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Can Theta Wave Induction Lower Inflammation and Alcohol Consumption in Mice?

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Abstract

Overconsumption of alcohol is one cause of chronic inflammation, which appears to be mediated by the canonical NF-kB pathway. TLR4 (a pathogen and damage associated molecular pattern receptor) and HMGB1 (a nuclear DNA binding protein and damage associated pattern molecule) levels are elevated in postmortem brains of chronic alcoholics. Meditation has been found to be effective in treating excessive alcohol drinking as it elicits both psychological and biochemical effects through theta wave induction throughout the brain. We propose that meditation lowers expression of inflammatory markers in the brain and systemically, as well as elicits behavioral changes that lead to a decrease in ethanol consumption. We will mimic a meditative state using optogenetics to stimulate rhythmic theta waves in discrete areas of the brain. We will then measure changes in expression of molecules associated with the inflammatory state and correlate them with behavioral changes. The study of these interactions may provide potential new targets for therapeutic intervention.

Background

Binge alcohol drinking is the most common form of excessive alcohol drinking and is associated with 88,000 deaths in the United States per year[1,2]. Chronic alcohol consumption triggers an inflammatory response which activates the neuroimmune system[3]. Both pharmacological and behavioral treatments have been shown to be effective in treating alcoholism[3,4,5]. These treatments converge on similar molecular regulators of behavior and downstream alcohol effects, presumably through toll-like receptor (TLR) signaling. Given the complexity of targeting components of the neuroimmune system without negatively affecting global immune function, an exploration of nonpharmacological interventions into excessive drinking is needed. Meditation has been found to be especially useful in treating excessive alcohol drinking as it elicits both psychological and biochemical effects [5, 6, 7]. Meditation has been shown to alter specific markers of inflammation, cell-mediated immunity, and anxiety-related behavior[7,8,9].

Hypothesis

Meditation, a non-pharmacological but well documented intervention that improves affective states, increases positive behavioral responses, and reduces alcohol consumption and binge drinking, works through increasing theta waves in brain areas central to affect and emotional regulation (ACC, amygdala, hippocampus). This leads to down-regulation of peripheral and central nervous system anti-inflammatory mediators, specifically TLR4 and its downstream signaling. partners, which are upregulated by chronic and acute alcohol consumption.

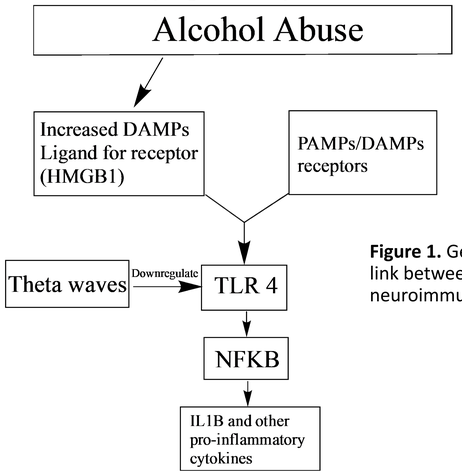

Figure 1. General overview of the mechanistic link between alcohol abuse and the neuroimmune system.

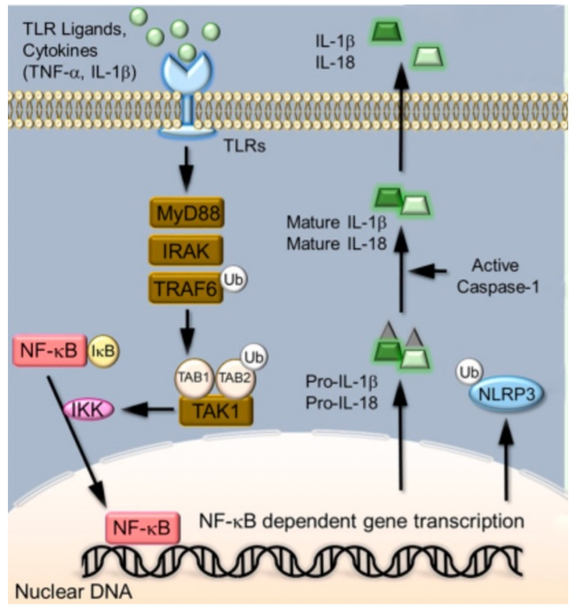

Figure 2. NF-kB signaling pathway.
Source: Liu, T.; Zhang, L.; Joo, D.; Sun, S.C. NF-KB Signaling in Inflammation. *Signal Transduction and Targeted Therapy,* **2017**, *2*: 1–9.

