ANXIETY—AN AMERICAN EPIDEMIC: THE ROLE OF PROBIOTICS IN ANXIETY MODULATION AND FUTURE MENTAL HEALTH TREATMENTS

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Submitted in partial fulfillment of the

requirements for Departmental Honors in

the Department of Psychology

Texas Christian University

Fort Worth, Texas

May 2, 2014

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ABSTRACT

Prior research suggests probiotic consumption provides many health benefits to the consumer. Evidence from recent studies indicates that probiotics may alter mood via the gut-brain axis. The alterations in mood shown in previous research are attributed to alterations in $GABA_A$ receptor subunit expression. GABA is the primary inhibitory neurotransmitter, and manipulation of ion flow through GABA receptors is a reliable way to manipulate anxiety. The goal of the current study was to investigate how daily administration of probiotics affected the expression of anxiety behaviors in the treated animals. We hypothesized that daily administration of *Lactobacillus reuteri* would result in decreased expression of anxiety behaviors. Newly weaned animals were fed daily either a 10% sucrose sterile broth solution, or a 10% sucrose broth solution containing *L. reuteri* (10^9 colony forming units per dose), until all animals were 70 days of age. Following the administration period, animals were tested over three days on measures of anxiety. On day 1, animals were tested on the elevated zero maze. On day 2 and 3, animals were tested in an open field apparatus. Following the behavioral tests, a corticosterone ELISA was performed. The results from our behavior tests support the hypothesis that chronic treatment with *L. reuteri* can reduce behavioral measures of anxiety behaviors. At this time, there were no statistically significant differences observed in the results from the corticosterone ELISA. With these results, the use of probiotics in relation with anxiety reduction seems promising.

ACKNOWLEDGMENTS

We would like to thank the following groups and individuals for the help and support that they have provided throughout the completion of this project:

- Supervising Professor
  - Dr. Gary Boehm
- Thesis Committee
  - Dr. Brenton Cooper and Dr. Michael Chumley
- Neuroscience Lab Graduate Students
  - Micah Eimerbrink
  - Jordon White
  - Marielle Khan
- Neurobiology of Aging Collaborative
  - Department of Psychology and Biology
    - Research Assistants at Texas Christian University
      - Samantha Hodges
      - Caleb St. Laurent
      - Lindsay Sadler
      - Ryan Madigan
      - Robert Pendry
      - Zach Kosmatka
      - Jenna Wiles

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INTRODUCTION

Anxiety—a word we toss around loosely, a feeling that may slightly rattle your mind or debilitate your body at its core. Its manifestation varies from person to person in frequency and saliency, yet we all share an unpleasant recall of its experience. Anxiety has plagued the human condition longer than recorded, yet advent of the first world exponentially increased the display of such stress within the population. This increase affects many within the mentally healthy faction of our society, but still does not touch upon the augmented suffering of the mentally ill. With about 40 million sufferers, anxiety disorders are the most common mental illness in the country. However, our nation leads the globe in health care technology and innovation; if any nation were equipped to handle this epidemic, it would be us.

Even while horribly underreported, anxiety diagnoses continue to top the charts of illnesses in America. In addition, rates of self-reported anxiety within the general population have risen steadily over the past few decades. The most cited causes fall upon finances, personal issues, and work—hallmarks of first-world society. With such saliency, one would assume a high accessibility of treatment options to combat anxiety. Unfortunately, myriad barriers have resulted in heavy underreporting, under diagnosing, and, subsequently, under-treatment of this widespread problem. Anxiety sufferers must scale walls of stigma, lack of time, lack of education, and financial costs to obtain relief. Furthermore, treatment is not easy. Psychotherapy, behavioral therapy, and pharmaceutical drugs are unpleasant, which makes adherence to them even more difficult. Treatment of any disease requires effective interventions that the patient uses religiously; anxiety treatment's multifaceted strain discourages compliance, which

decreases its effectiveness. Lessening of the negative aspects of treatment would amplify patient adherence, thus making treatment more successful.

We need a treatment filled with ease. Current steps to battle anxiety venture far from that ideal. They are hard on the body, mind, and one's wallet. This proves especially true with pharmaceutical drugs. Public opinion feels the same. A movement toward seemingly "natural" drugs has been building momentum for years. Many proponents tout the side effects, drug interactions, and unforeseen risks of synthetic prescriptions as fuel for their argument. On top of that, many interest groups concerned about over-medication have risen from the horizon; recent polls show considerable worry in our country about multiple psychiatric medications administered simultaneously. Lastly, the price of medications has ignited current events and political heat too scalding for the scope of this project. Overall, America wants an inexpensive, safe alternative to traditional psychiatric medicine. We think we have part of the answer.

What is more available, natural, and cheap than bacteria? Nothing. In all seriousness, the research surrounding bacteria maintaining beneficial symbiotic relationships with their hosts—particularly humans—has grown considerably. Studies on probiotics (meaning anything that encourages the growth of bacteria, including the bacteria itself) span a wide variety of medicinal disciplines, treading deeply into the realm of psychiatric health. More specifically, a plethora of studies has found significant regulatory influence by probiotics on a mechanism entitled the "Gut-Brain Axis". In the simplest of terms, this axis allows the intestines to produce varying effects on mental functioning. These noted effects include decreases in inflammation, mood swings, and many forms of psychological discomfort. If probiotic cultures could take a foothold in

helping to treat prevalent psychological distresses, such as anxiety, this would provide an alternative for which many advocates are yearning. These cultures encompass part of the healthy biome within the gut, and increasing their presence has yet to show consistently negative side effects. There have been virtually no reported detrimental interactions between probiotic cultures and pharmaceutical drugs. Bacteria-containing yogurts, smoothies, and dietary supplements make probiotic ingestion readily available to the average consumer, both temporally and financially. Furthermore, the ease of eating pleasurable foods with the cultures inside them makes adherence to probiotic ingestion simple. The possibility of probiotics becoming a part of the treatment plan for anxiety patients seems intriguing, but more research must be conducted to validate a strong causal relationship between these cultures and anxiety relief.

