the presence of glutamate transporter blockade.

BDNF enhances conditioned reward to cocaine and higher BDNF levels in mesolimbic structures during cocaine withdrawal are associated with relapse. Moreover, the BDNF gene polymorphism is linked to drug addiction. Augmentation of resting and depolarization-evoked glutamate release following the local application indicates that this protein modulates glutamatergic transmission. As cocaine upregulates BDNF expression, higher resting glutamate levels may occur independent of GLT-1-driven mechanisms. Absence of BDNF-induced enhancement of depolarization-evoked glutamatergic activity in 6-OHDA-lesioned animals further supports this assumption.

Repeated systemic administration of GLT-1 activator CTX reversed the effects of repeated cocaine administration on glutamate signal kinetics reflecting the involvement of GLT-1 in such modulatory effects. CTX also inhibited hyperactivity and attenuated the development of stereotypical sensitization to repeated cocaine exposure in rats. Moreover, GLT-1 expression in the dorsal striatum is increased by repeated CTX administration. It is not clear why CTX exposure in saline treated animals did not facilitate the clearance of potassium-evoked signals. As the kinetics of signals include both the terminal release and transporter-mediated uptake process, it is possible the former might have subsided the effects of CTX on glutamate clearance. However, reversal of decayed clearance of striatal glutamate by CTX in chronic cocaine treated animals provides strong support for the hypothesis that glial glutamate transporter is a critical player in cocaine-mediated effects on altered glutamate dynamics and behavioral changes.

Intrastriatal infusion of the AMPA receptor antagonist CNQX prevented the acquisition of behavioral sensitization to cocaine in rats supporting the notion that ionotropic glutamate receptors in the dorsolateral striatum are critically involved in the development of sensitization to repeated cocaine. Increased extracellular glutamate during chronic cocaine treatment may produce sustained activation of AMPA receptors and their trafficking in medial spiny neurons of the dorsolateral striatum leading to the sensitization of locomotor behavior. Future studies are warranted to determine the contribution of striatal NMDA glutamate receptors in cocaine-induced behavioral plasticity.

Taken together, our results suggest that behavioral sensitization to chronic cocaine involves alterations in striatal glutamate homeostasis and overactivation of AMPA receptors. Repeated systemic administration of a GLT-1 activator (β-lactam antibiotic CTX) attenuates the development of stereotypical and locomotor sensitization and normalizes resting glutamate levels following repeated cocaine exposure in rats.

Significance:

Chronic drug use leading to drug addiction is associated with impairments in reinforcement seeking behaviors. The pathological mechanisms associated with these maladaptive behaviors are not known. The present study suggests that chronic cocaine treatment perturbs glutamate homeostasis in the dorsal striatum presumably by inhibiting the glial transport process. As a consequence, resting striatal glutamate levels become elevated and overactivate inototropic (AMPA) glutamate receptors. It is also possible the augmented resting glutamate levels following cocaine withdrawal may also result from upregulated BDNF expression. These neuroadaptive changes in the dorsal striatum are associated with increased locomotor activity and stereotypy in rats following chronic cocaine use, as well as during drug challenge following the withdrawal state. As locomotor/stereotypic behavior involves activation of the dorsal striatum, and this brain region is also activated during habit learning, these results indicate that drugs of abuse hyperactivate this region during behavioral sensitization via glutamate-dependent mechanisms, and therefore promote habitual use of drugs. Taken together, our studies provide novel insights into the contribution of glutamatergic mechanisms that regulate cocaine-induced striatal plasticity and compulsive drug seeking and use. Furthermore, glial glutamate transporter and BDNF represent potential biological targets to be exploited for the development of therapeutic strategies for drug addiction.

Abstracts/Presentations:

1) Naughton SX, Shi X, Kelley LK, Rawls SM, Unterwald EM, Parikh V. Glutamatergic contributions to cocaine-induced neuroadaptations in dorsolateral striatum and behavioral sensitization. **Society for Neuroscience Abstracts** 2011; 41:99.
2) Naughton SX, Shi X, Kelley LK, Rawls SM, Unterwald EM, Parikh V. Glutamatergic contributions to cocaine-induced neuroadaptations in dorsolateral striatum and behavioral sensitization. Temple University **Center for Substance Abuse Research (CSAR) Retreat,** September 12, 2012; Philadelphia.

Figures

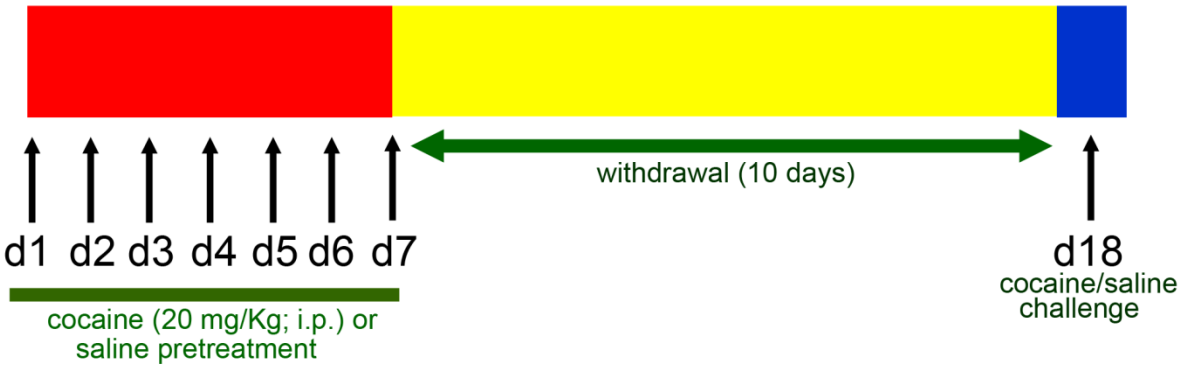

Figure 1: Rats were injected with cocaine (20 mg/Kg; i.p.) or saline once daily for 7 days. Following a 10-day withdrawal period, animals received a challenge dose of either cocaine or saline.

