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 – of the Commonwealth System of Higher Education

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** Germaine A Calicat, MLA

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

5. **Grant SAP Number:** 4100047651

6. **Project Number and Title of Research Project:** 6 - The Role of GSK3 in Psychostimulant-Induced Behaviors

7. **Start and End Date of Research Project:** 2/1/2009-7/31/2010

8. **Name of Principal Investigator for the Research Project:** Ellen M. Unterwald, PhD

9. **Research Project Expenses**.

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

   $ 25,000

   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
Enman	Graduate Assistant/PhD Student	75%	$25,000

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
Unterwald	Principal Investigator	5%

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_____ No____x_____

If yes, please describe your plans:

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

Based on the results of these studies, the plans are to concentrate our efforts in this area on cocaine, rather than on amphetamine. Results from these experiments show that the dependence on GSK3 is greater for cocaine than it is for amphetamine. We have obtained NIH funding for the further investigation of the role of GSK3 in cocaine-dependent actions (R01 DA09580; PI: Unterwald). The studies on cocaine were done by other lab personnel who were not supported by this stipend award to Ms. Nicole Enman.

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				
Female			1	
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 enhanced the research training of a PhD student at Temple University, thus leading to training of a new investigator in addition to completion of a research project.

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 application's strategic plan). Summarize the progress made in achieving these goals, objectives and aims for the entire grant award period. 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.

The Role of GSK3 in Psychostimulant-Induced Behaviors - Abuse of psychostimulants including cocaine and amphetamine continues to be a major public health problem that leads to loss of employment productivity, breakdown of families and communities, increases in crime and violence, and a variety of health issues not only for the individual but also for their children. Treatments for addiction to psychostimulants are lacking. As such, identification of potential novel targets for treatment of psychostimulant abuse is needed. This project will examine a novel cellular pathway that mediates some of the behavioral effects of cocaine and amphetamine. The role of the Akt-GSK3 pathway in psychostimulant-induced behaviors will be investigated, as will methods to regulate its function in order to reduce the addictive properties of these drugs.

Project Overview

Identifying the mechanisms underlying drug-induced reward and plasticity is critical for the design of therapeutics to treat psychostimulant addiction. One molecule that has received attention recently for its role in psychiatric disorders and the therapeutics used to treat them is glycogen synthase kinase-3 (GSK3). Evidence suggests that GSK3 is uniquely situated to modulate neuronal function and plasticity. Further, our published and preliminary data demonstrate that GSK3 activity is necessary for the manifestation of cocaine-induced behaviors and that the activity of GSK3 is regulated during cocaine exposure in a brain-region specific manner. The central hypothesis that will be investigated is that GSK3 is a critical molecular mediator of psychostimulant-induced actions including drug reward and drug-seeking behaviors. Further, we hypothesize that exposure to psychostimulants results in adaptations in GSK3 signaling and that these adaptations may underlie drug-induced neural plasticity contributing to the perpetuation of addictive disease.

The first research aim will be to determine the role of GSK3 in cocaine- and amphetamine-induced activity, sensitization, reward, and reinstatement. The contribution of GSK3 to the rewarding effects of cocaine and amphetamine will be determined using the conditioned place preference procedure in adult male mice. The role of GSK3 in the different phases of drug-induced reward will be evaluated including the development and expression of conditioned reward and the retrieval and reconsolidation of drug-associated contextual memories. The goal of this aim is to establish GSK3 as a target for new therapeutics for the treatment of addictive disease.

The second research aim will be to define the regulation of the GSK3 signaling pathway during cocaine and amphetamine exposure. The activity of GSK3 is regulated by its phosphorylation state. Changes in GSK3 phosphorylation, and hence activity, will be measured following acute and repeated administration of cocaine and amphetamine. Immunohistochemistry with antibodies that are selective for the phosphorylated state of GSK3 will be used to characterize the cellular and subcellular localization of drug-induced changes in GSK3 function. Identification of the cell types showing regulation of GSK3 activity will be determined, as will drug-induced changes in the subcellular distribution of GSK. GSK3 will be identified on tissue sections from mouse brain following administration of cocaine and amphetamine.

Expected Research Outcomes and Benefits

The goal of this project is to provide pre-clinical evidence to support the potential use of modulators of the GSK3 signaling pathway for the treatment of cocaine and/or amphetamine

addiction. These studies will determine if modulation of GSK3 can interfere with processes involved in addiction to these psychostimulants. In addition, this project will investigate how exposure to cocaine and amphetamine alters this important intracellular pathway in brain regions critically involved in mediating the rewarding and conditioned stimulus effects of drugs of abuse. Establishment of GSK3 as a novel target for the treatment of psychostimulant addiction could be a major public health advancement and significantly reduce the costs of addiction to the individual and to society.

