Appendices

Incubation of sucrose craving in animal models in press, Animal models of craving in press
APPLICATION FOR FEDERAL ASSISTANCE
SF 424 (R&R)

1. * TYPE OF SUBMISSION
   - [ ] Pre-application
   - [x] Application
   - [ ] Changed/Corrected Application

2. DATE SUBMITTED
   
3. DATE RECEIVED BY STATE
   
4. a. Federal Identifier
   
5. APPLICANT INFORMATION
   * Legal Name: Western Washington University
   * Department: Psychology
   * Division: Humanities and Social Sciences
   * Street1: 516 High Street
   * Street2: 
   * City: Bellingham
   * County / Parish: Whatcom
   * State: WA
   * ZIP / Postal Code: 98225
   * Country: USA: UNITED STATES
   * * Organizational DUNS: 019253134
   Person to be contacted on matters involving this application
   * Prefix: Dr
   * First Name: Ken
   * Middle Name: W
   * Last Name: Clark
   * Suffix: 
   * Phone Number: 360.650.4403
   * Fax Number: 360.650.6811
   * Email: ken.clark@wwu.edu

6. * EMPLOYER IDENTIFICATION (EIN) or (TIN): 916000562

7. * TYPE OF APPLICANT:
   - [ ] Other (Specify):
   - [ ] Women Owned
   - [ ] Socially and Economically Disadvantaged

8. * TYPE OF APPLICATION:
   - [x] New
   - [ ] Resubmission
   - [ ] Renewal
   - [ ] Continuation
   - [x] Revision
   If Revision, mark appropriate box(es).
   - [ ] A. Increase Award
   - [ ] B. Decrease Award
   - [ ] C. Increase Duration
   - [ ] D. Decrease Duration
   - [ ] E. Other (specify):

9. * NAME OF FEDERAL AGENCY:

10. CATALOG OF FEDERAL DOMESTIC ASSISTANCE NUMBER:

11. * DESCRIPTIVE TITLE OF APPLICANT'S PROJECT:
   Incubation of craving: Abstinence and Environmental Enrichment-mediated Molecular Adaptations

12. PROPOSED PROJECT:
   * Start Date: 04/01/2011
   * Ending Date: 03/31/2014

13. CONGRESSIONAL DISTRICT OF APPLICANT
   - WA-002

14. PROJECT DIRECTOR/PRINCIPAL INVESTIGATOR CONTACT INFORMATION
   * Prefix: Dr
   * First Name: Jeffrey
   * Middle Name: W
   * Last Name: Grimm
   * Suffix: 
   * Position/Title: Professor
   * Organization Name: Western Washington University
   * Department: Psychology
   * Division: Humanities and Social Sciences
   * Street1: 516 High Street
   * Street2: 
   * City: Bellingham
   * County / Parish: Whatcom
   * State: WA
   * ZIP / Postal Code: 98225
   * Country: USA: UNITED STATES
   * Phone Number: 360.650.3168
   * Fax Number: 360.650.7305
   * Email: jeffrey.grimm@wwu.edu
15. ESTIMATED PROJECT FUNDING

| a. Total Federal Funds Requested | 386,731.00 |
| b. Total Non-Federal Funds      | 0.00        |
| c. Total Federal & Non-Federal Funds | 0.00 |
| d. Estimated Program Income     | 0.00        |

16. * IS APPLICATION SUBJECT TO REVIEW BY STATE EXECUTIVE ORDER 12372 PROCESS?

- a. YES
  - [ ] THIS PREAPPLICATION/APPLICATION WAS MADE AVAILABLE TO THE STATE EXECUTIVE ORDER 12372 PROCESS FOR REVIEW ON:
  - DATE: [ ]
- b. NO
  - [x] PROGRAM IS NOT COVERED BY E.O. 12372; OR
  - [ ] PROGRAM HAS NOT BEEN SELECTED BY STATE FOR REVIEW

17. By signing this application, I certify (1) to the statements contained in the list of certifications* and (2) that the statements herein are true, complete and accurate to the best of my knowledge. I also provide the required assurances * and agree to comply with any resulting terms if I accept an award. I am aware that any false, fictitious, or fraudulent statements or claims may subject me to criminal, civil, or administrative penalties. (U.S. Code, Title 18, Section 1001)

* I agree

* The list of certifications and assurances, or an Internet site where you may obtain this list, is contained in the announcement or agency specific instructions.

18. SFLLL or other Explanatory Documentation

19. Authorized Representative

Prefix: Dr.  * First Name: Moheb  Middle Name: A.
* Last Name: Ghali
* Position/Title: Vice Provost for Research
* Organization: Western Washington University
Department: Research & Sponsored Programs  Division: 
* Street1: 516 High Street
Street2: 
* City: Bellingham  County / Parish: Whatcom
* State: WA: Washington  Province: 
* Country: USA: UNITED STATES  * ZIP / Postal Code: 98225-9038
* Phone Number: 360.650.2884  Fax Number: 360.650.6811
* Email: moheb.ghali@wwu.edu

* Signature of Authorized Representative

Moheb Ghali

* Date Signed

06/17/2010
# 424 R&R and PHS-398 Specific

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## Appendix

*Number of Attachments in Appendix: 2*
### Project/Performance Site Primary Location

- **Organization Name:** Western Washington University
- **DUNS Number:** 0792531340000
- **Street1:** 516 High Street
- **City:** Bellingham
- **State:** WA: Washington
- **Province:** 
- **Country:** USA: UNITED STATES
- **ZIP / Postal Code:** 98225-9038

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

### Project/Performance Site Location 1

- **Organization Name:**
- **DUNS Number:**
- **Street1:**
- **Street2:**
- **City:**
- **State:**
- **Province:**
- **Country:** USA: UNITED STATES
- **ZIP / Postal Code:**

I am submitting an application as an individual, and not on behalf of a company, state, local or tribal government, academia, or other type of organization.

### Additional Location(s)

- **Organization Name:**
- **DUNS Number:**
- **Street1:**
- **Street2:**
- **City:**
- **State:**
- **Province:**
- **Country:**
- **ZIP / Postal Code:**

---

Principal Investigator/Program Director (Last, first, middle): Grimm, Jeffrey, W
1. * Are Human Subjects Involved? □ Yes ☒ No
   1.a If YES to Human Subjects
      Is the Project Exempt from Federal regulations? □ Yes □ No
      If yes, check appropriate exemption number: □ 1 □ 2 □ 3 □ 4 □ 5 □ 6
      If no, is the IRB review Pending? □ Yes □ No
      IRB Approval Date: __________
      Human Subject Assurance Number: __________

2. * Are Vertebrate Animals Used? ☒ Yes □ No
   2.a If YES to Vertebrate Animals
      Is the IACUC review Pending? □ Yes ☒ No
      IACUC Approval Date: __________
      Animal Welfare Assurance Number: __________

3. * Is proprietary/privileged information included in the application? □ Yes ☒ No

4.a. * Does this project have an actual or potential impact on the environment? □ Yes ☒ No
   4.b If yes, please explain:
   4.c. If this project has an actual or potential impact on the environment, has an exemption been authorized or an environmental assessment (EA) or environmental impact statement (EIS) been performed? □ Yes □ No
      If yes, please explain:

5. * Is the research performance site designated, or eligible to be designated, as a historic place? □ Yes ☒ No
   5.a If yes, please explain:

6. * Does this project involve activities outside of the United States or partnerships with international collaborators? □ Yes ☒ No
   6.a If yes, identify countries:
   6.b Optional Explanation:

7. * Project Summary/Abstract 1235-project description.pdf
6. * Does this project involve activities outside of the United States or partnerships with international collaborators? □ Yes ☒ No
   6.a If yes, identify countries:
   6.b Optional Explanation:

8. * Project Narrative 1236-public health relevance statement

10. Facilities & Other Resources 1238-facilities.pdf
11. Equipment
12. Other Attachments

Tracking Number: GRANT10635432
Funding Opportunity Number: PA-10-070 Received Date: 2010-06-17T17:43:23-04:00
PROJECT DESCRIPTION

Relapse to drug and food seeking presents a significant public health concern as excessive preoccupation with drug or food seeking and taking contributes to numerous negative health outcomes. Using a rat model of reward seeking, this proposal aims to identify gene expression patterns in the brains of rats with a history of sucrose self-administration under short or long-term abstinence conditions known to produce low or high levels, respectively, of reward seeking (“incubation of craving”). In addition, some rats will experience abstinence in an enriched environment, a manipulation we have previously demonstrated to attenuate incubation of sucrose seeking. Gene expression will be visualized in the first Aim using Fos immunohistochemistry to map sucrose-cue activated regions. Aim 2 will utilize this mapping information to focus on discrete brain regions to isolate and quantitate dopamine D1 receptor signal cascade proteins, some in basal vs. phosphorylated states, as we have found that D1 receptor antagonism has time-dependent (incubation-dependent) effects on sucrose seeking. Beyond the goal of identifying neural substrates of addiction as a means to informing novel addiction therapies, the studies will be conducted as a means to expose undergraduate researchers to the scientific process. Engaging the students in this way will enhance their training experiences and the research environment at Western Washington University.
PUBLIC HEALTH RELEVANCE STATEMENT

Relapse to drug or food seeking presents a significant public health concern as excessive preoccupation with, and consumption of, drugs and food contribute to numerous negative health outcomes. The proposed studies aim to identify differential gene expression in the brains of rats related to relapse behavior with or without the relapse-attenuating pre-treatment of extended enriched environment living conditions. The results of these studies may lead to a better understanding of the molecular biology of relapse behavior and thus facilitate development of novel relapse treatment approaches.
FACILITIES AND OTHER RESOURCES

(laboratory of PI)

In the Academic Instruction Center, Western Washington University: 1 laboratory room (310 sq. feet), 2 connected procedure rooms (120 sq feet each) and 1 surgery room (120 sq feet) devoted to use solely by the PI. One procedure room contains 21 operant chambers in sound attenuating boxes connected to 2 computer interface systems (MED Associates). The other procedure room contains 8 operant boxes connected to 1 computer interface system and also has 2 place preference chambers. The main laboratory space is equipped with a fume hood and both wet and dry bench space. There are 4 computer workstations with internet access in the laboratory.

(shared space with Behavioral Neuroscience program)

Shared laboratory space includes a histology/microscopy room (300 sq. feet) with 2 fume hoods and wet and dry bench space. There is also a shared procedure room with a hood in the vivarium that is used for perfusions of animals.

(animals)

In the Academic Instruction Center, Western Washington University: 2 vivarium rooms (150 sq. feet each) devoted to use solely by the PI. The rooms have individual environmental control systems. Except for environmental enrichment studies, animals are housed individually in plastic cages with state-of-the-art ventilation and autowatering. Animal care and cage maintenance is performed by a full-time department animal technician. In-house breeding is done in one of the rooms.

(computer)

There are 3 PC computers devoted to the MED Associates operant systems. The PI has 4 PC desktop computers in the laboratory and 1 computer in his office. The PI also has a PC laptop computer. All computers have internet access. Computers are used for data collection, analysis, manuscript preparation, and literature searches. The PI has an office laser printer and there is a laser printer in the lab for manuscript preparation.

(office)

The PI has an office (150 sq. feet) in the Academic Instruction Center in close proximity to the laboratory.

(other)

In addition to the above facilities, WWU maintains extensive metal, wood, and electronic shops (Science and Technology Services) with labor available to the PI at no cost. The department of Psychology provides computer support and maintenance, animal care (no per diem costs are asked of the PI), and secretarial support.

(major equipment—PI)

Items in the laboratory of the PI include 29 MED Associates operant chambers (27 rat, 2 mouse) with infusion pumps for sucrose or drug delivery, computer control equipment for these systems, and 29 sound-attenuating chambers. Nine of the rat chambers and the 2 mouse chambers are also equipped with pellet dispensers. 12 of the rat chambers are equipped for intracranial self-stimulation studies. All operant chambers are equipped with locomotion monitoring infrared photobeams. The PI has a small locking refrigerator for storage of drugs and a larger refrigerator for storing sucrose and other solutions. Other equipment in the laboratory of the PI includes a dual-arm stereotaxic device, surgical instruments, a microinfusion pump for microinjection studies, a balance for measuring milligram quantities of drugs, balances for weighing animals, a pH meter, a heat/stir...
plate, a mini vortexer, a Vibratome ultrapro 5000 cryostat, a Vibratome 1000+ sectioning system, an Olympus BX41 upright light microscope with digital imaging, and an Olympus SZX7 stereoscope.