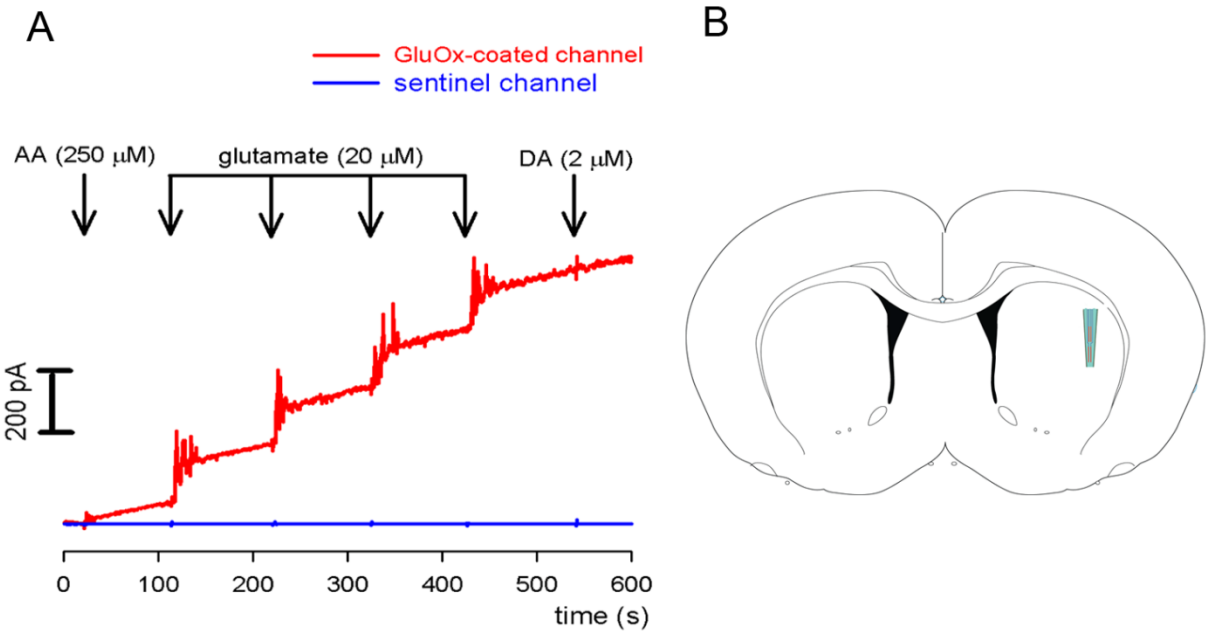

Figure 2: A) *In vitro* calibration of GluOx- and BSA-coated (sentinel) recording sites. Raw current traces depicting the responses to increasing concentrations of glutamate are shown. Note absence of current changes on the sentinel channel following glutamate additons. mPD-electroplating blocked the effects of interfering analytes on both channels. B) Illustration depicting the placement of the biosensor containing four recording sites when placed into the dorsolateral striatum.

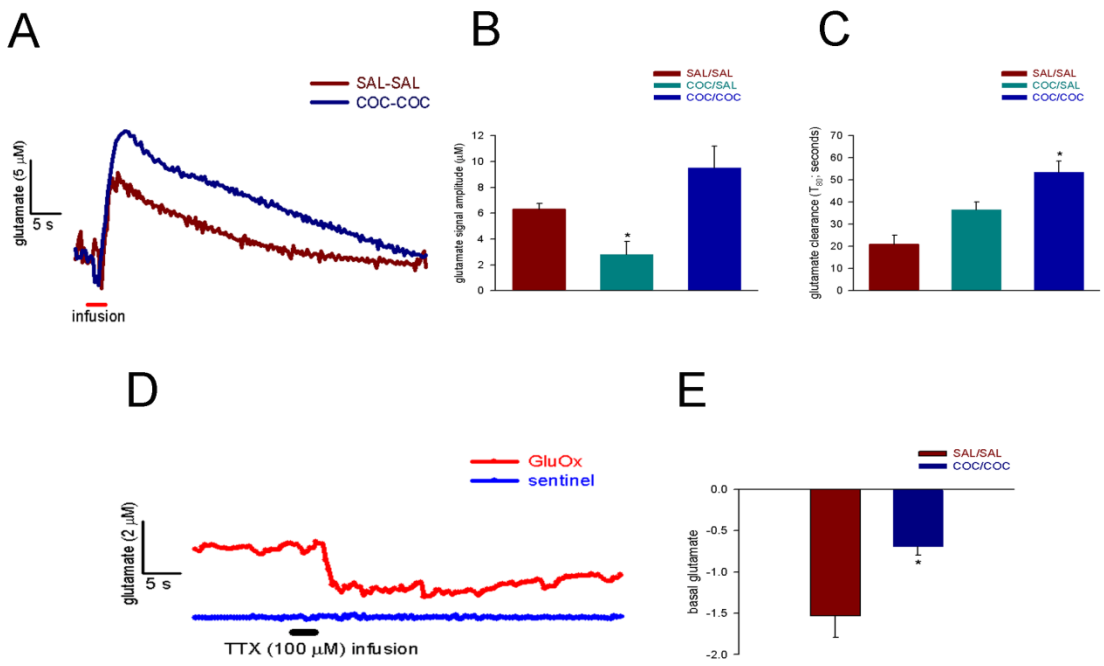

Figure 3: Effects of cocaine pretreatment on glutamate dynamics. A) Representative traces of glutamate signals following KCl (70mM; 1uL) infusions. B,C) Bar charts depicting potassium-evoked glutamate signal amplitudes and clearance. D) Electrochemical recordings from enzyme-coated and sentinel channel following TTX infusion. E) Resting neuronal glutamate levels were lower in COC/COC group. *, $p < 0.05$