ANXIETY DISORDERS

Prevalence

In the healthy individual, anxiety comes as a natural reaction to stress. Stress and anxiety in modulated amounts have evolutionarily kept humans alive. Small bouts of anxiety pushed us to escape predators, prepare for periods of famine, and protect our property and families. Even now, anxiety thrusts us to accomplish great feats and persevere through difficult situations. Anxiety fuels an athlete's desire to practice for their big competition, a student's long hours of studying before a midterm, and the minds of two people on a first date. However, too much of a good thing can prove to be detrimental. These feelings of anxiousness were only meant to permeate our psyche for short bursts of time until relief from the outcome occurred. Unfortunately, American society has drastically extended that time with long school semesters, work demands, and

familial responsibilities. Furthermore, certain individuals, for a variety of causes, are predisposed to unhealthy levels of stress and anxiety. Over 40 million Americans have this uncontrollable anxiousness as their reality. When the magnitude or frequency of anxiety interferes with one's ability to function at their highest level, this constitutes possible diagnosis of an anxiety disorder. There are specific illnesses underneath the umbrella of "anxiety disorders"; each has their own manifestation of unique symptoms as defined by the *Diagnostic and Statistical Manual of Mental Disorders*. In addition, the demographics affected and the time trends give an even clearer picture of how these illnesses are affecting America.

As described in the DSM-V, Post-Traumatic Stress Disorder's name explains the root of the ailment; the unhealthy stress levels stem from a traumatic experience and last for more than four weeks (DSM-V). Three main symptom groups define PTSD from most other mental health issues: re-experiencing symptoms, avoidance symptoms, and hyper-arousal symptoms (DSM-V). Re-experiencing may include flashbacks, nightmares, and scary thoughts that center around the traumatic event. Avoidance refers to the patient's tendency to avoid close contact with others, as well as emotional expression and any triggers that remind the sufferer of the said event. Hyper-arousal leads to insomnia, a tense demeanor, and being easily frightened. Similarly, Acute Stress Disorder exhibits almost identical symptoms, but must only last for a maximum of six weeks. Acute Stress Disorder ails a larger portion of those experiencing a traumatic event temporarily, while PTSD plagues a smaller group with longer-lasting symptoms (Fullerton et al., 2004).

Media attention surrounding military personnel and their propensity toward PTSD has increased recently since the conclusion of the United States' involvement in many

wars. Personal testimonies from affected war veterans and their families have gained nationwide response and sparked conversation on how to improve the conditions for those affected. Sufferers vividly describe the terrifying hallucinations, nightmares, stress, and the debilitating anxiety that they endure as part of their disorder. On top of that, family members of these sufferers feel an upturn of stress and anxiety themselves in regards to learning to cope with their loved one's ailment.

Between 4-20% of veterans from the recent operations in Afghanistan and Iraq have or will develop PTSD; over 30% of veterans of the Vietnam War have developed PTSD (Richardson et al., 2010). According to the National Institute of Mental Health (NIMH), 6.8% of all American adults will develop PTSD in their lifetime. Females are much more likely to experience PTSD than males after any given traumatic event, especially for individuals between the ages of 13-17. Approximately 6.6% of all female teenagers will develop this condition, compared to only 1.6% of male teenagers (NIMH). Overall, the rates of PTSD are higher among military personnel than the general population, yet those experiencing any form trauma will always be at an increased risk.

Another anxiety-related disorder widely discussed throughout our country is Major Depressive Disorder (MDD), commonly known as "depression". MDD is diagnosed when the patient simultaneously presents at least five symptoms of the disorder described in the DSM-V on a relatively daily basis for a period greater than two weeks. The possible symptoms of MDD include depressed/irritable mood, decreased pleasure and interest, significant weight/appetite change, change in sleep, change in activity, fatigue, feelings of guilt/worthlessness, diminished concentration, and suicidal thoughts. Though technically classified as a Mood Disorder, anxiety symptoms often

present with and exacerbate the severity of MDD. Furthermore, most treatments currently directed toward MDD also treat anxiety symptoms as well.

In the United States, 6.7% of adults will fall victim to Major Depressive Disorder at some point in their lifetime, almost the exact same amount as Post-Traumatic Stress Disorder (NIMH). Their demographic trends regarding sex also line up well; females are 70% more likely to develop MDD than males (NIMH). Additionally, the distribution of MDD across the country exhibits racial and ethnic disparities. For example, non-Hispanic Whites are 40% more likely to suffer from MDD than non-Hispanic Blacks (NIMH). Finally, people over the age of 60 are diagnosed with MDD at a strikingly lower rate.

Post-Traumatic Stress Disorder and Major Depressive Disorder encompass only two ways through which people may feel intense stress and anxiety. Anxiety disorders plague almost one-third of the adult population in America (see Figure 1 in Appendix A). However, that number only counts those who receive formal diagnosis; anxiety and stress adversely affect many more people within the healthy/normal range of mental functioning. To put these numbers into perspective, according to the Centers for Disease Control (CDC), one-third of all adult Americans currently qualify as obese, around the same proportion as anxiety disorders. Similarly, one in three Americans will develop skin cancer in their lifetime (CDC). These diseases receive much more ardent attention, and rightfully so since their prognoses often involve life-threatening outcomes. However, the obvious lack of focus on anxiety disorders makes sufferers feel as though they are alone, which only fuels the pathology. With over 40 million Americans (and their loved ones) suffering through a lifestyle of denoted by anxiousness, such a prevalent issue deserves more attentiveness amongst the public.

Treatment

As previously stated, the treatments aimed against anxiety often overlap with those for depression. Among prescription drugs, mental health professionals often prescribe Antidepressants, Benzodiazepines, Buspirone, and Beta-Blockers to combat feelings of anxiousness in their patients. Mental health professionals frequently recommend regular psychotherapy sessions with a clinical psychologist or psychiatrist, counseling sessions, and/or support group attendance in addition to prescriptions. These interpersonal sessions prove to be especially effective, and sometimes necessary, for more severe sufferers. Treatment plans rarely involve just one course of action; mental illnesses require multi-faceted treatments to heal the patient adequately. This wide range of options allows professionals to tailor treatment plans to the needs of each patient.

