Therapeutic Mitochondrial Delivery to Astrocytes for Ischemic Stroke

(Technical Paper)

The Effect of Environmental Factors on the Development of Major Depression and Schizophrenia

(STS Paper)

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On my honor as a University Student, I have neither given nor received unauthorized aid on this assignment as defined by the Honor Guidelines for Thesis-Related Assignments

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Introduction

In the United States alone, an estimate of 1.2 million people aged 18 or older are diagnosed with injury characterized, adult-onset brain disorders each year (Pal, n.d.). In addition, 11.2 million adults in the US aged 18 or older per year are diagnosed with a severe mental illness per year (National Institute of Mental Health, 2019b). Therefore, approximately 12.4 million new patients are affected by neurological or mental disorders each year.

Of the 1.2 million people affected by injury characterized brain disease, 51.3% are due to a stroke (Pal, n.d.). Worldwide, stroke is one of the leading causes of functional inability and death (Ramos-Lima, Brasileiro, de Lima, & Braga-Neto, 2018). These patients are susceptible to several post-stroke complications, such as serious falls, infections, blackouts, pains, and symptoms of depression and anxiety, that lead to an associated lower quality of life for them and their families (Langhorne et al., 2000). The high rates of complications and low quality of life following a stoke are due in part to the fact that current treatments of stroke do not address circulating dysfunctional mitochondria. Therefore, an improved therapeutic method is needed to treat ischemic stroke, specifically in regards to treating dysfunctional mitochondria. Consequently, the technical topic of this thesis is to design a new therapeutic method to treat ischemic stroke through the transplantation of exogenous mitochondria from healthy tissue to astrocytes.

Two commonly known mental illnesses are depression and schizophrenia. Therefore, the STS topic of this thesis is an investigation of the environmental factors that affect the development of major depression and schizophrenia and an analysis of the paradigm shift of the medical community in regards to diagnosis and treatment of these disorders. Major depression is the leading causes of disability for people aged 15-44 years in the US, and schizophrenia is one

of the top 25 leading causes of disability worldwide (Chong et al., 2016; Greenberg, Fournier, Sisitsky, Pike, & Kessler, 2015). The factors that lead to the development of these disorders have been researched and debated in regards to environmental, genetic, and biological risk factors. The conceptions on how environmental factors, such as socioeconomic status, affect the development of mental illness in turn affect the diagnosis and treatment of the disorders (Einstein & Klepacz, 2017).

Technical Topic

Stroke is the leading cause of physical and intellectual disability and has a high rate of mortality in the US (Yang, Mukda, & Chen, 2018). Approximately 795,000 people per year, in the US, have a new or recurrent stroke, and of this population 87% are classified as ischemic stroke caused by a blood clot lodged in an artery supplying blood to the brain (Roger et al., 2011).

The current standard of treatment for ischemic stroke primarily focuses on restoring blood flow to the brain by removing the blockage (Ciccone & Valvassori, 2013). However, with the current standard of treatment, more than half of the patients either die or do not fully recover (Bhatia et al., 2010). The lack of full recovery is because the current treatments do not address the mitochondria that become dysfunctional after an ischemic stroke.

In healthy brain tissue, astrocytes, glial cells of the central nervous system, transfer mitochondria particles to the neurons via the calcium-dependent mechanisms of CD38 and the cyclin adenosine diphosphate (ADP) ribose signaling (Hayakawa et al., 2016; Robinson et al., 2017). The transfer of mitochondria particles is to allow for cell-to-cell communication between astrocytes and neurons. As a result, ATP production and viability of neurons is additionally increased (Hayakawa et al., 2016). However, after ischemic stroke, the astrocyte mitochondria

become dysfunctional due to oxygen and glucose deprivation (Liu, Lu, Manaenko, Tang, & Hu, 2018). These dysfunctional mitochondria have abnormalities in mitochondrial membrane potential, produce increased amounts of reactive oxygen species (ROS), and have decreased adenosine triphosphate (ATP) production (Liu et al., 2018). When the dysfunctional mitochondria are circulated between astrocytes and neurons, it continues the ischemic cascade even after blood flow has been restored.