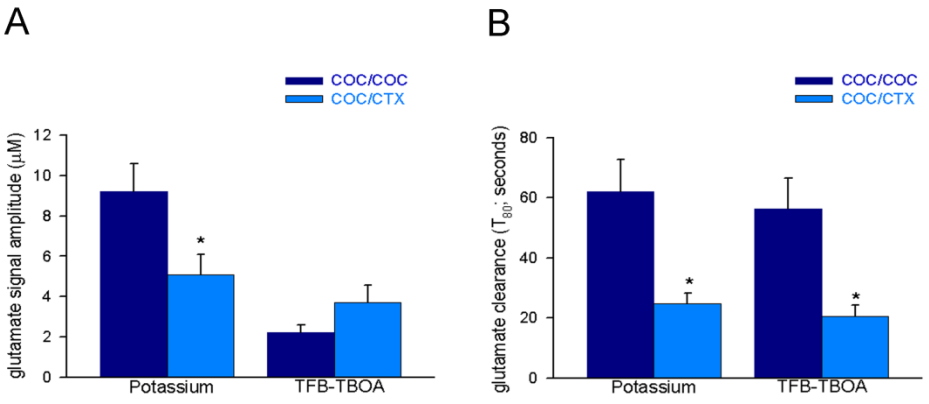

Figure 4: Effects of CTX on striatal glutamate levels following intrastriatal infusions of potassium and TFB-TBOA (a non-specific glial transporter blocker). CTX reduced depolarization-evoked glutamate release in chronic-cocaine treated animals (A). Furthermore, reduced clearance of potassium/TFB-TBOA-induced glutamate signals in cocaine pretreated animals returned to baseline levels with CTX coadminstration (*, $p < 0.05$ vs. COC/COC).

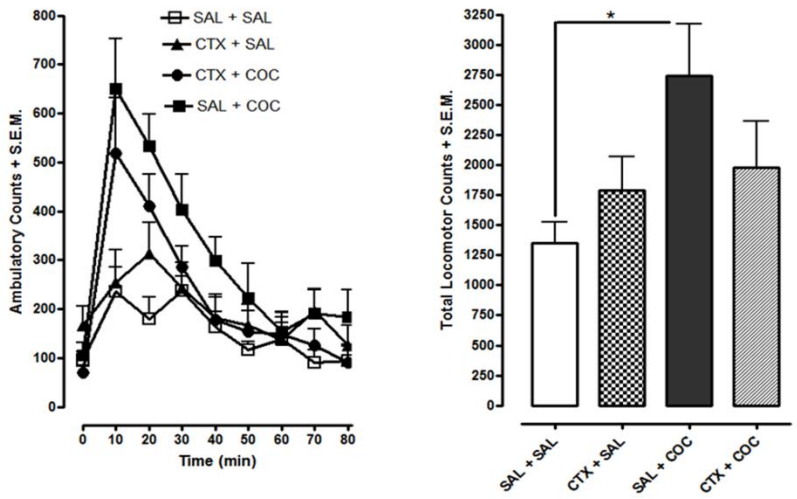

Figure 5: Effect of repeated CTX treatment on sensitization of ambulatory activity produced by repeated cocaine administration. Data are presented as mean ambulatory counts ± S.E.M on the day of cocaine challenge. N = 7-8 rats/group. *$p < 0.05$ compared to SAL + COC group in cumulative graph.

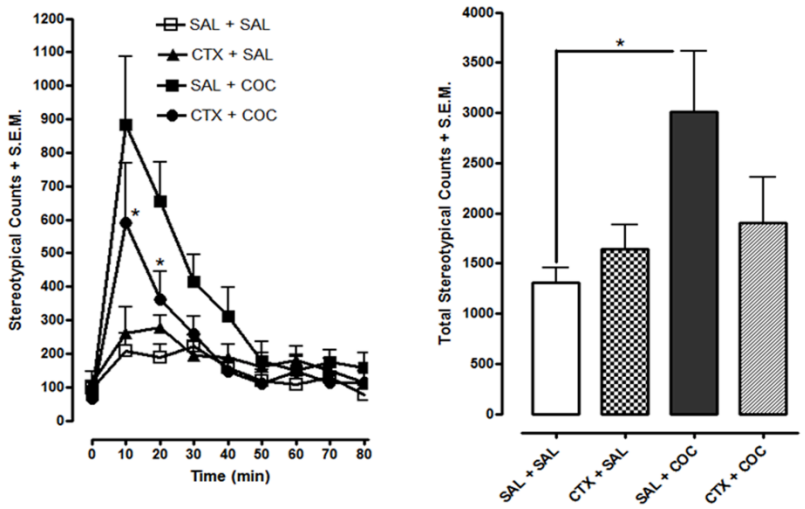

Figure 6: Effect of repeated CTX treatment on sensitization of stereotypical activity produced by repeated cocaine administration. *$p < 0.05$ compared to SAL + COC group in time course and cumulative graphs.