(major equipment—shared resources within Behavioral Neuroscience program)

Shared equipment includes a walk-in refrigerator and freezer, 2 ultrafreezers, 2 Milli-Q water purification systems, an automated open-field monitoring system, 4 serial reaction time operant chambers (2 rat, 2 mouse; Lafayette), 2 ESA HPLC systems, shakers, ultrasonic tissue homogenizers, a centrifuge, a Leica CM1950 cryostat, an Olympus BX51 upright microscope with fluorescence and motorized stage, an Olympus IX71 inverted microscope with fluorescence, an Olympus IX81 inverted scope with fluorescence and motorized stage, and a Olympus IX81/Fluoview FV1000 confocal microscope with motorized stage. All of the microscopes have digital imaging systems.

(other)

Profile of WWU and Students at WWU

For 11 years in a row, U.S. News and World Report has ranked Western Washington University as the best regional public university in the Pacific Northwest, and #2 in the western United States. Western is a predominantly undergraduate institution with 94% of the students enrolled in bachelor’s degree-granting programs.

In a recent survey, over 88% of all bachelor’s degree graduates reported immediately having found employment, about 28% of those in the science and healthcare sector. Another 18% of recent bachelor’s degree graduates report that they are already continuing their education, including graduate education in health-related sciences. Several students from the PI’s laboratory have been offered jobs in the biotechnology industry or have entered graduate training programs.

WWU has become a leader in the state of Washington in providing a quality college education and this is demonstrated by its popularity. In 2002, and in each year following, the quality of applicants for WWU have exceeded all other state 4-year institutions except the University of Washington. The tenure and promotion system at WWU values commitment to research that actively involves students as part of an emphasis on teaching and student contact. Research with students is valued as it provides an excellent complement to classroom teaching.

Profile of Department of Psychology, WWU

The Psychology department has 30 tenure-stream faculty, the majority of who are actively engaged in research projects. The department has bachelors and master’s degree programs including master’s degrees in general Experimental Psychology, mental health counseling, and school counseling. The majority of experimental Psychology master’s students go on to pursue doctoral degrees, as do many of the bachelor’s degree recipients (including from the new Behavioral Neuroscience program—see below). The department graduates over 300 Psychology undergraduate majors a year, making it the most popular undergraduate major at WWU. The department maintains an animal facility and technician and provides ample space for research in Behavioral Neuroscience. In addition, there is a full-time department computer technician.

The Psychology department encourages majors to participate in research projects—credit is given for research assistants, including over the summer quarter. There are currently 5 other active Behavioral Neuroscience faculty investigators in the department that are eager to facilitate the growth of the PI’s research.

Three recent developments that demonstrate the strengths of the Psychology department in training behavioral neuroscientists are the establishment of a bachelor of arts degree (and soon-to-be established BS) in Behavioral Neuroscience conferred as a joint degree between Psychology and Biology, opening of a new building in 2008 that houses the Psychology department including state-of-the-art laboratory spaces, vivaria, and wet/dry laboratory and classroom spaces exclusively for use by the Behavioral Neuroscience faculty and students, and the funding of the Biomedical Research Activities in Neuroscience (BRAIN)
initiative that provides funds for faculty lines and research experiences for students enrolled in the new Behavioral Neuroscience program.

The Behavioral Neuroscience Program was formally established in 2005, and received BRAIN funding to grow the program in 2007. The first student graduated in 2006 and, since then, a total of 23 students have graduated with a BA in Behavioral Neuroscience. There are currently 25 students in the program.

Likely Impact of renewal of an AREA Award

As per the R15 (AREA) Program Announcement, the three goals of the program are:

- to support meritorious research
- to strengthen the research environment of the institution
- to expose students to research (preferably undergraduates)

As detailed in the Biosketch and Research Strategy sections, the funding of the PI’s previous R15 parent grants has met the goals of the R15 program and the PI fully expects to continue to do so with renewed funding. AREA goals-specific details of the current proposal can be summarized as follows:

1. to support meritorious research  The application includes aims to identify brain region-specific gene expression related to incubation of craving and environmental enrichment. These studies may produce data that will have a high impact in the field of addiction.

2. to strengthen the research environment of the institution  The application will enhance the techniques available in the laboratory of the PI to explore molecular signaling related to behavior. This approach, and the equipment needed for it, will strengthen the research environment by enhancing the research capability of the institution. Also, as noted in the next item, the proposed studies were designed to be conducted with the participation of student research assistants. Increasing opportunities for students to be involved in research will directly enhance the research environment of WWU.

3. to expose students to research (preferably undergraduates)  There are currently 10 students working in the PI’s laboratory. All of them are planning to major in Behavioral Neuroscience and plan for further graduate study in the health sciences. Benefits of the AREA award would be to not only engage students in the research identified in the specific aims of the proposal, but also to increase the number and type of research questions that the laboratory could ask. As the PI has found from his previously funded applications, many of the laboratory’s published studies evolved from student independent research projects that were related to, but not specifically proposed by, the R15 parent grant.

Statement of Institutional Support

As part of this proposal, the PI has asked for some limited teaching release. This is reflected in the proposed budget. A letter signed by the chair of the Psychology department indicating this promised release time (course “buyout”) is attached to this proposal. It is also important to note here that the PI’s animal care and procurement is essentially “free” as the department has a full-time animal care technician and there are no per diem charges for subjects and that includes costs of in-house breeding. This amounts to an in-kind support from the department of thousands of dollars over the period of grant support requested.

Limited funds for travel are provided each year to faculty from the College of Humanities and Social Sciences and there are competitive funds available from the WWU grants office (Office of Research and Sponsored Programs) for small equipment and supply requests.

WWU maintains an electrical, metal, and wood shop with technicians to assist research projects on campus. Already, they have built exceptionally high quality sound attenuating chambers for the operant boxes used in the PI’s research as well as conditioned place preference chambers. WWU also has a well-trained staff to maintain the campus computer network.
## PROFILE - Project Director/Principal Investigator

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<td>Jeffrey</td>
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A. PERSONAL STATEMENT

I believe I have demonstrated that I have met the goals of the Academic Research Enhancement Award (R15) mechanism with the past 2 funded parent grant proposals. Specific details regarding this past success are detailed in the Research Strategy section of this proposal. Below are specific examples of how I believe I am uniquely qualified to be the PI for this proposal, based on the goals of the R15 program.

The three goals of the R15 program are:

• to support meritorious research
• to strengthen the research environment of the institution
• to expose students to research (preferably undergraduates)

1. support meritorious research

The proposed studies are to examine molecular signaling associated with the abstinence-dependent increase in sucrose seeking (incubation of craving) as well as environmental enrichment, a manipulation we have demonstrated to attenuate incubated sucrose seeking. The studies proposed are likely to be considered meritorious as they will explore neural substrates of relapse behavior. They are also quite novel as there are very few published studies on the neuro-signaling underlying incubation of craving and even fewer that have addressed environmental enrichment in this way. As PI, I have the requisite background for both the behavioral and gene expression protocols indicated in the proposal. Specifically, I have published several studies on the incubation of craving in both sucrose and cocaine self-administration experienced rats, I have collaborated on a Fos mapping study of contextual cue reactivity (unpublished), and I have published incubation of craving studies identifying protein expression (Western blot procedure) in cocaine or sucrose self-administration experienced rats. To make sure that I will be able to conduct these protein expression studies at WWU using the most state-of-the-art techniques, I have received enthusiastic consultation support from Drs. Shaham and Hope at NIDA intramural (see attached letters). Pending funding of the grant, I would travel to NIDA for refresher and updated training before starting the studies at WWU. I have also received a promise of consultation from Dr. Lattemann at the University of Washington (see attached letter) for conducting an initial evaluation of retroperitoneal fat pads in enriched vs. non-enriched rats as a measure of physiological fitness in these animals. I have collaborated with Dr. Lattemann extensively in the past, and I also serve as a consultant on relapse studies for her NIDA-funded work on the behavioral physiology of weight gain. All of these studies...
2. strengthen the research environment of the institution

WWU has been steadily cultivating a teacher-scholar culture for its faculty. Effective teaching is most valued, but the definition of teaching has shifted in recent years. More and more, faculty are seeking external funding in order to not only further their research program goals, but to better support involvement of students in research projects. Over the previous 2 periods of support for my parent R15 I have supported students in this way, helping to strengthen the research environment at WWU. My external funding was also important in the successful development and subsequent state-supported funding of a research-focused "high demand enrollment" initiative in Behavioral Neuroscience. The Biomedical Research Activities in Neuroscience (BRAIN) initiative includes annual funding for faculty positions and research experiences for the Behavioral Neuroscience BA program that is administered jointly between the Psychology and Biology departments. The initiative was successful because faculty in the program had demonstrated that high quality research could be conducted at WWU. The funding of the initiative and continued funding of grants such as my R15 have also encouraged other successful external funding applications. For example, we have just recently had 2 faculty members receive R15 funding. Dr. Finlay will be working with her students using a novel mouse model of schizophrenia. The other faculty member was awarded a R15 for her work with learning-related molecular signaling in C. elegans. Dr. Rose (the PI) and I have already discussed possible collaborative projects that incorporate drugs of abuse (e.g. nicotine) and I am serving as a mentor on a NARSAD application she submitted to examine dopamine transduction pathways related to learning and memory. Overall, the R15 has been and will continue to strengthen the research environment at WWU. Specific enhancements that will be brought by the funding of the current proposal will be the addition of molecular techniques (IHC, Western blot) combined with rodent behavior. At this time, there are no other laboratories at WWU conducting these types of analyses. The addition of these techniques is particularly salient considering that my laboratory is in the department of Psychology. Molecular techniques combined with psychological questions are extremely novel at WWU and would be a significant contribution to the research environment of the institution.

3. expose students to research (preferably undergraduates)

As noted in the previous section, despite the fact that WWU is a predominantly undergraduate and teaching-focused University, student-faculty interactions in scholarship are highly regarded. The R15 mechanism is ideally suited to allow exposure of students to research. I have documented examples of student involvement in my research program in the Research Strategy section of this proposal. The proposed R15 renewal would continue to facilitate student involvement in studies on the incubation of craving. With renewed funding, I plan to increase opportunities for students to be financially compensated for exceptional contributions to the lab. For example, I like to reward students for working on weekends and in the summer. As our behavioral studies do not take weekend or holiday breaks, we need all possible days covered. The funds are also extremely valuable for helping students travel to the annual Society for Neuroscience meeting. I had 5 students travel to the fall 2009 meeting. They had an amazing experience seeing posters and talks and also had brief introductions to Dr. Nora Volkow and Dr. Eric Kandel. The students also helped present our poster and this allowed them to see the important aspects of how to concisely present scientific information and to constructively take criticism. For the R15 renewal I would like to expand the travel opportunity by increasing the travel stipend for the most senior student members of the laboratory and also work to make the experience a more mentored trip where we meet as a group at the meeting to see specific talks/posters and then discuss what we have learned. Independent projects are also a key component of exposing students to research. All of my students become involved in the basic aspects of running a particular study, but more experienced students often take the initiative to either learn a more complicated procedure (e.g. cranial surgery or intracranial microinjection) or lead a completely novel study. A recent example is a 2009 publication on the effects of sucrose taste aversion transfer to a sucrose-paired cue. The student, John Harkness (see attached letter), worked out the design with me and then was responsible for conducting the entire study. He also was responsible for the manuscript production. The R15 renewal would be especially helpful in promoting these "side" projects because the funding amount is higher than the previous renewal (double) and we would have the new molecular techniques available to explore novel research questions. Overall and by definition, the
renewal of the R15 will be instrumental in exposing students to meritorious research and providing them opportunities to develop critical thinking and laboratory skills that will be of great benefit to their subsequent graduate training and/or career experiences.

