General Guidelines

This application is for a competitive grant. Always use complete sentences and a professional tone. Your audience is the Student Scholarly Activities subcommittee, an interdisciplinary group with faculty and student representatives from across campus. Be sure to explain concepts and define acronyms clearly that will not be familiar to a general collegiate audience. Applicants are expected to stay within the word limits of the field in the application. The process of completing this application increases your written communication skills and general understanding of the research process.

Prolonging the rapid antidepressant effect of ketamine

Tell Us About Yourself (cover letter/personal statement)

- Tell us about your background or experiences that lead you to this project. (300 word limit)
    - In this section, you should write a short personal statement that explains your background and interest in the project. Why you? Why this project? Why are you interested in this project? What personal or academic experiences lead you here?

My name is Andrew Ramirez, and I am a junior majoring in biomedical engineering. I love the critical thinking and problem-solving associated with engineering and I want to apply them to a problem that has significantly impacted my life: depression. I know what it's like to think you can't do anything right. I know what it's like to believe that things are never going to get better. I know what it's like to feel like you're isolated and alone from everyone else. These are all symptoms of depression; all things I don't want anyone to experience. That is my motivation for developing this proposal. Although I knew I wanted to create a project on depression, developing an idea for a project that I could feasibly conduct in the lab was challenging. Using my engineering knowledge, laboratory experience, and past experiences, I developed this project which I believe will elucidate some of the mechanisms of depression that could lead to better treatments. Although the idea of conducting my own experiment excites me, writing this proposal has been a bittersweet experience. It elicited painful memories, but it's those memories that inspired me to work hard on this proposal and continues to fuel my drive to create a world without depression; so no one has to feel like I did.

- How will this project help you reach your personal and/or career goals? (200 word limit)
    - Be specific about how this project fits into your broader educational and professional goals.

Conducting an independent research project would serve as a major stepping-stone in my professional career. After graduation, I plan to attend graduate school so that I can work toward my goal of becoming a professor because I enjoy helping people learn and I want to continue doing research on depression. I believe that this project will help me develop important analytical and critical thinking skills that will help me reach this goal. I already have some research experience by working in the Neural Engineering Laboratory run by Dr. Peixoto, and this project would deepen my understanding of the research process. Not only that, but this project would provide me with an opportunity to improve my verbal communication skills by disseminating the results to an audience. I love engineering and I believe that everyone interested in a research project should be able to understand and appreciate the results of it; so that people will be able to enjoy engineering as much as I do.

Project Description and Background Information

- Describe the established academic background for your project, as a brief literature review, with citations appropriate for your field. (300 word limit)
    - This section of the proposal should provide a discipline-specific context and rationale for the project. You should cite appropriate research and/or creative literature using in-text citations. You may end the section with the question you plan to answer. Creative and/or performance-based projects may describe the work of others as examples. What did you read, or watch, or listen to that lead you to your question or project?

Major Depressive Disorder (MDD) is a debilitating psychological disorder that affects millions of people worldwide and is characterized by symptoms such as fatigue, anhedonia, and low mood resulting in an overall decrease in quality of life [1]. Although much research has been done on MDD, there are few effective treatments for depression and the ones that do work often have a delayed effect [1]. Given this information, developing better treatments for MDD is of critical importance. With the low efficacy and delayed effect of traditional antidepressants,

researchers searched for alternative treatments for MDD until they eventually came upon ketamine. Ketamine has commonly been used as an anesthetic, but researchers have found it can also function as an antidepressant when administered in sub-anesthetic dosages [2]. Ketamine has garnered a lot of attention from clinicians and researchers due to its rapid and effective antidepressant effect, but its harsh side effects, transient antidepressant effect, and potential to be abused has restricted its use only to patients with treatment-resistant depression [3]. Currently, the only way to prolong the antidepressant effect of ketamine is through repeated dosing which is not ideal given the negative side effects such as hallucinations, confusion, and liver damage [4]. The aim of this proposed project is to answer the question: "How can the rapid antidepressant effect of ketamine be extended without repeated dosing?"

- Provide a brief overview of your project. (300 word limit)
    - Here is your opportunity to write a brief summary of what you are proposing to introduce the reviewers to your project. Briefly summarize the project, as if you were writing and abstract. What are you doing? How are you doing it?