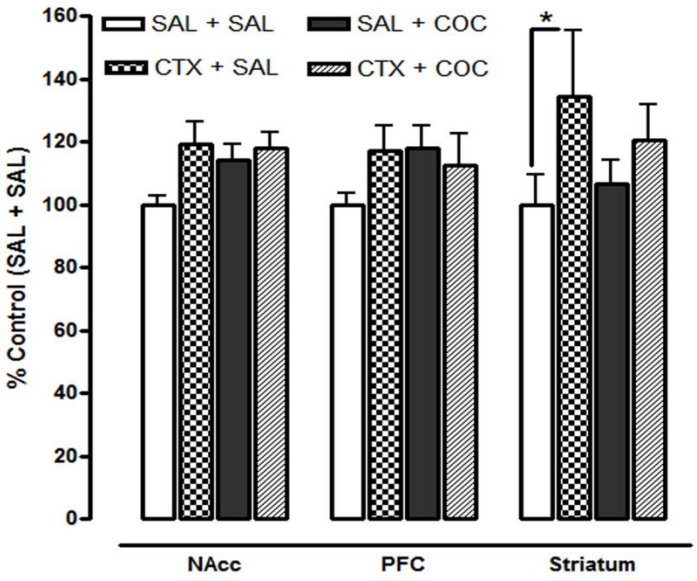

Figure 7: Effects of repeated CTX on basal and cocaine-induced changes in GLT-1 expression in the nucleus accumbens (NAcc), prefrontal cortex (PFC), and striatum of rats. Rats were euthanized 180 min following cocaine challenge and brains were extracted for Western blot analysis to quantify GLT-1 protein levels. *p < 0.05 compared to respective SAL + SAL group.

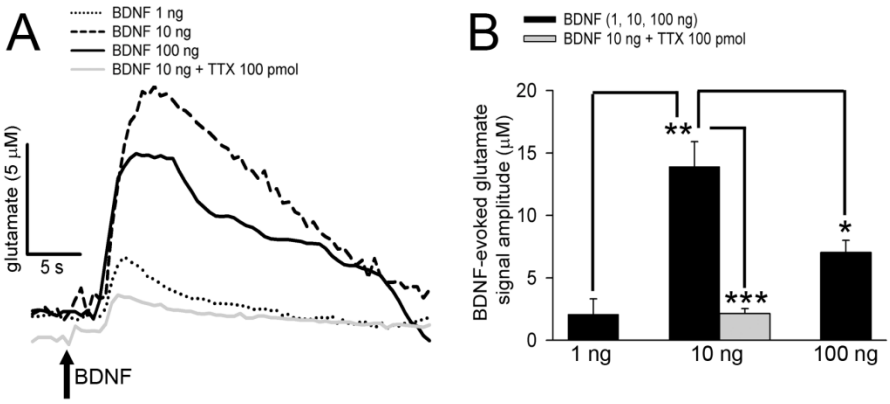

Figure 8: A) Representative traces of glutamate signals following local infusions of BDNF and attenuation with TTX. B) Bar charts depicting dose-dependent BDNF-induced increases in striatal glutamatergic activity. TTX administration profoundly reduced glutamate signal amplitudes. *, **, *** $p < 0.05$, 0.01, 0.001 *post hoc* comparisons.

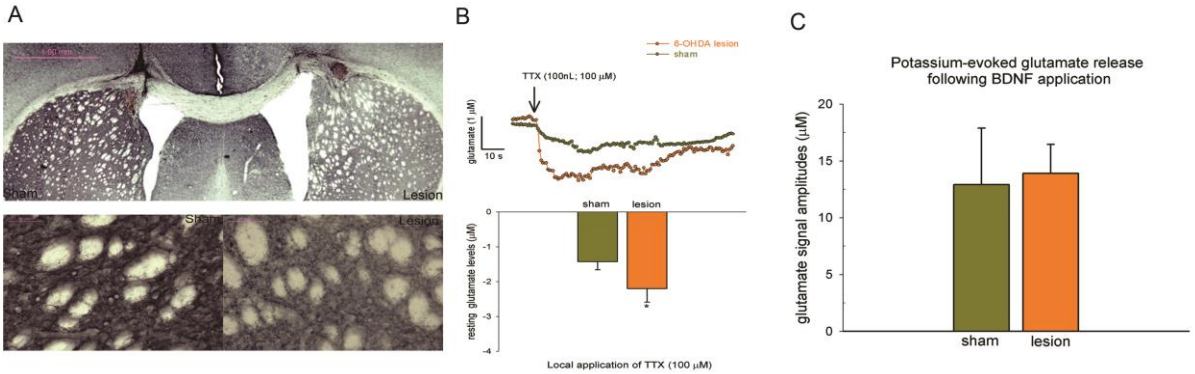

Figure 9: A) TH-stained coronal section illustrating the loss of dopaminergic inputs to dorsal striatum following 6-OHDA lesion. B) TTX-dependent resting glutamate levels were enhanced in dopaminergic deafferented animals (*, $p < 0.05$ vs. control). C) BDNF-induced increases in the amplitudes of potassium-evoked glutamate signals did not differ between the sham and 6-OHDA lesioned animals.

