<table>
<thead>
<tr>
<th>BUDGET ITEM</th>
<th>Department or College Funds</th>
<th>Outside Agency Funds</th>
<th>Personal Funds</th>
<th>Undergrad. Research Funds</th>
<th>GRAND TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Materials</td>
<td>Neuroscience department will provide surplus of 26 gage, 3/8in syringes. Use of HPHP biochemistry laboratory has been approved along with materials for performing assays which include: freezer for storage of samples until time to perform testing, micropipettes, beakers, fume hoods, test tubes, micro centrifuges, ELIZA 400nm-550nm reader and more.</td>
<td>Fluoxetine 1000mg @$45.80, 200 lancets @ $10, 200 capillary tubes for blood samples @ $9.2. 28 C57BL/6 mice, (14 male, 14 female) @ $27.10 each= $758.80 A basic mouse food pellet diet will be purchased through the zoology department and will cost $33.95 per 25lb bag. 2 Bags= $67.90. Bedding will be purchased by the same method, costing $33.95 per 40lb bag. 2 bags= $67.90</td>
<td></td>
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<td>$959.60</td>
</tr>
<tr>
<td>Equipment</td>
<td>Department of Zoology will provide room for housing mice along with cages/water bottles for housing mice. Micro Department will also provide professional trained in the handling of small animals to infect mice with pathogenic agent.</td>
<td>Strobe light @ 30$, 1 immunoassay kit for IL-6 measurements @$480.00 ELISA Testing through University of Maryland for IL-6 @ $10.50 per sample per test. 28 samples each with 3 tests = $882. 10 additional cages are needed for our study to house the appropriate number of mice individually. These will be purchased through Fisher Co. and will cost an additional $299.80</td>
<td></td>
<td></td>
<td>$1,691.90</td>
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<tr>
<td>Stipend:</td>
<td>Hrs @ $10/hr Benefits @ 8.5% Total</td>
<td></td>
<td></td>
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<tr>
<td>Mileage to gather Data (.36 per mile)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>$2,651.50</td>
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<tr>
<td>GRAND TOTAL</td>
<td></td>
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</table>
Body of Proposal

Project Description

Dealing with depression is difficult enough without the worry that it may place you at a greater risk of infection and illness. Recent research has found a significant link between the immune response and depression (Frank et al 1999, Kubera et al 2000, Schiepers, Wichers & Maes 2004, Cohen et al 2005). Researchers studying the links between immune response (IR) and antidepressants (AD) have hypothesized a new cause of depression known as the “cytokine model of depression”. Central to this hypothesis is the understanding that proinflammatory cytokines (IL-1 and IL-6 especially) are nearly always present in increased amounts in patients with depressive symptoms. Specifically patients receiving medication which includes administration of proinflammatory cytokines (e.g. cancer or hepatitis C) have been found to exhibit depressive symptoms, yet when medication has ceased the symptoms often fade away (Schiepers, Wichers & Maes 2004). Even more recent studies have shown that certain AD may work by decreasing the concentrations of proinflammatory cytokines and increasing anti-inflammatory cytokines in the blood (Janssen et al 2010). These findings suggest that depression may be caused by IR over activation, which causes a change in normal behavior which we identify as depression. This theory has large ramifications for the future treatment of depression, including the possibility that taking AD may be adversely affecting the IR, making treated patients more prone to sickness and infection. While these findings are significant in their scope, many questions still remain, including whether the mechanism of action of AD inhibits the immune response as a whole.

Our study hopes to contribute to the understanding of AD and IR by observing in vivo the effects of AD on chronically stressed mice and their IR. Mice models of chronic depression have been found to be both reliable and valid when modeling chronic human depression (Willner, 1997). Significant
amounts of research on this subject have used mice models of depression to allow for a more
controlled environment. This controlled environment allows us to observe changes in the concentration
of cytokines over the course of the experiment without worrying about the various lifestyles of human
subjects that may be contributing to the cytokine levels. By modeling our study after previous
examples of chronically stressed mice (e.g. Kubera et al 2001) we will have a consistent system to
collect valid data in our experiment.

In this experiment we will be testing the IR of chronically stressed mice by measuring cytokine
levels before and after the onset of depression, during administration of AD or placebo on
experimental and control groups respectively, and by a pathogenic challenge in vivo to all mice at the
end of the experiment. Our hope is to establish a quantifiable difference in the IR (either by cytokine
levels or by survivability of pathogenic challenge) between mice given AD and those given the
placebo (for a full outline of the project please see appendix). This knowledge will help future
researchers understand what differences there are in the IR of both groups, and where future research
may be focused to solve the complex questions regarding depression and IR.

Our role as students will be mostly independent. We will be working closely with Dr. Fowler,
Dr. Nakaoka, and Dr. Walker as mentors and will conduct each phase of the experiment, including the
necessary steps of stressing and immunoassays, with their supervision.