The transplantation of exogenous mitochondria from healthy tissue can help to reverse the effects of an ischemia-reperfusion injury, such as stroke, according to previous studies. In one clinical study of pediatric patients with myocardial ischemia-reperfusion injury following coronary artery occlusion and revascularization, transplantation of healthy mitochondria improved myocardial function within 24 to 48 hours after treatment (Emani & McCully, 2018). Another previous study has demonstrated that exogenous mitochondria injected systemically to a mouse with middle cerebral artery occlusion (MCAO), a model of ischemic stroke, can be taken up by neurons, astrocytes, and microglia (Liu et al., 2018). This treatment resulted in the upregulation of cell survival related signals in the MCAO mice, and postulated that astrocytes have the ability to transfer healthy mitochondria (if they possess them) to rescue damaged neurons after stroke due to their cell-to-cell communication.

However, none of the previous studies tested mitochondria from different sources. In all previous studies, mitochondria have been harvested from skeletal muscle sources. Yet, mitochondria can also come from different tissues such as adipose and cardiac muscle. Peridroplet mitochondria from adipose tissue have enhanced bioenergetic and low fatty acid oxidation capacity. Their fusion-fission properties also differentiate them from cytoplasmic mitochondria (Benador et al., 2018). Myocardium mitochondria have a larger reliance on aerobic

ATP when compared to skeletal muscle mitochondria (Miller, Rosenfeldt, Zhang, Linnane, & Nagley, 2003). Additionally, in a study completed by Song-Young Park, it was concluded that cardiac and skeletal muscle mitochondria have similar oxidative phosphorylation capacities but vary in terms of respiratory control rate and nonphosphorylating respiration (Park et al., 2014).

Therefore, our team, under the guidance of Richard Price and Catherine Gorick, will develop a method to obtain optimal uptake of, and ATP production by, exogenous mitochondria in human astrocytes to provide a framework for a new therapeutic method to treat ischemic stroke. This technical project will utilize experimental resources of Price lab, such as mice, cell culture materials, and reagents, and information gathered via literature review. In order to accomplish this goal, we will first extract mitochondria from the various tissue sources mentioned above. Then, we will measure the ATP production of each type of mitochondria alone and after being taken up by cultured astrocytes. Additionally, we will measure the amount of mitochondria taken up by cultured astrocytes by methods of fluorescence. The experimentation specified will be conducted throughout the 2019-2020 school year.

STS Topic

Major depression and schizophrenia are both severely debilitating mental illnesses that are associated with a poor quality of life. Major depression is among the most prevalent of psychiatric disorders, and symptoms can include problems with sleep, eating, energy, concentration, or self-worth (National Institute of Mental Health, 2019a; Schmitt, Malchow, Hasan, & Falkai, 2014). Patients with severe depression are associated with numerous adverse outcomes such as difficulties in role transitions, reduced role functioning, elevated risk of secondary disorders, and increased risk of early mortality due to physical disorders or suicide (Kessler & Bromet, 2013). Schizophrenia has a prevalence of about 1% and is characterized by

symptoms such as hallucinations, delusions, and asociality (Schmitt et al., 2014). Patients of schizophrenia are commonly homeless, victims of assaults or suicides, or end up in jail (Cernovsky, 2017). The prevalence and low quality of life of these debilitating disorders indicate the need for effective diagnosis and treatment procedures to address these illnesses.

Within the medical community, mental illness has historically been regarded solely as an imbalance of neurotransmitters, but in recent years several studies have demonstrated that the development major depression and schizophrenia can and are affected by environmental factors in addition to neurotransmitter imbalance (Einstein & Klepacz, 2017). For instance, those of low socioeconomic status are particularly considered to be at risk of developing these disorders (Saraceno, Levav, & Kohn, 2005). In connection to those of low socioeconomic status, a higher prevalence of the mental disorders have been found in certain racial minorities due to the fact that these minorities are typically also of low socioeconomic status (Chakraborty & McKenzie, 2002; Kwate & Goodman, 2015). Childhood and family factors are also potential factors to insight development of these disorders (Drake & Ginsburg, 2012). For example, studies have indicated that both childhood trauma and high rates of expressed emotion from family members are connected to the development of both depression and schizophrenia (Asarnow, Tompson, Hamilton, Goldstein, & Guthrie, 1994; Morgan & Fisher, 2007). The evidence presented indicates the connection between environmental risk factors and the development of depression and schizophrenia calls for a response from the medical community.