B. POSITIONS AND HONORS

**Positions and Employment**
Western Washington University
2010-present: Professor, Department of Psychology and Program in Behavioral Neuroscience
2005-2009: Associate Professor, Department of Psychology and Program in Behavioral Neuroscience
2001-2005: Assistant Professor, Department of Psychology

**Professional Memberships**
Faculty for Undergraduate Neuroscience, Food Addiction Institutes International Advisory Board, Sigma Xi, Society for Neuroscience, Northwest Medical Association honorary member (2007-2008)

**Other Experience**
NIH Study Section temporary member (BRLE) (Spring 2007, Spring 2008, Winter 2010, Summer 2010)
NIH Special Emphasis Study Section member (exercise and addiction) (Spring 2009)
NSERC-GSC12 (Canada) Discovery Grant reviewer (Winter 2009)
CNRS (France) ATIP Grant reviewer (Winter 2009)
Ad Hoc reviewers of 23 different journals, typically make 10-12 reviews a year

**Honors**
Behavioural Brain Research “Top Reviewers” of 2007, Spring 2008
Teaching and Learning Academy Faculty/Staff Choice Award, Spring 2006 (WWU)
WCBR Travel Fellow, Winter 2006
NIDA Early Career Investigators APA Poster Session Travel Award, Summer 2005
Teaching and Learning Academy Students Choice Award, Spring 2004 (WWU)
NIDA/NIH Career Development Award, Spring 2001 (NIDA)
Graduate Student Excellence Award, Spring 1999 (WSU)
Travel Grant, Graduate School, Fall 1995 (WSU)

C. 15 SELECTED PEER-REVIEWED PUBLICATIONS (IN CHRONOLOGICAL ORDER)
(Selected from 31 peer-reviewed publications)

**Most relevant to the current application**


Additional recent publications of importance to the field (in chronological order)


* in press chapters are included in Appendix of this application

D. RESEARCH SUPPORT

Ongoing Research Support

R15 DA016285-02  4/1/07-3/31/11 (in no-cost extension)  3 calendar
NIH/NIDA
Incubation of Craving: Neural Substrates
Supports investigation of contributions of nucleus accumbens subregions to the incubation of sucrose craving
Role: PI

R15 DA016285-02-S2  summer 2009, 2010  0 calendar
NIH/NIDA
NIH ARRA Summer Research Activities for Undergraduates Supplement to R15
Supports undergraduate research assistants for summers 2009 and 2010
Role: PI

R15 DA016825-02-S1  9/15/08-3/31/11  0 calendar
NIH/NIDA
Diversity supplement to R15
To support mentoring of an undergraduate, Christine Ratliff, in incubation of craving research
Role: PI

Completed Research Support

WWU Research and Sponsored Programs Mini Grant  Fall 2008
Support for book chapter preparation

WWU Research and Sponsored Programs Mini Grant  Fall 2007
Support to travel for collaboration in Seattle

WWU Foundation's Mentoring Undergraduates Grant  Spring 2007-Fall 2007
Supported mentoring undergraduate, John Harkness, in completing a research project investigating the effects of sucrose taste aversion on incubation of sucrose craving
Role: Mentor
Principal Investigator/Program Director (Last, first, middle): Grimm, Jeffrey, W

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<th><em>Prefix</em></th>
<th><em>First Name</em></th>
<th><em>Middle Name</em></th>
<th><em>Last Name</em></th>
<th><em>Project Role</em></th>
<th><em>Base Salary ($)</em></th>
<th><em>Cal. Months</em></th>
<th><em>Acad. Months</em></th>
<th><em>Sum. Months</em></th>
<th><em>Requested Salary ($)</em></th>
<th><em>Fringe Benefits ($)</em></th>
<th><em>Funds Requested ($)</em></th>
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<td>Jeffrey</td>
<td>W</td>
<td>Grimm</td>
<td>PD/PI</td>
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**Total Senior/Key Person: 132,776.00**

**B. Other Personnel**

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<th><em>Sum. Months</em></th>
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**Total Number Other Personnel: 3**

**Total Other Personnel: 67,320.00**

**Total Salary, Wages and Fringe Benefits (A+B): 200,096.00**
## C. Equipment Description

List items and dollar amount for each item exceeding $5,000

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<th>Equipment item</th>
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<td>2. 2 gel sets</td>
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<td>3. 2 transfer systems</td>
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<td>4. BCA System</td>
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<td>5. 2 computer systems</td>
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<td>7.</td>
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<td>9.</td>
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<td>10.</td>
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<tr>
<td>11. Total funds requested for all equipment listed in the attached file</td>
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### D. Travel

#### Domestic Travel Costs (Incl. Canada, Mexico and U.S. Possessions)

Funds Requested ($) $12,000.00

#### Foreign Travel Costs

Funds Requested ($)

### E. Participant/Trainee Support Costs

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<th>Support Costs</th>
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<td>2. Stipends</td>
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<td>3. Travel</td>
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<td>4. Subsistence</td>
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<tr>
<td>5. Other</td>
<td></td>
</tr>
<tr>
<td>Number of Participants/Trainees</td>
<td></td>
</tr>
</tbody>
</table>

Total Participant/Trainee Support Costs
F. Other Direct Costs

1. Materials and Supplies
2. Publication Costs
3. Consultant Services
4. ADP/Computer Services
5. Subawards/Consortium/Contractual Costs
6. Equipment or Facility Rental/User Fees
7. Alterations and Renovations

8. 
9. 
10. 

Total Other Direct Costs 20,404.00

G. Direct Costs

Total Direct Costs (A thru F) 300,000.00

H. Indirect Costs

Indirect Cost Type | Indirect Cost Rate (%) | Indirect Cost Base ($) | * Funds Requested ($) |
--- | --- | --- | --- |
1. S&W | 53.10 | 163,335.00 | 86,731.00 |
2. 
3. 
4. 

Total Indirect Costs 86,731.00

Cognizant Federal Agency DHHS, Wallace Chan, (425) 437-7820

(Agency Name, POC Name, and POC Phone Number)

I. Total Direct and Indirect Costs

Total Direct and Indirect Institutional Costs (G + H) 386,731.00

J. Fee

Funds Requested ($) 

K. * Budget Justification 1247-budget justification.pdf

(Only attach one file.)
BUDGET JUSTIFICATIONS

Personnel

Principal Investigator

Jeffrey Grimm, PhD will devote 4.25 calendar months (35.4% effort) to the project. The budget includes 3 months of summer salary and an annual one course teaching buyout (typical teaching load is 6 courses per 9 month academic year). Dr. Grimm will be responsible for coordinating the studies described in the Specific Aims and for training the undergraduate research assistants. He will also be responsible for organizing the results of the studies and will lead the production of research reports related to the studies.

Undergraduate Research Assistants

The budget includes funding for 2,040 annual hours of time-slip undergraduate research assistant work. This will be divided to provide 3, ¼ time (10 h/wk) positions during the academic year and 2 full-time (40 h/wk) positions during the summer. This is equivalent to 3 individuals at 2.25 calendar months (18.75% effort) and 2 individuals at 3 calendar months effort (25% effort) on the project. Other students may work for research credit.

Students will be selected for the paid positions from Dr. Grimm’s laboratory group (currently there are 10 students) based on seniority, research interest, and availability. Undergraduate research assistants will be responsible for helping to schedule experiments, running behavioral studies, collecting tissue, and conducting some aspects of the molecular assays. Select students (based upon skill set and interest) will be trained to conduct more detailed aspects of the molecular studies (e.g. quantitation steps). Research assistants will also be responsible for general laboratory maintenance duties, and will be involved in the general design of studies as well as interpretation of results and presentation of results at meetings and as part of manuscript development.

The 3 students identified to be the first research assistants will be Jesse Barnes, Kindsey North, and Stefan Collins. These students have all been members of the laboratory for 2 years and will be working in the laboratory the summer of 2010 funded by an ARRA (government stimulus funds) “Summer Research Experiences for Undergraduates” supplement to the current R15.

Equipment

Several equipment items are included in the proposed budget as the lab will be setting up 2 assays not currently conducted in the laboratory. For immunohistochemistry, most of the equipment is already available (microscopes with digital imaging, etc.) but for Western blotting there will be a need to purchase many items including a microplate reader for total protein determination (BCA assay), gel apparatuses, a transfer system, and an imaging system. Feedback from Dr. Bruce Hope at NIDA has led the PI to consider purchasing an infrared detection system to use with fluorescent-labeled antibodies instead of a traditional imaging system to use with chemiluminescence. The infrared procedure is more sensitive for detection of low-abundance proteins and also allows simultaneous probing for phosphorylated and non-phosphorylated protein in the same band. This would be a valuable tool for our Specific Aim 2. An infrared detection system would be the most expensive single piece of equipment in the budget.

Supplies

The assays in the proposal require many consumable items (pre-cast gels, transfer paper, reagents, antibodies). Based on an initial calculation of our projected throughput, the PI is requesting approximately $550/month for supplies. Note that there is no requesting for specific funds for animals as the PI is able to breed and maintain rats in the research facility at no cost.
Travel

$2500 per year is requested to support the travel of the PI to the annual Society for Neuroscience meeting. Any remaining funds would be supportive to other travel, including travel to NIDA in Baltimore for training on Fos IHC and Western blotting methods (see attached consultant letters). $1500 per year is requested to help support undergraduate travel to the annual society for Neuroscience meeting. This will be divided into 3, $500 awards per year to be given based upon seniority, interest, and availability to travel.

Consultants (see attached letters)

Yavin Shaham, PhD
Behavioral Neuroscience Branch
IRP/NIDA/NIH

Dr. Shaham is a leading expert on the neurobiology of addiction and includes studies of the incubation of craving in his research program. He was a mentor of the PI when the PI was a post-doctoral fellow at NIDA.

Services: Dr. Shaham will train the PI on the current Fos IHC protocol in his laboratory at NIDA IRP in Baltimore. Dr. Shaham will continue to serve as a consultant as the proposed project is completed at WWU.

Bruce Hope, PhD
Behavioral Neuroscience Branch
IRP/NIDA/NIH

Dr. Hope is an expert on molecular techniques, especially in the context of addiction behaviors. He first-authored the first study of long term cocaine-induced delta Fos B adaptations with Dr. Eric Nestler. Dr. Hope taught the PI how to conduct Western blot analyses in his laboratory when the PI was a post-doctoral fellow at NIDA.

Services: Dr. Hope will train the PI on the current Western blotting protocols in his laboratory at NIDA IRP in Baltimore. Dr. Hope will continue to serve as a consultant as the proposed project is completed at WWU.

Dianne Lattemann, PhD
Dept of Psychiatry & Behavioral Sciences
University of Washington

Dr. Lattemann is a leading expert on the neurophysiology of body weight and has also published studies on the neurobiology of sucrose seeking. The PI has collaborated on research and educational outreach projects with Dr. Lattemann.

