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Assessing the Generalizability of Early Life Stress Effects on Aggression

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The project entitled Assessing the Generalizability of Early Life Stress Effects on Aggression aims to enhance our understanding of the intricacies of human aggression associated with childhood trauma. Early life stress is a reliable predictor of aggression in adults. Previous studies have used rodent models to establish a link between early life stress and aggression, but many of these studies employed stressors that are not commonly experienced by humans, such as foot shock. This project intends to focus on more human-relevant stressors like social isolation and social defeat that will provide a better understanding of the brain pathways of human aggression associated with childhood trauma. This research may lead to more targeted clinical interventions.

Cover Sheet

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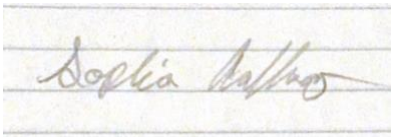
Student Applicant Signature/Date  1-24-2023

Faculty Mentor Signature/Date  1/24/2023

Background Statement

I first heard about Dr. Nordman's lab at a meeting for a pre-health registered student organization, and upon reading about his research, I immediately became interested. I found it fascinating that he has found several correlations between early-life stress and aggression, as well as reverse strategies for the aggression caused by early-life stress. As a society, we consistently hear about several psychological conditions, and the struggle to find the right medications and treatments, and I believe that this research will allow us to better understand stress-induced aggression, and what parts of the brain are driving this behavior.

I decided that I wanted to become a physician during my sophomore year of high school, after completing a science fair project about blood clotting and hemophilia. For the project, I created a blood simulation to investigate how an anticoagulant can affect coagulation, and how disrupting coagulation can lead to several blood disorders such as hemophilia. What started out as a science project I was not looking forward to doing, became something I devoted hours of my time trying to learn and understand. I knew that if people didn't know much about this one disease, what other diseases still need to be better understood? From then, I began building my knowledge to one day reach my goal of becoming a physician and understanding more about the medical field.

When starting my first semester at SIU, I had heard from other students and advisors that joining a research lab would be a great way to get hands-on experience and apply the information I was learning in my classes. When hearing about this, I knew research was something I wanted to pursue. I spent a lot of time reading about different labs, but none of them piqued my interest or seemed like something I wanted to be a part of. Many labs expected students to understand everything already and know how to do everything in their labs. When I heard about Dr. Nordman's lab, I was told that he enjoys having undergraduate students in his lab, and truly cares about everyone understanding the goal of his lab, and though I have only been in his lab for a few months, I have found that to be more than true. Dr. Nordman immediately had me read research papers, that he would discuss with me and help me understand, as well as had me shadow other students in the lab to learn how the research is conducted. I believe that with Dr. Nordman as my mentor, I will be able to grow as a researcher and contribute to the field of healthcare.

Research/Creativity Statement

Trauma caused in childhood is a strong and reliable predictor of violent aggression seen in adults. It has been found that 38% of people who are physically abused in childhood are arrested for violent crimes later in their lives (Carson 2015). The CDC uses the phrase Adverse Childhood Events or ACEs to describe early life stress. It has been found that those who experience ACEs throughout their early life have higher rates of violence with intimate partners as well as other forms of aggression (McKinney et al 2009). In our lab, we developed an early-life stress protocol that is used on mice to reflect ACEs experienced by humans. While it is well understood that early life stress does in fact lead to increased aggression, the impact of stress on the brain that causes the aggression is poorly understood. Due to this, there is a lack of clinical treatments available for excessive and recurrent aggression such as is experienced in some psychiatric diseases. Further research on this topic is crucial in order to better understand aggression pathways, so clinical interventions may be developed in order to treat patients with these symptoms.

Background

We recently developed a two-hit model for early-life stress in which rodents are subjected to early adolescent social isolation accompanied by an acute traumatic stressor in the form of non-contingent foot shock (Nordman et al, 2020 and 2022). The early adolescent social isolation portion of the model is intended to imitate the stress caused by being socially rejected or excluded during a critical time of development. The acute traumatic stressor that follows is used to further aggravate the negative effects of isolation. We find that this model causes long-lasting anxiety-like behavior, depressive-like behavior, fear, and pathological aggression primarily by strengthening the neural pathways arising from the amygdala. The amygdala is an essential area of the limbic system that displays increased activity in highly aggressive children and adults exposed to traumatic stress.

While this model is effective in inducing the desired behaviors, it is not completely ethologically applicable to humans. Humans experience a vast scope of stressors both socially and physically throughout their lives. The stress experienced by humans is very intricate and the isolated and traumatic character of stressors used in this model does not entirely capture the intricacy of that stress. In order to address this limitation, we aim to generalize this model using other forms of acute traumatic stressors including social defeat and restraint stress. These stressors are ethologically more justifiable and better grasp the complexity of stress experienced by humans. We hypothesize these substitute acute stressors will cause an increase in pathological aggression in mice similar to the previous stressors.