This project will test the hypothesis that cortisol, the chemical associated with stress, is the key to extending the antidepressant effect of ketamine. Although stress has an especially heavy impact on people with MDD, there is evidence that overcoming difficult situations can lead to resilience [5]. Establishing the relationship between cortisol concentration and the antidepressant effect of ketamine will be done using microelectrode arrays. Microelectrode arrays work by detecting when neurons spike, or activate, using electrodes. This project will use neurons from the hippocampus, the part of the brain responsible for learning and memory, because of its implications in MDD [6]. In depressed patients the hippocampus is less active, but an antidepressant can make it more active [7]. Microelectrode arrays can detect this increase in neuronal spiking and track how long it takes to return to baseline with varying cortisol concentrations. In addition to cortisol, this project will also use both enantiomers of ketamine: R-ketamine and S-ketamine. They have the same chemical structure but have a mirror arrangement of atoms which makes their effects vastly different. In fact, the FDA recently approved S-ketamine as a treatment for MDD, but some studies suggest that R-ketamine is safer and more effective as an antidepressant making it important to learn more about both enantiomers of ketamine [8]. In addition to using microelectrodes, immunofluorescence will be performed. Immunofluorescence is a technique that can be used to image neurons and astrocytes which are cells that support neurons. Astrocytes are negatively affected by MDD, and immunofluorescent images will be analyzed to see how ketamine and cortisol physically alter astrocytes [9]. After that, a computational model will be developed that shows how cortisol concentration affects the duration of increased neural spiking caused by ketamine.

- What research or creative question will your project work to address? What is the knowledge or creative gap that you are filling? (150 word limit)
    - This paragraph should clearly define the scholarly question. Make sure to connect the question to the established background described previously. The applicant should identify how this work will add to the "big picture" by contributing to the knowledge of the discipline. How will this original project address a knowledge gap in your field? Why is your project significant?

Much research has focused on elucidating the causes of the rapid antidepressant effect of ketamine. Ketamine works by activating a complex signaling pathway that leads to rapid synaptic plasticity which is how the brain changes [10]. Researchers have found that the antidepressant effect of ketamine starts working in a few hours and typically lasts for a few days but can occasionally last up to two weeks [2]. This proposed project will focus on extending the antidepressant effect of ketamine which has not been researched as much. This will be done by equating MDD to a decrease in hippocampal neural spiking and monitoring how cortisol levels affect the duration of increased neural spiking caused by ketamine. Learning more about ketamine is important for the development of a safer, more effective, and longer lasting antidepressant and making it accessible to more people.

Process

- Describe the creative process, research design, and/or methods you will employ to complete the project. (200 word limit)
    - In this section, you should describe the creative process, research design, and/or methods you will employ to complete the project. A list of steps may be appropriate. Please include only work you will do during the time during which you are funded. What will you do during the semester or summer? What will you do to answer your question or complete your creative activity?

The first step in this project will be to euthanize the rats and extract the hippocampus from each brain. The hippocampus neurons and astrocytes will be cultured onto microelectrode arrays where they will grow for a month before taking any measurements. From there, the cell cultures will be split into two groups: the control group and the experimental depressed group. A chemical called carbenoxolone will be added to the experimental group because it is known to negatively affect neurons and astrocytes like in MDD [11]. After that, R-ketamine and S-ketamine will be administered to each half of the experimental group. In addition, increasing

concentrations of cortisol will be added to each culture in the experimental group. The MC_Rack program will record neurons spiking from the microelectrode arrays. After all measurements have been taken, they will be analyzed using the NeuroExplorer program and MATLAB. Once that is complete, immunofluorescence will be performed on each culture, and the images will be analyzed using ImageJ. Once all the data has been collected, a computational model will be developed in MATLAB establishing the relationship between cortisol concentration and the duration of the increased neural spiking caused by ketamine.

- Describe how your mentor will support your project goals (150 word limit)
    - Explain why your mentor is the mentor of choice for your project. How will they support you, for example, with regular meetings, guidance on project execution, or direct, personal mentoring in a laboratory or field project? You will need to discuss this with your mentor before completing this part of the proposal. Why did you pick this mentor?

My mentor, Nathalia Peixoto, has supported me ever since I transferred to George Mason. She gave me an opportunity to work in her lab where I have learned about the research process and gained experience working with the equipment and software that will be used in this project. She is an expert in the field of neuroscience and her expertise will help me in completing this project. She will help me with the immunofluorescence and computational modeling parts of this project since I have limited experience with these techniques. Not only that, but she is also a kind and pragmatic person who enjoys helping others. For these reasons, I have chosen Dr. Peixoto as my mentor.

- Include a description or list of materials and equipment necessary to complete the project, if applicable. (150 word limit)
    - A description of materials and equipment necessary to complete the project should be included. If you need resources not available at Mason (e.g. a unique instrument or manuscript) to complete the project, you should obtain access to those resources before submitting your proposal and include a statement to that effect in this section. If you don't need anything you may put N/A in the box.