Services: Dr. Lattemann will train the PI and his students on the proper procedure for dissecting and quantifying retroperitoneal fat pads from rats. Dr. Lattemann will continue to serve as a consultant as the proposed project is completed at WWU.
# RESEARCH & RELATED BUDGET - Cumulative Budget

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<td>Section B, Other Personnel</td>
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<th>Total Salary, Wages and Fringe Benefits (A+B)</th>
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<th>Section D, Travel</th>
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| 1. Domestic                                   | $12,000.00  |
| 2. Foreign                                    |             |

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<tr>
<th>Section E, Participant/Trainee Support Costs</th>
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| 1. Tuition/Fees/Health Insurance             |             |
| 2. Stipends                                  |             |
| 3. Travel                                    |             |
| 4. Subsistence                               |             |
| 5. Other                                     |             |
| 6. Number of Participants/Trainees           |             |

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| 1. Materials and Supplies                    | $20,404.00  |
| 2. Publication Costs                         |             |
| 3. Consultant Services                       |             |
| 4. ADP/Computer Services                     |             |
| 5. Subawards/Consortium/Contractual Costs    |             |
| 6. Equipment or Facility Rental/User Fees    |             |
| 7. Alterations and Renovations               |             |
| 8. Other 1                                   |             |
| 9. Other 2                                   |             |
| 10. Other 3                                  |             |

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# 1. Project Director / Principal Investigator (PD/PI)

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<tr>
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<tbody>
<tr>
<td>* First Name:</td>
<td>Jeffrey</td>
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<tr>
<td>Middle Name:</td>
<td>W</td>
</tr>
<tr>
<td>* Last Name:</td>
<td>Grimm</td>
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# 2. Human Subjects

- Clinical Trial: No
- * Agency-Defined Phase III Clinical Trial: No

# 3. Applicant Organization Contact

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<tr>
<td>* First Name:</td>
<td>Ken</td>
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<tr>
<td>Middle Name:</td>
<td>W</td>
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<tr>
<td>* Last Name:</td>
<td>Clark</td>
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</table>

| Phone Number: | 360.650.4403 |
| Fax Number: | 360.650.6811 |
| Email: | ken.clark@wwu.edu |

* Title: Director, Research & Sponsored Programs

| * Street1: | 516 High Street |
| Street2: | |
| * City: | Bellingham |
| County/Parish: | Whatcom |
| * State: | WA: Washington |
| Province: | |
| * Country: | USA: UNITED STATES |
| * Zip / Postal Code: | 98225-9038 |
4. Human Embryonic Stem Cells

* Does the proposed project involve human embryonic stem cells?  ☒ No  ☐ Yes

If the proposed project involves human embryonic stem cells, list below the registration number of the specific cell line(s) from the following list: http://stemcells.nih.gov/research/registry/. Or, if a specific stem cell line cannot be referenced at this time, please check the box indicating that one from the registry will be used:

Cell Line(s):  ☐ Specific stem cell line cannot be referenced at this time. One from the registry will be used.

[Cell Line(s) listed]
# PHS 398 Research Plan

## 1. Application Type:

From SF 424 (R&R) Cover Page. The response provided on that page, regarding the type of application being submitted, is repeated for your reference, as you attach the appropriate sections of the Research Plan.

*Type of Application:

- [x] New
- [ ] Resubmission
- [ ] Renewal
- [ ] Continuation
- [ ] Revision

## 2. Research Plan Attachments:

Please attach applicable sections of the research plan, below.

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SPECIFIC AIMS

Drug abuse continues to contribute to negative health and social outcomes (De Alba et al. 2004; Rehm et al. 2006). Attention has recently been turned to excessive food consumption (“food abuse”) as obesity rates have doubled in regions of the US between 1999-2008 (Flegal et al. 2010). It has been suggested that disordered eating and drug addiction share common neurobehavioral features (Wang et al. 2004; Volkow and Wise 2005; Nair et al. 2009). High rates of relapse to drug use following prolonged forced abstinence periods characterize the behavior of experienced cocaine users (O’Brien et al. 1988; Mendelson and Mello 1996) and nearly all obesity patients return to pre-treatment weights 5 years post-treatment (Brownell and Wadden 1992; Wadden 1993) while 90% of dieters fail to achieve weight loss goals (Grodstein et al. 1996). In cocaine-free individuals, drug craving and relapse to drug use can be triggered by exposure to stimuli previously associated with drug taking (Childress et al. 1999; Carter and Tiffany 1999; Epstein et al. 2009). For individuals dieting, food-paired stimuli can lead to increased food craving and intake (Jansen et al. 2003; Sobik et al. 2005).

In a rat model of drug relapse, we and others have found that rats will respond progressively more for a reward-paired cue over a period of several weeks of abstinence from self-administration. This “incubation of craving” (Grimm et al. 2001) has been observed in human addicts (Piasecki et al. 1998; Piasecki et al. 2000; Kosten et al. 2005; Nava et al. 2006; Marrone et al. 2009) and may be a key factor in high rates of relapse to drugs and food in the clinical population. Using the animal model, one of our findings in our current R15 funding period was that systemically-delivered dopamine D1 antagonist (SCH23390) attenuation of responding for a sucrose-paired cue was more effective at an early (1 day) vs. a later (1 month) period of forced abstinence (see preliminary data). SCH23390 also attenuated cue reactivity when administered directly to either the core or shell of the nucleus accumbens, but the effect was not abstinence-dependent (see preliminary data). In a separate study, we found that one month of environmental enrichment blocked the development of the incubation of sucrose seeking in rats (Grimm et al. 2008, preliminary data). Aims of the current proposal are to integrate questions that followed from these two lines of research. First, we plan to identify regions of the brain preferentially activated by a sucrose-paired cue following 1 month of forced abstinence from self-administration. Second, we will determine how the activity expression profile differs in rats that have been environmentally enriched. Third, given that the expression technique we plan to use (expression of Fos, an immediate-early gene product) is mediated by dopamine D1 receptors, we plan to use the Fos expression findings to select regions for secondary analyses of signaling cascade proteins and transcription factors in the D1 → Fos pathway. Analyses of these proteins will give us detailed insight into molecular adaptations that may accompany incubation of sucrose seeking and/or environmental enrichment. These analyses will also help us to determine why SCH23390 has abstinence-dependent effects upon sucrose seeking in rats.

Specific Aim 1. This Aim is to examine regional cue-induced Fos expression after either 1 or 30 days of forced abstinence from sucrose self-administration with or without environmental enrichment during forced abstinence.

Specific Aim 2. This Aim is to examine regional basal and cue-induced dopamine D1 receptor signal cascade and related transcription protein expression after either 1 or 30 days of forced abstinence from sucrose self-administration with or without environmental enrichment during forced abstinence. The proteins of interest for Specific Aim 2 are to be initially limited to those directly in the D1 receptor → Fos expression pathway.

Impact

The results of these studies will provide information regarding molecular adaptations in the brain that relate to reward seeking behavior. Results of these studies will be critical for subsequent functional follow-up studies wherein the possible causal role of the identified brain regions and protein adaptations on relapse behavior may be determined. Overall, the results of these studies will provide information of great interest to basic addiction researchers and also to clinicians, in particular as the studies are designed around modeling an intervention strategy for relapse. The other key element of impact of these studies is that the research program is conducted with undergraduate research assistants at a traditionally non-research focused institution. Carrying out these studies will expose students to meritorious research and improve the research environment of Western Washington University.
RESEARCH STRATEGY

(a) Background and Significance

Relapse behaviors are a major public health problem

Drug abuse continues to contribute to negative health and social outcomes (De Alba et al. 2004; Rehm et al. 2006). Attention has recently turned to excessive food consumption (“food abuse”) as obesity rates have doubled in some regions of the US between 1999-2008 (Flegal et al. 2010). It has been suggested that disordered eating and drug addiction share common neurobehavioral features (Wang et al. 2004; Volkow and Wise 2005; Nair et al. 2009). High rates of relapse to drug use following prolonged forced abstinence periods characterize the behavior of experienced cocaine users (O'Brien et al. 1988; Mendelson and Mello 1996) and nearly all obesity patients return to pre-treatment weights 5 years post-treatment (Brownell and Wadden 1992; Wadden 1993) while 90% of dieters fail to achieve weight loss goals (Grodstein et al. 1996). In cocaine-free individuals, drug craving and relapse to drug use can be triggered by exposure to stimuli previously associated with drug taking (Childress et al. 1999; Carter and Tiffany 1999; Epstein et al. 2009). For individuals dieting, food-paired stimuli can lead to increased food craving and intake (Jansen et al. 2003; Sobik et al. 2005).

Time-dependent increase in relapse behaviors—incubation of craving

Gawin and Kleber (1986) proposed, based upon clinical observations, that abstinent cocaine addicts become increasingly sensitive to the craving-inducing effects of cocaine-paired environmental stimuli over the course of abstinence. Such an effect has been demonstrated in a clinical study (Kosten et al. 2005). “Incubation of craving” has also been observed in human heroin and cigarette-smoking addicts (Piasecki et al. 1998; Piasecki et al. 2000; Nava et al. 2006; Marrone et al. 2009). As with drug relapse, the power of food cues to direct food-seeking behavior could “incubate” over time.

We have observed an analogous time-dependent increase in cue-induced cocaine seeking in rats withdrawn from cocaine self-administration (Grimm et al. 2001). This phenomenon generalizes to rats with a history of sucrose self-administration (see preliminary data). As this incubation of reward seeking occurs for a variety of drugs (Bienkowski et al. 2004; Lu et al. 2004), as well as food and non-nutritive rewards (e.g. Splenda®, unpublished observations), there is likely a common neural circuit mediating its expression. Our studies so far suggest that it is not simply a sensitization phenomenon akin to psychostimulant sensitization. For example, incubation of cocaine seeking is not accompanied by a time-dependent increase in cocaine-induced locomotor activity (unpublished observations), nor is it accompanied by a time-dependent increase in cocaine-primed reinstatement of cocaine seeking (Lu et al. 2004). Furthermore, incubation of sucrose seeking is also not accompanied by a time-dependent increase in cocaine-induced locomotion and the abstinence-dependent effects of acute cocaine on sucrose cue reactivity are the opposite of what would be expected with sensitization (Grimm et al. 2006). Instead, our studies in the past several years have focused on a critical role for motivational processes in the incubation of craving (Grimm et al. 2005; Grimm et al. 2008; Harkness et al. 2009) with recent focus on the nucleus accumbens (NAcc).

The NAcc appears to function as a “limbic-motor interface” as it receives projections from several limbic areas (including the basolateral amygdala and ventral tegmental area) and projects to motor output areas (Mogenson et al. 1980; Mogenson and Yang 1991). The NAcc may modulate the elasticity of “demand” (Aberman and Salamone 1999) or “reward cost” (Bowman and Brown 1998) for a stimulus. For example, the NAcc has been shown to control the magnitude of responding a subject elicits to produce a conditioned reward (Burns et al. 1994). Given our hypothesis regarding a potential critical role for the NAcc in incubation, we were surprised to find an abstinence-dependence to the effects of D1 antagonism on sucrose cue-reactivity when the drug was administered systemically, but not when administered into either the core or shell of the NAcc (see preliminary data). It could be that the D1-mediated effect on incubation is mediated by other brain regions that communicate with the NAcc. For example, NAcc AMPA receptors lacking the gluR2 subunit appear to mediate incubation of cocaine seeking (Conrad et al. 2008) and activation and inactivation studies of ventral medial prefrontal cortex (VMPfc) indicate that this structure can modulate incubation of cocaine seeking (Koya et al. 2009). It could be that glutamate arising from this region of the cortex mediates the AMPA effect identified by Conrad et al. It is also possible that incubation of reward seeking is not mediated by the NAcc at
all. For example, decreasing glutamate release in the central nucleus of the amygdala (CeA) reduces incubation of sucrose (Uejima et al. 2007) or cocaine seeking (Lu et al. 2007). Clearly, more information is needed to determine the critical brain regions involved in the incubation of craving effect and the neuroanatomical site(s) where D1 antagonism has time-dependent effects on the incubation of reward seeking.

Environmental enrichment functions as a relapse prophylactic

Impacts of lower socio-economic status (SES) include drug use (Winstanley et al. 2008) and obesity (Drewnowski et al. 2007). Although the relationship between SES and these health outcomes, outcomes that have learning and motivational components (Wise 2004; Volkow and Wise, 2005), is complex it would be reasonable to explore how ameliorating aspects of a “deprived” environment might affect these outcomes. To explore the impact of enrichment on behavior and neurobiological indices related to learning and motivation (Nithianantharajah and Hannan, 2006), a simple animal model was developed comparing rats living in isolated or normal (relative term) conditions to those in comparatively “enriched” conditions (Rosenzweig, 2003). In this model enrichment typically involves more space, greater social contact, and the opportunity to explore novel objects and engage in exercise. Environmental enrichment has repeatedly been shown to increase problem solving abilities in rats demonstrated as enhanced performance in the radial arm (Hellemans et al. 2004) and water mazes (Daniel et al. 1999; Pham et al. 1999), and increased efficiency in solving Hebb-Williams problems (Will et al. 1977; Murtha et al. 1990).