Summary of Completed Research

Aim 1: Determine the role of GSK3 in amphetamine-induced activity, sensitization, and reward

Materials and Methods

Study 1: The effect of valproic acid (VPA) and SB 216763 on acute amphetamine-induced hyperactivity.
Subjects: Male CD-1 mice (8 weeks old) were obtained from Charles River Laboratories (Wilmington, MA). Mice were housed five per cage on a 12-h light/dark cycle with *ad libitum* access to food and water. Animals were allowed to acclimate to the animal facility for seven days prior to behavioral testing, and were weighed and handled daily. All animal testing was conducted in accordance with the National Institutes of Health guidelines for the Care and Use of Laboratory Animals. All experimental protocols were approved by the Institutional Animal Care and Use Committee of Temple University School of Medicine.
Drugs: Sodium valproate (Sigma Aldrich; St. Louis, MO) and *D*-amphetamine (NIDA; Bethesda MD) were dissolved in sterile saline (0.9% NaCl). SB 216763 (Tocris Bioscience; Ellisville, MO) was dissolved in 3.6% DMSO (Sigma) and brought up to volume in Tocrisolve 100 (Tocris Bioscience).
Locomotor activity: Locomotor activity was evaluated in automated monitors (AccuScan Instruments, Inc., Columbus, OH). The number of photocell beam breaks in each chamber was recorded by a computer equipped with the Digiscan DMicro system. The system differentially measures horizontal movement and repetitive beam breaks, which characterize ambulatory activity and stereotypic activity, respectively. Mice (n=11/group) were placed in the activity monitors for 30 minutes to acclimate, and then pretreated with saline or valproate (50-300 mg/kg, i.p.). Thirty minutes later mice were injected with saline or amphetamine (2 mg/kg, i.p.), and activity was measured for 60 minutes. Separate sets of mice (n=12/group) were pretreated with SB 216763 (2.5-5.0 mg/kg, i.p.) or vehicle 20 minutes prior to saline or amphetamine (2 mg/kg, i.p.), and locomotor activity was recorded for 60 minutes.
Data analysis: Ambulatory and stereotypy data were analyzed for significance using two-way ANOVA and Bonferroni post-hoc analysis.

Study 2: The effect of VPA and SB 216763 on the development of amphetamine-induced behavioral sensitization.
Subjects: See Study 1.
Drugs: Sodium valproate (Sigma Aldrich; St. Louis, MO) and *D*-amphetamine (NIDA; Bethesda MD) were dissolved in sterile saline (0.9% NaCl). SB 216763 (Tocris Bioscience; Ellisville,

MO) was dissolved in 3.6% DMSO (Sigma) and brought up to volume in Tocrisolve 100 (Tocris Bioscience).
Behavioral Sensitization: Mice (n=10-12/group) were pretreated once daily with VPA (300 mg/kg, i.p.) or saline 30 minutes prior to amphetamine (2 mg/kg, i.p.) or saline for five days. All mice were challenged with amphetamine (1 mg/kg, i.p.) in the absence of VPA seven days later. Ambulatory and stereotypic activities were measured for 30 minutes following amphetamine challenge. Separate sets of mice (n=8-12/group) were pretreated with vehicle or SB 216763 (5 mg/kg, i.p.) 20 minutes prior to amphetamine (2 mg/kg, i.p.) or saline once daily for five days. All mice were challenged with amphetamine (1 mg/kg, i.p.) in the absence of SB 216763 seven days later. Ambulatory and stereotypic activities were measured for 30 minutes upon amphetamine challenge.
Data Analysis: Ambulatory and stereotypy data were analyzed for significance using two-way ANOVA and Bonferroni post-hoc analysis.

Study 3: The effect of VPA and SB 216763 on the development of amphetamine-conditioned reward.
Subjects: See Study 1.
Drugs: Sodium valproate (Sigma Aldrich; St. Louis, MO) and *D*-amphetamine (NIDA; Bethesda MD) were dissolved in sterile saline (0.9% NaCl). SB 216763 (Tocris Bioscience; Ellisville, MO) was dissolved in 3.6% DMSO (Sigma) and brought up to volume in Tocrisolve 100 (Tocris Bioscience).
Conditioned Place Preference: An unbiased CPP procedure was used. On days 1-4, mice (n=8-11/group) were pretreated with saline or valproic acid (300 mg/kg, i.p.) in their home cages. Thirty minutes post-injection, mice received saline or amphetamine (2 mg/kg, i.p.) and were confined to one side of the CPP chamber for 30 minutes. On day 5, animals had free access to both sides of the conditioning chamber in a drug-free state and time on each side of the chamber was recorded for 30 minutes. Separate sets of mice (n=8/group) were pretreated with vehicle or SB 216763 (5 mg/kg, i.p.) in their home cages. Mice received saline or amphetamine (2 mg/kg, i.p.) 20 minutes later, and were confined to one side of the chamber for 30 minutes. On day 5, time spent on each side of the conditioning chamber in a drug-free state was recorded.
Data Analysis: Time spent on each side of the conditioning chamber was analyzed for significance using two-way ANOVA and Bonferroni post-hoc analysis.