There has been a recent push in the medical field for mental disorders to be evaluated with consideration to the environmental factors that lead to their development in addition to the associated neurotransmitter imbalance and biology of each disorder (Einstein & Klepacz, 2017). This perspective is as a result of increased research indicating the role of environmental factors

and the general public belief by those with mental illnesses that environmental factors played a role in the development of their diseases (Jorm et al., n.d.). The evolution of perceiving mental illness as purely biological and genetic, to also considering environmental factors is best described as a paradigm shift. This paradigm shift is important to society because **when the general public and the medical community are unaware and uneducated on the environmental risk factors associated with the development of major depression and schizophrenia, more individuals are at risk of developing these disorders and diagnosis and treatment procedures are not as effective.**

According to Thomas Kuhn, an American philosopher, a paradigm shift occurs when the experts in a certain field can no longer describe scientific advances and new information with their current viewpoints, so communities with new ideas and viewpoints take over to describe the advances in the field (Ridgway, Baker, Woods, & Lawrence, 2019). Historically, the definition of paradigm has been controversial (Orman, 2016). Additionally, the theory of paradigm shift has been critiqued an "oversimplified" explanation and that it therefore cannot be applied to all scientific revolutions (Adams, n.d.). In response, Kuhn has revised his definition of paradigm over the years to address criticisms associated with the theory (Orman, 2016). As a result, in the current case of a paradigm shift toward the medical view of the factors that lead to the development of mental illness, this theory is applicable despite the historical critiques.

This STS research topic will increase awareness of the role environmental factors play in the deterioration of mental health. Several of the environmental risk factors associated with the development of depression and schizophrenia could be mitigated or prevented with increased awareness and education among the general public on this problem (Schmidt, 2007). In addition, increased research on the environmental risk factors and the paradigm shift in the medical

community could result in better treatment for individuals with major depression and schizophrenia as a result of more members of the medical community adopting the paradigm shift. Therefore, synthesized research on this topic could help lead to the prevention and improved treatment and diagnosis of both schizophrenia and major depression.

Research Question and Methods

The research question to be investigated is **"How do environmental factors affect the development of major depression and schizophrenia?"** In order to examine this question, documentary research methods will be used. Google Scholar will be used as a comprehensive database for the literature search due to its large range of access. Additionally, PubMed will be used as a more detailed database to supplement the literature search because of its relevance of literature in the public health field. Keywords used to search these databases will include development of major depression and *, development of schizophrenia and *, environmental factors and development of mental illness, and public or medical views of the development of schizophrenia and major depression. The * indicates a specific environmental factor that is searched such as childhood trauma, socioeconomic status, or prenatal nutrition. Additionally, some further variants of these keywords scheme will be used to obtain a larger pool of literature. The documentary research began in Fall 2019, and the STS thesis will be written in the Spring 2020 semester.

The method of documentary research is most applicable to investigate the research question for several reasons. First, documentary research provides the opportunity for the synthesis of several types of research methods for this topic (i.e. survey, interview, literature review). Second, a large and diverse population of individuals with major depression and/or

schizophrenia is not readily available to survey. Therefore, documentary research provides both a more feasible and unbiased option.

Conclusion

Stroke, major depression, and schizophrenia are all serious disorders that affect a large number of the population worldwide. To address the detrimental post-stroke complications, a therapeutic method will be developed by determining the mitochondria with the most ATP production and uptake potential by astrocytes. This project will provide the beginning *in vitro* framework for the therapy to later be developed for *in vivo* models and ultimately human use. To address the debate among the medical community over the environmental factors that lead to the development of major depression and schizophrenia, the STS research paper will provide a synthesized analysis of the environmental factors that are postulated to lead to the development of these disorders. Furthermore, the paradigm shift of the medical community and public beliefs towards environmental factors role in the development of mental illness will be analyzed and outlined. The produced research will provide awareness for better prevention, diagnosis, and treatment of both major depression and schizophrenia.

