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REGULAR DECISION

Dear Admissions Committee:

I have enclosed my abstract and scientific research paper *Promoting Myelination as a Strategy to Reverse Depression-like Behavior in Socially Isolated Mice*. I won the fourth place award in the Cellular and Molecular Biology category of the Westchester Science and Engineering Fair (WESEF) in the spring of 2016 with my project and have recently submitted my paper and abstract to the following competitions: Regeneron Science Talent Search (STS) and Junior Science and Humanities Symposia (JSHS). For JSHS, I was selected as one of 30 students out of a total of over 300 research paper submissions to be a PowerPoint Presenter. As a Presenter (in the Neurobiology category), I have the opportunity to advance to the state level competition of JSHS in Albany based on my performance in this regional competition.
Kindly include my research paper and abstract with my file. Thank you.

Sincerely,
Komal Keerthy

Briarcliff High School

Promoting Myelination as a Strategy to Reverse Depression-like Behavior in Socially Isolated Mice

ABSTRACT

Objectives: Depression affects 350 million people globally every year. The current treatment for depression is antidepressant medication but, only 30-50% of this medication is effective. We sought to explore a new method to reverse depression by promoting myelination of axons in the prefrontal cortex (PFC). This was achieved by administering the antihistamine drug, clemastine, in a mouse model.

Methods: In this experiment, the adult male mice were placed in three different groups. Two of the groups were isolated for eight weeks to induce depression, and then either treated with vehicle (placebo) or clemastine for the last two weeks. The third, the control group, was group-housed for all 10 weeks. A social interaction test, which used a video-tracking device to measure social avoidance and interaction behavior of each test subject, was then performed on all groups, after which the mice were sacrificed. RNA extraction and analysis was done, as well as electron microscopy, to mount the PFC regions of the mice onto slides for further analysis. Immunohistochemistry was then performed. The slides were stained with primary antibodies, CC1 (marker for differentiated oligodendrocytes, myelin-producing neural cells), MBP (marker for myelin), and NG2 (marker for oligodendrocyte progenitor cells).

Results: Results of the social interaction test showed that clemastine was found to reverse social withdrawal behavior in adult male mice. Also, increased myelination and oligodendrocyte differentiation was detected in the clemastine treated group.

Conclusions: Enhanced myelination and OPC differentiation are beneficial for reversing depressive-like behavior in socially isolated adult mice. Clemastine successfully enhanced myelination, OPC differentiation, and reversed social avoidance behavior in the socially isolated mice. Through staining of secondary antibodies, we were able to deduce that the prevalence of OPCs among the groups was not correlated to the reversal of depression in the mice, but that the differentiation of OPCs is what contributed to the phenomenon. This research confirms a possible method of treatment for socially-isolated or depressed adult male mice; therefore, moving forward, this research may be applicable to the adult brain. This suggests that promoting myelination is a potential strategy to reverse depression.

Promoting Myelination as a Strategy to Reverse Depression-like Behavior in Socially Isolated Mice

REVIEW OF LITERATURE

Depression is a common illness worldwide, with an estimated 350 million people affected. Depression is different from usual mood fluctuations and short-lived emotional responses to challenges in everyday life (Sanchez et al., 1998). Especially when long-lasting and with moderate or severe intensity, depression may become a serious health condition. It can cause the affected person to suffer greatly and function poorly at work, at school and in the family. At its worst, depression can lead to suicide. Over 800 000 people die due to suicide every year. Suicide is the second leading cause of death in 15-29-year-olds (Teicher et al., 2004; Mehta et al., 2012). Although there are known, effective treatments for depression, fewer than half of those affected in the world (in many countries, fewer than 10%) receive such treatments (Sanchez et al., Makinodan et al., 2012). Barriers to effective care include a lack of resources, lack of trained health care providers, and social stigma associated with mental disorders. Another barrier to effective care is inaccurate assessment. (Gibson et al., 2014). In countries of all income levels, people who are depressed are often not correctly diagnosed, and others who do not have the disorder are too often misdiagnosed and prescribed antidepressants (Pigott et al., 2010; Gibson et al., 2014).