Results of Aim 1
Study 1: The effect of valproic acid (VPA) and SB 216763 on acute amphetamine-induced hyperactivity.
In our first study, we investigated the effect of a non-selective inhibitor of GSK3, valproic acid, as well as a selective inhibitor of GSK3, SB 216763 on acute amphetamine-induced activity (Figure 1). Mice were pretreated with valproic acid (50-300 mg/kg, i.p.) prior to amphetamine, and activity was measured for 60 minutes. Two-way ANOVA of the ambulatory data showed significant interaction, pretreatment, treatment effects (Interaction: $F(3,80) = 2.757$, $p = 0.0477$; Pretreatment: $F(3,80) = 3.758$, $p = 0.0140$; Treatment: $F(1,80) = 12.64$, $p = 0.0006$). Bonferroni post-hoc analysis showed that saline-amphetamine significantly increased ambulatory counts compared to saline-saline treated controls (**p<0.01). Also, pretreatment with valproic acid significantly attenuated amphetamine-induced ambulation (***p<0.001). Analysis of the stereotypy data revealed significant interaction, pretreatment, and treatment effects (Interaction:

$F(3,80) = 2.857$, $p = 0.0422$; Pretreatment: $F(3,80) = 4.258$, $p = 0.0076$; Treatment: $F(1,80) = 14.23$, $p = 0.0003$). Bonferroni post-hoc analysis indicated that pretreatment with each dose of VPA significantly attenuated stereotypic activity (50 mg/kg, *p<0.05; 150 mg/kg, **p<0.01; 300 mg/kg, ***p<0.001).

Separate sets of mice were pretreated with SB 216763 (2.5-5.0 mg/kg, i.p.) prior to amphetamine, and activity was measured for 60 minutes (Figure 2). Two-way ANOVA of the ambulatory data revealed significant pretreatment and treatment effects (Interaction: $F(2,62) = 1.092$, $p = 0.3419$; Pretreatment: $F(2,62) = 3.470$, $p = 0.0373$; Treatment: $F(1,62) = 35.01$, $p < 0.0001$). Bonferroni post-hoc analysis showed increased ambulatory activity in mice treated with vehicle-amphetamine compared to vehicle-saline treated controls (***p<0.001). Pretreatment with SB 216763 significantly attenuated ambulation compared to mice treated with vehicle-amphetamine (2.5 mg/kg, *p<0.05; 5 mg/kg, *p<0.05). Two-way ANOVA of stereotypic activity revealed significant treatment effects (Interaction: $F(2,62) = 1.480$, $p = 0.2356$; Pretreatment: $F(2,62) = 1.092$, $p = 0.3418$; Treatment: $F(1,62) = 38.84$, $p < 0.0001$). Bonferroni post-hoc analysis indicated a significant increase in stereotypy in mice treated with vehicle-amphetamine compared to those treated with vehicle-saline (***p<0.001). Pretreatment with 5 mg/kg of SB 216763 significantly attenuated amphetamine-induced stereotypy (*p<0.05).

Study 2: The effect of VPA and SB 216763 on the development of amphetamine-induced behavioral sensitization.

The second study examined the effect of daily pretreatment of valproic acid on the development of amphetamine-induced behavioral sensitization to stereotypy (Figure 3). Two-way ANOVA of the stereotypy data revealed significant interaction, pretreatment, and treatment effects (Interaction: $F(1,42) = 8.73$, $p = 0.0051$; Pretreatment: $F(1,42) = 6.956$, $p = 0.0117$; Treatment: $F(1,42) = 11.46$, $p = 0.0016$). Bonferroni post-hoc analysis revealed a significant increase in stereotypy counts (***$p < 0.001$) in mice treated with daily saline-amphetamine compared to saline-saline controls, indicating the development of amphetamine-induced sensitization. Analysis also showed daily pretreatment with valproic acid (300 mg/kg) significantly attenuated the development of amphetamine-induced sensitization of stereotypy in mice treated with VPA-amphetamine compared to saline-amphetamine controls (***$p < 0.001$).

