

The Cognitive-Enhancing Effects of Lion's Mane in A Rodent Model of Attention-Deficit Hyperactivity Disorder (ADHD): A Research Protocol



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Abstract

The intersection between mycology and modern medicine has captivated the scientific community, with a growing focus on fungus potential to address symptoms of mental health disorders. Lion's Mane mushroom, renowned for cognitive enhancement attributed to hericenones and erinacines stimulating the nerve growth factors (NGF), has emerged as a subject of significant interest. Recent studies suggest its potential antidepressant and anxiolytic properties, broadening its relevance to various mental health challenges. Attention-deficit/hyperactivity disorder (ADHD), characterized by executive functioning difficulties, notably in attention and memory, is often linked to working memory deficits. Numerous investigations indicate that a substantial proportion of individuals with ADHD exhibit impaired working memory. Working memory, responsible for temporarily holding and processing information, plays a pivotal role in encoding memories for long-term storage or extinction. This paper suggests that Lion's Mane may offer therapeutic benefits by enhancing working memory, thereby positively influencing the day-to-day executive functioning of individuals grappling with ADHD. This will explore the potential benefits of utilizing Lion's Mane with a specific focus on working memory, using a spontaneously hypertensive rat (SHR) model, which presents ADHD-like symptoms. The potential implications of such findings underscore the promising role of Lion's Mane in addressing cognitive challenges associated with ADHD.

Keywords: lion's mane; attention-deficit/hyperactivity disorder; radial arm maze; spontaneously hypertensive rats; working memory; executive functioning

Introduction

Lion's Mane mushroom (*Hericium erinaceus*) is renowned for its cognitive-enhancing properties, being extensively researched in the realm of medicinal mushrooms. Studies target its effects on cognitive function, memory, learning, and brain health, with a focus on neurogenesis and neuroplasticity. Preliminary research indicates potential applications in mental health challenges, including antidepressant and anxiolytic properties. Attention-deficit/hyperactivity disorder (ADHD) is a mental health disorder characterized by symptoms like inattention, hyperactivity, and impulsivity, posing challenges in professional, academic, and daily pursuits [1]. ADHD manifests in three main types: predominantly inattentive, predominantly hyperactive/impulsive, and combined presentations [2]. Inattentiveness involves difficulties in task focus, attention to detail, and organizational challenges, notably in working memory [3]. Hyperactivity entails excessive energy, talkativeness, and an inability to stay still [4]. ADHD is associated with altered brain structures and activation during tasks [5]. Non-genetic factors include premature birth,

low birth weight, prenatal exposure to alcohol or tobacco, and extreme maternal stress [6].

The active compounds in Lion's Mane, hericenones and erinacines, stimulate nerve growth factor (NGF), promoting neuronal survival and growth to sustain healthy cognitive function [7]. NGF plays a role in reducing chronic inflammation, impacting focus and concentration, while also increasing acetylcholine levels, a neurotransmitter crucial for learning and memory [8]. Lion's Mane has demonstrated stress-reducing effects, enhancing cognitive functioning. Individuals with ADHD face challenges in executive functioning, encompassing planning for consequences and short/long-term actions [9]. Daily executive functioning challenges include distractibility, forgetfulness, organizational difficulties, lateness, frequent losses, and motivation deficits [10]. This study investigates Lion's Mane's therapeutic potential in enhancing working memory in ADHD, focusing on addressing the pronounced impairment in working memory compared to other memory deficits [11].

Spontaneously hypertensive rats (SHR) are typically used as a model for studying ADHD due to their hyperactive

and impulsive behaviors, which resemble the symptoms of ADHD in humans [12]. This model is relevant for evaluating the effects of Lion's Mane on ADHD because hericenones and erinacines have shown potential neuroprotective and neurotrophic properties that may improve cognitive function and behavior.