Prescription drugs act on various mechanisms and pathways in the body to relieve anxiety and stress. Psychiatrists prescribe Benzodiazepines (i.e., tranquilizers, anti-anxiety drugs) for anxiety more than any other drug type. This class of medication decreases feelings of stress and anxiousness by depressing the central nervous system. Benzodiazepines work very quickly and effectively after ingestion. However, their potent effects contribute to the many unintended side effects and other drug interactions, such as nausea, depression, double vision, and memory loss. These adverse properties may outweigh, or even eliminate, the benefits of Benzodiazepine administration. In these cases, antidepressants often become the viable options. Antidepressants, such as Selective-Serotonin Reuptake Inhibitors, Monoamine Oxidase Inhibitors, and Tricyclics, have a much lower risk of dependency, abuse, and side effects than Benzodiazepines. These lower risks make antidepressants much safer for long-term management of anxiety

issues. Unfortunately, antidepressants do come with their own slew of negative consequences. In younger patients, antidepressants have a heavy correlation with suicidal thoughts and withdrawal issues. Buspirone is a newer medication with a low risk of side effects, but only treats low-grade General Anxiety Disorder. This drug decreases anxiety by increasing Serotonin and decreasing Dopamine. It can be taken for long periods of time with little chance of dependency or abuse, but has not been shown to be effective for severe anxiety. In addition, unlike the more powerful medications, it has a long latency period of about two weeks before benefits become apparent. Finally, Beta-Blockers are a widely distributed treatment option for high blood pressure that is off-label prescribed to treat anxiety. Beta-Blockers work by decreasing norepinephrine, which modulates the sympathetic nervous system, to decrease stress levels across the body. This low-potency drug does not alter the emotional aspects of worry, just the physiological manifestations of stress. Similar to the other drugs mentioned, Beta-Blockers also carry some slight side effects, such as dizziness, nausea, slow pulse, and sleepiness. All these medications can provide relief for symptoms without much effort on the patient's part. However, lack of adherence and a plethora of unintended side effects easily decrease effectiveness and use.

Along with prescription medications, a treatment plan often includes some form of interpersonal therapy. This therapy may be administered intensively, such as regular one-on-one sessions with a mental health professional, or leisurely, such as non-therapist-facilitated support groups. Severe disorders typically require consistent sessions with a clinical psychologist or psychiatrist. Mental health professionals may employ a variety of behavioral or talk therapies to help their patients. However, these professionals openly trust and steadily use Cognitive-Behavioral Therapy (CBT) as the primary behavioral

treatment for anxiety disorders. Cognitive-Behavioral Therapy differs from traditional "talk-therapy" in that it fosters a more collaborative therapist-client relationship, uses reinforcement to increase positive thoughts while punishing negative thoughts, and may directly expose the client to the experiences that trigger his or her anxiety. CBT lasts for twelve weeks, yet provides long-lasting improvements for anxiety disorders. The positive outcome of CBT can be further increased by congruent administration of a psychiatric medication. Of course, even CBT has its negatives. Successful completion of the treatment requires consistent adherence to these emotionally-trying sessions for three consecutive months and the financial resources to pay for the therapy; unfortunately, any client can easily fail either of these requirements.

When a mental health professional diagnoses a client with a disorder, this professional will recommend or prescribe a certain level of treatment(s) to relieve the symptoms of the illness. From this point on, the patient holds the responsibility of seeking treatment. According to the National Institute of Mental Health, just under 40% of those diagnosed with these disorders currently seek health care treatments (e.g., psychiatric visits, prescription drugs, etc) (see Figure 2 in Appendix A). Furthermore, a majority of anxiety sufferers currently do not participate in any treatment measures (see Figure 2 in Appendix A). The American Psychological Association (APA) conducted a poll in 2004 to ask Americans about their opinions toward mental health treatments. When asked reasons why they would choose to not seek treatment, the respondents primarily cited time-consumption, stigma, and cost as reasons. The mental health expenditures around the time of this poll also shifted from more expensive measures, such as specialty hospitals, to less expensive and less time-consuming options, such as

prescription medications (see Figure 3 and 4 in Appendix A). Though this may seem to solve the issue, many people who solely choose to use medications also require interpersonal therapy as well. This decision to under-treat exhibits how negative aspects of treatment may be decreasing the quality of life of anxiety patients.

The Issue

Anxiety disorders affect a considerable portion of our nation's populace; furthermore, this large group of sufferers deserves attention on how to improve their treatment options. We need a treatment option that combats the pitfalls of current remedies. Prescription drugs may induce a variety of unwanted side effects, interact with other drugs, and overall lack an enjoyable form of administration. Interpersonal therapies encompass mentally challenging sessions and mandate consistent attendance for effectiveness. Moreover, all types of treatments accrue major monetary costs that may also form a barrier between the patient and proper treatment. In spite of the negatives, current psychiatric medications and interpersonal therapies show remarkable effectiveness and should not be removed from practice. However, a treatment plan has multiple components, and incorporating easy-to-follow parts into this plan makes the plan as a whole more effective. We believe developing and integrating low cost, readily accessible treatments with minimal side effects would result in greater obedience to treatment, thus providing an improved quality of life for affected individuals.

GUT-BRAIN AXIS

The "Good" Bacteria

The primary function of the gastrointestinal tract is to digest food and absorb nutrients into the body. The functions of this major body system would prove impossible,

or at least absurdly inefficient, without the resident bacteria present throughout. The resident bacteria here may collectively be called “Commensal Bacteria,” “Flora,” “Microbiota,” or “Microbiome.” Commensal bacteria line the epithelium of the entire alimentary canal and participate in a mutually beneficial symbiotic relationship with our bodies. The bacteria receive a warm, nutritious, safe place to live and reproduce, while the human body receives help in breaking down nutrients and warding off pathogenic bacteria. Commensal bacteria often contain enzymes that the human body does not naturally produce, which is how these microorganisms aid in the digestion of materials humans cannot normally break down. On top of that, these bacteria tightly pack across the epithelial lining, which prevents pathogenic bacteria—bacteria that cause disease—from taking a foothold within the intestines. Antibiotics, a very common medication for most bacterial infections, prevent reproduction of the “good” bacteria in your gastrointestinal organs as well. This non-specific combating of all bacteria leads to gastrointestinal distress as a common side effect of these medications. In contrast, not taking antibiotics can lead to a stronger relapse of the infection for a variety of reasons. One reason is the commensal bacteria that would normally shield the epithelium from pathogenic bacteria have been taken away. This allows the small amount of leftover pathogenic organisms to plant themselves throughout the GI tract more easily, contributing to a more potent second attack. Overall, the bacteria that make up the microbiome in the gut (intestines) tend to be the most impactful and beneficial. These bacteria do not simply keep to themselves; they interact with a major organ system and enable our bodies to function at a highly efficient level. Interestingly though, their impact has been shown to extend far beyond the reach of the intestinal epithelium.