Separate sets of mice were pretreated daily with SB 216763 prior to amphetamine to test the effect of selective inhibition of GSK3 of the development of amphetamine-induced sensitization to stereotypy (Figure 4). Two-way ANOVA of the stereotypy data indicated significant treatment effects (Interaction: $F(1,36) = 0.5903$, $p = 0.4473$; Pretreatment: $F(1,36) = 0.8391$, $p = 0.3658$; Treatment: $F(1,36) = 25.67$, $p < 0.0001$). Bonferroni post-hoc analysis revealed a significant increase in stereotypy upon amphetamine challenge in mice treated daily with vehicle-amphetamine compared to vehicle-saline controls (***$p < 0.001$). Mice pretreated daily with SB 216763 (5 mg/kg) also exhibited significantly greater stereotypic counts than saline treated control mice (**p <0.01). Analysis indicated no significant difference between mice treated with daily SB 216763-amphetamine and those that received vehicle-amphetamine ($p > 0.05$).

Study 3: The effect of VPA and SB 216763 on the development of amphetamine-conditioned reward.

Our third study investigated the effect of pretreatment valproic acid on amphetamine-induced place preference (Figure 5). Analysis of the results revealed that mice treated with saline-amphetamine (***p<0.001) and VPA-amphetamine (***p<0.001) spent significantly more time on the drug paired side of the chamber as opposed to the saline paired side of the chamber compared to the saline pretreated controls. These results indicate the development of amphetamine-induced place preference in all mice conditioned with amphetamine, and VPA did not attenuate amphetamine-induced reward.

Separate sets of mice were pretreated with SB 216763 prior to amphetamine and confined to one side of the conditioning chamber (Figure 6). Analysis of the results indicated that mice treated with vehicle-amphetamine spent more time on the drug paired side than the saline paired side of the chamber (*p<0.05) indicating the development of amphetamine-conditioned reward. Mice treated with SB 216763-amphetamine also spent more time on the drug paired side than the saline paired side of the chamber, indicating the development of amphetamine-induced place preference (***p<0.001).

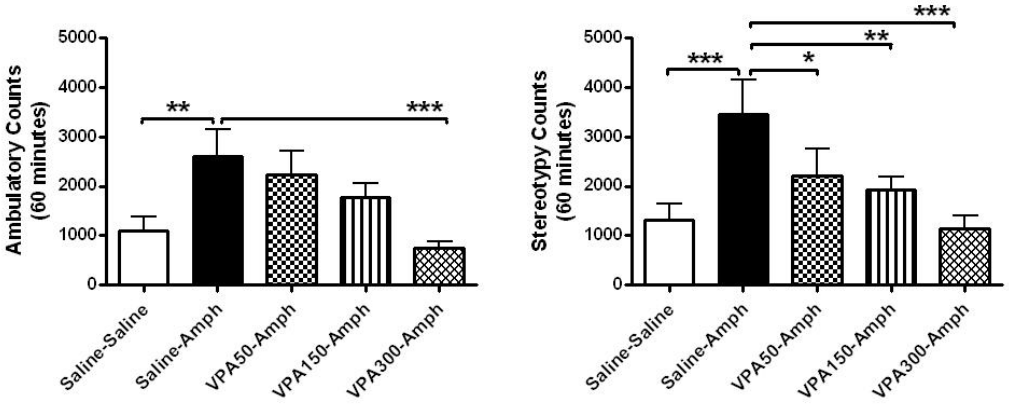

Figure 1. Amphetamine-induced ambulatory (left) and stereotypy (right) in mice pretreated with VPA (50-300 mg/kg). VPA significantly attenuated ambulatory activity (300 mg/kg, ***p<0.001) and stereotypy (50 mg/kg, *p<0.05; 150 mg/kg, **p<0.01; 300 mg/kg, ***p<0.001).