References

Adams, A. J. (n.d.). Objections to Kuhn's theory of scientific progression - Hektoen International. Retrieved October 21, 2019, from https://hekint.org/2017/01/22/objections-to-kuhns-theory-of-scientific-progression/

Benador, I. Y., Veliova, M., Mahdaviani, K., Petcherski, A., Wikstrom, J. D., Assali, E. A., … Shirihai, O. S. (2018). Mitochondria Bound to Lipid Droplets Have Unique Bioenergetics, Composition, and Dynamics that Support Lipid Droplet Expansion. *Cell Metabolism*, *27*(4), 869-885.e6. https://doi.org/10.1016/J.CMET.2018.03.003

Bhatia, R., Hill, M. D., Shobha, N., Menon, B., Bal, S., Kochar, P., … Demchuk, A. M. (2010). Low Rates of Acute Recanalization With Intravenous Recombinant Tissue Plasminogen Activator in Ischemic Stroke Real-World Experience and a Call for Action. https://doi.org/10.1161/STROKEAHA.110.592535

Cernovsky, Z. (2017). Quality of life in persons with schizophrenia. *Mental Illness*. Page Press Publications. https://doi.org/10.4081/mi.2017.7052

Chong, H. Y., Teoh, S. L., Wu, D. B. C., Kotirum, S., Chiou, C. F., & Chaiyakunapruk, N. (2016, February 16). Global economic burden of schizophrenia: A systematic review. *Neuropsychiatric Disease and Treatment*. Dove Medical Press Ltd. https://doi.org/10.2147/NDT.S96649

Ciccone, A., & Valvassori, L. (2013, March 7). Endovascular treatment compare to intravenous thrombolysis for acute ischemic stroke. *… England Journal of …*. https://doi.org/10.1056/NEJMoa1213701

Drake, K. L., & Ginsburg, G. S. (2012). Family Factors in the Development, Treatment, and

Prevention of Childhood Anxiety Disorders. *Clinical Child and Family Psychology Review*, *15*(2), 144–162. https://doi.org/10.1007/s10567-011-0109-0

Einstein, E. H., & Klepacz, L. (2017). What Influences Mental Illness? Discrepancies Between Medical Education and Conception. *Journal of Medical Education and Curricular Development*, *4*, 2382120517705123. https://doi.org/10.1177/2382120517705123

Emani, S. M., & McCully, J. D. (2018). Mitochondrial transplantation: applications for pediatric patients with congenital heart disease. *Translational Pediatrics*, *7*(2), 169–175. https://doi.org/10.21037/tp.2018.02.02

Greenberg, P. E., Fournier, A. A., Sisitsky, T., Pike, C. T., & Kessler, R. C. (2015). The economic burden of adults with major depressive disorder in the United States (2005 and 2010). *Journal of Clinical Psychiatry*, *76*(2), 155–162. https://doi.org/10.4088/JCP.14m09298

Hayakawa, K., Esposito, E., Wang, X., Terasaki, yasukazu, liu, yi, Xing, C., … lo, E. H. (2016). Transfer of mitochondria from astrocytes to neurons after stroke. *Nature Publishing Group*, *535*. https://doi.org/10.1038/nature18928

Jorm, A. F., Korten, A. E., Jacomb, P. A., Christensen, H., Rodgers, B., & Pollitt, P. (n.d.). Public beliefs about causes and risk factors for depression and schizophrenia. *Social Psychiatry and Psychiatric Epidemiology*, *32*(3), 143–148. https://doi.org/10.1007/bf00794613

Kessler, R. C., & Bromet, E. J. (2013). The Epidemiology of Depression Across Cultures. *Annual Review of Public Health*, *34*(1), 119–138. https://doi.org/10.1146/annurev-publhealth-031912-114409

Langhorne, P., Stott, D. J., Robertson, L., MacDonald, J., Jones, L., McAlpine, C., … Murray, G. (2000). Medical complications after stroke: A multicenter study. *Stroke, 31*(6), 1223–1229. https://doi.org/10.1161/01.STR.31.6.1223