Biological Components

Per the name, the "Gut-Brain Axis" involves a line of communication between the intestinal tract and the central nervous system (see Figure 5 in Appendix A). This particular system is an extension of the central nervous system into the intestines that provides reciprocal information between the two networks. The Vagus Nerve (X) innervates the gut from the brainstem and creates this two-way mechanism. There are certain basic messages that need to be received by the brain, such as when the colon is full and needs to be relieved. However, we are focusing on the axis for its higher-order functions. The research regarding these neural connections shows considerable capacity for the intestinal canal to alter functions in the central nervous system. This remarkable capability has been extensively studied concerning Irritable Bowel Syndrome, Crohn's Disease, and other intestinal disorders to investigate how these ailments modulate different central nervous system functions, especially regarding stress (Fichna & Storr, 2013). Researchers consider inflammation within the intestines as the reason behind these specific effects. The microbiome has been shown to modulate this inflammation, and thus the brain; however, our aspect of interest lies specifically within bacteria involved.

All bacteria release compounds from their natural metabolic reactions, including the commensal microorganisms in the gut. These compounds have shown to cause widespread effects in relation to brain functions. Microbiota studies show myriad results, including decreased self-reports of stress in cancer patients, modulated characteristics of Autism Spectrum Disorder, and improved sensorimotor behaviors (Yang et al., 2014; Abdulrahman, 2013). With such positive data, focusing on how alterations in the

intestinal bacteria could lead to new treatments has become a primary goal in this realm of scientific research.

Antibiotics, as previously stated, decrease the level of bacteria in the body. However, probiotics encourage proliferation of bacterial colonies and introduce new species within the body. Probiotics cause alterations in the microbiota of the GI tract. Since we know the state of the microbiome can readily result in changes of the central nervous system, bacterial alterations in the microbiome due to probiotic administration should lead to similar effects.

PROBIOTICS

Commercial Use

Within the public, most people already consume bacterial cultures absentmindedly. Yogurt and other cultured dairy products contain healthy bacteria that may take a foothold in the gastrointestinal tract once it is ingested. Even though most people consume probiotics without direct intention, certain yogurts do explicitly advertise the presence of probiotic substances as digestion aids. Getting individuals with these digestive issues to use this non-prescribed form is particularly easy because of low cost, easy accessibility, and an enjoyable method of administration. Since the methods of probiotic consumption already lack the pitfalls of current treatments for mental health, investigating their potential role as a component in anxiety treatment plans may prove particularly fruitful.

Previous Literature and Research

Lactobacillus strains (the most common bacterial strains used as probiotics) decrease anxiety as measured by various behavior tests including the elevated plus maze

and Stress-Induced Hyperthermia tests (Bravo et al., 2011; Luo et al., 2014; Ohland et al., 2013; Yang et al., 2014). The mechanisms behind this effect have been attributed to Vagus Nerve-mediated changes in $GABA_A$ receptor subunit expression, glucocorticoid levels, and other fear-involved circuits (Bravo et al., 2011; Goehler et al., 2007). GABA receptors are the primary inhibitory neuronal receptors in the central nervous system. The administration of probiotics has been shown to alter expression of the $GABA_A$ receptor subunits along with displaying anxiolytic properties in the brain. This reputable relationship has led to anxiety attributions to manipulations of ion flow through these receptors (Nasehi et al, 2014; Earnheart et al., 2007). The Hypothalamic-Pituitary-Adrenal Axis leads to the production of glucocorticoids that produce stress throughout the mammalian body (Sudo N, et al., 2004; Brinks et al., 2009). Cortisol is the primary stress-induced glucocorticoid released in humans, and the rodent equivalent is corticosterone. The anxiolytic effects from probiotic administration are often accompanied by decreased corticosterone expression (Gareau et al., 2007; Bravo et al., 2011; Ait-Belgnaoui et al., 2014). Further research to establish these relationships is ongoing and moving toward pinpointing exact mechanisms. The research in this field is quite new, yet the effects of probiotic administration on stress and anxiety appear consistent.

THE EXPERIMENTAL PROCESS

Experimental Subjects

The test subjects used in our experimental tests were naïve male C57L/6J mice bred in the Texas Christian University vivarium from a breeding stock obtained from the Jackson Laboratory (Bar Harbor, ME). After the animals were weaned from their mother,

the animals were housed in groups ranging from two to four animals per standard polycarbonate mouse cage (dimensions: 30 x 20 x 16 centimeters). Light and dark cycles were automatically regulated, and animals were provided access to food and water *ad libitum*. All animals were treated in compliance with the *Guide for the Care and Use of Laboratory Animals*, and both the behavioral and biological experiments were conducted in accordance with the protocol approved by the Institutional Animal Care and Use Committee at Texas Christian University.

Treatment Conditions

The selected animals involved in this study were randomly divided and assigned within each cage group into one of two treatment options—a sugar broth solution, which served as the control, or the probiotic solution, which served as the independent variable of the experiment. For the animals selected to be in the control condition, the newly weaned animals were fed daily a 10% sucrose sterile broth solution. For the experimental condition, newly weaned animals were fed a 10% sucrose broth solution containing *Lactobacillus reuteri* (10^9 colony forming units per dose). All animals were fed daily the assigned solution until the youngest animal of their cage group reached 70 days of age.

Part I: Behavioral Tests

Following the sucrose-sterile and probiotic administration period, the animals were tested over a period of three days on measures of anxiety. The first behavioral test that was performed was an elevated zero maze, which lasted for one day. An elevated zero maze consists of an elevated circular platform with two opposite enclosed sections and two open quadrants (see Figure 6 in Appendix A). Animals, such as mice, have an instinctual desire to be in closed, dark spaces—a natural protective instinct to survive

against predators. Additionally, mice are highly sensory-based creatures via their whiskers, and therefore prefer areas such as walls and enclosed spaces, which allow their whiskers to have surfaces to assess. Because of these natural instincts, animals, such as mice, tend to find open and exposed areas to be stressful and aversive. Therefore, anxiety-like behaviors in this experimental design would be expressed by a decreased amount of time spent in the open quadrants and a decreased number of entries into the open areas. For our experimental purposes, animals from both control and probiotic groups were selected for this behavioral test to observe if probiotic administration would have a statistically significant effect on the exploratory and anxiety behaviors as compared to the animals in the control group.