The research question focuses on whether Lion's Mane could have therapeutic effects on improving working memory functioning in attention-deficit/hyperactivity disorders. The hypothesis is that both male and female mice given the Lion's Mane will have greater concentration and focus on behavioral tasks, specifically, the radial arm task. If the group that is exposed to the Lion's Mane treatment improves managing the symptoms of ADHD, then Lion's Mane can be considered as a potential long-term treatment.

The expected results are that the Lion's Mane male and female groups will demonstrate an improvement in working memory, allowing them to perform better on the radial arm maze task.

Furthermore, this research addresses a significant knowledge gap in the literature regarding the impact of medicinal mushrooms on cognitive disorders like ADHD. By exploring the effects of Lion's Mane on working memory in a rodent model, this study could help support novel natural therapeutic strategies. The implications of such findings could extend to developing more holistic and personalized approaches to managing ADHD, potentially benefiting a broad spectrum of individuals struggling with this disorder.

Methods

One hundred twenty-eight spontaneously hypertensive rats (SHR), consisting of sixty four males and sixty-four females, aged eight weeks, will be selected for this study. This study uses a four-group, between-subjects and longitudinal design. The group is first split into males and females to account for gender differences. Then, each group is divided into a group of either receiving the Lion's Mane treatment in their diet or receiving a regular diet. The dependent variable is the improvement in symptoms related to ADHD measured by an automated video tracking system with a radial arm test. This quantitative measure demonstrates the percentage of mice exhibiting reduced ADHD symptoms throughout this longitudinal study. The SHR strain is valuable for studying ADHD symptoms due to its traits of hyperactivity, impulsivity, and deficits in sustained attention. The animals will be housed under standard conditions with a 12-hour light-dark cycle at a temperature of 22°C, with ad libitum access to food and water. Bedding and enrichment materials will be provided to ensure animal welfare. The hundred and twenty-eight mice will be divided into two main groups based on sex. Each sex group will be randomly assigned into two subgroups for the independent variable treatment. This separation is done to account for the sex differences between the groups. The Lion's Mane experimental group will receive a daily dose of one mg of Lion's Mane powder supplement from Host

Defense Mushrooms (Host Defense Mushrooms, Fungi Perfecti, LLC., Olympia, WA, USA) mixed into their wet pellet food. The control group will receive wet pellet food without the Lion's Mane supplement [13]. The treatment will be administered for two months. Weekly weight measurements will be taken to monitor any significant variations due to dietary differences.

Prior to the experimental trials, all rodents will undergo a one-week habituation period to the radial arm maze environment. During this period, they will be allowed to freely explore the maze without food rewards to familiarize themselves with the layout and reduce potential stress associated with novel environments. This step ensures that subsequent performance reflects executive functioning rather than maze unfamiliarity. Any rodents displaying signs of stress or illness during habituation will be excluded from the study to maintain data integrity. The radial arm maze will be employed to assess executive function, particularly spatial working memory and cognitive flexibility [14]. The maze consists of twelve arms radiating from a central point, with food rewards placed in four of the arms. A pre-trial initial radial arm maze test will be conducted to establish baseline performance. Then, a four-week treatment period with daily Lion's Mane administration and weekly weight monitoring will take place. Radial arm maze testing at the end of the four-week period. Lastly, an eight-week treatment period with continued Lion's Mane administration will take place with a final radial arm maze testing at the end.

For the radial arm maze procedure, the rodents will be placed in the center of the maze, and the time taken to find the arms containing the food rewards will be recorded [15]. The number of times an animal revisits an arm that was previously baited is recorded as a working memory error because the status of the arm changed from baited to not baited [14]. Each rodent will undergo the radial arm procedure to evaluate the effect of Lion's Mane during three time periods, at beginning, at four weeks, and at 12 weeks. An automated video tracking system will be used to collect qualitative data on behavioral symptoms of ADHD, including the number of errors and time taken to complete the maze. The time it takes to see results from medicinal mushrooms varies, but some studies have indicated noticeable changes in four weeks [16].