Finally, we will determine if these acute stressors activate the medial amygdala (MeA), a region in the brain known to regulate attack behavior and behavioral responses to stress (Nordman et al. 2020). Previous studies by Dr. Nordman's lab have established that early-life stress promotes long-lasting excessive attack behavior by activating MeA neurons and strengthening MeA pathways in mice (Nordman et al. 2020) (Fig. 1A-C). Weakening these pathways can suppress the aggression increase for the life of the animal, establishing the MeA as a key nucleus in stress and aggression and a possible clinical target for treating unrestrained anger and violence

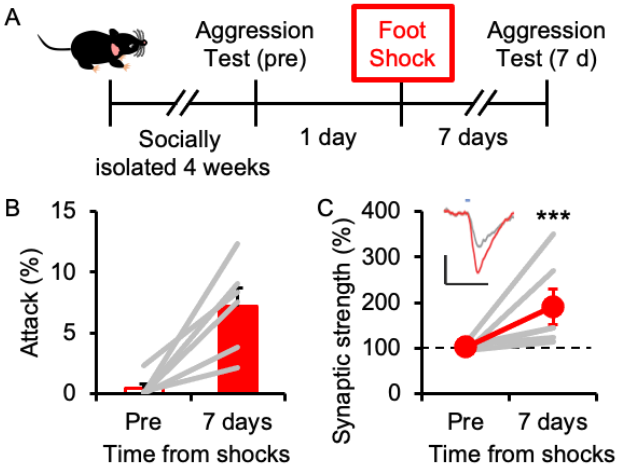


Fig. 1: Early stress promotes long-lasting attack behavior by activating and strengthening MeA pathways. (A) Experimental schedule for testing aggression before and after early life stress. (B) % of time spent attacking in the aggression test 1 day before (Pre) and 7 days after foot shocks. (C) % change in synaptic strength (excitatory postsynaptic field potential) at MeApv-VmHvl synapses 30 min before (Pre) and 7 days after foot shock. Traces represent synapatic signal before (grey) and after (red) foot shock. MeA pathways are significantly strengthened after early life stress. Mean +/- SEM. ***p < 0.001.

in humans. These findings suggest that if the alternate acute stressors we propose in this study can be substituted in our early life stress paradigm to promote long-lasting attack behavior, they will operate through a similar MeA-dependent mechanism.

Finally, by generalizing our model and the underlying the neural mechanisms, we hope to better understand the role of early life stress on pathological aggression in humans.

Methodology

Experiment 1: Assess the generalizability of early life stress-induced aggression. Three-week-old mice will be socially isolated for four weeks. The mice will then be divided into three groups (**n = 8 mice per condition, 3 conditions = 24 mice**): 1) mice exposed to acute social defeat stress; 2) mice exposed to acute restraint stress; 3) a control condition where mice are not exposed to either stress.

For the acute social defeat experiments, the mice will be placed in a novel cage with a larger, more aggressive conspecific, and allowed to freely interact for 10 min. The larger mice are expected to attack the smaller experimental mice, as observed in many of our previous studies. For the acute restraint stress experiments, the mice will be placed in a restraint tube containing a 50 mL falcon tube with holes drilled into the bottom to allow for breathing, and into the cap to hold the tail. Mice will be left in the restraint tube for 60 min, as done in previous studies.

Seven days later, aggression testing will take place. Experimental mice will be placed into a novel arena with a smaller, non-aggressive conspecific and allowed to freely interact for 10 min. The test will be recorded using a ceiling-mounted camera, and then hand-scored for aggressive and non-aggressive social behavior.

Experiment 2: Determine if the medial amygdala neurons mediate generalized early life stress. In order to test if the medial amygdala neurons regulate generalized early-life stress, we will use immunohistochemistry. Three-week-old socially isolated mice will be exposed to social defeat stress, restrain stress, or the control condition as described above (**n = 6 mice, 3 conditions, total = 18 mice**). One hour after the acute stress, mice will be euthanized, and their brains fixed in 4% paraformaldehyde. Control animals will be euthanized at the same time point. Brains will then be sliced into 40-um sections and prepared for immunolabeling. Brain slices containing the medial amygdala will be labeled with primary antibodies against the neural activity marker c-Fos (Bullitt, 1990). c-Fos is a protein that is only expressed in cells that were recently activated, which allows us to detect cells that were recently activated by acute stress. Alexa fluor 488 secondary antibodies will be used to visualize the primary antibodies. Images will be captured with a confocal microscope.

Statistical analysis and anticipated outcomes

Analyzation of the experiments will be done using One-way ANOVAs, comparing all three groups. Post-hoc Tukey's tests will be used for comparison within the groups. For experiment 1, we are expecting the acutely stress socially isolated mice to be more aggressive than the non-acutely stressed mice, which indicates the generalizability of our early-life stress model. For experiment 2, we are expecting the medial amygdala to have more fluorescently labeled cells (c-Fos+) after acute stressors, indicating that the cells of the medial amygdala are activated after general acute stress. The expected results for these two experiments suggest that social isolation followed by acute stress during adolescence promotes long-lasting pathological aggression through the activation of medial amygdala neurons.

Timeline

Test 1		**Test 2**	
Social isolation (4 weeks)	Aug. 2023	Social isolation (4 weeks)	Jan. 2024
Acute stress (social defeat or restraint stress)	Early Sep. 2023	Acute stress (social defeat or restraint stress)	Early Feb. 2024
Aggression testing	Late Sep. 2023	Immunohistochemistry (3-4 days)	Feb. 2024
Data Analysis (3 weeks)	Oct. 2023	Microscopy (2-3 weeks)	Late Feb. – Mar. 2024
Repeat Test 1	Nov. – Dec. 2023	Data analysis (3 weeks)	Mar. – Apr. 2024

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