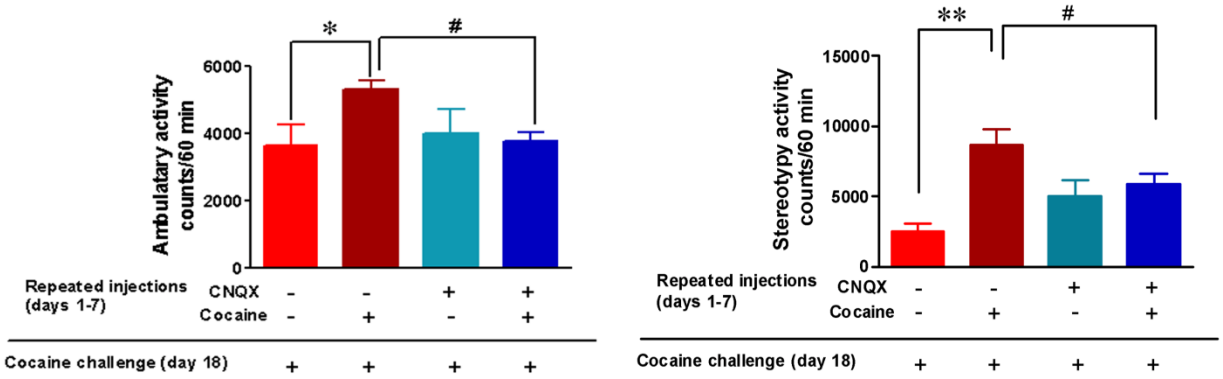

Figure 10. Left) Effect of AMPA receptor blockade on the acquisition of *ambulatory* behavioral sensitization to cocaine. Data are presented as mean ± SEM; n = 5–7 rats/group (*p < 0.05, #p < 0.05). Right) Effect of AMPA receptor blockade on the acquisition of *cocaine*-induced behavioral sensitization to *stereotypy* in rats. Data are presented as mean ± SEM; n = 5–7 rats/group (**p < 0.01, #p < 0.05). Bilateral intrastriatal infusion of CNQX blocked the sensitized behavioral response in rats injected with repeated cocaine.

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, the number of the publication and an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two

publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

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.	D'Amore DE, Tracy BA, Parikh V.	Exogenous BDNF facilitates strategy set-shifting by modulating glutamate dynamics in the dorsal striatum	June, 2013	☐Submitted ☐Accepted ☒Published
2.				☐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:

We have 1 research manuscript based on these data that is currently under preparation (below). We anticipate submitting this papers for publication within the next few months.

1. **Parikh V, Tracy B, Yegla B, Naughton SX. Discrimination learning and flexible cognitive control in BDNF heterozygous mice: effects of striatal dopamine depletion *(in preparation).***

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.

Drug addiction is a complex psychiatric relapsing disorder that affects emotional, psychological and social functions. Currently, over 23 million Americans are affected by this disease. However, the neurobiological basis of drug addiction still remains unknown. Drugs highjack the brain by affecting cognitive control processes. A major outcome of this research was that we were able to demonstrate that chronic drug use affects glutamate signaling in the brain circuits that are critical for cognitive control processes and decision-making. These findings have major implications not only for understanding the neurobiological basis of drug addiction but also to develop therapeutic approaches to treat this pathology.

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.

Our study shows that chronic cocaine may affect decision-making abilities by producing neuroadaptations in the corticostriatal circuits by perturbing glutamate signaling in the dorsal striatum. Mechanistically, these alterations involve inhibition of the glial transport process and consequently, overactivation of AMPA glutamate receptor signaling by augmented baseline glutamate during the abstinence phase of addiction. These novel findings indicate that heightened glutamate activity in the striatum may interfere with the cognitive control processes thereby promoting compulsive and habitual drug use. More importantly, the data generated from this research led us to propose that glutamatergic system may be a potential target for drug development research on addiction and drugs that stabilize glutamatergic signaling seem to hold promise in treating this disorder. Specifically, glial glutamate transporters and AMPA receptors appear to be viable biological targets that may be exploited for developing to control compulsive drug use.

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. *For Nonformula grants only – include information*

for only those key investigators whose biosketches were not included in the original grant application.

A 2-pg biosketch of all PIs (Parikh, Unterwald and Rawls) is attached below.

BIOGRAPHICAL SKETCH: VINAY PARIKH, Ph.D.

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person.

NAME	POSITION TITLE
Vinay Parikh, Ph.D.	Assistant Professor of Neuroscience
eRA COMMONS USER NAME (credential, e.g. agency login) parikhv	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
Dr. H.S.Gour University, India	B.Pharm	1992	Pharmaceutical Sciences
Gujarat University, India	M.Pharm	1994	Pharmacology
Punjabi University, India	Ph.D.	1999	Pharmacology
Medical College of Georgia, USA	Post-doc	2001-2003	Neurochemistry/Psychopharmacology
Ohio State University, USA	Post-doc	2003-2004	Neuroscience/Psychopharmacology
University of Michigan, USA	Post-doc	2004-2007	Neuroscience/Psychopharmacology

A. Position and Honors:

Positions and Employment

1992-1994: Junior Research Fellow, Gujarat University, India
1994-1998: Senior Research Fellow, Department of Pharmaceutical Sciences and Drug Research, Punjabi University, India
1998-2001: Executive Scientist, Department of Biological Research, Sun Pharma Advanced Research Center, Sun Pharmaceutical Industries Ltd., India
2001-2003: Postdoctoral Fellow, Department of Psychiatry and Health Behavior, Medical College of Georgia, Augusta, GA, USA
2003-2004: Postdoctoral Researcher, Department of Psychology, Psychobiology and Behavioral Neuroscience Area, Ohio State University, Columbus, OH, USA
2004-2005: Postdoctoral Research Associate, Department of Psychology and Neuroscience program, University of Michigan, Ann Arbor, MI, USA
2005-2009: Assistant Research Scientist, Department of Psychology and Neuroscience program, University of Michigan, Ann Arbor, MI, USA
09/2009 - Assistant Professor (tenure-track), Department of Psychology and Neuroscience Program, Temple University, Philadelphia, PA, USA