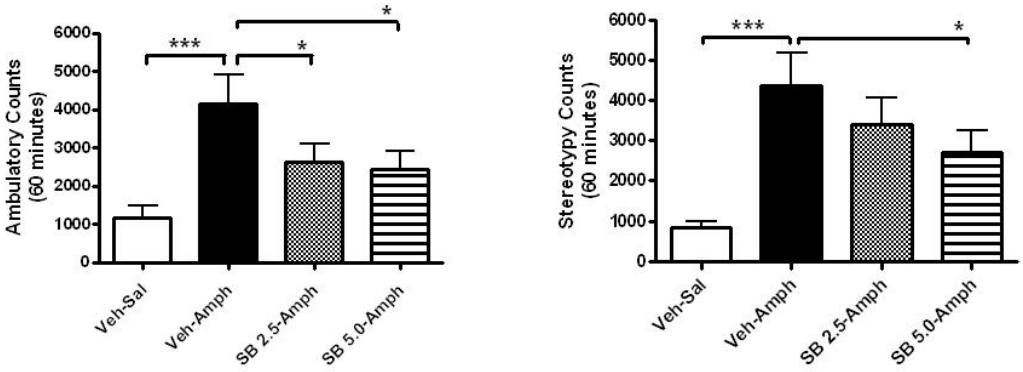

Figure 2. Amphetamine-induced ambulation (left) and stereotypy (right) in mice pretreated with the selective GSK3 inhibitor SB 216763 (2.5-5 mg/kg). SB 216763 significantly attenuated ambulation (2.5-5 mg/kg, *p<0.05) and stereotypy (5 mg/kg, *p<0.05).

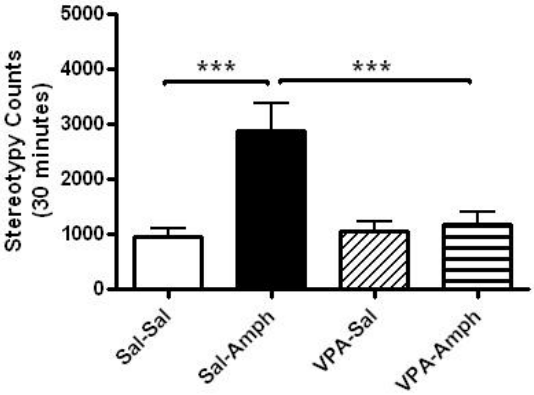

Figure 3. Pretreatment with VPA significantly attenuated the development of amphetamine-induced sensitization to stereotypy after repeated amphetamine administration (***p<0.001).

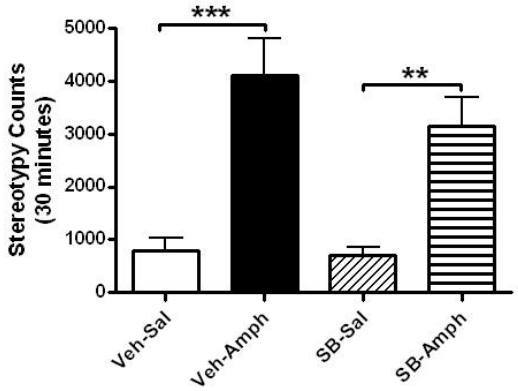

Figure 4. Amphetamine-induced sensitization to stereotypy in mice administered repeated amphetamine injections. All mice treated with repeated amphetamine developed significant sensitization to stereotypy compared to saline counterparts (**p<0.01, ***p<0.001). Pretreatment with SB216763 (5 mg/kg) did not significantly alter amphetamine-induced

sensitization.

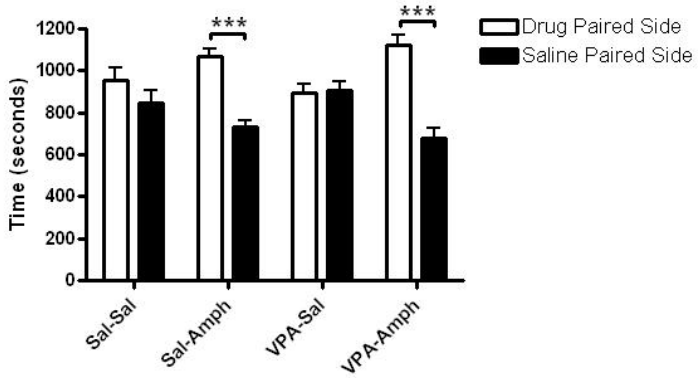

Figure 5. The effect of VPA on the development of amphetamine-induced place preference. All mice treated with amphetamine spent more time on the drug paired side of the chamber than saline paired side (***p<0.001). Pretreatment with VPA did not alter amphetamine conditioned reward.

