The Effect of Acute Stressors on the Mushroom Body Health of Drosophila Melanogaster

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Abstract:

This study analyzes the relationship between the mushroom body function of drosophila melanogaster and 3 acute stressors: sleep deprivation, starvation, and social isolation. With stress becoming an increasingly large issue, and little research on acute stress in current literature, this study aims to highlight implications of acute stress using drosophila as a model for humans. The three parts of methodology include the stress/control conditions, the conditioning, and the maze trials. Using the Ethoscope's "Decision Making Arena" canton wild type flies were either stressed or not stressed, prepped on the maze with positive reinforcement, and then put through the arena, final time, for a time trial. The time in the maze is indicative of the drosophila's mushroom body function, similar to the hippocampus in humans. This methodology was set up to test the memory and learning of flies, using spatial navigation. This methodology was repeated on flies with an ADHD VPS4 related gene mutation, to ensure conditioning was successful. After 80 flies were tested, results were analyzed in Data Classroom. Using a 2 way ANOVA, a P-Value of 0.03 was found, showing there was strong confidence there was a difference between the stressors. Further research needs to be conducted with a larger sample size to prove a statistically significant relationship. No notable difference was shown between males and females regarding performance in the maze. Further research must be done to add to the literature on stress. In our busy, fast paced society stress seems to manifest itself in everyday life. This study aimed to shed light on the impact of every day stress. Education on stress and its harmful effects will help the informed to combat it and find better preventative methods.

Introduction:

Stress varies from person to person and does not hold one "official" clinical definition; It looks different for everyone. In minimal amounts, stress can be beneficial (11). That little twist

in your stomach before the big game could even help you perform better, but more than that leads into dangerous territories. Harmful stressors may present themselves in school, work, personal relationships, or even for no clear reason. The intangibility of stress, and inconsistency depending on the person both contribute to its lack of credibility. Especially in the United States, stress should not lack credibility.

Review of Literature:

Even with high prevalence of stress in the United States—with 33% of people reporting extreme stress, 77% of people reporting stress that affects their physical health, and 73% of people reporting stress that affects their mental health—it is still neglected (10). There are emerging stress management strategies, and awareness for the topic, there is still not enough emphasis on its harmful outcomes. Emotional stress is a major contributing factor to the six leading causes of death. Work-related stress is a direct factor in around 120,000 deaths yearly (2). Stress may lead to death in extreme cases, but even minimal stress can have long-lasting impacts on your health.

Stress can be deadly, but that's not the only possible outcome. A number of psychopathologies in humans—including anxiety, PTSD, depression, schizophrenia, and drug use relapse—are linked to stress (6). It has been found that these disorders are all bonded by one common thread: alterations in cognitive processes. Main alterations to cognitive processes include memory and learning (7). So, in laymen's terms, learning and memory are both indicators of stress related alterations (6). The hippocampus is a complex structure of the limbic system deeply embedded in the temporal lobe (11). It has a major role in learning and memory, and has been shown to get affected in a variety of psychiatric and neurological disorders (11). More specific functions of the hippocampus include: relational memory organization, short term

memory, and memory consolidation (4). The hippocampus is a hub for these psychopathologies, and further research has made it clear why. The hippocampus has been found to be highly susceptible to stress, but many still pondered what made it this way (4). Recent studies have found that the hippocampus has an abundant amount of cortisol receptors covering its surface (15). Cortisol is the primary stress hormone. It increases glucose in your blood stream, and curbs functions that would be nonessential or harmful in a fight or flight situation (3). Cortisol also regulates your body metabolism, suppresses inflammation, regulars blood pressure, and helps control your sleep-wake cycle (3). Too much or too little cortisol can be harmful, and with all of those functions, it's understandable why stress can be so debilitating (3). Recent studies have shown the hippocampus to be less functional in times of both acute and chronic stress, and additionally it's been shown to shrink in size due to stress (15). Although both learning and memory tasks are impaired during times of stress, it's mainly the hippocampal-dependent memory tasks (5). Spatial mapping and navigation are both part of relational memory organization, a function of the hippocampus. Therefore, one way to track hippocampal damage is through navigational ability (4).

In most literature surrounding stress, it is administered over a long time period. Because of the serious effects of chronic stress, this is no surprise, but that doesn't mean acute stress has no ramifications. Every day stressors have become so common for humans, that they blend into what is expected and normal. The lack of research on every day stress impact has driven this study and the main questions making up its foundation: How can every day stress affect us? How do varying types affect us differently? What similarities and differences do men and women have? And why is no one doing research on every day/acute stress?

Hypothesis/Purpose:

The purpose of this study is to investigate how different variations of acute stressors affect the developing mushroom bodies of Drosophila Melanogaster, more commonly known as fruit flies. In Drosophila, the mushroom body is the closest equivalent of a human hippocampus. Since navigation is a function of the hippocampus, if drosophila are exposed to different stressors, then their resulting navigation skills will be reflective of the hippocampal health of the flies. Using a maze to test this navigation, the time to make it through the maze will reflect the hippocampal health. If the fly takes a longer time, it is correlated to a poorly functioning hippocampus, in comparison to the flies who made it through the maze faster. 3 different stressors will be used on the flies: sleep deprivation, social isolation, and starvation. If drosophila are exposed to these 3 acute stressors, the sleep deprivative will be the most detrimental causing less flies to make it through the maze, in a shorter period of time.