Behavioural data will be collected using the automated video tracking system. Performance metrics include the time taken to find the food rewards and the number of working memory errors [14]. Data will be recorded at three points: pre-trial baseline, four weeks, and eight weeks following initial administration. An Analysis of Variance (ANOVA) will be performed to compare the means of both groups (male and female, treated and control) at different time points. A Pearson Correlation Coefficient test will be used to evaluate the association between Lion's Mane treatment and ADHD symptoms, specifically focusing on working memory improvement.

This study will obtain consent and adhere to the animal care expectations set by the Institutional Animal Care and Use Committee (IACUC). All procedures involving animals

will comply with ethical guidelines to ensure their welfare throughout the study.

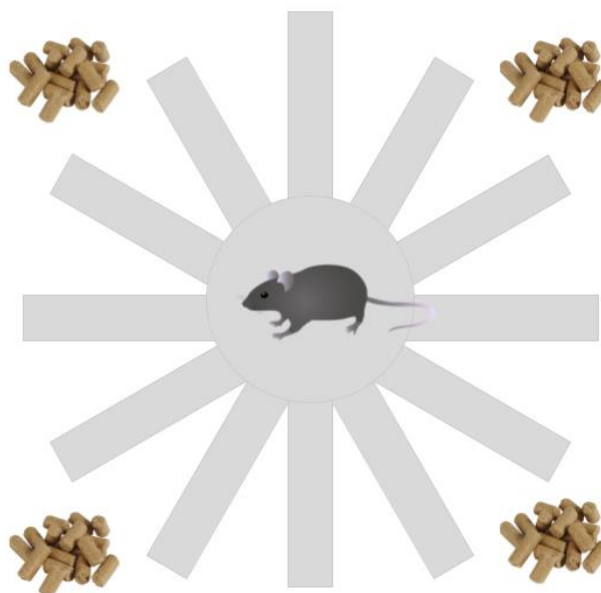


Figure 1. Twelve arm radial maze. The above image illustrates the radial arm maze test utilized in rodent studies. The food represents visual cues in the surrounding environment. Created using [BioRender.com](https://www.biorender.com).

Results

The study is completed in a specific time frame. First, permission must be acquired to use one hundred twenty-eight SHR mice from the IACUC, which will be sorted into the four groups randomly post-approval. After approval, habitation of the mice to the radial arm maze must take place in addition to completing the first radial arm maze test to gain a baseline. The experimental male and female SHR will receive a four-week treatment of one mg of Lion's Mane powder supplement dosages in their wet pellet food daily. After this initial treatment period, the second radial arm maze test will be conducted to measure any immediate effects on working memory.

The treatment will then continue for an additional eight weeks, maintaining the same dosage. Throughout the study, the radial arm maze test will be utilized to assess working memory performance at multiple points. Each utilization of the radial arm maze includes the use of an automated video tracking system to collect qualitative data regarding the behavioral symptoms of ADHD, such as hyperactivity, impulsivity, and inattention. The data obtained from these tests will be analyzed using an ANOVA to compare the means between the control and experimental groups.

The behavioral symptoms of ADHD, including the number of correct and incorrect arm choices, time spent exploring, and latency to make decisions, will be carefully recorded and analyzed. Changes in these metrics will provide insights into the effects of Lion's Mane on working memory

and ADHD symptoms. Simultaneously, a Pearson Correlation Coefficient test will be conducted to evaluate the association between Lion's Mane treatment and the observed behavioral changes. This analysis will help determine the strength and direction of the relationship between Lion's Mane supplementation and improvements in working memory.

Preliminary results are expected to show that the experimental groups receiving Lion's Mane supplementation will demonstrate significant improvements in working memory performance compared to the control groups. Specifically, it is predicted that the Lion's Mane groups will make fewer errors in the radial arm maze and exhibit reduced ADHD-like behaviours such as issues with working memory which impacts impulsivity and hyperactivity. By comparing the pre- and post-treatment data, this study aims to investigate the potential of Lion's Mane as a therapeutic intervention for ADHD, particularly in enhancing working memory. The findings could provide valuable insights into the neuroprotective and cognitive-enhancing effects of Lion's Mane, contributing to the current evidence supporting its use in mental health and cognitive disorders.

