The Regulation of Sleep and Circadian Rhythms: The Role of Melatonin and Adenosine in Zebrafish

Thesis by

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For Veronica

&

My Parents

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Abstract

Sleep is a highly conserved behavioral state whose regulation is still unclear. In this thesis I first briefly introduce the known sleep circuitry and regulation in vertebrates, and why zebrafish is seen as a good model to study sleep-regulation. I describe the existing two-process model of sleep regulation, which posits that the two processes C (circadian) and S (homeostatic) control timing of sleep-wake behavior. I then study the role melatonin plays in the circadian regulation of sleep using zebrafish. First, we find that the absence of melatonin results in a reduction of sleep at night, establishing that endogenous melatonin is required for sleep at night. Second, melatonin mutants show a reduction in sleep in animals with no functional behavioral rhythms suggesting that melatonin does not require intact circadian rhythms for its effect on sleep. Third, melatonin mutants do not exhibit any changes in circadian rhythms, suggesting that the circadian clock does not require melatonin for its function. Fourth, we find that in the absence of melatonin, there is no rhythmic expression of sleep, suggesting that melatonin is the output molecule of process C. Finally, we describe a connection between adenosine signaling (output molecules of process S), and melatonin. Following this we proceed to study the role adenosine signaling plays in sleep-wake behavior. We find that first, adenosine receptor A1 and A2 are involved in sleep- wake behavior in zebrafish, based on agonist/antagonist behavioral results. Second, we find that several brain regions such as PACAP cells in the rostral midbrain, GABAergic cells in the forebrain and hindbrain, Dopamine and serotonin cells in the caudal hypothalamus and sox2 cells lining the hindbrain ventricle are activated in response to the A1 antagonist and VMAT positive cells are activated in response to the A2A agonist, suggesting these areas are involved in adenosine signaling in zebrafish. Third, we find that knocking out the zebrafish adenosine receptors has no effect on sleep architecture. Finally, we find that while the A1 agonist phenotype requires the zfAdora1a receptor, the antagonist and the A2A agonist behavioral phenotypes are not mediated by the zfAdora1a, zfAdora1b and zfAdoraA2Aa, zfAdora2Ab receptors respectively.

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