Other Experience and Professional Memberships

2004-present: Member, Society for Neuroscience
2005-present: Member, International Behavioral Neuroscience Society
2006-present: Member, American Society for Neurochemistry
2006-present: Member, International College of Neuropsychopharmacology
2007-present: Review Editor – Frontiers in Integrative Neuroscience
2009-present: Adhoc Grant Reviewer – Netherlands Organization for Scientific Research
2010- present: Adhoc Grant Reviewer - Alzheimer's Association
2010-present: Scientific Review Associate – European Journal of Neuroscience

Honors

1992: Magna cum laude, Dr. H.S.G University, India
1992: G. P. Nair Award, Indian Drugs Manufacturer's Association, India
1992-1994: Junior Research Fellowship, University Grants Commission, India
1995-1998: Senior Research Fellowship, Council of Scientific and Industrial Research, India
1996: Achari Award, Indian Pharmacological Society, India
1997: Young Scientist Award, Indian Science Congress Association, India
2003: Young Investigator Award, International Congress of Schizophrenia Research, USA
2006: Travel Award, Winter Conference of Brain Research, USA
2006: Rafaelson Young Investigator Award, International College of Neuropsychopharmacology,USA
2008: Research Faculty Recognition Award, University of Michigan, USA
2010: New Investigator Award in Alzheimer's disease, American Federation for Aging Research, USA
2010: NARSAD Young Investigator Award, USA

B. Selected peer-reviewed publications (in chronological order; out of 44 total):

1) Parikh V, Evans DR, Khan MM, Mahadik SP. Nerve growth factor levels in never medicated first episode psychotic patients and medicated chronic schizophrenic patients. *Schizophrenia Research* 2003; 60:117-123
2) Parikh V, Terry AV, Khan MM, Mahadik SP. Modulation of nerve growth factor and choline acetyltransferase expression in rat hippocampus after chronic exposure with haloperidol, risperidone and olanzapine. *Psychopharmacology* 2004; 172:365-374.
3) Sarter M, Parikh V. Choline transporters, cholinergic transmission and cognition. *Nature Reviews Neuroscience* 2005; 6:48-56.
4) Parikh V, Kozak R, Martinez V, Sarter M. Prefrontal acetylcholine release controls cue detection on multiple time scales. *Neuron* 2007; 56: 141-54.
5) Parikh V, Man K, Decker MW, Sarter M. Glutamatergic contributions to nAChR agonist-evoked cholinergic transients in the prefrontal cortex. *The Journal of Neuroscience* 2008; 28: 3769-3680.
6) Parikh V, Ji J, Decker MW, Sarter M. β2 subunit-containing and α7 nAChRs differentially control prefrontal glutamatergic and cholinergic signaling. *The Journal of Neuroscience* 2010; 30:3518-3530. PMCID: PMC2864641
7) Howe WM, Ji J, Parikh V, Williams S, Sarter M. Enhanced shifting from endogenous to exogenous attention by selective stimulation of α4β2* nAChRs: underlying cholinergic mechanisms. *Neuropsychopharmacology* 2010; 35:1391-1401. PMCID: PMC2855755
8) Ortega LA, Tracy BA, Gould TJ, Parikh V. Effect of chronic low- and high-dose nicotine on cognitive flexibility in C57BL/6J mice. *Behavioral Brain Research* 2013; 238: 134-145.
9) Parikh V, Howe WM, Welchko R, Naughton SX, Han D, D'Amore DE, Turner DL, Sarter M. Diminished trkA receptor signaling reveals cholinergic-attentional vulnerability of aging. *European Journal of Neuroscience* 2013; *37: 278-293*. PMID: 23228124

D. Research Support

Ongoing Grant Support

1) NARSAD *Parikh (PI)* 07/15/2011-07/14/2014
Functional interactions between BDNF and glutamatergic signaling in fronto-striatal circuits. Role: PI
2) *1R01MH086530-01A1 Sarter (PI)* 07/15/2010 - 06/30/2015
NIH/NIMH
Choline transporter capacity limits motivated behavior in mice, rats, and humans
Role: Co-Investigator

BIOGRAPHICAL SKETCH

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

NAME	POSITION TITLE
Unterwald, Ellen M.	Professor of Pharmacology and Director, Center for Substance Abuse Research
eRA COMMONS USER NAME ellen01	

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

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Rhode Island, Kingston, RI	BS, RPh	1980	Pharmacy
Boston Univ School of Medicine, Boston, MA	PhD	1985	Pharmacology
Albert Einstein College of Medicine, Bronx, NY	Post-doc	1985-1988	Neuroscience

Positions

1981-1985 Graduate Research Assistant, Laboratory of Behavioral Pharmacology (Dr. Conan Kornetsky) Boston University School of Medicine, Boston, MA

1985-1988 Post-doctoral Research Fellow, Department of Neuroscience (Dr. R. Suzanne Zukin) Albert Einstein College of Medicine, Bronx, NY

1988-1994 Research Associate, Laboratory of the Biology of Addictive Diseases (Dr. Mary Jeanne Kreek) The Rockefeller University, New York, NY

1994-1998 Assistant Professor, Dept. Psychiatry, New York University Medical Center, New York, NY

1994-present Guest Investigator, Lab of the Biology of Addictive Diseases, Rockefeller Univ, New York, NY

1998-2006 Associate Professor, (with tenure 7/01) Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA

2006-present Professor, Department of Pharmacology, Temple University School of Medicine, Phila, PA

2007-present Professor, Center for Substance Abuse Research, Temple University, Phila, PA.

2008-present Director, Center for Substance Abuse Research, Temple University, Phila, PA.

