I. Cover Sheet

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Department: Biology
Previously funded by CURCA? _X_Yes ____No

Title of Proposal: Evaluating Gene Level Alterations in the Brain After Adolescent Methylphenidate Exposure
End date for proposed activities: May 2013

II. Narrative

Description of proposed research project or creative activities.

This project stems from mouse behavioral studies conducted with the support of previous CURCA awards. In these studies, we observed significant increases in adult sensitivity to a small methamphetamine dose in female but not male adult mice that were exposed to methylphenidate (MPD; marketed as Ritalin^{TM} and Concerta^{TM}) during adolescence. This small dose does not elicit a response in saline treated animals. A response to the small dose is indicative of increased susceptibility to addiction. Adolescence in mice has been well defined in the literature to represent a 10-day period of time following weaning (separation of pups from their mom). We know that these behavioral changes are occurring, but we do not know the molecular basis for these changes in the brain. Students will begin to answer this question by treating male and female adolescent mice with MPD or an equal amount of saline over a 5-day or a 10-day period after weaning. Students will also treat male and female mice with MPD during adolescence and then a small dose of methamphetamine in adulthood. After the final injection on the fifth and tenth day of adolescence or before and after the adult small dose of methamphetamine, students will microdissect several key brain regions. RNA will be isolated from these key brain regions for analysis using real-time PCR. Real-time PCR analysis provides students with the ability to quantitate the activity (expression) of specific genes responsible for key regulatory roles in the development and long-term function of these brain regions. Analysis of gene activity comparing saline (control) to MPD-treated animals as well as male and female differences during adolescence and in adulthood will provide insight into the mechanism underlying the development of addictive behavior in humans. Genes will be chosen based on their relevance to the function of these brain regions and the literature (explained in the "involvement of undergraduates" section). The importance of this line of research is two-fold. First, our behavioral data has never been reported in the literature and provides our lab with a novel opportunity to fill an important gap in the literature involving the mechanism of this behavior. Second, using real-time PCR analysis of specific genes throughout the adolescent dosing of MPD and in adulthood is an innovative research design to investigate how and when specific genes can lead to increases in addictive behaviors. This information from our animal model will provide direct insight into human addiction as it relates to adolescent exposure to MPD.

Significance of the proposed work.

MPD (marketed as Ritalin^{TM} and Concerta^{TM}) is a psychostimulant drug commonly prescribed for the treatment of ADHD and less commonly prescribed for the treatment of narcolepsy, depression, and other psychiatric conditions. There are recent reports of large increases in MPD misuse, diversion, and abuse, which coincide with clinical trends in ADHD overdiagnosis and misdiagnosis. Furthermore, adolescent abuse of stimulants such as MPD has increased in response to the pressures of academic success. Of key importance, we have found that adolescent exposure to MPD in a non-ADHD model organism leads to increased sensitization to a small dose of methamphetamine that would not typically cause behavioral changes in adulthood. These behavioral studies indicate that adolescent exposure to the drug causes long-term alterations in the function of brain regions involved in addiction. Although these behavioral data are novel and important in-and-of themselves, determining the mechanism of these alterations is necessary in identifying potential therapeutic targets, which also provides a greater understanding of specific mechanisms of addictive behavior.

Our data indicate that female mice are more susceptible to addictive behaviors after adolescent exposure to MPD. There is a paucity of data with respect to sex differences following MPD exposure. The mechanism behind these sex differences will provide insight to fields beyond addiction. For example, adolescence is a key developmental time in the brain. Understanding how gene activity differs in response to a drug provides new information regarding the normal development of the brain. Furthermore, an understanding of how MPD effects females more than males may be of clinical importance when deciding on which medication is prescribed with regards to treatment of conditions such as ADHD.