Environmental enrichment in rats tends to result in decreased self-administration of amphetamine (Bardo et al. 2001; Green et al. 2002). Its effect on food consumption has been equivocal so far, with environmental enrichment decreasing sucrose intake in one study (Brenes and Formaguera, 2008), but increasing sucrose self-administration in another, if only temporarily (Bardo et al. 2001). However, discovered during the current R15 funding period, we recently reported (preliminary data) the first example of environmental enrichment attenuated relapse behavior. Specifically, we found that one month of environmental enrichment blocked the incubation of sucrose seeking (Grimm et al. 2008). The effect was subsequently generalized to cocaine by another research group (Chauvet et al. 2009). It is possible that enriched rats are more adept at learning and relearning the significance of stimuli paired with reward or punishment. For example, enriched rats demonstrate enhanced place preferences (Bowling and Bardo, 1994; Bardo et al. 1995; Smith et al. 2005) and aversion (Smith et al. 2003), accelerated extinction of conditioned fear (Pietropaolo et al. 2006), and accelerated extinction of lever pressing previously associated with either amphetamine or sucrose (Stairs et al. 2006). We conclude that enriched rats might be at an advantage, from a decreased vulnerability perspective, at avoiding conditioned addiction behaviors such as cue-induced “relapses” characteristic of both eating (Marlatt, 1990) and drug addiction (Gawin, 1991; Childress et al. 1999). Environmental enrichment appears to function as a prophylactic against incubation of reward seeking. Much more research needs to be conducted to identify the neuronal substrates of this effect.

Fos indicates neuronal activation and D1 receptor-mediated signaling cascades

Rationale was provided in the 2 preceding sections to further examine how D1 receptor antagonism and environmental enrichment affect the incubation of sucrose craving. One initial step is to identify brain regions that are constitutively altered and/or selectively activated by sucrose-paired cues in rats that have been enriched vs. control animals. Expression of the immediate-early gene c-Fos indicates neuronal activation (Herrera and Robertson, 1996). Compared to rats that have not been made abstinent to sucrose, such a study would provide a map of brain regions that are potential critical sites in the effects of enrichment and/or the incubation of craving effect on sucrose cue reactivity. We propose below to conduct this study (Specific Aim 1) using immunohistochemistry (IHC) for the immediate-early gene product Fos. Fos has been used extensively as an indicator of acute and conditioned neuronal activation (e.g. Shalev et al. 2003; Konkle and Bielajew, 2004; Hamlin et al. 2006; Mattson et al. 2008; Kufahl et al. 2009) related to rewards. Changes in Fos expression have also been shown to track with the time-dependent expression of behavioral sensitization to cocaine (Crombag et al. 2002; Hope et al. 2006) supporting the feasibility of the time course (forced abstinence) studies we propose below.
Of critical relevance to the results of our preliminary studies with the D1 antagonist, D1 receptor activation is critical for the expression of Fos (Ciccocioppo et al. 2001; Hamlin et al. 2006; Hu et al. 2008). Fos expression from Specific Aim 1 will therefore not only give us insight into brain regions involved in the effects of enrichment and conditioned cue reactivity, but will point to the most likely post-synaptic signaling cascade targets of systemically-administered D1 antagonist. We propose below (Specific Aim 2) to conduct a second study using Western blot analysis of total and phosphorylated proteins that have been identified as key steps in molecular signaling cascades linking D1 receptor activation and Fos expression. Identification of phosphorylated proteins provides more information, compared to identifying total protein alone, on the dynamic state of the transduction pathway. In addition to D1 receptor, we propose below to examine several constitutively expressed proteins in the D1 → Fos signaling cascade in both basal and phosphorylated states (PKA, DARPP-32, ERK, CREB, and Elk1, see Figure 4 below). A bias towards or against the phosphorylated state of one of the signaling proteins (PKA, DARPP-32, ERK) or transcription factors (CREB, Elk1) would provide some explanation of any differential expression of Fos by incubation and/or enrichment observed in Specific Aim 1. Specific alterations in the transcription factors would also indicate that other gene targets may have altered expression by incubation and/or enrichment (Nestler 2001) (e.g. for CREB: brain derived neurotrophic factor, synaptic transmission related proteins such as syntaxin 1A, ion channel proteins) (McClung and Nestler, 2003). Such targets could be the focus of future studies. As our design has comparison groups to assess the effects of abstinence, cues, and incubation, alterations in protein expression and phosphorylation state related to incubation and/or environmental enrichment would provide more detailed evidence for neuroplasticity related to abstinence and/or environmental conditions, respectively, while cue-related changes would indicate adaptive changes in the sensitivity of the transduction pathways to acute stimulation. We plan to use Western blot analysis as a more quantitative technique, compared to IHC, for identifying protein levels. There will also be a benefit to undergraduate research assistants by having 2 techniques in the laboratory, namely more opportunities for their laboratory technique development.

Summary of proposed studies

The finding of an incubation of reward seeking for both cocaine and sucrose-experienced rats indicates that there is likely a single behavioral mechanism common to both reward classes. These time-dependent increases in reward seeking suggest neuroplastic changes. Environmental enrichment attenuates the development of the incubation of craving effect so it is possible that environmental enrichment either directly or indirectly counteracts the molecular changes underlying incubation. The purpose of this proposal is to first identify brain regions that are selectively activated in relapse conditions (Specific Aim 1) and then identify levels of D1 signaling cascade proteins expressed in the identified regions (Specific Aim 2).

It is important to note here that the studies proposed in these aims are quite novel. Very little is known about the expression of genes related to the incubation of sucrose seeking (Grimm et al. 2002) and only a handful of studies have examined gene expression patterns in animals following environmental enrichment (Thiriet et al. 2008; Solinas et al. 2009). As a means to provide an understanding of brain mechanisms underlying addiction, and the prevention thereof, the proposed studies may have significant impact in the field of addiction. This is in addition to the impact they will provide in allowing innovative, exciting, significant research training opportunities for undergraduates. We also plan to conduct supplementary analyses with the subjects from Specific Aim 2 to further enhance research training opportunities. Specifically, we plan to dissect and weigh retroperitoneal fat pads of the rats and may develop a plan for specialized behavioral observations to provide insight into how environmental enrichment affects the “fitness” of the animals. These analyses will be conducted under consultation with Dr. Dianne Lattemann (see attached letter of support).

(b) Innovation

The proposal is to explore regional gene expression and molecular signaling adaptations due to a novel relapse prevention strategy (environmental enrichment). Examining these patterns of gene expression and signaling adaptations within the incubation paradigm is a novel approach that aims to provide information not only about how environmental enrichment reduces relapse, but what neuroadaptations are related to relapse itself. The proposal is also extremely innovative within the context of the PI’s institution as gene expression studies coupled with behavioral outcomes are not currently conducted. Undergraduate involvement in conducting these innovative studies will strengthen the research environment of the institution.
As per the R15 (AREA) Program Announcement, the three goals of the program are:
- to support meritorious research
- to strengthen the research environment of the institution
- to expose students to research (preferably undergraduates)

Reflecting the Goals of the R15 program, the 2007-2010 R15 funding of my laboratory allowed the following accomplishments:

- my laboratory has published research in high quality journals and has presented results of studies at international meetings
- the strength of the research environment has increased over the years of funding in that more students have had access to research training experiences and to the enhanced depth of the behavioral neuroscience degree program brought about by having NIH-funded equipment (e.g. operant boxes, intracranial self-stimulation, cryostat, microscope) available for student research projects
- in the most recent R15 grant support period I have supervised 11 independent studies projects. Twenty students (18 of the 20, undergraduates) have been active research assistants in my laboratory (see detail below). Four students went to work in the biotechnology/health industry, one works as a fellow at the NIDA/NIH, one entered a master’s program in Clinical Psychology, one entered a PhD program in Clinical Psychology, and two have been admitted to PhD programs in Neuroscience

Specific examples of outcomes related to the 3 R15 goals are embedded in the following sections. The Progress Report section indicates how the 3 goals have been met in the current funding period. The Preliminary Data section also looks back at what has been accomplished but also lays out the studies planned for the grant renewal. The end of that section contains a brief plan for how students will be involved in conducting the proposed studies. All of these sections are considered aspects of “Approach” as they are provided as means to achieve the goals of the R15 mechanism.

Progress report

Involvement of students in basic behavioral neuroscience research

The R15 mechanism has been instrumental in allowing me to open the lab to student research assistants. Funds from this grant have allowed me to not only involve students in the studies directed by the Specific Aims of the parent grant, but to develop other related lines of study in many cases directed by student ideas (see list of projects below). I was also awarded supplemental funds including an Underrepresented Minority Student Supplement for Christine Ratliff and an ARRA stimulus fund Administrative Supplement to support undergraduate research in the summer.

Student involvement in my Behavioral Neuroscience laboratory (2007-2010):

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<td>Carl Buse</td>
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<td>John Harkness</td>
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<td>Meghan Manaois</td>
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<td>Dan Osincup</td>
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<td>Ryan Pemberton</td>
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<td>Christine Ratliff</td>
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Projects supported by the current R15 (2007-2010); students and research training opportunities indicated:

Effects of nicotine on sucrose self-administration and sucrose seeking  Spring 2009-present
- Student tasks/skill building: handling rats, running experiments, collecting data, data entry, drug
  injections, preparing drug
- Students involved: Christine Ratliff, Addison Tice, Clarence Holmes, Jason Tsukahara, Jesse Barnes,
  Kindsey North, Lauren Thompson, Rachel Weber, Stefan Collins

Effects of systemic or intra-nucleus accumbens core or shell dopamine D1 antagonist on incubation of sucrose
  craving in rats  Fall 2007-present
- Student tasks/skill building: handling rats, running experiments, collecting data, data entry, surgical
  experience (select students), histology including microscope work, drug injections, preparing drug
- Students involved: Addison Tice, Christine Ratliff, Clarence Holmes, Jason Tsukahara, Jesse Barnes,
  Kindsey North, Lauren Thompson, Rachel Weber, Stefan Collins, Meghan Manaois, Amber Fyall,
  Sierra Webb

Effects of taste aversion to sucrose on incubation of sucrose craving in rats  Fall 2007-Spring 2009
- Student tasks/skill building: handling rats, running experiments, collecting data, data entry, drug
  injections, preparing drug, data analysis, manuscript preparation
- Students involved: John Harkness, Addison Tice, Christine Ratliff, Clarence Holmes, Jason Tsukahara,
  Jesse Barnes, Kindsey North, Lauren Thompson, Rachel Weber, Stefan Collins, Meghan Manaois, Amber Fyall,
  Sierra Webb

Effects of satiety and habituation on incubation of sucrose craving in rats  Fall 2006-Spring 2008
- Student tasks/skill building: handling rats, running experiments, collecting data, data entry, data
  analysis
- Students involved: Jason Wells, John Harkness, Sierra Webb, Amber Fyall

Effects of environmental enrichment on incubation of sucrose craving in rats  Spring 2006-Winter 2008
- Student tasks/skill building: handling rats, running experiments, collecting data, data entry, enriching
  rats, preparing drug, data analysis, manuscript preparation
- Students involved: Carl Buse, Meghan Manaois, Dan Osincup, Barbara Wells, Amber Fyall, Ryan
  Pemberton, Ryan Reese, John Harkness

Influence of sugar, fat, and flavor on the rewarding value of highly palatable foods  Spring 2006-Winter 2007
- Student tasks/skill building: handling rats, running experiments, collecting data, data entry, data
  analysis, preparing sugar, fat, flavor solutions, experimental design, manuscript preparation
- Students involved: Jason Wells

Effects of sucrose intake on brain stimulation reward threshold in rats  Spring 2005-Spring 2008
- Student tasks/skill building: handling rats, running experiments, collecting data, data entry, surgical
  experience, histology including microscope work, drug injections, preparing drug, experimental design,
  data analysis
- Students involved: Amber Fyall (Master’s degree project)

Effects of naloxone (opiate antagonist) on incubation of sucrose craving in rats  Spring 2005-Winter 2007
- Student tasks/skill building: handling rats, running experiments, collecting data, data entry, drug
  injections, preparing drug, experimental design, data analysis, manuscript preparation
- Students involved: Meghan Manaois (Master’s degree project), Dan Osincup, Amber Fyall, Barbara
  Wells
Progress report publications 2007-2010 (student names in bold)

Peer reviewed publications


Posters/presentations—scientific meetings


Preliminary data related to proposed studies

As indicated by the list of publications and presentations listed above, we have been very productive in 2007-2010. Our productivity is especially salient considering that the current R15 is relatively modest in dollar amount ($150,000) and that we are at an institution that prioritizes teaching. I typically carry a teaching load of 6 courses a year (2 per academic quarter). The following section will indicate preliminary data relevant to this proposal. Due to space limitations, details of the publications and presentations from 2007-2010 are only indicated as relevant to the proposal.