This research will culminate in a scholarly paper intended to be presented at the WSU
Undergraduate Research Symposium and possibly at regional and national conferences.

Qualifying experiences include laboratory experience in classes of General Chemistry, Organic
Chemistry, Biochemistry I, and Microbiology 2054. Matt Fullmer has experience working in
controlled substances as a pharmacy technition, and Sterling Haws has experience in wound care, and
working in sterile environments as a certified nurse’s assistant.
References


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Project Methods & Timeline

**For a full description of the methods and timeline please see appendix.**

Institutional Animal Care and Use Committee approval will be obtained over the summer of 2012. This will include training by the veterinarian in the handling and treatment of the mice.

Mice will be purchased in August, and cytokine levels will be taken initially for baseline data. In September the mice will be subjected to a depression model which consists of an alternating schedule of various stressors including: Overnight lighting, forced swimming, twelve hour food/water fasts, forty-five degree cage tilts, and twelve hour strobe light illumination.

The model of depression depends on measurement of sucrose solution intake which is a measure of anhedonia (a loss of pleasure in things one used to enjoy) a major symptom of depression. As the stressing schedule progresses depression will be established by a significant decrease in sucrose solution intake. The effects of the model should be seen within four weeks.

In October the depression model should be in effect, and administration of the SSRI antidepressant to the experimental group will commence. The SSRI will be administered while the Control group is administered a placebo. The depression model continues while the anti-depressant regimen is given.

After five weeks of SSRI administration, the immune response will be tested of both groups. This will be done by measuring cytokine levels and by inoculation of the mice with an infectious agent (non-lethal to humans) which will be administered to both groups. Rates of mortality in both groups will be used to determine the effects of antidepressants on the mice immune response. A laboratory professional experienced in animal handling will perform the pathogenic challenge at the end of the experiment. This service will be provided free of charge by Lynn Moyes, lab manager of the
Microbiology Department. He will inoculate each mouse with a pathogen to establish survivability. This will take place in November. Disposal and final data collection will start in December.

Budget Explanation

The total grant amount requested for this research is $2,651.50
All materials and/or equipment left over or not used in this experiment will remain the property of Weber State University.

Materials:

The project requested amount for this category is $959.60, which does not include materials offered by the HPHP lab. This amount includes the cost of buying the mice used for the study. C57BL/6 mice are the mouse most commonly used in experiments such as this because of the control of their genetic make-up. Each gene is known and therefore contributes to the control of the experiment, allowing us to disregard any genetic variability that may influence our data in our test group. Each mouse will be purchased from Jackson Laboratories and costs $27.10 each, for both male and female mice. We will be purchasing 14 male and 14 female mice to evenly represent each sex, and to allow for a large enough group to eliminate random error. Twenty-eight mice at $27.10 a piece amounts to $758.80. Also listed in this amount is the cost of food. For standardized food pellets that provide all necessary nutrients for mice a twenty-five pound bag costs $33.96 each. This will be purchased through the Zoology Department, but we must provide the funds ourselves. Two bags at $33.96 each equals a total of $67.92. Along with food bedding for the mice is also required and will be purchased through the Zoology Department, but again we must provide the funds. A forty pound bag of bedding costs $33.95 each. Two bags of bedding are requested. Two bags at $33.95 each equals $67.90. Specialized lancets, and capillary tubes used for submandibular bleeds will be purchased online from MEDIpoint.com. 200 lancets cost $10, and 200 capillary tubes will cost $9.20. The project lists having a professional experienced in animal handling perform the pathogenic challenge at the end of the experiment. This service will be provided free of charge by Lynn Moyes, lab manager of the Microbiology Department. He will inoculate each mouse with a pathogen (non-lethal to humans) to establish survivability.

Equipment:

The project requested amount for this category is $1,691.90. A strobe light is included in this section due to its use in previous successful experiments including mice models of depression. Its total cost will be $30. One immunoassay kit used for detecting levels of IL-6, a proinflammatory cytokine, is also included. This kit is professionally made and will contain special enzyme linked reagents that are very sensitive to the detection of the target molecules. The kit will contain all materials needed for the assays that are not provided by the HPHP lab. We are requesting this kit to allow us to perform our own testing to compare with the results we will obtain from sending our samples to University of Maryland. University of Maryland offered the lowest cost pricing for professional cytokine testing. We are requesting these professional tests in the event that something goes wrong with our own tests performed by the immunoassay kits. This will also offer a second source to support our data giving validity to our results at the end of the study. University of Maryland quotes a price of $10.50 per test, per sample. With twenty-eight samples for each of the three periods when cytokines will be measured this equals $882.00 for the total amount needed for testing. Finally, each mouse will need to be housed separately. The Zoology animal lab has about thirty total cages available for use, however due to other possible studies during the fall of 2012, and the need to house the mice while cages are being cleaned
regularly 10 additional cages are requested and will be purchased through the Zoology department at a cost of $299.80.

