Modeling Alzheimer's Disease Under Non-invasive Brain Stimulation: Effects of Prior Methylmercury Exposure on Oxidative Stress in iPSC-Derived Cortical Neurons

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Abstract:

Methylmercury is a wide-ranging and highly toxic compound that is associated with neurotoxicity in the brain. It can also be linked to neurodegenerative diseases, including Alzheimer's disease, where oxidative stress pathology is a cause of its progression and can be suppressed using non-invasive brain stimulation techniques. This study aims to measure how high methylmercury exposure in Alzheimer's disease-related cell models will intensify oxidative stress levels following non-invasive brain stimulation. Using iPSC-derived cortical neurons that exhibit amyloid-beta and tau pathologies, simulation of methylmercury exposure and non-invasive brain stimulation will be conducted to measure the intensity of oxidative stress. Results can enhance future non-invasive brain stimulation treatments for efficacy and safety in Alzheimer's disease patients.

Introduction:

Methylmercury (MeHg) is a widespread pollutant that is mostly found in contaminated fish and marine animals, with approximately 19 million people worldwide exposed to its neurotoxic effects (Budnik & Casteleyn, 2019). Methylmercury exposure can lead to adverse effects in the central nervous system and can contribute to the development of neurological diseases, including Alzheimer's disease (AD) (Antunes Dos Santos, 2016). Moreover, non-invasive brain stimulation (NIBS) is a safe, cost-effective approach that is often used to enhance neural plasticity and cognitive function and to also suppress oxidative stress in AD patients. Since emerging research is dedicated to the implementation of NIBS for reducing cognitive impairment in people with AD, the effect of NIBS on AD patients who are previously exposed to MeHg remains unexplored, and whether undesirable results, such as the increase of oxidative stress, are encountered from NIBS treatment. This proposal aims to fill in these gaps by addressing this specific question: Does prior high methylmercury exposure intensify oxidative stress in iPSC-derived cortical neurons modeled Alzheimer's disease undergoing simulated NIBS? The exploration of this topic is crucial for optimizing NIBS treatment efficacy for AD patients and improving patient safety from the impact of intensified oxidative stress by running MeHg exposure tests beforehand. In this proposal, we expect that MeHg exposure will contribute to the increased intensity of oxidative stress in the iPSC-derived cortical neurons that are going through non-invasive brain stimulation.

Literature Review:

Previous studies have highlighted a relationship between neurotoxicity and mercury exposure, Alzheimer's disease, oxidative stress, and non-invasive brain stimulation. An Alzheimer's disease brain is already vulnerable to oxidative stress since it is one of the major effects of the pathophysiological mechanisms that occur in AD. Accumulation of reactive oxygen species (ROS) primarily in the mitochondria, which can lead to oxidative stress, is an effect of MeHg exposure (Fujimura & Usuki, 2020; de Almeida et al., 2022). Moreover, mercury poisoning in Alzheimer's disease patients is associated with multiple central nervous system disorders such as oxidative stress and mitochondrial malfunctions, which can significantly add on to the naturally occurring oxidative stress in AD (Paduraru et al., 2022). Non-invasive brain stimulation techniques, specifically transcranial direct current stimulation (tDCS), have been proven to reduce ROS levels and overall oxidative stress in a transgenic mice AD model and a vascular

dementia animal model in recent studies (Ri Xu et al. 2026; Guo et al., 2020). However, previous studies have failed to address the specific population of AD patients with MeHg exposure who seek to use NIBS treatment to mitigate its effects, and whether NIBS will still work efficiently in reducing oxidative stress. Therefore, this proposal aims to address these gaps by conducting a study that connects all these variables.

Methodology:

Somatic cells, specifically blood cells, from Alzheimer's disease patients will be collected, after informed consent, then reprogrammed into induced pluripotent stem cells (iPSCs) using Yamanaka Factors (Oct4, Sox2, Klf4, c-Myc). The iPSCs will then be differentiated into cortical neurons using NGN2 induction followed by maturation (30–60-day post-differentiation) to ensure exhibition of AD-phenotypes, such as amyloid-beta and tau pathologies (Mungenast et al., 2016). The iPSCs will be divided into two groups, one with simulated methylmercury exposure and one without simulated methylmercury exposure.

To simulate long-term methylmercury exposure in the experimental group, methylmercury chloride dissolved in water will be exposed to the cell culture media using concentrations ranging from 0.1 μM to 1.0 μM for 6-10 days to model chronic toxicity (Prince et al., 2021).

For both the control and experimental groups, transcranial direct current stimulation (tDCS) will be applied through electrodes in a microelectrode array (MEA) chamber for only 10-20 minutes a day to minimize cell death (Lu et al., 2023). Total intracellular reactive oxygen species (ROS) will be measured in each group using DCFH-DA (2′,7′-dichlorodihydrofluorescein diacetate) assay, which uses a non-fluorescent probe that is oxidized by ROS to emit green fluorescence. Lastly, the intensity of the emitted green fluorescence in both groups will be measured using a microplate reader then compared for higher levels of ROS using an independent samples t-test for the MeHg- exposed neurons and the non-exposed neurons under identical NIBS conditions (Kim & Xue, 2020).

Conclusion:

Determining the outcomes of oxidative stress in Alzheimer's disease-modeled iPSCs with prior MeHg exposure after NIBS stimulations can potentially increase AD patient safety and efficacy in real-life settings. However, this proposal has certain constraints, such as the possibility of genetic variance in the iPSCs which may result in high biological variability. Additionally, the simulation of methylmercury exposure in the proposal is more simplified, which fails to accurately model long periods of neurotoxicity in AD patients. Future studies should consider varied cell lines and more accurate MeHg exposure depending on the results of this study.

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