This project will need the following chemicals: R-ketamine, S-ketamine, carbenoxolone, and cortisol which will be obtained from external vendors. The rats that will be used will be bred before the start of the project. Antibodies will be needed for immunofluorescence, and they will be bought from external vendors. Cell culture medium will be needed for the general maintenance of each culture, and it will be bought from online vendors. The remaining materials and equipment such as microelectrode arrays, other miscellaneous cell culture supplies, and the accompanying software are already available in the Neural Engineering Laboratory.

Timeline

- If applicable, briefly explain any preliminary work you have done for this project. (50 word limit)
    - Write N/A in the box if you haven't done any preliminary work. Your answer here will not affect your funding.

Before this project can begin, a proposal will be submitted to the Institutional Animal Care & Use Committee (IACUC) since this project will use animals.

- Write your expected timeline in the format of schedule, every two weeks, for the length of the program. (200 word limit)
    - Please explain how you will invest your time during the semester to complete the project. Consult with your faculty mentor regarding the different sections of the project and provide the review committee with a plan of work including detailed timetables for the semester.

Weeks 1 and 2: Order R-ketamine, S-ketamine, carbenoxolone, and cortisol. Euthanize rats and extract hippocampus from each rat. Culture the hippocampus neurons and astrocytes onto microelectrode arrays.

Weeks 3 and 4: Continue to monitor the growth of each cell culture. Learn more about modeling hippocampus neurons and neural networks.

Weeks 5 and 6: Add carbenoxolone to the experimental group. Administer R-ketamine and S-ketamine with varying cortisol concentrations to each half of the experimental cultures. Record the electrical activity of control and experimental cultures using the MC_Rack program.

Weeks 7 and 8: Analyze the data obtained from microelectrode arrays using Neuroexplorer. Use MATLAB to filter data and perform statistical tests. Perform immunofluorescence on each dish and analyze immunofluorescent images using ImageJ. Use data gathered from this experiment to create a computational model relating cortisol concentration and the antidepressant effect of ketamine.

Week 9: Make conclusions from the results. Create a poster that will be shown at the OSCAR Celebration of Student Scholarship. Draft a paper that could be submitted to the George Mason Review and possibly other peer reviewed journals.

- How do you anticipate working with your mentor? (100 word limit)
    - For Example: frequency of meetings

I expect to meet with my mentor once a week to make sure everything is going well. As there are always unexpected events that can occur in the lab, she will be there to help me navigate through any obstacles that may come up.

Expected outcomes

- Please explain what you will produce as a result of your project. (250 word limit)
    - Describe the anticipated outcomes, products and/or results of the project. It should be clear to the review committee how these results will contribute to the scholarly and/or creative community. Please mention any products, deliverables, presentations, papers, art objects, etc. that you will be creating as a result of this project. If you know, identify where the project outcomes may be shared, e.g. a Mason undergraduate research symposium, a professional conference, a submitted publication, juried show, and/or performance, etc. What will you produce? How will your results/creative activity be communicated in your field?

This project will establish the relationship between the antidepressant effect of ketamine and cortisol concentration. It will help elucidate some of the mechanisms of how ketamine, both R-ketamine and S-ketamine, produces its rapid antidepressant effect and how it may be extended. The first deliverable will be produced by analyzing the data collected from the microelectrode arrays and drawing conclusions using statistical tests. In addition, the immunofluorescence images will be analyzed to determine any significant changes in length of astrocytes and number of neurons present in each culture. The last deliverable will be a computational model developed to show how cortisol levels can affect the duration of the increased neural spiking caused by ketamine. This project will be shown at the OSCAR Celebration of Student Scholarship. It is also possible that the results from this project will be submitted to the George Mason Review and other journals in the field of neuroscience and psychology. By the end of this project, I will have further developed my engineering and research skills which will help me reach my goal of becoming a professor. Additionally, continuing to do research on MDD will lead to better treatments and be one step closer to developing a world without depression.

- **BIBLIOGRAPHY (350 word limit):** Include a list of every reference cited in the application narrative. Use the style most appropriate to the discipline. You may also include a selection of works consulted as space allows.

[1] C. Otte *et al.*, "Major depressive disorder," *Nat Rev Dis Primers*, vol. 2, no. 1, p. 16065, Dec. 2016, doi: 10.1038/nrdp.2016.65.

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[7] A. J. Eisch and D. Petrik, "Depression and Hippocampal Neurogenesis: A Road to Remission?," *Science*, vol. 338, no. 6103, pp. 72–75, Oct. 2012, doi: 10.1126/science.1222941.

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