Federal Government Public Advisory Committees (selected):

1998-2003 Member, Training & Career Development Subcommittee, NIDA Initial Review Group (NIDA-K)

2001 Ad Hoc Reviewer, Research Centers in Minority Institutions Review Committee, NCRR Initial Review Group (RIRG-M 02)

2001-2005 Reviewer for NIDA "Cutting Edge Basic Research Award" (CEBRA) Program

2004 Special Emphasis Panel ZDK1 GRB03 "Training for a New Interdisciplinary Workforce; Roadmap initiative"

2004-2006 Ad Hoc Member of Biobehavioral Regulation, Learning and Ethology (BRLE) Study Section

2006-2007 Member, Biobehavioral Regulation, Learning and Ethology (BRLE) Study Section

2009-2011 Member, Training & Career Development Subcommittee, NIDA-K, NIDA/NIH

2012 Reviewer for Centers of Biomedical Research Excellence (COBRE) grants, ZRR1 RI-B 01.

2012 Editorial Board Member for Stage 2 review of Specialized Center of Research (SCOR) on Sex Differences, ZRG1 EMNR-Q(50)R
2009-2013 Member, Molecular Neuropharmacology and Signaling (MNPS) review panel Chair, 2011-2013

Membership in Professional Societies (selected):
1993-present Member, College on Problems of Drug Dependence Program Committee, 2006-2010
1994-2000 Associate Member, American College of Neuropsychopharmacology
1984-present Society for Neuroscience Vice President of the Philadelphia Chapter of SFN, 2002-2006; President of the Philadelphia Chapter of SFN, 2006-2009
1985-present International Narcotics Research Conference; Executive Committee, 2009-2012
2002-present American Society for Pharmacology and Experimental Therapeutics

Peer-reviewed publications (selected)

Perrine SA, Sheikh IS, Nwaneshiudu C, Schroeder JA, and Unterwald EM, Withdrawal from chronic cocaine decreases delta opioid receptor signaling and increases anxiety- and depression-like behaviors in the rat. Neuropharmacology, 54:355-364, 2008. PMID: 18045627

Soderman AS, Unterwald EM. Cocaine reward and hyperactivity in the rat: Sites of mu opioid receptor modulation. Neuroscience, 154(4):1506-16, 2008. PMID: 18550291

Niculescu, M., Perrine, S.A., Ehrlich, M.E., Unterwald, E.M. TrkB: a neuromodulator of age-specific behavioral responses to cocaine in mice, Journal of Neuroscience, 28:1198-1207, 2008. PMID: 18234897

Perrine SA, Miller JM, Unterwald EM. Cocaine regulates protein kinase B and glycogen synthase kinase-3 activity in selective regions of rat brain. Journal of Neurochemistry, 107:570-577, 2008. PMID: 18717814

Miller JS, Tallarida RJ, Unterwald EM. Cocaine-induced hyperactivity and sensitization are dependent on GSK. Neuropharmacology, 56(8):1116-23, 2009. PMID: 19328817

Nwaneshiudu CA, Unterwald EM. NK-3 receptor antagonism prevents behavioral sensitization to cocaine: a role of glycogen synthase kinase-3 in the nucleus accumbens. Journal of Neurochemistry, 115(3):635-42, 2010. PMID 20807318

Randall-Thompson JF, Pescatore KA, Unterwald EM. A role for delta opioid receptors in the central nucleus of the amygdala in anxiety-like behaviors. Psychopharmacology 212:585-595, 2010. PMID: 20730419

Carvalho AF, Reyes AR, Sterling RC, Unterwald E, Van Bockstaele EJ. Contribution of limbic norepinephrine to cannabinoid-induced aversion. Psychopharmacology, 211:479-491, 2010. PMID: 20602088

Rasmussen B, Unterwald EM, Rawls SM. Glutamate transporter subtype 1 (GLT-1) activator ceftriaxone attenuates amphetamine-induced hyperactivity and behavioral sensitization in rats. Drug and Alcohol Dependence, 118:484-488, 2011. PMID:21524862

Enman NM, Unterwald EM. Inhibition of GSK3 attenuates amphetamine-induced hyperactivity and sensitization in the mouse. Behavioral Brain Research 231(1):217-25, 2012. PMID: 22649795

Reith ME, Ali S, Hashim A, Sheikh IS, Theddu N, Gaddiraju NV, Mehrotra S, Schmitt KC, Murray TF, Sershen H, Unterwald EM, Davis FA. Novel C-1 substituted cocaine analogs unlike cocaine or benztropine. Journal of Pharmacology and Experimental Therapeutics 343(2):413-25, 2012. PMID: 22895898

BIOGRAPHICAL SKETCH

Provide the following information for the key personnel and other significant contributors in the order listed on Form Page 2.
Follow this format for each person.

NAME Scott Manning Rawls	POSITION TITLE Associate Professor of Pharmacology Department of Pharmacology and Center for Substance Abuse Research Temple University School of Medicine
eRA COMMONS USER NAME srawls01	

EDUCATION/TRAINING *(Begin with baccalaureate or other initial professional education, such as*

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
East Carolina University	BS	1990	Chemistry
East Carolina University School of Medicine	PhD	1999	Neuroscience
Temple University School of Medicine	Post-doc	2000-02	Pharmacology

A. POSITIONS AND HONORS

Positions

2002-2003 Assistant Professor of Biology, Department of Biology, Washington College (MD)

2003-2009 Assistant Professor of Pharmacology, Department of Pharmaceutical Sciences, Temple University School of Pharmacy

2009-2011 Associate Professor of Pharmacology (tenured), Department of Pharmaceutical Sciences, Temple University School of Pharmacy

2011-present Associate Professor of Pharmacology (tenured), Department of Pharmacology, Center for Substance Abuse Research, Temple University School of Medicine

B. SELECTED PUBLICATIONS (from 73 peer-reviewed publications)

Rawls S.M., Tallarida R.J., Robinson W. and Amin M.: The β-lactam antibiotic, ceftriaxone, attenuates morphine-evoked hyperthermia in rats. *Br. J. Pharmacol.* **151** 1095-1102 (2007).