The second behavioral test was conducted for a testing period of two days. An open field apparatus is a box with a designated center zone surrounded by four raised walls (see Figure 7 in Appendix A). Once the animal enters the maze, the animal is tracked and monitored by infrared beams. These beams monitor the amount of time the animal spends in the center zone, near the walls, etc. As previously explained, animals, such as mice, have a natural instinct to prefer enclosed and dark spaces. Hence, anxiety-like behaviors in the open field maze are indicated by decreased numbers of entries into the center area of the maze, as well as a decreased amount of time spent in the center area of the maze. For our experimental purposes, animals from both control and probiotic groups were selected to undergo the two-day open field maze test. The data collected on the first day of testing for the open field maze was used to establish a baseline for the control and probiotic groups. On the following day, the animals were subjected to the

open field again to see whether the animals associated or recalled the situation of the open field from the previous day to be aversive or stressful.

Part II: Biological Test

After both behavioral measures were performed on the animals, we conducted one biological test, a corticosterone enzyme-linked immunosorbent assay (ELISA). Because corticosterone is a glucocorticoid secreted by the adrenal gland and is the major stress steroid produced in non-human mammals, we believed that measuring the presence of this hormone in our animals would be a major indicator of stress and a measure of anxiety. In this test, the amount of color that is expressed in the testing wells determines how much of the selected compound is present in the serum—a standard curve relating optical density to the amount of corticosterone was used to identify the hormone levels in the serum. We hypothesized that since corticosterone is released due to activation of the HPA Axis, the axis' function should be diminished with the administration of probiotics, and thus more corticosterone would be found in the control group animals rather than the animals in the probiotic condition.

Results

In the elevated zero maze, the probiotic-fed animals had significantly more entries into the open quadrants of the maze than did the broth-fed animals (see Figure 8 in Appendix B). Additionally, the animals that were administered probiotics spent a significantly longer amount of time in the open arms of the maze than did the control group of broth-fed animals (see Figure 9 in Appendix B).

For the open field maze, the first day of testing showed no statistically significant difference between the control group of broth-fed animals and the experimental condition

of the probiotic-fed animals. In regards to the number of entries and the amount of time spent in the center of the maze, day one of testing showed no major differences between the groups. However, on the following day of testing for the open field maze, there were statistically significant differences between the groups. On day two of the open field test, the probiotic-fed animals had a significantly greater amount of entries into the center of the maze than did the control group (see Figure 10 in Appendix B). Furthermore, the animals that were administered probiotics spent a significantly longer amount of time in the center of the maze than did the broth-fed animals in the control group (see Figure 11 in Appendix B).

Unfortunately, in the corticosterone ELISA, there was no statistically significant difference in corticosterone levels of control broth-fed animals and probiotic-fed animals.

DISCUSSION

The results from both behavioral measures of the elevated zero and the open field mazes support the hypothesis that chronic administration of *L. reuteri* can help to reduce the expression of anxiety behaviors, as seen with the animals of this experiment. In both behavioral measures, the animals exposed to the probiotic treatment remained more exploratory in the approach-avoidance measures, therefore suggesting that animals in probiotic treatments did not recall the scenario set forth by the maze to be as aversive. This implies that these animals also had lower levels of anxiety in the potentially stressful situation. Since the corticosterone ELISA did not produce statistically significant results at this time, this may suggest that corticosterone may not be a related mechanism in explaining the behavioral results of this experiment. However, it should be noted that there are daily fluctuations of corticosterone levels in relation to varying wake and sleep

cycles, thus this variation could have affected the biological test results. Another point to be considered is that adrenaline could be a potential mechanism involved as it is related with stress and anxiety, or the responsible biological mechanisms involved in explaining the behavioral results rely solely on the GABA receptor expression and neurological processes.

Overall, such results provide support for the basis that these probiotic-fed animals contain lower levels of GABA receptor expression in the brain. These results further suggest that bacterial cultures, such as probiotics, help to reduce stress and anxiety levels in mice, and possibly within humans. Further studies should investigate minimum and maximum dosage levels of probiotics that would be necessary for similar results to take effect, and other studies should further investigate the actions of probiotics on the immune system in relation to anxiety and stress.

Furthermore, interest in probiotics may continue to grow across the nation, possibly becoming a major point of interest for popular culture with the prevailing problem of anxiety and stress affecting all. With public attitude shifting toward “natural” supplements over synthetic medicines, the notion that bacterial cultures may alleviate psychological discomfort could add even more attention to probiotics in the future.

APPENDIX A: SUPPORTING MATERIAL

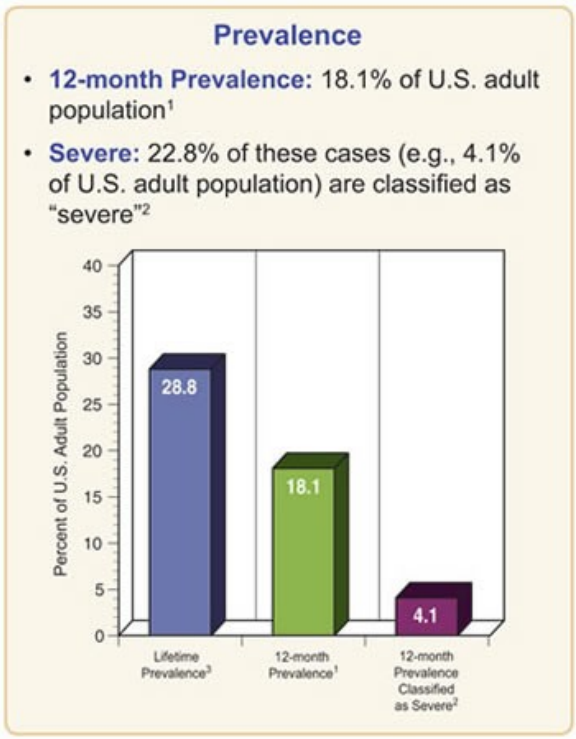

Figure 1.
Source: Kessler RC, Chiu WT, Demler O, Walters EE. Prevalence, severity, and comorbidity of twelve-month DSM-IV disorders in the National Comorbidity Survey Replication (NCS-R). Archives of General Psychiatry, 2005 June, 62(6):617-27