The finding of an incubation of effect for both cocaine and sucrose seeking may indicate that there is a single behavioral mechanism common to both reward classes. These time-dependent increases in reward seeking suggest neuroadaptations in brain regions involved in mediating reward seeking behaviors. Environmental enrichment appears to attenuate the development of the incubation of sucrose seeking, so it is possible that environmental enrichment either directly or indirectly counteracts the molecular changes underlying incubation.
The purpose of this proposal is to first identify brain regions selectively activated in relapse conditions with or without an environmental enrichment experience (Specific Aim 1), and then identify levels of D1 signaling cascade proteins expressed in those regions and their projections areas, as appropriate (Specific Aim 2).

1. Incubation of sucrose craving

As discussed previously in this proposal, cocaine seeking in rats progressively increases over the first 2 months of forced abstinence from self-administration (Grimm et al. 2001, for example). We have also demonstrated an incubation of craving effect in animals following forced abstinence from sucrose self-administration (Figure 1, from Grimm et al. 2005; see also Grimm et al. 2006, 2007, 2008; Harkness et al. 2009). It could be that a general behavioral mechanism is responsible for the incubation of craving effect in both cocaine and sucrose-experienced animals. Perhaps a second mechanism is responsible for the more pronounced effect in cocaine-experienced animals.

Figure 1. Means (+SEMs) of responses on lever previously associated with sucrose in a 1-h session of access to a tone+light stimulus previously associated with sucrose self-administration.

2. Systemic, not NAcc, D1 antagonist selectively reduces sucrose seeking in early forced-abstinence

In the current grant period we have continued to explore the pharmacology of the incubation of sucrose seeking including mediation of the effect by opiate receptors (Grimm et al. 2007). Following positive findings with dopamine agonism (Grimm et al. 2006), we have been recently focusing on a potential role for dopamine D1 receptors. D1 antagonism has been demonstrated to decrease drug cue reactivity in other relapse paradigms (Ciccocioppo et al. 2001; Crombag et al. 2002; Bossert et al. 2009). We have recently found that low dose D1 antagonism (SCH23390) selectively decreased responding for a sucrose-paired cue after 1, but not 30 days of forced abstinence (Figure 2A as reported at Society for Neuroscience, 2009, Grimm et al.). NAcc core or shell site-directed D1 antagonism also decreased cue reactivity, but this effect was not abstinence-dependent (Figure 2B as reported at Society for Neuroscience, 2009, Grimm et al.). More studies are required to determine the critical brain regions involved in the incubation of craving effect and the site(s) where D1 antagonism has time-dependent effects on the incubation of reward seeking.

Figure 2. A: Lower doses of systemic SCH23390 selectively reduced responding for a sucrose-paired cue after 1, but not 30 days of forced abstinence. B. SCH23390 delivered directly to the NAcc core or shell reduced cue reactivity as well, but the effect was not abstinence-dependent. Microinjection doses were bilateral Lo (.3 µg/site) or Hi (.6 µg/site). Control studies indicated that, except for the 25 ug/kg systemic dose and the Hi NAcc Shell dose, the drug effects were not due to non-specific motor impairment (not shown). Means (±SEMs) indicated on figure.
3. Environmental enrichment blocks development of incubation of sucrose seeking

We recently found that one month of environmental enrichment (4 rats co-housed in large, multi-level environment with novel toys 3 days/week) protected rats from developing an abstinent-dependent increase in sucrose seeking (Figure 3, from Grimm et al. 2008). A similar effect was subsequently reported by another laboratory with rats that had self-administered cocaine (Chauvet et al. 2009). These exciting results point towards novel therapeutic approaches for addiction. To this end, it will be critical to further explore behavioral parameters of the enrichment effect and to explore its neurobiological substrates. Currently there is very little known about how enrichment attenuates reward seeking.

**Figure 3.** Rats self-administered sucrose for 10 days and then were tested for sucrose seeking after 1 AND 30 days of forced abstinence. Half of the rats were Enriched during the 30 days of forced abstinence. Means (±SEMS) are indicated on the figure.

Responding for Cue

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</tbody>
</table>

* significantly different from Day 1 of forced abstinence, \( p < 0.05, n=8 \) per group.

3. Environmental enrichment blocks incubation of sucrose seeking

Environmental enrichment protects against the development of the incubation of sucrose seeking and this has important clinical implications. Further study is required to identify brain regions that mediate this effect.

As described in the Background and Significance section above, the current proposal is to build on our preliminary findings to map out brain regions related to incubation of sucrose seeking and environmental enrichment (Specific Aim 1). Fos expression will provide brain activation signatures revealed with IHC. As Fos expression has been shown to depend on dopamine D1 receptor activation, the mapping study may also provide insight into brain regions that mediate the abstinent-dependent effect of SCH23390 on sucrose cue reactivity. Specific Aim 2 is to explore signaling cascade proteins including transcription factors that mediate D1 receptor activation \( \rightarrow \) Fos expression. Completion of both Aims will allow us to develop a picture of regional and molecular targets for future functional studies of the incubation of sucrose seeking and environmental enrichment effects. Also, as noted in other sections, the studies in this proposal will allow undergraduate research assistants to gain important laboratory skills that bridge behavior and molecular biology.

Research Design and Methods (General Methods follow this section)

The general design depicted in this table is to allow examination of effects of responding for a sucrose-paired cue on gene expression (including phosphorylation of signal cascade proteins) in rats following prolonged forced abstinence under either control or enriched environment conditions.
Experimental groups n=10 per group (see power analysis in Vertebrate Animals section).

<table>
<thead>
<tr>
<th>Training (operant box)</th>
<th>Forced Abstinence (home cage)</th>
<th>Sucrose Seeking (operant box)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 Day (no enrichment)</td>
<td>2 h contingent cues or 2 h no cues</td>
<td></td>
</tr>
<tr>
<td>30 Days Control (no enrichment)</td>
<td>2 h contingent cues or 2 h no cues</td>
<td></td>
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<tr>
<td>30 Days Enriched</td>
<td>2 h contingent cues or 2 h no cues</td>
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</tr>
</tbody>
</table>

**Specific Aim 1.** This Aim is to examine regional cue-induced Fos expression after either 1 or 30 days of forced abstinence from sucrose self-administration with or without environmental enrichment during forced abstinence. Six groups of rats are required for this Aim (10 rats/group X 6 groups = 60 rats required).

**Rationale.** Fos is a marker of neuronal activation (Herrera and Robertson, 1996) and Fos expression appears to be mediated by dopamine D1 receptors (Ciccocioppo et al. 2001; Hamlin et al. 2006; Hu et al. 2008). D1 receptors have been demonstrated to be involved in reactivity to both drug and food-paired cues including the incubation of sucrose seeking (Ciccocioppo et al. 2001; Crombag et al. 2002; Bossert et al. 2009; preliminary studies, above). Specific Aim 1 will provide data that may yield insight into a role for differential activation within and/or between brain regions that could contribute to relapse, incubation, and their attenuation by environmental enrichment.

**Expected outcomes, potential limitations, and alternative approaches.** We expect that responding for sucrose-paired cues will lead to pronounced Fos expression in brain regions that have been shown to be critical in the processing of reward-paired stimuli (e.g. basolateral amygdala and NAcc). It is also likely that there will be measurable activation in other regions known to be involved in the processing of reward value (prefrontal and orbitofrontal cortices). We are not certain as to which brain regions might have differential Fos expression due to incubation and/or environmental enrichment other than it is likely there will be an incubation-related effect on expression, presumably a potentiation, in the CeA and VMpfc (Lu et al. 2007; Koya et al. 2009) and an enrichment-related change in expression in hippocampus and other regions of the cortex (perhaps a decrease in activation) (Ekstrand et al. 2008). Due to this general uncertainty, we plan to cast a somewhat wide net by planning to examine Fos expression in several brain regions (see general methods, below) typically assessed in similar mapping studies of cue reactivity (e.g. Kufahl et al. 2009). It is possible, however, that even this extensive survey approach will miss a key region. Also, other approaches might include adding a water self-administration group to control for the effects of a sucrose self-administration history. However, since rats would likely not respond much for water-paired cues (we have unpublished data indicating this to be the case), the added groups would not add much to our understanding of the neurobiology of sucrose-cue reactivity. We feel that our relatively simple research design will yield fairly clear and important initial mapping data that will be of use for follow-up studies such as described in Specific Aim 2.

**Specific Aim 2.** This Aim is to examine regional basal and cue-induced dopamine D1 receptor signal cascade and related transcription protein expression after either 1 or 30 days of forced abstinence from sucrose self-administration with or without environmental enrichment during forced abstinence. Six groups of rats are required for this Aim (10 rats/group X 6 groups = 60 rats required).

**Rationale.** Differential Fos activation by incubation and/or environmental enrichment identified in Specific Aim 1 within and/or between brain regions would suggest potential neuroadaptive changes in the dopamine D1 signaling pathway. Specific Aim 2 will provide data that might illuminate molecular signaling adaptations critical for sucrose cue seeking and its exaggeration or attenuation by incubation or environmental enrichment, respectively. Specific adaptations identified with Specific Aim 2 will be informative to subsequent functional follow-up studies. For example, if a particular signal has been identified to be up-regulated by incubation, pretreatment with its antagonist during forced abstinence might be effective at blocking the development of incubation. Signals altered by enrichment could similarly be targeted, perhaps as a way of pharmacologically introducing the beneficial effects of enrichment. The proteins of interest for Specific Aim 2 are to be initially limited to those directly in the D1 receptor → Fos expression pathway. As illustrated in Figure 4, these include the D1 receptor, PKA, DARPP-32, ERK, CREB, and Elk1. As noted in sections above, we plan to examine
both basal and phosphorylated levels of the post-receptor proteins as this level of regulation might be more informative of the actual activity of the signal cascade vs. examining total protein alone (regardless of level of phosphorylation). As indicated in the Figure, glutamate and BDNF interact with the signaling cascade we propose to examine. Targeting of glutamate and/or BDNF for expression or functional studies could be the focus of a future proposal.

**Figure 4.** Some detail of the D1 receptor \(\rightarrow\) Fos expression pathway. This figure is from a Lu et al. (2006) review in *Trends in Neurosciences.*

**Expected outcomes, potential limitations, and alternative approaches.** It is likely that we will observe changes in levels of constituents (overall and/or phosphorylated levels) of the D1 signaling cascade in regions that are identified by Fos expression in Specific Aim 1. In the unlikely event that we do not find differential Fos expression by incubation and/or enrichment in Specific Aim 1, or if there are unforeseen technical difficulties with the IHC approach, we will continue to Specific Aim 2 with a focus on regions that would be most likely to be involved in incubation and/or enrichment, based on published literature. For example, we would likely choose to first examine the CeA and VMPfc (incubation sites: Lu et al. 2007; Koya et al. 2009) as well as the hippocampus and other regions of the prefrontal cortex (enrichment sites: Ekstrand et al. 2008). We realize that the list of proteins and brain regions of interest is long and that this may decrease our potential throughput. For this reason, we will proceed by selecting 3 brain regions for analysis at the start (the remaining micropunched tissues from other brain regions will be stored for later analyses) and select 3 or 4 proteins for conducting Western blot analyses. Likely we will examine D1 receptor protein, one or two of the post-receptor signals (PKA, DARPP-32) and a transcription factor (e.g. CREB). The list for these initial studies will be determined after consultation with Dr. Bruce Hope at NIDA as, at this point, we are not sure how much tissue we will have to work with (i.e. need information from Specific Aim 1 regarding brain region targets, some could be relatively small) and it could be that by the time the studies are conducted there will be advances in the field (novel proteins identified and/or better antibodies available) that could bias our selection one way or the other. Regardless, our throughput will be enhanced for post-receptor proteins as the fluorescent probe technology allows determination of both basal and phosphorylated levels of a protein in the same blot (Marin et al. 2009).