Control of genes takes place in response to signals from both inside and outside of the cell. These signals cause specific genes to be activated or inhibited. The effect of the control of gene activity is altered cellular activity. This cellular activity in turn controls specific behaviors associated with particular regions of the brain. Therefore, our investigation of gene activity is, in effect, a secondary measure of the behavioral

alterations we have already observed. Beyond providing therapeutic targets and an understanding of behavior, our studies will also provide insight into how specific genes can alter long-term functions of cells, which can be both local and systemic. Since all cell types share the genes we will measure, we anticipate that the data collected from this study will also inform other fields of biology. Therefore, long-term changes caused by a gene in the brain may relate to other systems where developmental alterations lead to dysfunction in adulthood.

Taken together, I believe elucidating the mechanism of gene activity alterations following adolescent MPD exposure has significant biological, clinical, and therapeutic impacts.

Goals and expected products.

This research is a piece of a larger on-going project whose data will be submitted to a peer-reviewed neuroscience journal (e.g., Brain Research) to follow the publication of the behavioral data described above. Currently there are 4 students who are also involved in the research project described here. Several of these students will be co-authors on the publication of the behavioral data, which is currently in progress. Elucidating the mechanism of these behavioral differences is a natural progression of this research. It is our anticipation that students will provide intellectual input into the publication of their gene activity data as co-authors.

Students will present their data at the regional conferences of the Association of Southeastern Biologists in Charleston, WV and the Southeastern Psychological Association in Atlanta, GA during the Spring of 2013. Furthermore, students will participate in the North Georgia Research Conference locally.

Plan for involvement of undergraduates in the project or activity.

Our lab uses a "see one, do one, teach one" approach to student development. Students new to the lab begin their involvement as helpers (see one) on their way to developing the skills they need (do one) which is facilitated by more seasoned students (teach one). These student leaders practice high-level science while developing leadership skills in the lab through personnel and project management. The research plan detailed above will involve 5 student leaders in the Fall semester, each responsible for their own project. 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 me 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 research detailed above will be presented at least one regional conference (either the Southeastern Psychological Association or the Association for Southeastern Biologist) and one local conference (the North Georgia Research Conference). Each student will be expected to and encouraged to apply for available internal and external travel awards (Council of Undergraduate Research Travel Awards) and research grants (BBB Honor's Society Research Awards). 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.

This model of undergraduate research has served us well in the past in the development of students. It ensures that students understand the broader impact of their specific project to the long-term goals of the research by having the time to participate in projects leading into their own. It also ensures that students have the time to develop their own creative, intellectual input into their project. For the research plan described above, each student working this academic year will be selecting the gene or genes they would like to investigate using the techniques they have now had the time to master. The proposals they have written for this Fall semester, clearly demonstrate how their gene of interest makes sense to investigate in the adolescent MPD dosing paradigm. This creative input ensures ownership of their work, and has led to a significant increase in the excitement and work ethic in the lab. Simply stated, students are technically trained, have an understanding of the lab's goals and history, and are now providing their own ideas of how to proceed. This model of undergraduate research also ensures that CURCA monies will sustain future projects since students working with these student leaders will be ready to take their place with their own creative ideas in the future.

Productivity from previous CURCA funded projects

During the past 3½ years, my CURCA funding has supported my direction of 43 independent study projects through the Department of Biology. These projects have resulted in 55 student presentations at national (2), regional (33), and local (20) research conferences. Furthermore, 6 invited presentations at research and leadership conferences have involved the direct participation of these research students. The high level of research these student-focused projects entail is evidenced by the awards and honors these students have received. These include selection for the CUR Posters on the Hill, 6 student authored research grants from the □□□ national biology honors society, 3 Psi Chi national psychology honors society awards at the Southeastern Psychological Association Conference, 2 □□□ awards at t Conference, 2 awards at the Georgia Academy of Sciences meetings, and multiple awards at NGCSU. The list below represents the presentations and awards supported by last year's CURCA funding along with 2 student co-authored peer-reviewed manuscripts published this past year (* represents NGCSU students):