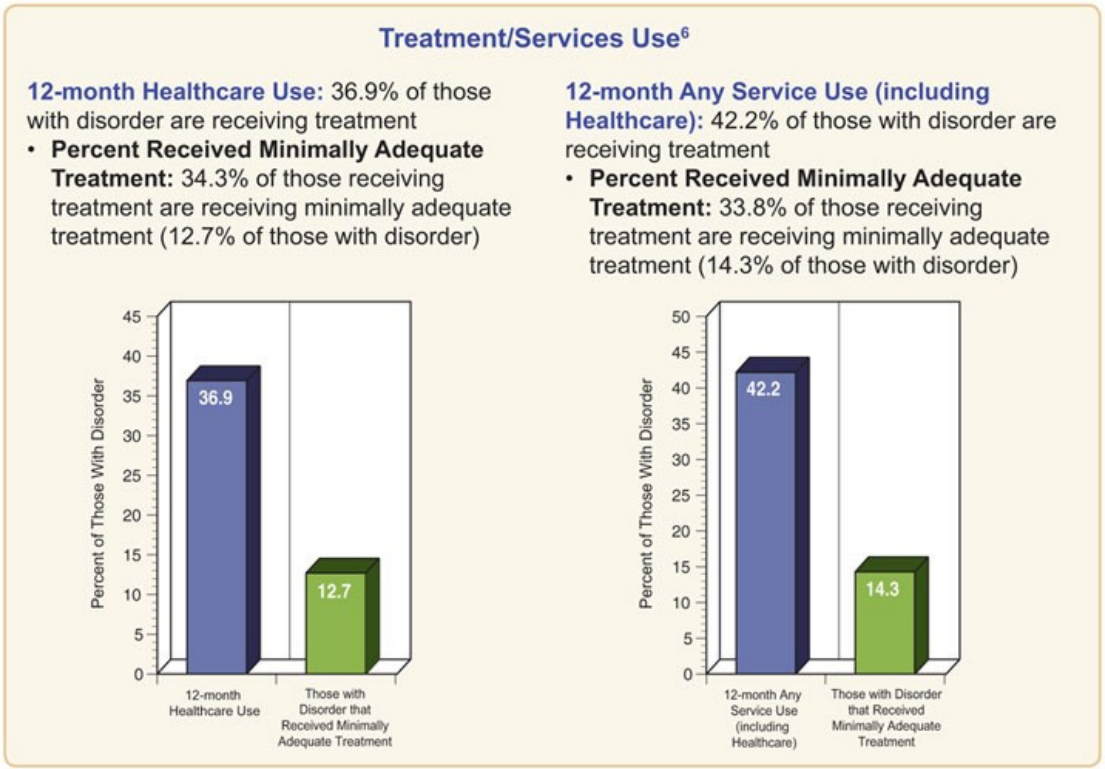

Figure 2.
Source: Wang PS, Lane M, Olfson M, Pincus HA, Wells KB, Kessler RC. Twelve month use of mental health services in United States. Archives of General Psychiatry. 2005 June, 62(6):629-640.

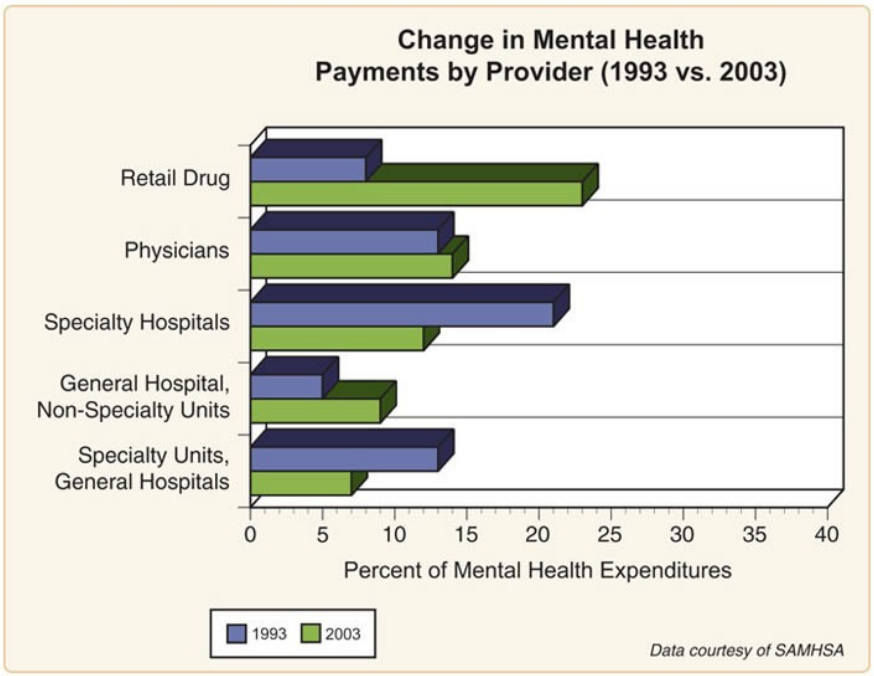

Figure 3.
Source: National Institute of Mental Health: Agency for Healthcare Research and Quality's (AHRQ's) Medical Expenditure Panel Survey (MEPS)

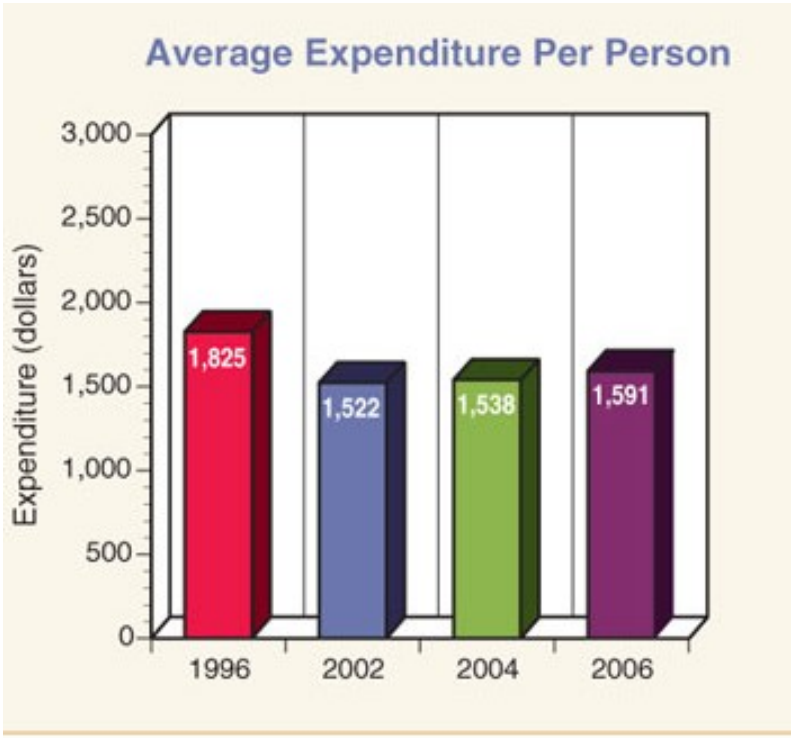

Figure 4.
Source: National Institute of Mental Health: Substance Abuse and Mental Health Services Administration