Methodology:

This study consists of three parts: the stress or control exposure, the operant conditioning and then the maze trials. An isogenic wild type strain and an attention deficit hyperactive disorder strain with a missing VPS4A gene was used. This missing gene codes for reward anticipation and reward processing. The ADHD flies will be used as a test of the conditioning accuracy, while the wild type strain is meant to simulate “an everyday, regular person”. There was a total of 80 flies used in this study: 40 canton wild-type flies, and 40 ADHD with missing VPS4A gene. The groups consisted of control, starvation stress, sleep deprivation stress, and social isolation stress group. The sleep deprivation stress consists of two things to guarantee the flies cannot rest. They will be placed under a constant light source, and then they will also be moved around by a vibrator in intervals. The next experimental stressor group will be starved.

They will still be given water for hydration, and so they don't dry out. The last experimental group will be the social isolation flies. The flies will be placed in separate viles, that are capped, and then placed in a dark room or cabinet. In less complicated terms, there will be 4 groups of 10 flies each, for the wild type flies. There will be another set of 4 groups of 10 flies each for the ADHD flies. Stressors and control conditions were administered for 24 hours prior to conditioning and maze trials. This allows stress to be considered acute rather than chronic.

The flies need motivation to go through the maze. Instead of using a food or odor to "lure" the flies through the maze, this study uses operant conditioning. The purpose is not to test the flies' scent smelling ability; it's to test the flies navigational memory. Conditioning occurred for 2 rounds in the maze, and is followed by a 3rd, where time is recorded. The flies were placed under a regular visible light and a UV light. When exposed to UV light, and there is successful completion of the maze, a puff of vinegar was administered, as a reward. When exposed to the visible light, and there is successful completion of the maze, the flies received no reward. This was a form of a positive reinforcement. Next a period of 3 minute rest occurred. This will make it so that when the maze trial occurs, completion won't because of sheer proximity to the last round; it will be an actual test of navigational memory. Then the 3rd round, and maze trial occurred, while the UV light shined.

The conditioning and maze trials was conducted using the "Ethoscope". The Ethoscope is made up of 3D printed parts, and a raspberry pi, connected to an overhead camera. The benefits of using an Ethoscope rather than regular drosophila testing methods is the size, accessibility, and the different arenas. The Ethoscope comes with several arenas (14). They serve different purposes but for this experiment, the "Decision Making Arena" was used. The arena served as a "maze" to test the navigational abilities of the drosophila. The "Decision Making Arena" is a

more advanced version of a “Y” maze. The arena was inserted into the Ethoscope and the flies were put through, one by one. The time in the 3rd round was collected. Materials in this study will require minimal disposable. The flies will die off naturally, after they have been experimented on, and they will be able to be thrown away. This study was conducted in the Science Research Drosophila Lab. Data analysis took place through *Data Classroom* where graphs were be made, and p-values were calculated with a 2 way ANOVA.

Results:

The third time trial through the maze was recorded on Data Classroom, along with the sex, type of stress/control condition, and fly type. A 2 way ANOVA was used to calculate P-Values of the relationships within this study. There was a P-Value of 0.03, or a ***strong confidence,*** between the type of stressor and the time in maze. Showing that there was a notable difference between the type of stressor and time within the maze. However, there was no proof between what stressor proved to be “worse”. There was only a strong confidence in the fact that they are different. This alone isn’t statistically significant and further research would need to be done to prove why this relationship exists. There was no statistical significance between the sex of the flies and the time in maze. There was an error or residual of 72.

Discussion:

This study had a very high error or residual, 0 meaning the model is perfect. Overall, further research needs to be done to analyze the implications of acute stress. My initial hypothesis that sleep deprivation would be the most detrimental stressor was not supported. The lack of statistical significant relationships does not mean there are none. The sample size may have contributed to the lack of significance, along with human error. This study did however show that there is strong confidence in differences between: sleep deprivation, social isolation,

and starvation. Further research needs to be done to analyze what differences there are and why these exist, but this study is a starting place. This study shows that further analysis really should be done, because something big could be discovered.

Conclusion:

With 5 million stress related deaths per year, I think the significance of this study is pretty self-explanatory. Your brain continues to grow well into your 20s, so exposure to every day stress may cause deficits to your hippocampal health. There isn't much research about this possible problem, and that's a problem. Knowing how certain types of stress affect the hippocampus is beneficial to understanding how to prevent and treat those stressors. Not only is it important to understand the different types of stress, but it is also important to see how acute stress is impactful. Acute or "everyday stress" is rarely as researched as chronic stress. Although the stress may be acute, we really don't know how powerful it is. Because so many people experience "every-day" or acute stress it is that much more important to uncover its implications. Finding a correlation between poor hippocampal health and every day stressors could be beneficial to the world of science. If a prevalent relationship is found, scientists may want to work to find ways to limit this stressor in your adolescent years. If a treatment or medication is necessary it is important that we refine it so it is fixing the exact problem necessary. Above all, educating ourselves on the impact of acute stress can allow for preventative measures; we would rather learn we need to prevent something rather than learn how to treat it. If this study prevents an unsupported hypothesis, it will still be adding to the literature on acute stress.

This study uses a 3 part methodology—stressor, or control, conditions, operant conditioning, and maze trials—to examine how acute stress affects hippocampal function, but

mushroom body function in drosophila. Due to the hippocampus' involvement in navigational memory, a fly's ability to make it through a maze will be indicative of hippocampal health. The Ethoscope, Decision Making Arena, will be used to perform conditioning and maze trials.

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