Peer Reviewed Publications:

1) Shanks RA, Anderson J*, Taylor JR*, Lloyd SA (*in press 2012*). Amphetamine and methamphetamine have a direct and differential effect on BV-2 microglia cells. *Bulletin of Experimental Biology and Medicine.*

2) Shanks RA, Southard EM*, Tarnowski L*, Bruster M*, Wingate SW*, Dalman N, Lloyd SA (*2012*). A vodcasted, cross-disciplinary, behavioral neuroscience laboratory exercise investigating the effects of methamphetamine on aggression. *Bioscene, 37*(2): 10-17.

The Association of Southeastern Biologists Conference, Athens, GA.

1) Bryant S*, Schulz J*, Tavares C*, Doyle HH*, Lloyd SA & **Shanks RA** (2012). Effects of adolescent methamphetamine exposure on methamphetamine sensitization in adult mice.
2) Phillips B*, Herdliska A*, Lloyd SA & **Shanks RA** (2012). An assessment of a novel behavioral neuroscience laboratory exercise.
3) Pass J*, Gonzalez E*, Helton A*, Herdliska A*, Lloyd SA & **Shanks RA** (2012). The effects of adolescent methamphetamine exposure on executive functions in adult mice.
4) Helton A*, Schulz J*, Lloyd SA & **Shanks RA** (2012). The effects of methamphetamine on PRX antioxidant proteins in a cultured microglia cell line and a mouse model.

The Annual Meeting of the Southeastern Psychological Association, New Orleans, LA.

1) Schulz J*, Tavares C*, Lloyd SA & **Shanks RA** (2012). Adolescent methylphenidate leads to methamphetamine cross-sensitization in adult mice.
2) Tavares C*, Schulz J*, Bryant S*, Lloyd SA & **Shanks RA** (2012). Adolescent d-amphetamine leads to methamphetamine cross-sensitization in adult mice.
3) Bryant S*, Schulz J*, Tavares C*, Doyle HH*, Lloyd SA & **Shanks RA** (2012). Effects of adolescent methamphetamine exposure on methamphetamine sensitization in adult mice.
4) Phillips B*, Herdliska A*, Lloyd SA & **Shanks RA** (2012). An assessment of a novel behavioral neuroscience laboratory exercise.
5) Pass J*, Gonzalez E*, Helton A*, Herdliska A*, Lloyd SA & **Shanks RA** (2012). The effects of adolescent methamphetamine exposure on executive functions in adult mice.
6) Bruster M*, Lloyd SA & **Shanks RA** (2012). The effects of methamphetamine on aggression.

The Annual North Georgia Academic Research Conference, Dahlonega, GA

1) Schulz J*, Tavares C*, Lloyd SA & **Shanks RA** (2012). Adolescent methylphenidate leads to methamphetamine cross-sensitization in adult mice. *****Oral Presentation Award**
2) Tavares C*, Schulz J*, Bryant S*, Lloyd SA & **Shanks RA** (2012). Adolescent d-amphetamine leads to methamphetamine cross-sensitization in adult mice.
3) Bryant S*, Schulz J*, Tavares C*, Doyle HH*, Lloyd SA & **Shanks RA** (2012). Effects of adolescent methamphetamine exposure on methamphetamine sensitization in adult mice. *****Poster Presentation Award**
4) Phillips B*, Herdliska A*, Lloyd SA & **Shanks RA** (2012). An assessment of a novel behavioral neuroscience laboratory exercise.
5) Pass J*, Gonzalez E*, Helton A*, Herdliska A*, Lloyd SA & **Shanks RA** (2012). The effects of adolescent methamphetamine exposure on executive functions in adult mice.
6) Bruster M*, Lloyd SA & **Shanks RA** (2012). The effects of methamphetamine on aggression. *****Oral Presentation Award**
7) Helton A*, Schulz J*, Lloyd SA & **Shanks RA** (2012). The effects of methamphetamine on PRX antioxidant proteins in a cultured microglia cell line and a mouse model.