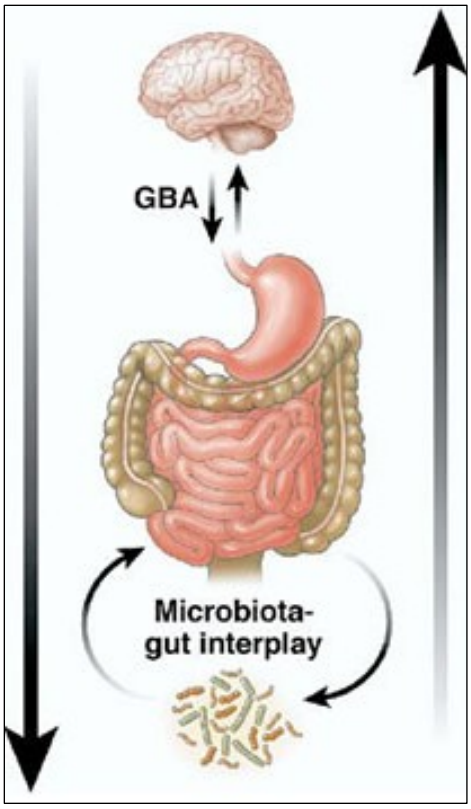

Figure 5.
Source:
http://jasoncholewa.com/2012/07/22/considering-probiotics-for-health-and-performance/

Figure 6. Elevated Zero Maze
Source: Berlin Mouse Clinic for Neurology and Psychiatry

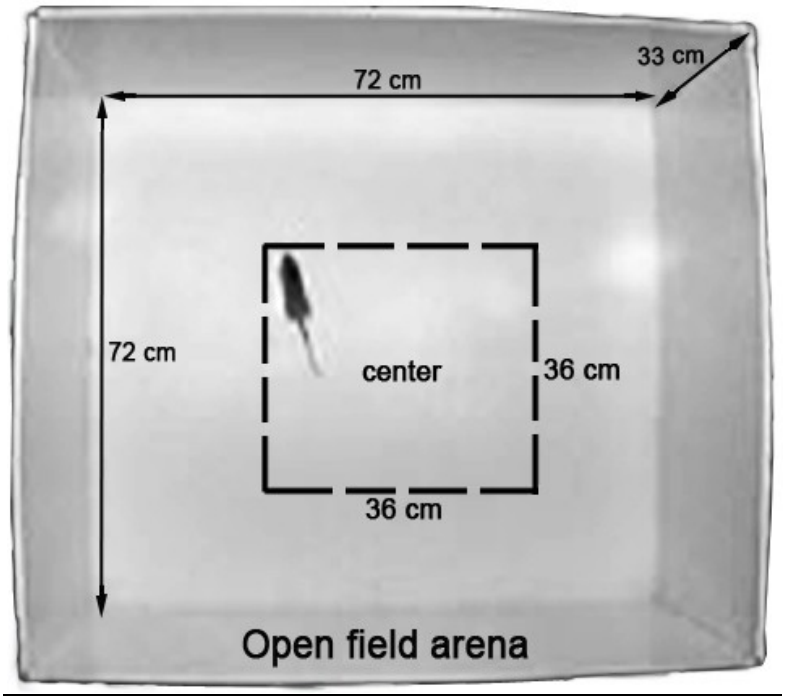

Figure 7. Open Field Maze Source: Lad HV, Liu L, Paya-Cano JL, Parsons MJ, Kember R, Fernandes C, Schalkwyk LC. Behavioral battery testing: evaluation and behavioral outcomes in 8 inbred mouse strains. Physiol Behav. 2010 Mar 3, 99(3):301-16. Epub 2009 Dec 2

