I. Cover Sheet

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Title of Proposal:

A Cellular Analysis of the Effects of Methamphetamine on the Neuroimmune System

End Dates of Proposed Activities:

May 2012

II. Narrative

Methamphetamine (METH) is one of the most commonly abused drugs in the US. METH is a psychostimulant, which acts as a powerful indirect agonist of dopamine in the nervous system. The resulting “dopamine dumping” produces overactivation and oxidative damage at dopamine terminals, both of which are upstream events leading to apoptosis (i.e., programmed cell death) and help to explain METH’s neurotoxic profile.

Microglia are phagocytic cells found in the CNS and are descendant from the same myeloid lineage as macrophages found in

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vulnerable to opportunistic infection and exacerbated secondary damage after primary focal insults. It is of no surprise that microglia activation states and cytokine/chemokine profiles are altered by neurotoxic doses of METH in the striatum (a region in the brain which receives a dense dopamine innervation from the midbrain substantia nigra). Is the effect of METH on microglia the result of indirect dopaminergic toxicity or the result of direct actions of METH on the microglia? If so, what are the mechanisms by which this is occurring? Finally, are microglia in other dopaminergic and non-dopaminergic regions of the brain also affected?

1. **Analysis of the effect of METH on Microglia Using *In Vitro* Techniques.**

To create a cell line of a non-cancerous cell, one must isolate the cell type of interest (e.g., microglia) and use molecular techniques to alter the native cell cycle. This now immortal cell line has the advantage of self-renewal, while also maintaining many of the characteristics of the primary cell from which it was developed (e.g., phagocytosis, migration, and cytokine and chemokine release). BV-2 is a murine microglia cell line useful for studying the direct effects of METH on microglia in the absence of other cell types. Our lab, under CURCA funding, has previously demonstrated a direct, dose-dependent, inhibitory effect of METH on BV-2 phagocytosis as well as an alteration in the microglia cytokine/chemokine profile. Importantly, these effects were the result of functional alterations and not an effect on cell viability. Based on these initial observations, several additional studies logically follow:

A. Are other characteristic microglia functions also altered by METH?

Microglia have multiple pseudopodia, which “feel” their extracellular environment. When stimulated they actively recruit other nearby microglia to also respond to the stimulus through chemical signals (i.e., chemokines). The motility of the microglia can be assessed using a migration assay, whereby “clumps” of BV-2 cells are counted and overall activated movement is measured in the presence or absence of METH and cellular debris. Under the right conditions, BV-2 exist as a uniform layer of cells. Cellular debris (i.e., homogenized mouse brain), mimicking METH induced neuronal damage, is added to some plates, which stimulates BV-2 cells and in turn stimulates active

would indicate that METH not only inhibits function (i.e., phagocytosis) but also recruitment of microglia in the presence of neuronal damage thereby potentially exacerbating the METH induced neurodegeneration.

B. What cellular mechanisms are affected by the METH?

Our data suggest that the effects of METH on microglia

These student leaders practice high-level science while developing leadership skills in the lab through personnel and project management. The projects detailed above will involve 5 student leaders in the Fall semester, each responsible for their own project. Furthermore, other students have chosen to pursue scientific evaluation of novel labs they have developed and run in the classroom using the knowledge and skill sets developed while working on these studies. These educational research projects have been planned for both the fall and spring semesters and will offer invaluable teaching experience for our students interested in education while enhancing the overall academic experience at NGCSU. Additional student leaders will carry on these projects in the Spring. Student leaders must submit detailed project proposals before they begin their work that include an extensive literature review, detailed protocols related to their project and a timeline for completion. Student leaders manage all aspects of their projects and receive as much attention and help from PIs as necessary. All projects and proposals are developed in collaboration with the PIs, who are careful to ensure that each project will have a definitive and realistic product at the end of the semester. For example, the 5 projects detailed above will be presented at at least one regional conference (either the Southeastern Psychological Association (SEPA) or the Association for Southeastern Biologist (ASB)) and one local conference (the North Georgia Academic Research Conference (NARC)). Each student will be expected to and encouraged to apply for available internal and external travel awards and research grants. Our students have been successful in obtaining both internal and external funding in the past as they are able to draw heavily from the research proposals they have already developed.

Each of the student projects described above is a piece of a

the CUR-sponsored Posters on the Hill event in Washington, DC. We expect our students to accumulate similar accolades this year in recognition of their hard work and scientific achievements. However, it is critical to note that these students would not have had the opportunities described above without the financial support of CURCA. I hope you see that the CUCRA money received went to good use.

III. Budget and Projected Timeline

In the past year, our lab has been extremely productive. We anticipate the completion and publication of several of the projects outlined above within the next year. However, as we empirically test some hypotheses, others arise to provide further avenues of research for our students. With research data supported by prev[(I)w -3.31 <</MCITu(e)-0 T1

Category	Description	Project	Price	Total
Mice	*Per diem for *in vivo* studies for 3 months (food, bedding, caging supplies, and animal facility maintenance fees)	D-E 30 mice	3.82 per diem (3 months)	343.80
Cell Culture	**Per diem for *in vitro* studies for 9 months (media, flasks, slides, sterilization equipment, CO2, plates, pipette tips, gloves)	A-C	1.92 per diem (9 months)	518.40
Inert Beads	Phagocytosis assay beads	A-B	150.00	150.00
Chemicals	Cell signaling inhibitors (H89, Oakadic Acid, LY, Forskolin, IBMX)	B	~400.00	400.00
Western blot Analysis				

projects are a part of a larger line of experimentation that will continue indefinately. Therefore, students work often work on their projects in subsequent semesters while training new students to carry on their line of questioning. For most projects the student begin by generating the necessary animals and/or practicing cell culture techniques. They then engage in their manipulation (drug exposures) followed by data collection and analysis and a formal write-up for presentation. Each step is carefully planned with the student leader to avoid other time conflicts (school, holiday, work, etc.) and to be completed within a given semester.

Additional Core Equipment/Supplies needed, but already obtained by Drs. Lloyd and Shanks:

1. Cell Culture Facility
2. BV-2 Cell Line
3. Real Time RT-PCR