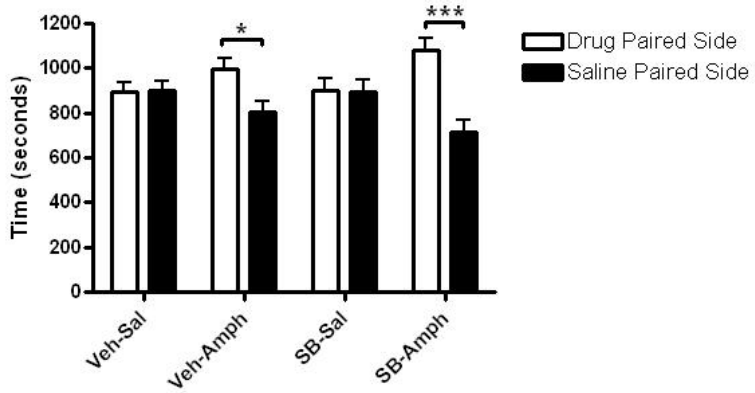

Figure 6. The effect of GSK3 inhibition by SB 216763 on the development of amphetamine-induced reward. All mice treated with amphetamine spent more time on the drug paired side of the chamber than saline paired side (*p<0.05, ***p<0.001). Pretreatment with SB 216763 did not alter amphetamine conditioned reward.

Aim 2: Regulation of phosphorylation of glycogen synthase kinase 3 (GSK3) in brains from amphetamine-injected mice.

Materials and Methods

This study investigated the regulation of the phosphorylation of GSK3 by amphetamine. Adult male CD-1 mice were pretreated with valproic acid (300 mg/kg, i.p.) or saline followed by acute amphetamine (2 mg/kg, i.p.) or saline 30 minutes later. The caudate putamen, nucleus accumbens, and frontal cortex were removed by gross dissection 60 minutes following

amphetamine injections. Brain tissues were sonicated in 1% SDS with 1mM NaF and 1mM $NaVO_4$, boiled for 5 minutes, aliquotted, and stored at -80oC until assayed. Protein concentrations were determined by a modified Lowry assay. Protein samples were subjected to SDS-polyacrylamide gel electrophoresis (7.5% Tris-HCl BioRad Ready-gels, Hercules, CA) and transferred to nitrocellulose membranes for 95 minutes. Membranes were blocked for 1 hour in 5% nonfat dry milk and Tween-TBS solution prior to incubation overnight at 4oC in phospho-GSK3α/β (1: 2,000, Cell Signaling, Beverly, MA) and total GSK3α/β (1: 5,000, Santa Cruz, Santa Cruz, CA) primary antibodies. Membranes were also incubated in anti-tubulin antibody (1: 300,000, Sigma, St. Louis, MO) at room temperature for 90 minutes to correct for protein loading or transfer differences. Membranes were subsequently washed in Tween-TBS and incubated for 1 hour at room temperature with infrared anti-rabbit or anti-mouse secondary antibodies (1: 10,000, Licor Biosciences, Lincoln, NE). Nitrocellulose membranes were imaged and analyzed using the Odyssey infrared imaging system (Licor Biosciences, Lincoln, NE).

Results of Aim 2

Mice were pretreated with valproic acid (VPA; 300 mg/kg, i.p.) or vehicle 30 minutes prior to amphetamine (2 mg/kg, i.p.), and brain tissue was obtained 60 minutes later. Protein extracts from the caudate putaman, nucleus accumbens, and frontal cortex were analyzed by immunoblot for levels of phosphorylated and total GSK3α and β and tubulin (Fig. 7). Analysis by two-way Analysis of Variance (ANOVA) showed that levels of phosphorylated ser^{21}-GSK3α in the caudate putamen were similar among experimental groups (Interaction: $F(1, 36) = 3.454$, $p<0.05$; Pretreatment: $F(1, 36) = 0.895$, $p>0.05$; Treatment: $F(1, 36) = 2.164$, $p>0.05$) (Fig. 7A). Significant interaction and pretreatment effects were detected for levels of phosphorylated ser^{9}-GSK3β in the caudate putamen (Interaction: $F(1, 36) = 5.225$, $p<0.05$; Pretreatment: $F(1, 36) = 3.685$, $p<0.05$; Treatment: $F(1, 36) = 6.380$, $p>0.05$) (Fig. 7B). Post-hoc analysis showed significant elevations in phosphorylated ser^{9}-GSK3β in mice injected with VPA-amphetamine compared to vehicle-amphetamine (vehicle-amphetamine vs. VPA-amphetamine, $p<0.01$) or valproate-saline (vehicle-amphetamine vs. VPA-saline, $p<0.05$).

In the nucleus accumbens, levels of p-ser^{21}-GSK3α (Interaction: $F(1, 34) = 3.025$, $p>0.05$; Pretreatment: $F(1, 34) = 0.542$, $p>0.05$; Treatment: $F(1, 34) = 2.636$, $p>0.05$) (Fig. 7C) and p-ser^{9}-GSK3β (Interaction: $F(1, 34) = 1.286$, $p>0.05$; Pretreatment: $F(1, 34) = 0.080$, $p>0.05$; Treatment: $F(1, 34) = 0.120$, $p>0.05$) (Fig. 7D) were not significantly different between groups.