**Timeline for studies identified in the specific aims.** We believe that we can have the IHC results from the first Aim 1.5 years into the grant. Running of subjects for Specific Aim 2 can actually start prior to Specific Aim 1 being complete as the brains from Specific Aim 2 will be frozen as subjects complete the study. As regions of interest are identified from Specific Aim 1, we can start micropunching from the frozen brains from Specific Aim 2. These punches can then be stored until needed for the Western blot analyses. These analyses would start approximately at the beginning to middle of the second year of the grant and then continue through year 3. The planned travel of the PI to NIDA for consultation on molecular techniques would occur at the beginning of the funding period. If needed, a follow-up trip to NIDA would be arranged later in the grant funding period.

**Future directions and other possible studies.** As noted in the sections above, the correlational findings of the Specific Aims in this proposal could inform subsequent functional studies. For example, brain regions identified with Fos expression could be targeted for inactivation in behavioral studies. Furthermore, proteins of interest from Specific Aim 2 could be targeted with agents to inhibit their activity, also as part of behavioral studies. Such studies would expand the understanding of the dynamic molecular changes underlying addiction behaviors and could inform future treatment approaches for addiction. Funding of the proposal will also allow other meritorious research to be conducted with undergraduate researcher involvement. As with the current R15, student input has driven studies in other directions (e.g. the published environmental enrichment study was a student project, as was the recent taste aversion study). Overall, the studies supported by the R15 will allow many students to gain skills behavioral analysis and molecular biology. We also plan to conduct some initial physiology analyses of the fitness of rats that are enriched. This will be an analysis of retroperitoneal fat pads under consultation with Dr. Dianne Lattemann. Related to this, we are considering structured video analysis of rats during enrichment to gauge their social interactions and general activity levels.
Plan for involvement of students in conducting proposed studies. The PA for the R15 indicates that the Budget Justification section should have detail on student involvement in the proposed studies. Detail has therefore been provided in that section. Briefly, the plan is for undergraduates to be involved in all aspects of the proposed studies. This level of involvement, up to helping with manuscript writing, has been the level engendered by the previous R15 grants to the laboratory. Students that will be in the laboratory as of the earliest funding date of the current proposal include: Jesse Barnes, Stefan Collins, David Goodman, Clarence Holmes, Kindsey North, Christine Ratliff, Lauren Thompson, Addison Tice, Jason Tsukahara, and Rachel Weber. Students identified for the first 3, ¼ time appointments will be Kindsey North, Jesse Barnes, and Stefan Collins. These students have seniority in the laboratory.

General Methods

Subjects and housing. 3 months old Simonsen-derived male Long-Evans rats will be used for all experiments. Rats will be bred in the Western Washington University vivarium. They will be housed individually (16x8x8 inch cages except for environmental enrichment conditions) on a 12-h reverse day/night cycle (lights off at 7 AM) with Purina Mills Inc. Mazuri Rodent Pellets and water available ad libitum (except for water deprivation as noted below). Rats will be weighed each Monday, Wednesday, and Friday for the duration of the experiment. Immediately prior to the self-administration training phase, the animals will be deprived of water for 17 h to encourage sucrose self-administration on the first day of training. Rats will then return to ad libitum water access (both in home cage and in operant conditioning chamber). All procedures follow the guidelines outlined in the “Principles of laboratory animal care” (NIH publication no. 85-23) and are approved by the Western Washington University Institutional Animal Care and Use Committee. Justification for use of males only is to avoid additional comparison groups to control for the estrous cycle. Sex difference and estrous cycle comparisons may be of interest in a future study.

Sucrose self-administration and sucrose seeking. Self-administration and seeking procedures will be similar to our previously published studies (e.g. Grimm et al. 2008). Operant training and testing will take place in operant conditioning chambers (30 x 20 x 24 cm; Med Associates) containing two levers (one stationary and one retractable), a tone generator, a white stimulus light above the active retractable active lever, and a red house light on the opposite wall. An infusion pump will deliver sucrose into a reward receptacle to the right of the active lever. Operant conditioning chambers are enclosed in sound-attenuating cabinets with ventilation fans. Rats will spend 2 h/day for 10 consecutive days in operant conditioning chambers where they will be allowed to press the active lever for a 0.6 ml delivery of 10% sucrose solution into the receptacle to the right of the lever. This response will also activate a compound stimulus consisting of a tone (2 kHz, 15 dB over ambient noise) and the white light. The compound stimulus will last for 5 s and will be followed by a 40-s time out, during which presses on the active lever will be recorded but have no programmed consequence. A response on the inactive (stationary) lever will not have a programmed consequence, but presses will be recorded. Four photobeams crisscross the chamber. The total number of beam breaks will be recorded during cue-reactivity testing. At the end of each training session, rats will be returned to home cages. The sucrose seeking session will be identical to training conditions, but sucrose syringes will be absent.

Environmental Enrichment. The enriched environment consists of a large wire mesh environment (83.8 cm by 88.9 cm by 45.7 cm from Quality Cage Company, Portland, OR). Rats (4 per group) will begin enrichment the afternoon of the final day of sucrose self-administration and remain enriched for the duration of the study. PVC pipe (20.3 cm length, 10.2 cm diameter) will be provided at all times and a novel “toy” will be exchanged with a used toy every Monday, Wednesday, and Friday. Toys will be purchased at a local pet store and include items designed for rodents but also toys typically used for cats and dogs.

Immunohistochemistry. Immediately following the sucrose seeking session, rats will be deeply anaesthetized with sodium pentobarbital and perfused transcadially with 100 ml of 0.1M phosphate-buffered saline (PBS) followed by 400 ml of 4% paraformaldehyde in 0.1M sodium phosphate (pH 7.4). Brains will be removed and post-fixed in 4% paraformaldehyde for 2 hours before transfer to 30% sucrose in 0.1 M sodium phosphate (pH 7.4) for 48 hours at 4 °C. Brains will be subsequently frozen at -80 °C until brain sectioning. Coronal sections (40 µm), separated by 120 µm and centered at +3.2, +1.6, -2.56, -5.8 relative to bregma (as per Kufahl et al. 2009), will then be cut in a cryostat, collected in cryoprotectant (20% glycerol and 2% dimethylsulfoxide in 0.1 M sodium phosphate, pH 7.4) and stored at -80 °C. Sections will be thawed and
rinsed three times for 10 min each in PBS and placed in blocking buffer (3% normal goat serum [NGS], 0.20% Triton X-100 in PBS) for 1 hour at room temperature. Sections will then be incubated overnight at 4°C in a 1:4000 dilution of Fos antibody (Fos sc-52, Lot F2209, Santa Cruz Biotechnology) in blocking buffer. Sections will then be washed in PBS and incubated for 2 h at room temperature with a 1:600 dilution of biotinylated anti-rabbit IgG secondary antibody (BA-1000, Vector Laboratories, Burlingame, CA, USA) in 1% NGS and 0.20% Triton X-100. Sections will be washed in PBS and processed for 1 h with avidin-biotin-peroxidase complex (ABC Elite kit, PK-6100, Vector Laboratories). Sections will be washed again in PBS and developed in a solution containing 0.035% DAB and 0.04% hydrogen peroxide in PBS for approximately 2 min. Sections will then transferred into PBS and mounted onto chrom-alum/gelatin-coated slides. Once dry, the slides will be dehydrated through a series of alcohol (30, 60, 90, 95, 100, 100% ethanol) and cleared with Citrasolv (Fisher Scientific) before coverslipping. Brightfield images of regions of interest, (as per Kufahl et al. 2009 and Koya et al. 2009) (cortex (VL, VM, PrL, IL, Orb, Cg2, AgI and Lent), basal ganglia (NAccC, NAccS, dCPu, BTA and SNr), amygdala (BlA, CeA and LA), dorsal hippocampus (dCA1, dCA2, DG and dS) and ventral hippocampus (vCA1, vCA3 and vS), will be imaged with the 5x objective on an Olympus BX51 upright microscope with CCD camera. Labeled Fos-immunoreactive nuclei will be quantitated using the Neurolucida computer-aided plotting system (Microbrightfield) by an investigator blind to experimental condition.

Western blot. Immediately after the sucrose seeking session, rats will be rapidly decapitated and brains will be frozen by immersion into −40 °C isopentane. One mm thick coronal brain sections will be cut on a cryostat at the levels determined by Fos expression in Specific Aim 1 and micropunches taken using 14- or 12-gauge needles and stored at −70 °C. Tissue punches will then be homogenized in 1% SDS, and protein concentrations assayed using the BCA assay (Pierce Chemical Company; Rockford, IL). Protein concentrations will be standardized by diluting with 1% SDS. Samples will then be subjected to SDS-polyacrylamide gel electrophoresis (10% acrylamide/0.27% N, N’-methylenebisacrylamide resolving gel) for three h at 150V and transferred electrophoretically to Immobilon-P transfer membranes (Millipore Corp; Bedford, MA) at 0.3A for 2 h. Immunoblotting for proteins will follow methods as described in Marin et al. (2009). Briefly, membranes will be incubated for 1 h in blocking buffer and then incubated overnight at 4 °C containing either one (D1 receptor) or two primary antibodies (for total and phosphorylated proteins). Specific antibodies will be selected under consultation from Dr. Bruce Hope. After primary antibody incubation, blots will be washed and incubated for 1 h with appropriate anti-mouse and/or anti-rabbit secondary antibodies labeled with IRDyes 680 and/or 800 (Li-Cor Biosciences). Fluorescence from fluorophores will be assessed with an Odyssey IR fluorescence scanner (Li-Cor Biosciences) and quantified using ODYSSEY 2.0 software.

Fat pad analysis. Retroperitoneal fat pads (representative of total body adiposity) will be dissected as per Wolden-Hanson et al. (1999) from rats in Specific Aim 2 and weighed immediately.

Data analysis

Behavior. Repeated measures analysis of variance (RMANOVA) will be made for active lever responding over training days to ensure that groups to be placed into different treatment conditions are initially statistically identical. The main factors will be Forced Abstinence (1 day with no enrichment, 30 days with no enrichment, 30 days with enrichment) and Sucrose Seeking (2h contingent cues, 2h no cues). Active lever responding during the sucrose seeking session will be compared between groups with ANOVA using these same factors. Appropriate post-hoc comparisons will be made with the Fisher PLSD test and significant differences will be indicated if p<0.05. Inactive lever responding and locomotor activity (total photobeam breaks) during the sucrose seeking session will be compared between groups using the same statistical procedure.

Proteins. For Fos expression, counts of Fos-labeled nuclei will be compared between groups using ANOVA with the factors identified above. The selected brain regions (see General Methods, below) will be examined separately. For Western blot identified proteins, protein concentrations will be converted to percent of the 1 day forced abstinence (no enrichment group) and then compared between groups using ANOVA with the factors identified above.

Fat pad weights will be compared between groups using ANOVA. Other statistical evaluations may be conducted post-hoc. For example, we may examine Pearson’s r (or Spearman’s rho if the data are not normally distributed) correlations between behavioral measures and protein values.
PROGRESS REPORT PUBLICATIONS


*in press publications are included in Appendix
VERTEBRATE ANIMALS

1. Adult (3 months), male Long-Evans rats will be used in all experiments of this proposal. The total number of rats to be used over a 3-year span is 120.