Liu, F., Lu, J., Manaenko, A., Tang, J., & Hu, Q. (2018). Mitochondria in Ischemic Stroke: New Insight and Implications. *Aging and Disease*, *9*(5), 924–937. https://doi.org/10.14336/AD.2017.1126

Miller, F. J., Rosenfeldt, F. L., Zhang, C., Linnane, A. W., & Nagley, P. (2003). Precise determination of mitochondrial DNA copy number in human skeletal and cardiac muscle by a PCR-based assay: lack of change of copy number with age. *Nucleic Acids Research, 31*(11), 61e – 61. https://doi.org/10.1093/nar/gng060

Nabeshima, T., & Kim, H.-C. (2013). Involvement of genetic and environmental factors in the onset of depression. *Experimental Neurobiology*, *22*(4), 235–243. https://doi.org/10.5607/en.2013.22.4.235

National Institute of Mental Health. (2019a). Major Depression. Retrieved September 22, 2019, from https://www.nimh.nih.gov/health/statistics/major-depression.shtml

National Institute of Mental Health. (2019b). NIMH » Mental Illness. Retrieved October 22, 2019, from https://www.nimh.nih.gov/health/statistics/mental-illness.shtml

Orman, F. T. (2016). *"Paradigm" as a Central Concept in Thomas Kuhn's Thought*. *International Journal of Humanities and Social Science* (Vol. 6). Retrieved from www.ijhssnet.com

Pal, S. (n.d.). Incidence and Prevalence of Major Neurologic Disorders. Retrieved October 22,

2019, from https://www.uspharmacist.com/article/incidence-and-prevalence-of-major-neurologic-disorders

Park, S.-Y., Gifford, J. R., Andtbacka, R. H. I., Trinity, J. D., Hyngstrom, J. R., Garten, R. S., … Richardson, R. S. (2014). Cardiac, skeletal, and smooth muscle mitochondrial respiration: are all mitochondria created equal? *American Journal of Physiology. Heart and Circulatory Physiology*, *307*(3), H346-52. https://doi.org/10.1152/ajpheart.00227.2014

Ramos-Lima, M. J. M., Brasileiro, I. de C., de Lima, T. L., & Braga-Neto, P. (2018). Quality of life after stroke: Impact of clinical and sociodemographic factors. *Clinics*, *73*. https://doi.org/10.6061/clinics/2017/e418

Ridgway, E., Baker, P., Woods, J., & Lawrence, M. (2019, March 1). Historical developments and paradigm shifts in public health nutrition science, guidance and policy actions: A narrative review. *Nutrients*. MDPI AG. https://doi.org/10.3390/nu11030531

Robinson, K. G., Scott, R. A., Hesek, A. M., Woodford, E. J., Amir, W., Planchon, T. A., … Akins, R. E. (2017). Reduced arterial elasticity due to surgical skeletonization is ameliorated by abluminal PEG hydrogel. *Bioengineering & Translational Medicine*, *2*(2), 222–232. https://doi.org/10.1002/btm2.10060

Roger, V. L., Go, A. S., Lloyd-Jones, D. M., Benjamin, E. J., Berry, J. D., Borden, W. B., … Turner, M. B. (2011). AHA Statistical Update Heart Disease and Stroke Statistics-2012 Update A Report From the American Heart Association. https://doi.org/10.1161/CIR.0b013e31823ac046

Saraceno, B., Levav, I., & Kohn, R. (2005). The public mental health significance of research on socio-economic factors in schizophrenia and major depression. *World Psychiatry : Official*

Journal of the World Psychiatric Association (WPA), *4*(3), 181–185. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/16633546

Schmidt, C. W. (2007). Environmental connections: a deeper look into mental illness. *Environmental Health Perspectives*, *115*(8), A404, A406-10. https://doi.org/10.1289/ehp.115-a404

Schmitt, A., Malchow, B., Hasan, A., & Falkai, P. (2014). The impact of environmental factors in severe psychiatric disorders. *Frontiers in Neuroscience*, *8*, 19. https://doi.org/10.3389/fnins.2014.00019

Yang, J.-L., Mukda, S., & Chen, S.-D. (2018). Diverse roles of mitochondria in ischemic stroke. *Redox Biology*, *16*, 263–275. https://doi.org/10.1016/j.redox.2018.03.002