In the frontal cortex, significant interaction effects were detected for levels of phosphorylated ser^{21}-GSK3α (Interaction: $F(1, 36) = 10.14$, $p<0.01$; Pretreatment: $F(1, 36) = 1.309$, $p>0.05$; Treatment: $F(1, 36) = 0.0001$, $p>0.05$) (Fig. 7E), and post-hoc analysis showed significantly higher levels of GSK3α in the frontal cortex of mice pretreated with valproate compared to vehicle prior to amphetamine (vehicle-amphetamine vs. VPA-amphetamine, $p<0.01$). Significant interaction effects were also found for levels of phosphorylated ser^{9}-GSK3β in the frontal cortex (Interaction: $F(1, 36) = 0.021$, $p<0.05$; Pretreatment: $F(1, 36) = 0.077$, $p>0.05$; Treatment: $F(1, 36) = 0.488$, $p>0.05$) (Fig. 7F). Post-hoc analysis determined significantly higher levels of phosphorylated ser^{9}-GSK3β in the frontal cortex of mice administered valproate-amphetamine compared to vehicle-amphetamine (vehicle-amphetamine vs. VPA-amphetamine, $p<0.05$).

Total levels of GSK3α and GSK3β in each of the three brain regions tested were similar in all experimental groups (data not shown). Because GSK3 is inactivated by phosphorylation on ser[21] (α) or ser[9] (β), these results demonstrate that valproic acid inhibited GSK3α and GSK3β activity in the frontal cortex, as well as GSK3β activity in the caudate putamen of amphetamine-injected animals.