It is known that white matter deficits are associated with depression (Covington et., al 2005). White matter consists of enriched glia cells such as oligodendrocytes (Sanchez et al., 1998). Oligodendrocytes generate myelin, the insulating membrane that covers many neuronal axons and facilitates the propagation of electrical signals along neuronal circuits. Although oligodendrocytes and the myelin they create were assumed to be static cites, recent studies indicate that myelin is much more malleable than once thought (Covington et al., 2005; Liu et al, 2012). Imaging studies have shown that various forms of learning correlate with structural changes in the human brain's white matter, and animal studies have demonstrated oligodendrocyte and myelin changes in response to social and environmental conditions (Liu et., al 2012). These observations raise the possibility that myelin is highly dynamic and that changes in myelin are important components of brain plasticity (De Angelis et., 2012; McKenzie et al., 2014). According to fMRI imaging in depression patients, white matter structure and a lower count of oligodendrocytes in human brains was observed (De Angelis et., 2012). Studies have also shown a high co-mobility or co-occurrence between depression and multiple sclerosis (Liu et., al 2012).

Juvenile social isolation and neglect influence adult cognitive function and social interactions (Covington et al., 2010). Studies of children raised in institutions where neglect was rampant showed that deprivation also correlates with the medial preforontal cortex (mPFC), which are not reversed by subsequent foster care placement (Covington et al., 2010; Makinodan et., al 2012). Similarly, rhesus monkeys isolated as juveniles, have white matter disturbances and impaired working memory in adulthood, suggesting that juvenile social experience and forebrain white matter development are linked (Makinodan et., al 2012). Studies indicate that experience and neuronal activity influence central nervous system (CNS) myelin. However, it remains unclear whether any effect of social experience on oligodendrocytes is important for the establishment of normal adult neuronal circuits and their function, what aspects of oligodendrocyte development are regulated by social experience, and which molecular mechanisms underlie these events (McKenzie et., al 2014).

It is known that there are myelination defects associated with depression (Liu et al., 2012; Liu et al., 2015) but the goal of this study was to see whether or not enhancing myelination would prove to be beneficial, in other words, reverse depressive-like symptoms, in mice that express depressive-like behavior. In order to test this, we socially isolated adult male mice to mimic depression over the course of 10 weeks and looked for increased levels of NG2 cells (markers for oligodendrocyte progenitor cells/OPCs) and MBP (Myelin Basic Protein: markers for myelin). Clemastine, a widely available first-generation antihistamine with a favorable safety profile for use, is used primarily for symptomatic treatment of allergies, and also exhibits antimuscarinic properties. Previous studies show that it realty enhanced oligodendrocyte differentiation and wrapping of micropillars (Deshmukh et., al 2013). Clemastine also showed to be most effective in enhancing differentiation and myelination of oligodendrocytes (Deshmukh et., al 2013). Therefore, in this study, clemastine was used to promote myelination

in one of the three groups of adult male mice of the experiment.

RESEARCH QUESTION

Will promoting myelination reverse depressive-like symptoms in socially isolated mice?

HYPOTHESIS

H1. Inducing myelination will promote the reversal of depressive-like symptoms in the PFC of socially isolated mice.

H0. Inducing myelination will not promote the reversal of depressive-like symptoms in the PFC of socially isolated mice.

METHODS AND MATERIALS

In this study, our research models were adult male mice. A total of 16 mice were divided into three separate groups. Group A, the control, consisted of five adult male mice, labeled A6-10. A6-A10 were group-housed, so no depressive-like symptoms were induced. Group B consisted of six mice, labeled B7-12, were socially isolated for eight weeks. After this isolation period, they were gavaged with vehicle (ddH2O) for two weeks. Group C consisted of six mice as well, labeled C7-12. They were socially isolated for eight weeks and then gavaged with clemastine for two weeks following isolation.

A social interaction test that entailed a video tracking device was performed on all subjects after week 8 and week 10 of the experiment.

All sixteen mice were sacrificed after this 10 week time frame. My part in this project started at this point in the experiment. RNA was extracted from all subjects to test for expression of several myelin genes. The cryostat was utilized to slice the brain into several sections. The sliced PFC brain regions were mounted onto slides and were frozen to proceed lab work. All slides were stained with DAPI (4',6-diamidino-2-phenylindole), a blue fluorescent stain, to locate the nuclei in the PFCs. NG2, MBP, and CC1 antibodies were pipetted onto all of the slides to locate the presence of oligodendrocyte progenitor cells (OPCs), myelin, and differentiated oligodendrocytes, respectively. Green fluorescent secondary antibody (MBP) and red fluorescent secondary antibody (NG2) were pipetted onto all the slides to clearly visualize the distinction between the NG2 cells and MBP. CC1 was also pipetted onto all the slides. The slides were refrigerated overnight. Images were scanned to the confocal microscope for immunohistochemistry (using the same resolution) to be further quantified.