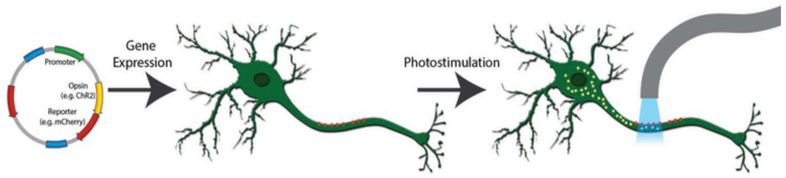

Figure 3. Example schematic of an optogenetics procedure. A channel rhodopsin, fused to mCherry, is expressed in neurons (red dots). When exposed to light of the correct wavelength, the pore opens, cations flow into the cell (yellow dots), and the neuron is activated.
Source: Optogenetics Guide. https://www.addgene.org/guides/optogenetics/ (accessed Jan, 2020).

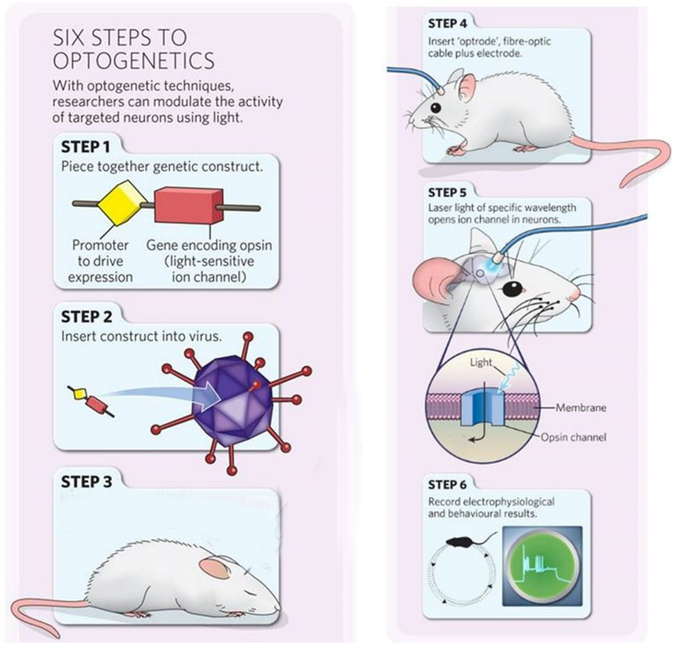

Figure 4. Six steps to optogenetics.
Modified from: Buchen, L. Neuroscience: Illuminating the Brain. *Nature* **2010**, *465*, 26–28.

Rationale

We propose to test the effectiveness in mice of a demonstrated proxy for meditation to reduce brain cell signaling through the NF-kB pathway, thus reducing inflammatory biomolecule production while concomitantly producing behavior changes that reduce alcohol consumption. We will do this by inducing rhythmic activity (theta waves) in the brains of mice using optogenetics. Optogenetics is a technique that uses light to control the activity of cells in living tissue that has been genetically modified to express light-sensitive ion channels[10]. These light-gated ion channels are opsin-variants that are G-protein coupled receptors (GPCR) involved in signaling across the membrane[10]. Upon targeted light stimulation, the receptors open or close to permit the movement of ions into or out of cells. We will target three brain regions, the anterior cingulate cortex (ACC), the central nucleus of the amygdala (CeA), and the hippocampus. Three types of mice (two optogenetically responsive at different frequencies and one nonresponsive control) will be used for our experiment. Optogenetic effects on markers of inflammation and mouse affect/behavior will be measured. ELISA measurements will be used to quantitate inflammatory markers, and location and levels of TLR4 and HMGB1 will be quantitated using Western blots of brain tissues and immunocytochemistry on targeted brain slices. Additionally, mRNA levels of TLR4 and other expressed inflammatory genes will be determined by reverse transcriptase-PCR.

Significance and Innovation

Our hypothesis is innovative in nature due to its divergence from traditional alcohol abuse treatments based solely on pharmacological interventions. We propose to explore the mechanistic link between alcohol drinking, addiction, and the body's neuroimmune response on a molecular level through seeking to target the neuroimmune system's negative inflammatory response. Through optogenetics, we can study the biochemical correlations and behavior changes induced by a proxy for meditation with the important social and economic goals of decreasing alcohol consumption, alcoholism, and the huge societal, medical, and economic cost that it arises from. Through doing so, we can save people and multigenerational effects of excessive alcohol consumption.

Acknowledgements

We would like to thank Dr. Henry Jakubowski and Dr. Edward McIntee for mentoring us through this process.

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