2. The studies described in this proposal necessitate the use of animals in order to test a model of relapse in humans. The experimental protocol calls for the use of post-mortem analyses in order to examine neural substrates of behavior. Thus, the research techniques cannot be used on humans, nor is there any feasible non-animal alternative. The rat species has been chosen based on its availability, size, and the extensive rat literature relevant to the proposed research. We have selected 10 animals per group to maximize the power to detect significant differences between treatment conditions yet minimize the number of animals needed for the studies. We have found in our previous published research that we can see an effect of environmental enrichment on the incubation of sucrose seeking with n=8 per group with a power value of .9, and can see a difference between drug (cocaine) treated groups for levels of the dopamine transporter protein with n=6 per group with a power value of .76. Considering the need to identify both a behavioral and a molecular effect in the same animals, 8 per group could therefore be a minimal n size per group. However, given that we will be looking for differences in amounts of proteins due to behavioral manipulations (not drug) we feel that we need to increase n sizes to increase statistical power. Also, we will likely have a small percentage of rats (10% or so) that do not reliably acquire sucrose self-administration. On balance, this has led us to choose n=10 per group.

3. Animal care is provided by a full-time trained staff member in the Department of Psychology, Academic Instruction Center, Western Washington University. Additional laboratory animal care support is provided by a visiting veterinarian from the University of Washington.

4. All experimental procedures have been approved by the WWU IACUC and follow the guidelines outlined in the "Principles of laboratory animal care" (NIH publication no. 85-23). Animals are weighed 3 times a week and are monitored daily for signs of illness. None of the behavioral procedures involve stress or discomfort.

5. Rats in Specific Aim 1 will be deeply anaesthetized with sodium pentobarbital prior to perfusion. Appropriate depth of anesthesia will be indicated by lack of foot and tail pinch withdrawal reflex. Rats in Specific Aim 2 will be rapidly decapitated without prior anesthesia or CO2. This will be performed by experienced personnel trained by the PI. The rationale for sacrifice without use of anesthetic or CO2 is that drugs or CO2 might alter the proteins to be analyzed. The rapid decapitation technique has been used in the PI's laboratory previously. Euthanasia of any animal that becomes ill during the course of a study will be performed using CO2 in accordance with the recommendations of the Panel on Euthanasia of the American Veterinary Medical Association.
LITERATURE CITED

Aberman JE, Salamone JD (1999) Nucleus accumbens dopamine depletions make rats more sensitive to high ratio requirements but do not impair primary food reinforcement. Neuroscience 92: 545-52


Bossert JM, Wibbey KA, Pickens CL, Nair SG, Shaham Y (2009) Role of dopamine D(1)-family receptors in dorsolateral striatum in context-induced reinstatement of heroin seeking in rats. Psychopharmacology (Berl) 206: 51-60


Chauvet C, Lardeux V, Goldberg SR, Jaber M, Solinas M (2009) Environmental enrichment reduces cocaine seeking and reinstatement induced by cues and stress but not by cocaine. Neuropsychopharmacology 34: 2767-78


References Cited
Smith MA, Chisholm KA, Bryant PA, Greene JL, McClean JM, Stoops WW, Yancey DL (2005) Social and environmental influences on opioid sensitivity in rats: importance of an opioid’s relative efficacy at the mu-receptor. Psychopharmacology (Berl) 181: 27-37


June 12, 2010

To whom it may concern,

With respect to the NIH R15 grant renewal application by Jeff W. Grimm at Western Washington University, I would like to share how I have benefited from Dr. Grimm’s mentorship and funding targeted at undergraduate research and training. I had the opportunity to work with Jeff Grimm for nearly 4 years while an undergraduate student in behavioral neuroscience at Western Washington University. Since graduating in June 2009, I have enrolled as a graduate student at Oregon Health and Science University in the behavioral neuroscience doctoral program. The level of attention that I received from Dr. Grimm as an undergraduate student is unmatched by the experiences of many classmates in my program. I attribute much of my success at both Western and OHSU to the mentorship of Dr. Grimm and the opportunities that his lab afforded me.

My training prepared me to be a successful graduate student by exposing me to the rigors of scientific study and techniques of behavioral research. While working with Dr. Grimm, travel funds available from his grant made it possible for me to twice attend and present work at the national conference of the Society for Neuroscience, in addition to several local conferences. After presenting these data, I was able to publish one paper as a contributing author, and another paper as the primary author. Dr. Grimm trained me in techniques that I now use at OHSU, such as stereotaxic intracranial cannula implantation, systemic injection, intracranial microinjections, operant box self-administration models, and intracranial self-stimulation. Additionally, as a result of Dr. Grimm’s mentorship and undergraduate funding, I developed experimental protocols, performed data analyses, and engaged in scientific writing. Furthermore, Dr. Grimm guided me through several funding applications and I was awarded an internal undergraduate research grant funded by the Western Washington University Foundation. Without the support of an hourly wage for time spent in the lab, it would have been necessary for me to work a second job, thereby taking time from my projects and research. These experiences were invaluable to me as an undergraduate student, but were time and financially intensive for Dr. Grimm.

Funding from Dr. Grimm’s NIH R15 grant is directly responsible for many career opportunities I have had beginning as an undergraduate student. Ultimately, these undergraduate experiences prepared me for the expectations of a graduate student and as a scientist. Without doubt, Jeff Grimm is an excellent mentor and has contributed to the success of many students in top-ranking graduate programs. I hope that future undergraduate students will continue to benefit from Dr. Grimm’s outstanding training and mentorship.

Sincerely,

[Signature]

John Harkness

Oregon Health and Science University
harknesj@ohsu.edu
(425) 503-4593
May 25, 2010

Bruce Hope, PhD
Behavioral Neuroscience Branch
IRP/NIDA/NIH
251 Bayview Blvd
Baltimore, MD 21224

Jeffrey W. Grimm, PhD
Department of Psychology and Program in
Behavioral Neuroscience
Western Washington University
516 High Street
Bellingham WA, 98225

RE: Incubation of craving: Abstinence and Environmental Enrichment-mediated Molecular Adaptations

Dear Jeff,

I am writing to confirm my enthusiasm to serve as a consultant on your R15 grant that proposes to identify molecular signaling associated with sucrose seeking and environmental enrichment. As we have discussed, the Western blot procedure will provide a sensitive measure of abundance of specific proteins, including phosphorylated proteins. These measures could provide evidence for neuroplasticity in the D1 → c-FOS signaling pathway that you are studying.

You first became proficient in the Western blot procedure in my laboratory at NIDA and data from an “incubation” study resulted in a publication we co-authored in 2002. I have no doubt that you will be successful in setting up and conducting similar studies at WWU. Nonetheless, I would be happy if you need to visit NIDA again to learn our most recent procedures. We have streamlined some of the protocols and have also been working with a fluorescent label approach that facilitates simultaneous assessment of phosphorylated and total levels of a given protein in the same band. These changes in protocol could be really helpful for addressing your specific research question.

We will arrange for your visit if/when your grant is funded. The cost of supplies while you are here will be taken care of by our intramural funds. I will happy to be a consultant after you return to do the research at Western Washington University.

Sincerely,

Bruce Hope, PhD
May 27, 2010

Professor Jeffrey W. Grimm, Ph.D.
Dept of Psychology and Program in Behavioral Neuroscience
Western Washington University
516 High Street
Bellingham WA 98225-9172

Dear Jeff,

I am writing to confirm that I will be delighted to be a consultant on your R15 proposal “Incubation of craving: Abstinence and Environmental Enrichment-mediated molecular adaptations”. As we’ve discussed, some evaluation of the body composition and/or metabolic status of your animals experiencing environmental enrichment will be valuable information to go along with the behavioral and molecular measurements you are proposing. Measurement of fat pads is a quick and validated surrogate for total body composition measurements. My lab is experienced in the dissection technique and we will happy to train you and/or your students in this. Best wishes for success with your proposal, and we look forward to working with your group in the near future.

Sincerely,

Dianne Figlewicz Lattemann, Ph.D.
Research Career Scientist, VAPSHCS
Research Professor, Dept of Psychiatry & Behavioral Sciences,
University of Washington
May 21, 2010

Dr. Yavin Shaham  
Behavioral Neuroscience Branch  
IRP/NIDA/NIH  
251 Bayview Blvd  
Baltimore, MD 21224

Jeffrey W. Grimm, PhD  
Department of Psychology and Program in  
Behavioral Neuroscience  
Western Washington University  
516 High Street  
Bellingham WA, 98225

Dear Jeff,

I am writing to express my enthusiasm to serve as a consultant for the c-FOS mapping studies described in your R15 proposal to NIDA entitled "Incubation of craving: Abstinence and Environmental Enrichment-mediated Molecular Adaptations".

As we recently discussed, an initial c-FOS expression IHC study would yield critical information regarding the neuroanatomical substrates of incubation of sucrose cue reactivity as well as sites that mediate the effects of environmental enrichment on the incubation effect. Although you became quite familiar with the c-FOS IHC protocol while working at NIDA (unpublished collaboration with Bruce Hope and Hans Crombag) I invite you to come back to NIDA for a working visit to learn the details of our c-FOS IHC current standard operating procedure. The technique was a valuable tool in previous published studies from our section, and continues to be used in our ongoing studies. Pending funding of your R15, we will make arrangements for your visit. We will cover all the expenses of the laboratory supplies and reagents involved in your training in our place from our intramural funds. I will continue to serve as a consultant on the project as you conduct the studies at Western Washington University.

I look forward to working together on these experiments.

Sincerely,

Yavin Shaham, PhD  
Section Chief
To Whom It May Concern:

I am writing in support of the NIH grant renewal submitted by Dr. Jeffrey Grimm entitled *Incubation of Craving: Abstinence and Environmental Enrichment-mediated Molecular Adaptations*. The purpose of the research that forms the basis for this proposal is to (1) identify brain regions that are selectively active in relapse conditions and (2) identify levels of D1 signaling cascade proteins expressed in the identified regions and their projection areas. Dr. Grimm has an outstanding record of publications in this area. I believe that the proposed study will be a significant addition to the field of addiction by providing an understanding of the brain mechanisms that underlie addiction.

Thus, the Department of Psychology and the College of Humanities and Social Sciences have agreed to a funded one-course reduction for each year of the grant proposal to provide Dr. Grimm with additional time to pursue this research. If you have questions or concerns, please feel free to contact me via e-mail (dale.dinnel@wwu.edu) or telephone (360-650-3522).

Sincerely,

Dale L. Dinnel
Professor and Chair, Psychology
1. Application Type:
From SF 424 (R&R) Cover Page. The responses provided on the R&R cover page are repeated here for your reference, as you answer
the questions that are specific to the PHS398.

* Type of Application:
  - [ ] New
  - [ ] Resubmission
  - [ ] Renewal
  - [ ] Continuation
  - [ ] Revision

Federal Identifier:

2. Change of Investigator / Change of Institution Questions

☐ Change of principal investigator / program director

Name of former principal investigator / program director:

  Prefix: ____________________________
  * First Name: _____________________
  Middle Name: ____________________
  * Last Name: _______________________
  Suffix: ____________________________

☐ Change of Grantee Institution

* Name of former institution:

3. Inventions and Patents  (For renewal applications only)

* Inventions and Patents:  Yes [ ]  No [ ]

If the answer is “Yes” then please answer the following:

* Previously Reported:  Yes [ ]  No [ ]
4. * Program Income

Is program income anticipated during the periods for which the grant support is requested?

☐ Yes  ☒ No

If you checked "yes" above (indicating that program income is anticipated), then use the format below to reflect the amount and source(s). Otherwise, leave this section blank.

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5. * Disclosure Permission Statement

If this application does not result in an award, is the Government permitted to disclose the title of your proposed project, and the name, address, telephone number and e-mail address of the official signing for the applicant organization, to organizations that may be interested in contacting you for further information (e.g., possible collaborations, investment)?

☐ Yes  ☒ No