Yulin W., Chen Y., Xu W., Lee D., Rawls S.M., Cowan A., and Liu-Chen L. 2-methoxymethyl-salvinorin B is a potent kappa opioid receptor agonist with longer-lasting action *in vivo* than Salvinorin A. *J. Pharmacol. Exp. Ther.* **324** 1073-1083 (2008).

Rawls S.M., Cavallo F., Ding Z., and Raffa R.B.: A β-lactam antibiotic (ceftriaxone) inhibits physical dependence and abstinence-induced withdrawal in planarians. *Eur J Pharmacol.* **584** 278-284 (2008).

Rawls S.M., Robinson W., Patel S. and Baron A.: β-lactam antibiotic prevents kappa opioid tolerance by a glutamate-dependent mechanism. *Neuropharmacology*, **55**:865-70 (2008).

Ward S.J., Whiteside G., Kim J., Rawls S.M. and Walker E.A.: Effects of cannabinoid CB_1 receptor antagonist SR 141716A on feeding behavior across development. *Psychopharmacology*, **206**:155-165. (2009).

Wu H., Tzeng N., Qian L., Wei S., Hu X., Rawls S.M., Flood P., Hong J.S. and Lu R.: Glia mediate memantine-elicited neuroprotection in midbrain neuron/glia cultures: beyond NMDA receptors. *Neuropsychopharmacology*, **34**:2344-2357 (2009).
Rawls S.M., Patel H., Zielinski M., Sacavage S., Baron D.A., and Patel D.: β-lactam antibiotic reduces morphine analgesic tolerance in rats through GLT-1 transporter activation. *Drug and Alcohol Dependence*, 107:261-263 (2010).
Rasmussen B.A., Unterwald E.M. and Rawls S.M.: Methanandamide blocks the development of amphetamine-induced behavioral sensitization in rats. *Eur. J. Pharmacol*., 627:150-155 (2010).
Rawls S.M., Baron D.A., and Kim J.K.: β-lactam antibiotic inhibits development of morphine physical dependence in rats. *Behav. Pharmacol.,* 21:161-164 (2010).
Rawls S.M., Patil T., Kim J., and Raffa R.B.: First evidence that drugs of abuse produce behavioral sensitization and cross-sensitization in planarians. *Behav. Pharmacol.,* 21:301-13 (2010).
Rawls S.M., Karaca F., Madhani I.C., Bhogani V., Abou-Gharbia M., and Raffa R.B.: β-lactamase inhibitors display anti-seizure activity in invertebrates. *Neuroscience*, 169:1800-1804 (2010).
Rasmussen B.A., Baron D.A., Kim K., Unterwald E.M., and Rawls S.M.: β-lactam antibiotic produces a sustained reduction in extracellular glutamate in the nucleus accumbens of rats. *Amino Acids*, 40:761-764. (2011).
Rasmussen B.A., Unterwald E.M., and Rawls S.M.: GLT-1 transporter activator ceftriaxone inhibits amphetamine-induced hyperactivity and behavioral sensitization in rats. *Drug and Alcohol Depend.* 118 484-488 (2011).
Tallarida C., Song K., Raffa R.B., and Rawls S.M.: GCPII inhibitor displays anti-glutamate and anti-cocaine effects in an invertebrate assay. *Amino Acids* 42 2521-2524 (2012).
Ramoz L., Lodi S., Bhatt P., Reitz A., Raffa R.B., and Rawls S.M.: Mephedrone (bath salt) pharmacology: clues from an invertebrate assay. *Neuroscience* 208 79-84 (2012).
Kovalevich J., Corley G., Azdemir A., Yen W., Kim J., Rawls S.M., and Langford D.: Cocaine Decreases Expression of Neurogranin via Alterations in Thyroid Receptor/Retinoid X Receptor Signaling. *J. Neurochem.* 121 302-313 (2012).
Owaisat S., Raffa R.B., and Rawls S.M.: In vivo assessment of harmine efficacy against psychostimulants: preferential inhibition of cocaine-induced stereotypical activity through a glutamatergic mechanism. Neurosci. Lett. 525 12-16 (2012).
Lisek R., Xu W., Yuvasheva E., Chiu Y., Reitz A.B., Liu-Chen L.Y., and Rawls S.M.: Mephedrone ('bath salt') elicits conditioned place preference and dopamine-sensitive locomotor activation in rats. Drug and Alcohol Depend. in press (2012).
Kovalevich J., Corley G., Yen W., Rawls S.M., and Langford D.: Repeated cocaine exposure produces changes in white matter proteins in the nucleus accumbens that are normalized by a glutamate transport activator. Am. J. Pathol. in press (2012).

D. CURRENT RESEARCH GRANTS

R21DA032718-01A1 Role: Principal Investigator 8/15/12 – 8/14/14
NIH/NIDA Costs: $412,500
"Mephedrone and addiction: clues from animal models"
R21DA030676-01A1 Role: Principal Investigator 09/15/11 - 09/14/13
NIH/NIDA "Clavulanic acid: an abuse-deterrent and CNS-active therapeutic"
Costs: $420,750