Discussion

NGF is a neurotrophin, meaning it has a significant role in aiding specific neuron populations survive and helps promote growth and axon branching [17]. NGF plays a crucial role in the growth, differentiation, survival, and

plasticity of cholinergic neurons found in areas like the septum, striatum, and nucleus basalis of Meynert [18]. Dysfunction in NGF metabolism is thought to contribute to Alzheimer's disease by leading to the degradation of NGF, which is essential for the proper functioning of cholinergic neurons [18]. This disruption affects neurotrophin-related signaling pathways, contributing to cognitive decline, and is evidenced by reduced NGF levels in the brains of Alzheimer's patients, even in those without dementia symptoms but with amyloid plaques [18]. Hericenones and erinacines are low-molecular-weight compounds that can interact at the molecular level by regulating cytokines, protein kinases, and transcription factors, and effectively cross the blood-brain barrier [18]. They have demonstrated neuroprotective and neurotrophic effects in both in vitro and in vivo studies involving animal models of peripheral nerve injury, stroke, and Alzheimer's disease [18]. Hericenones and erinacines reduce neuroinflammation and provide antioxidant protection against oxidative stress, which supports neurogenesis and synaptic plasticity, which are crucial for cognitive functions. These compounds act on the hippocampus to improve memory and the cerebral cortex which is associated with executive function [18]. There is preliminary evidence in human studies of potential cognitive and mood benefits of Lion's Mane, but more research is needed to fully understand their efficacy and potential risk factors.

One limitation of this study is that the SHR model does not fully encompass the complexities of the different types of ADHD, as they exhibit all of the behavioral symptoms and are more reflective of the combined type. It is also important to acknowledge the limited translatability of rodent experiments to humans. Clinical trials must be completed to verify the findings. Another limitation is the lack of extended follow-up periods to evaluate the long-term potential side effects of Lion's Mane. Furthermore, due to the cyclical hormone release in female rodents, behavioral changes may be more variable in females, affecting the consistency of Lion's Mane's impact on working memory. Future research could include conducting vaginal lavages to determine the estrous state of female rats, assessing whether the effects of Lion's Mane vary with hormone fluctuations.

Conclusions

In summary, this study highlights Lion's Mane's potential in ameliorating ADHD-related working memory deficits, suggesting a promising supportive treatment and contributing to medicinal mushrooms' evolving role in addressing cognitive challenges. The results indicate that Lion's Mane treatment could be utilized in managing the symptoms of ADHD and be considered a potential long-term treatment. This study is significant because it addresses a prevalent neurodevelopmental disorder using a holistic and personalized approach that can be tailored to individual needs.

Future research should validate these findings and explore long-term effects in clinical trials, utilizing neuroimaging techniques for deeper insights into associated neural changes. Another line of investigation could evaluate how different mushrooms, such as the reishi mushroom, might improve executive functioning. Additionally, future research could explore how Lion's Mane mushroom affects males and females differently, to determine if it could benefit different facets of executive functioning for each sex.

List of Abbreviations Used

ADHD: Attention-deficit/hyperactivity disorder
IACUC: Institutional animal care and use committee
NGF: Nerve growth factor
SHR: Spontaneously hypertensive rats

Conflicts of Interest

All author(s) declare that they have no conflicts of interest.

Ethics Approval and/or Participant Consent

Our manuscript did not require ethics approval because it was a research proposal. The proposed procedure would require approval from the Institutional Animal Care and Use Committee and the Research Ethics Board.

Authors' Contributions

JVP: Made contributions to research, drafted the methods and results, completed citations, revised the manuscript critically and gave final approval of the version of the manuscript to be published.

LK: Made contributions to research, contributed to drafting the discussion and methods, drafted conclusions, completed citations, revised the manuscript critically and gave final approval of the version of the manuscript to be published.

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