Major depression is one of the most common mental disorders in the United States [1]. It is characterized by low mood and sadness for extended periods of time. Those affected experience interference with daily life, including their ability to work, eat, sleep, study, connect, and enjoy once-pleasurable activities [1]. Many studies have provided insight into the biological and genetic mechanisms of depression, including linking its symptoms to the circadian rhythm. Circadian rhythm is mediated by the daily light/dark cycle via cellular proteins called circadian clocks [3]. Circadian clocks can be modulated by antidepressant drugs that often help depressive symptoms [3]. Several studies show that natural products may act as antidepressants. An herb, valerian, has been shown to exhibit antidepressant effects by acting on neurotransmitters in the brain [7]. Depression has been studied in many laboratories using the model organism, zebrafish. Zebrafish exhibit depression-like behaviors, which include stopping swimming and hiding [4]. Although a study has been done to compare the gene expression of the light-responsive circadian clock genes in zebrafish and in blind cave fish, *this study neglected to determine the gene expression of dopamine receptors during this time* [5, 6]. In addition, *studies have not been conducted to determine how natural treatments such as valerian affect the gene expression in zebrafish.* Zebrafish and blind cavefish provide a unique approach to identify factors that regulate circadian rhythm via dopamine receptors.

Genomic and proteomic techniques as well as behavior observation will provide insight on these variables' effects on depressive-like symptoms in fish. Since the zebrafish, blind cave fish, and human PER2 protein share a high percent identity between themselves, they will be useful in studying such effects.

The **primary goal** of this study is to determine how the light/dark cycle affects *dopamine receptor* expression as well as how natural products such as valerian affect gene expression. The secondary goal is to determine whether the fish with changes to their light/dark cycle exhibit depression-like symptoms like stopping swimming and hiding.

Since light cycle and circadian rhythm studies have only investigated the psychological and behavioral effects in humans in relation to depression, this study will help to understand the biological and genetic effects of such cycles on dopamine receptors in the brain. This, as well as results from the valerian and chocolate experiments could lead to future therapies for depression, including circadian rhythm synchronization, diet change, and pharmacological drugs.

Specific Aim 1: Determine how well conserved the human, zebrafish, and blind cavefish PER2 proteins are.
Approach: I will use online databases such as BLAST, Homologene, and Clustal Omega to investigate similarities and differences between said proteins.
Hypothesis: The proteins will share domains such as the PAS domain and the Period C domain.

Specific Aim 2: Determine the gene expression of *PER2* and D2 dopamine receptors in light/dark environments. Approach: I will use RNA-seq to identify gene transcripts that are found in the brains of wild type zebrafish and blind cavefish and *PER2* mutant zebrafish and blind cavefish. I will also measure receptor levels using Western blots of postmortem fish brains. To determine if fish with abnormal levels of dopamine receptors indicate depression-like symptoms, we will observe the fish for behaviors like stillness and hiding.
Hypothesis: Fish with disrupted circadian rhythms will exhibit abnormal levels of *PER2* expression and also dopamine receptors. We hypothesize genes with the following GO terms to be expressed, though at differing times in the circadian rhythm: signal transducer activity, transcription factor activity, circadian rhythm, transcription, nucleus, cytoplasm. Fish with abnormal dopamine receptors levels will exhibit depression-like behavior such as stillness and hiding.

Specific Aim 3: Determine how the natural product, valerian, affects protein expression in the brains of wild type and mutant zebrafish. Approach: Label free quantification and mass spectrometry of proteins in the brains will be conducted to quantify valerian's effect on the model organisms' brains. Samples will also be subjected to LC-MS/MS to identify any phosphopeptides. These experiments will be compared to a control group, which is not fed valerian.
Hypothesis: Valerian fed fish and control fish brains will exhibit differences in protein expression and phosphorylation. Valerian fed fish may exhibit expression of proteins with the following GO terms: neurotransmission, GABA receptor activity (positive regulation), synaptic cleft

References

[1] National Institute of Mental Health http://www.nimh.nih.gov/health/topics/depression/index.shtml?utm_content=buffera397a&utm_source=buffer&utm_medium=twitter&utm_campaign=Buffer#part4
[2] *Web*MD. Causes of Depression. Retrieved January 27, 2014, from http://www.webmd.com/depression/guide/causes-depression
[3] Golombek, D., Bussi, I., and Agostino, P. (2014). Minutes, days, and years: molecular interactions among different scales of biological timing. Philosophical Transactions of the Royal Society. B 369. doi: 20120465
[4] Society for Neuroscience. (2010, November 18). Depression-like behavior identified in zebrafish; Inability to cope with stress may play role in depression. Science Daily. Retrieved March 10, 2014 from http://www.sciencedaily.com/releases/2010/11/101116101736.htm
[5] Mejia, R. (2011). Cave-dwelling Fish Provide Clues to the Circadian Cycle. PLos Biol. 9(9) e1001141. doi: 10.1371/journal.bio.1001141
[6] Cavallari, N., et al. A Blind Circadian Clock In Cavefish Reveals that Opsins Mediate Peripheral Clock Photoreception. PLoS Biol. doi: 10.1371/journal.pbio.1001142
[7] Müller, L. G. et al. (2011). Antidepressant-like effect of *Valeriana glechomifolia* Meyer (Valerianaceae) in mice. Progress in Neuro-Psychopharmacology & Biological Psychiatry 36(2012): 101-109. Doi: 10.1016/j.pnpbp.2011.08.015