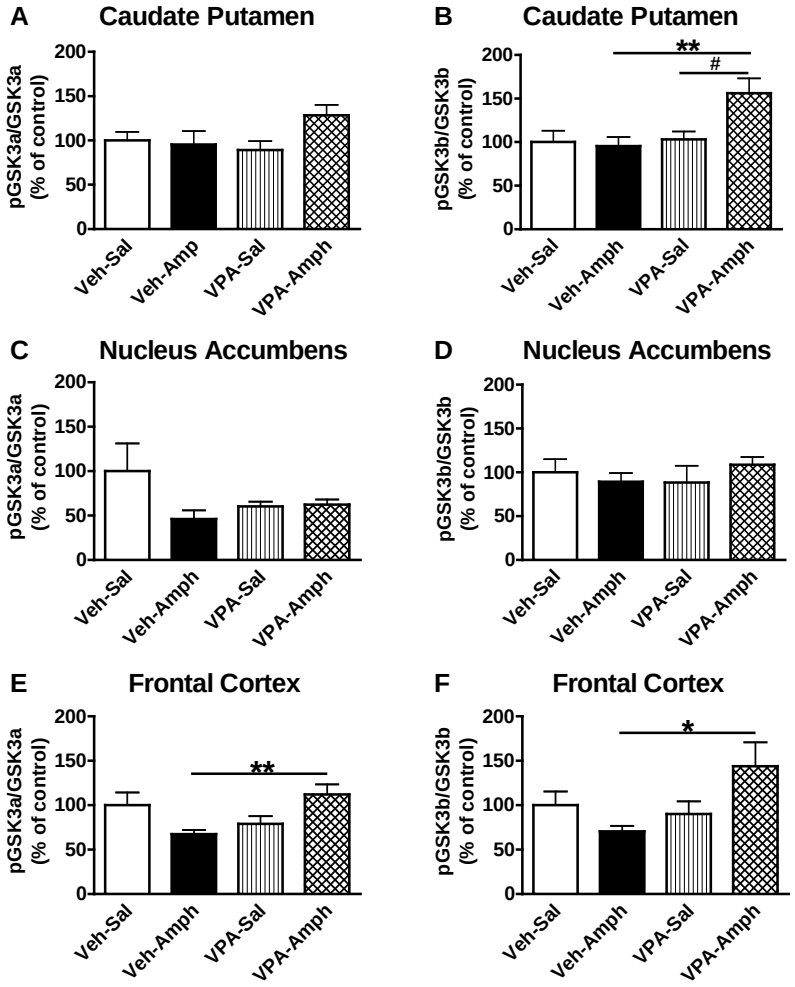

Figure 7. Brain tissue was obtained 60 minutes following amphetamine (2 mg/kg) or saline administration from mice pretreated with valproic acid (300 mg/kg) or vehicle. Western blots of protein extracts obtained from caudate putamen, nucleus accumbens, and frontal cortex were analyzed. (A) Pretreatment with valproic acid did not significantly alter levels of phosphorylated GSK3α in the caudate putamen. (B) Phosphorylated GSK3β in the caudate putamen was significantly increased in mice pretreated with valproic acid compared to vehicle prior to amphetamine (**$p<0.01$) and saline (#$p<0.05$). (C) Administration of valproic acid prior to amphetamine did not significantly alter phosphorylation of GSK3α or (D) GSK3β in the nucleus accumbens. (E) Pretreatment with valproic acid significantly increased levels of phosphorylated GSK3α in the frontal cortex of amphetamine treated mice compared to pretreatment with vehicle (**$p<0.01$). (F) Levels of phosphorylated GSK3β in the frontal cortex were significantly greater in mice pretreated with valproic acid prior to amphetamine (*$p<0.05$). Data points represent the mean + SEM (n=9-10/group).

Abstracts and Scientific Meeting Presentations

1. GSK3 is necessary for acute amphetamine-induced hyperactivity, but not sensitization or reward. Society for Neuroscience, November 2010, San Diego, CA (poster)
2. Enman, N. and Unterwald, E.M. Valproic acid attenuates amphetamine-induced behavior in mice. The College on Problems of Drug Dependence, June 2010, Scottsdale, AZ (oral presentation)
3. Possible role of GSK3 in amphetamine-induced behaviors. Philadelphia Chapter of Society for Neuroscience, April 2010, Philadelphia, PA (poster)
4. Effect of valproic acid on amphetamine-induced behaviors in mice. Frontiers in Addiction Research: NIDA Mini-Convention, October 2009, Chicago, IL (poster)
5. Effect of valproic acid on amphetamine-induced behaviors in mice. Society for Neuroscience, October 2009, Chicago, IL (poster)
6. Valproic acid attenuates acute amphetamine-induced locomotion in mice. The College on Problems of Drug Dependence, June 2009, Reno, NV (poster)

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, listed in the table, in a PDF version 5.0.5 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.Inhibition of GSK3 attenuates amphetamine-induced hyperactivity in the mouse	N.M. Enman and E.M. Unterwald	Behavioural Brain Research	08/2011	☐ Submitted ☐Accepted ☒Published

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

Yes_____ No__x_____

If yes, please describe your plans:

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.

The results generated by this pre-clinical research project provide important insights into the signaling pathways engaged by amphetamine and their role in amphetamine-induced behaviors. These data provide a basis for further experiments to determine if GSK3 can be targeted to affect psychostimulant actions.

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.

These data demonstrate a role for GSK3 in amphetamine-induced behaviors and identify some importance differences between amphetamine and cocaine in this regard.

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.*

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 Initiative.

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)

Ehrlich, M.E., Sommer, J., Canas, E., Unterwald, E.M. Periadolescent mice show enhanced □FosB up regulation in response to cocaine and amphetamine. Journal of Neuroscience. 22:9155-9159, 2002. PMID: 12417638

Perrine SA, Hoshaw BA, Unterwald EM. Delta opioid receptor ligands modulate anxiety-like behaviors in the rat. British Journal of Pharmacology, 147:864-872, 2006. PMID: 16491101

Tropea TF, Guerriero RM, Willuhn I, Unterwald EM, Ehrlich ME, Steiner H, Kosofsky BE. Augmented D1 dopamine receptor signaling and immediate early gene induction in adult striatum after prenatal cocaine. Biological Psychiatry, 63(11):1066-74, 2008. PMID: 18275938

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

Trecki J, Unterwald EM. Modulation of cocaine-induced acitivty by intracerebral administration of CXCL12. Neuroscience, 161:13-22, 2009.PMID: 19303923

Zakharova E, Miller J, Unterwald E, Wade D, Izenwasser S. Social and physical environment alter cocaine conditioned place preference and dopaminergic markers in adolescent male rats. Neuroscience 163:890-897, 2009. PMID: 19580849

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

Trecki J, Brailoiu GC, Unterwald EM. Localization of CXCR4 in the forebrain of the adult rat, Brain Research, 1315:53-62, 2010 (including journal cover illustration). PMID: 20026091

Rasmussen BA, Baron DA, Kim JK, Unterwald EM, Rawls SM. Beta-lactam antibiotic produces a sustained reduction in extracellular glutamate in the nucleus accumbens of rats. Amino Acids 40:761-764, 2011. PMID: 20383795

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 (including journal cover illustration). PMID: 22895898

Craige CP, Unterwald EM. Serotonin (2C) receptor regulation fo cocaine-induced conditioned place preference and locomotor sensitization. Behavioral Brain Research 238:206-210, 2013. PMID: 23103406