**Stipend:**

No stipend is requested for those students involved in this experiment.

**Mileage:**

No mileage budget is requested in this study.

**UNDERGRADUATE RESEARCH LONG TERM GRANT APPLICATION**

**Additional Questions**

1. What funding have you received from OUR in the past? Where has your previous project been disseminated?
   
   a. Neither Sterling Haws, nor Matt Fullmer have received any OUR grants in the past.

2. Is this project part of a required course? If so, please indicate the support (monetary and in-kind) provided for this project by the academic department.
   
   a. Yes, it is required for academic credit for Sterling Haws as a Capstone Project where he will receive a letter grade according to the quality, and outcome of the project.

3. What additional sources of funding have been solicited? Is your department willing/able to fund any equipment they will be retaining?
a. The Department of Neuroscience has offered materials for mice injections.

b. The HPHP lab has been solicited for use in the development of Immunoassays and sample storage. This includes materials such as micropipettes, test tubes, fume hoods, assay readers, deep freezers, centrifuges, and sterile working environment.

c. Department of Zoology has offered use of animal lab with available cages and working/storage space for mice.

4. Where do you plan to disseminate the results of this project?

   a. WSU research symposium, as well as any state, and national conventions which we are able to present at.

5. If you are requesting a stipend, please list all significant time commitments (5+ hours per week) that you expect to maintain over the duration of your project including, for example, class and work schedules.

   a. No Stipend is requested.
Appendix

Phase I: Weeks 1-4:

Each mouse will be housed in its own separate cage equipped with bedding and 2 water bottles (one with regular water, and one with a 0.2% sucrose water solution). Mice will be randomly separated into two groups at this time. One group will be the control group and the other will be our experimental group. Stressing will start week one and will follow a randomized schedule of stressors which will include:

- Two periods of twelve to fourteen hour food or water fasting
- Two twelve to fourteen hour periods of forty-five degree cage tilt.
- Two periods of over-night illumination.
- One twelve hour period of strobe light illumination.
- One five minute forced swim test in room temperature water.

Stressors will all be administered each week with Sunday being the only day without stressors being administered. During the course of the experiment proper bedding, housing and feeding will be observed (except in cases of fasting).

Preliminary blood samples will be taken week one by submandibular bleed to establish baseline cytokine levels in mice.

Weeks 4-9:

Depression status will be determined by measuring a noticeable decrease in the consumption of 0.2% sucrose solution (a measure of anhedonia). Once depression is established cytokine levels will be evaluated again by submandibular bleed. Antidepressants will then be administered on a regular routine to the experimental group while the control group will experience depression without treatment. Stressing will continue as before on a randomized schedule during the duration of the test. Once antidepressants have proven to start working (by sucrose-water intake returning to normal in experimental group) cytokine levels will be taken a final time.

Phase II: Weeks 9-11

At this time all testing will have taken place. Data collection and analysis will begin and the experiment will move to its final phase.

The final phase will incorporate a mortality test for the evaluation of overall immune response. This test requires that we allow a fully functional pathogenic infection to take place and run its course in the mice. Experimental and control conditions will remain the same with both groups continuing to receive the previously explained system of stressing. The experimental group will continue to receive antidepressants and the control group will continue without treatment for depression. Whether there is a difference in mouse mortality rates in either group will provide evidence for the effects that antidepressants play in the immune response. The pathogen used in the mortality challenge will be provided by the Microbiology Department and will be staphylococcus aureus. This strain of bacteria was chosen because it is the easiest strain to culture and administer at WSU. Necessary precautions will be taken to ensure safety to those taking part in the study. The staph strain will be administered subcutaneously by Lynn Moyes, an experienced professional in the Microbiology Department. This disease will not be communicable to humans from mice.

After the mortality test has been completed all data will be reviewed and analyzed. Should any of the animals remain after the mortality test they will be properly, and humbly disposed of.
according to protocol from the Animal Care and Use Committee. Frozen blood samples will be sent to University of Maryland for cytokine analysis, and we will perform our own cytokine assays to compare results. The results will be presented in a statistical chart detailing the levels of cytokines in comparison between the control and experimental groups at each of the three times established in the experiment (baseline levels, the initiation of depression, and after antidepressants have began working). The mortality data will be expressed on graph detailing time till death for each mouse, and grouped into experimental and control groups.

**Conclusion:**

The data collected for the levels of cytokines throughout the study will add experimental data to the growing body of evidence relating cytokines to depression. The purpose of the mortality test will be a unique look at the overall effects of antidepressants upon the immune system. By performing these two tests in conjunction with each other this study seeks to establish a quantifiable relationship between immune response and antidepressants.