



CALIFORNIA STATE SCIENCE FAIR 2015 PROJECT SUMMARY

Name(s) Rebecca M. Sine	Project Number S0527
Project Title Allopregnanolone Site of Action for Promoting Regeneration of Human Neural Stem Cells: Uncovering the Paths	
<div>Objectives/Goals<p>This project's overall objective is twofold: (1) to optimize the treatment of allopregnanolone/Allo (a neurosteroid proven to reduce Alzheimer's symptoms in mice) with human neural stem cells for the accurate detection of proliferation (as a transition from treatments with rat neural stem cells), and (2) to elucidate the GABAergic site of action required for Allo to have an optimal effect in the promotion of regeneration of human neural stem cells in the model of Alzheimer's disease (using structural analogues of Allo to target specific GABA subunit conformations). The main experimental parameters that have required optimization for human neural stem cells are growth factor concentration, incubation and treatment time, Allo concentration, and cell-seeding density.</p></div> <div>Abstract<p>MTS colormetric assays were used to quantify cell proliferation for experimental optimization, and western blot (which quantifies protein expression) and immunocytochemistry (which provides qualitative analysis of protein expression) were used for preliminary testing with Allo analogues.</p></div> <div>Methods/Materials<p>Optimization data shows that seeding human neural stem cells in a 96-well plate at a density of 10,000 cells per well, growing them for five days, starving them for 48 hours (which resets the cells' mitotic cycles), treating them with an Allo concentration of 50nM and a growth factor concentration of 100%, and allowing them to incubate with their given treatments results in an optimal and accurate detection of neurogenesis prompted and upregulated by Allo. Data regarding analogue efficacy is currently preliminary.</p></div> <div>Results<p>My current goal now is to use the newly found experimental parameters to find the Allo analogue required to induce optimal neurogenesis, and perform genetic analysis to determine the GABA site of action conformation required for this effect. Knowing this will allow me know exactly which types of neurons that a particular analogue is targeting. It is also important to point out that Alzheimer's disease is not the only disease that can benefit from the optimization of Allo experimentation like this one; Allo plays a role in the treatment of epilepsy, depression, stress, and much more. Although optimization may not be the final solution endpoint, it definitely paves the way to get there.</p></div> <div>Conclusions/Discussion</div>	
Summary Statement <p>In my project, I have optimized the experimental parameters required for the treatment of human neural stem cells with allopregnanolone, a neurosteroid proven so far to reverse Alzheimer's symptoms in mice only, but not yet in humans.</p>	
Help Received <p>Used lab equipment at the University of Southern California under the supervision of Christine Solinsky</p>	