APPENDIX B: RESULTS

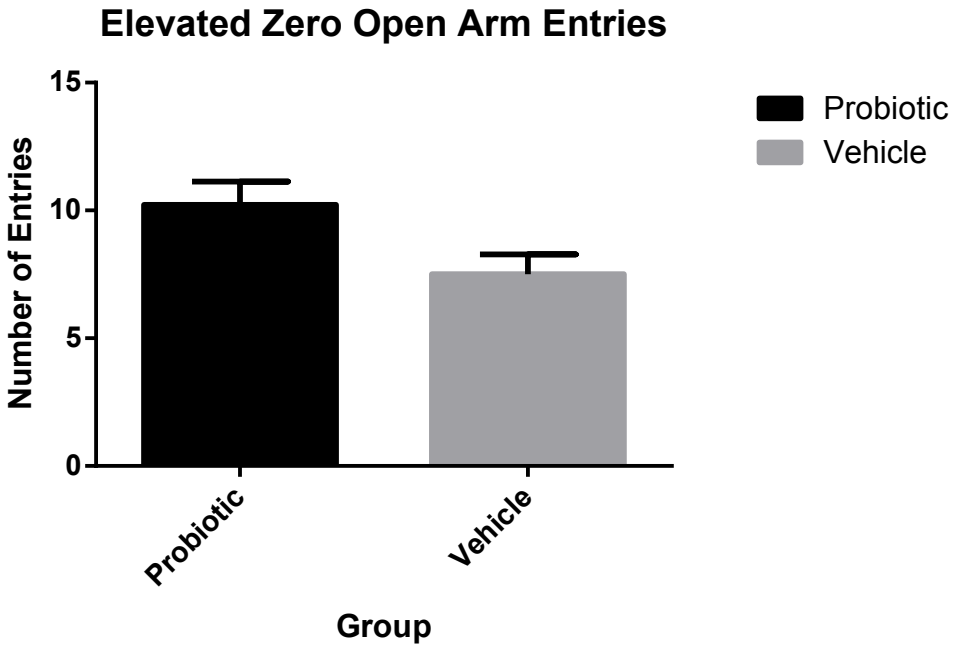

Figure 8. Elevated Zero Maze Results: number of entries into the open arm

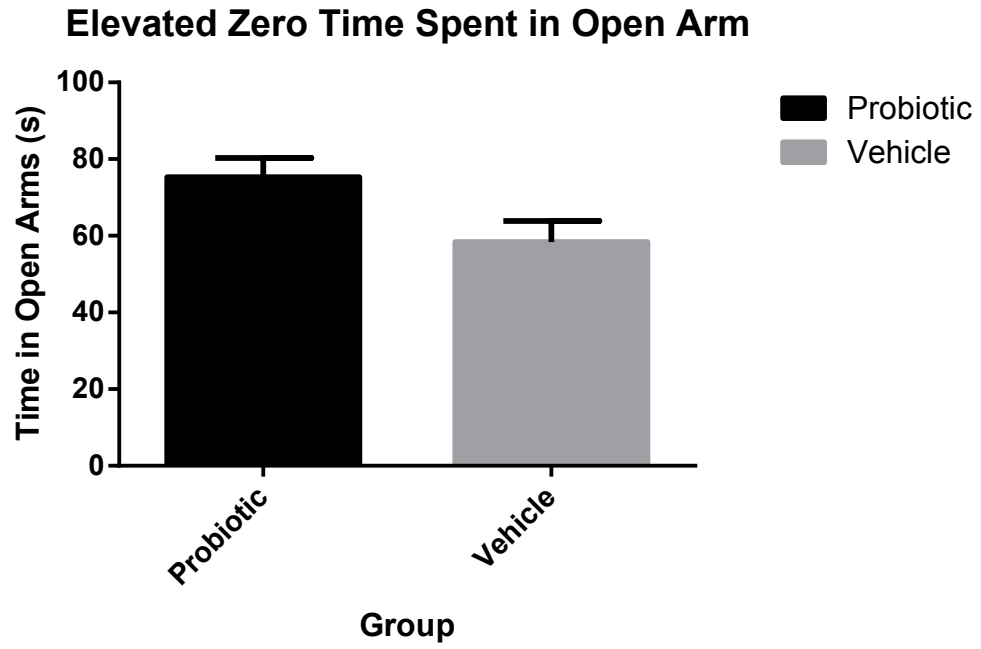

Figure 9. Elevated Zero Maze Results: amount of time spent in open arm

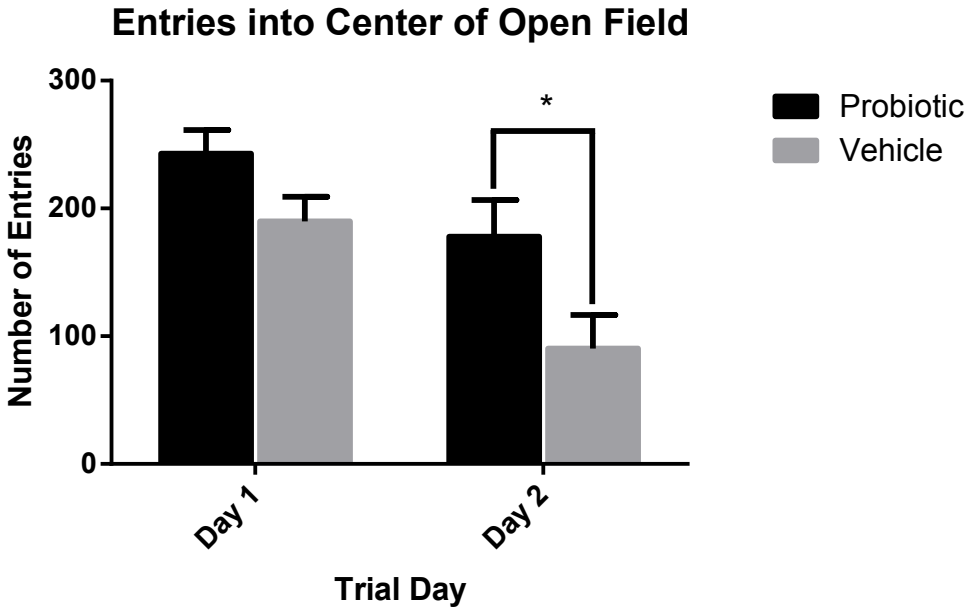

Figure 10. Open Field Maze Results: number of entries into the open field

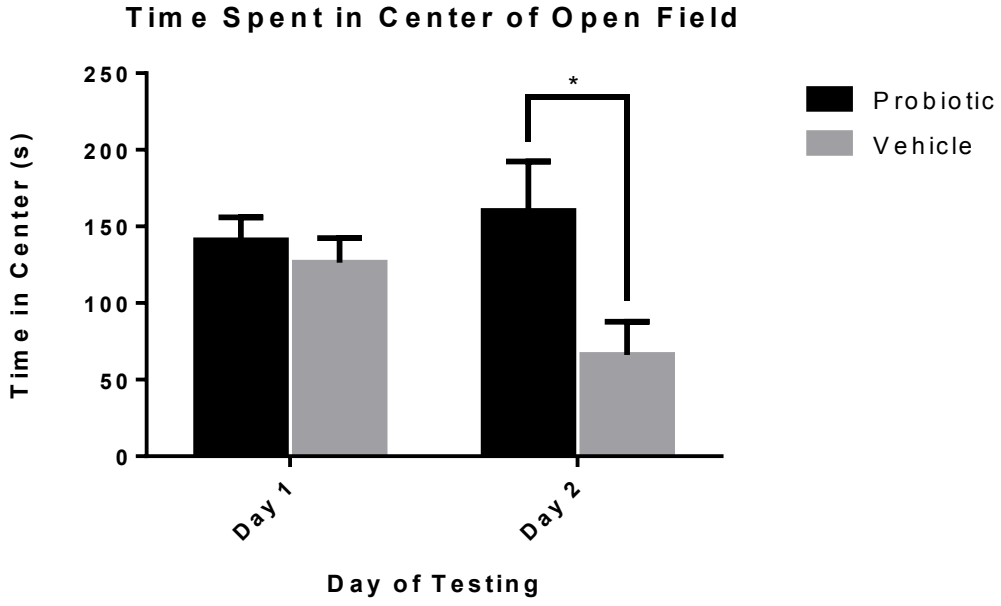

Figure 11. Open Field Maze Results: amount of time spent in the open field

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