RESULTS

Fig. 1 Time Spent in Interaction zone for Group Housed, Vehicle-Treated, and Clemastine treated mice

Results of the social interaction test showed that there were no differences detected in locomotion among all groups. The isolated group treated with vehicle (iso+veh) showed signs of social withdrawal with reduced interaction time with a conspecific mouse compared to the group-housed mice. The isolated group treated with clemastine (iso+clem), however, was indistinguishable from the group-housed controls, indicating that clemastine was sufficient to reverse social withdrawal behavior in socially isolated mice.

Prevalance of Myelin Genes between Iso+veh and Iso+clem

Prevalance of Myelin Genes between Iso+veh and Iso+clem

Visual representation of Myelin among group housed, iso+veh, and iso+clem
Visual representation of Myelin among group housed, iso+veh, and iso+clem

Fig. 4 Comparison of g-ratios among the three groups

Fig 5. Visual representation of OPCs among the groups

Prevalence of OPCs among the three groups
Prevalence of OPCs among the three groups
Visual representation of OPCs among the groups
Visual representation of OPCs among the groups

Fig. 7 Visual representation of differentiated OPCs among the groups

Visual representation of differentiated OPCs among the groups
Visual representation of differentiated OPCs among the groups

Prevalence of differentiated OPCs among the three groups
Prevalence of differentiated OPCs among the three groups

The graphs that tested for myelin using MBP marker showed significant green fluorescence in the group-housed mice, significantly less green fluorescence in the iso+veh group, and significant expression of green fluorescence in the mice that were administered clemastine. To further validate myelin expression within the groups, several myelin genes such as *Mog, Mag, Mbp, Mobp,* and *Plp* were tested for. The RNA extracted from the mice after sacrifice were prepped using the RNeasy kit and was amplified using PCR (polymerase chain reaction). After doing qPCR and analyzing the expression of the genes using a statistical analysis program, all data was synthesized into the first graph below. There was a trend in the qPCR results but no statistical significance level. However, the g-ratio was measured by dividing the diameter of the axon by the diameter of the entire myelinated fiber (therefore, the lower the g-ratio value, the thicker the myelin is around the axon and vice versa) The g-ratio value for the iso+veh group was significantly less compared to the iso+clem. Iso+ clem group and the group-housed mice shared about the same g-ratio value.
To quantify the OPCs in the images, nuclei encircled by myelin (indicated by increased concentration of red fibers surrounded a blue dot) were counted. This visual indicated the presence of an OPC because according to Liu et. al, when mice are isolated, their chromatin structures change and resemble the stage of an OPC. According to the last image, the greatest expression of OPCs was in the mice that were administered with vehicle. However, a statistical significance was not reached to conclude that the prevalence of OPC's among the groups was what caused the reversal of depression. Thus, the PFC slides were stained with CC1 as well. Results showed that there was a significant increase in the amount of differentiated oligodendrocytes in the iso+clem group compared to the iso+veh group, indicating that the increased count of oligodendrocytes was what was responsible for rescuing the depression in the socially-isolated mice.

DISCUSSION AND CONCLUSION

The novel finding of this research is that enhanced myelination and OPC differentiation are beneficial for reversing depressive-like behavior in socially isolated adult mice was found as a result of this experiment. Clemastine successfully enhanced myelination, OPC differentiation, sufficiently rescued social avoidance behavior in the socially isolated mice. Through staining of secondary antibodies, MBP, NG2, and CC1, we were able to deduce that the prevalence of OPCs among the groups was not correlated to the reversal of depression in the mice, but that the differentiation of OPCs is what contributed to the phenomenon.

However, before fully accepting these results, it is important to consider the limitations. Seventeen adult male mice were used in this research. Thus, results could show variation if female or juvenile mice are included in the study. Greater sample size of test subjects would also contribute to more dependable results.

This research confirms a possible method of treatment for socially-isolated or depressed adult male mice; therefore, moving forward, this research could be translated to the adult brain. Through imaging studies and further screenings of new depression specific medication, it will be possible to use this knowledge of clemastine's mechanisms to find a potential medication, and thus, a potential cure for depression.

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