BBB Honors Society □Undergraduate Research Grants:

1) Helton A*, Lloyd SA, Shanks RA. " The effects of methamphetamine on PRX antioxidant proteins in a cultured microglia cell line and a mouse model " **Total Award: $355**
2) Pass TJ*, Lloyd SA, Shanks RA. " The Effects of Adolescent Methamphetamine Exposure on Executive Functions in Adult Mice " **Total Award: 350**

III. Budget and project timeline

In the past year, our lab has been extremely productive. We anticipate the completion and publication of several of the projects 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 previous CURCA support, Drs. Shanks and Lloyd have been awarded one external grant. We anticipate submitting more applications this year using CURCA-supported preliminary data. Given our focus on the undergraduate research experience we are considering REU awards as well as foundation, NSF (TUES) and NIH (R15, AREA) basic science awards. The expansion of student participation and research capabilities necessitates that we obtain external funding support. However, continued research until that funding is obtained will serve to enhance our ability to do so. We have included the following budget to conduct this research over the next academic year.

Category	Description	Price	Total
Mice	*****Per diem for *in vivo* studies for 3 months (food, bedding, caging supplies, and animal facility maintenance fees) ~30 mice	3.82 per diem (6 months)	787.60
PCR Primers	Primers are purchased based on student interest in specific genes. These primers also require some adjustment during a validation period, so often there must be several primers for each gene purchased.	15 primer sets for specific genes	225.00
RNA isolation kit	1 kit provides 50 isolations for brain regions sufficient for this study	150.00	150.00
Drugs	Methylphenidate (Ritalin) and methamphetamine + processing, shipping and licensing fees.	573.21 MPD 240.11 METH	813.32
Real Time RT-PCR kit	Kit provides all materials to analyze gene activity using the real-time PCR machine	297.00 (ea)	891.00
Equipment	Surgical equipment (scalpels, forceps, scissors, brain matrix)	~150.00	150.00
TOTAL			**3016.92**

*** Per diem costs** - inclusive of all costs associated with maintaining the animals including food, bedding, caging supplies, cleaning supplies, and other animal facility maintenance fees. Please note that this per diem is well under national averages.
The mouse animal facility and real-time RT-PCR machine have already obtained by the lab for use on this project.

Timeline:

As described above, all student leaders submit their own, individual project proposal complete with a project timeline. We work closely with the students to ensure that their project and the product it produces are completed within the Fall or Spring semester. These student projects are a part of a larger line of experimentation that will continue indefinitely. 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 students begin by generating the necessary animals and/or practicing RNA isolation and real-time PCR 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. Please note that 4 student leaders are already into the 4th week of the timeline below and are on track to complete the rest of the proposed tasks pending CURCA funding.

Fall Semester: Bi-weekly lab meetings are a required part of participation as well as weekly individual meetings with me
Week 1-2
Proposals of student leaders are in their final format following an iterative process of revisions.
Week 3-4
Validation of primers used on the real-time PCR machine provides valuable technical experience and is a necessary step in the use of this technology.
Week 5-7
Animals for the 5 and 10 adolescent exposures to MPD and saline are generated.
Week 7-9
Injections and dissection of brain sections take place (these are staggered and overlap the previous timeframe to avoid unnecessary time demands in the lab during one day. RNA isolation and measurement of the RNA.
Week 10-12
PCR analysis specific to each student project will take place. Statistical analysis of the results will take place for the rest of the semester.

Spring Semester: Bi-weekly lab meetings are a required part of participation as well as weekly individual meetings with me
The spring semester schedule will repeat the same timeline with the following additions:
Week 3-4
Students will have a draft of a presentation to present at a lab meeting.
Week 5-7
Animals for the 5 and 10 adolescent exposures to MPD as well as adult exposure to methamphetamine and saline are generated.