Chapter 1

Introduction

Introduction

Melatonin and adenosine are two small molecules that have been reported to have an important effect on sleep wake behavior. This study examines how these two molecules individually contribute to the regulation of sleep-wake behavior and proposes a model by which one regulates the other to bring about the sleep/wake states. In order to study their role on sleep, a scientist's definition of sleep is first required. Sleep can be a defined as a rapidly reversible state of behavioral quiescence which must have the following features to be called sleep: (1) consolidated periods of immobility exhibiting circadian rhythmicity (2) a specific posture, specific to individual species (3) a decrease in sensory responsiveness/ or an increased arousal threshold (4) a homeostatic regulatory mechanism or a mechanism by which deprivation of the behavior results in an accompanying rebound once the deprivation stimulus has been removed (Campbell and Tobler, 1984; Hendricks et al., 2000).

Using these criteria, sleep has been observed in a variety of the members of the animal kingdom including humans, zebrafish and fruit flies (Campbell and Tobler, 1984; Hendricks et al., 2000). Sleep deprivation assays have shown that prolonged deprivation of sleep results in deterioration of performance in memory related tasks. The absence of sleep can results in deterioration in performance and eventually prolonged deprivation can result in death (Everson et al., 1989; Li et al., 2009; Seugnet et al., 2008; Shaw et al., 2002). These results have lead scientists to theorize that during sleep episodes maintenance processes must be occurring in the brain, which are crucial to brain performance. One theory suggests that the purpose of sleep is to replenish depleted brain energy stores such as glycogen (Benington and Craig Heller, 1995). This theory places

low the low energy molecule adenosine in a central role, as a readout of the brain energy levels, and a regulator of sleep wake circuits (Benington et al., 1995). Another theory suggests that the purpose of sleep may be to trim the majority of the connections formed in the brain over the course of the day in order to be able to absorb new information the next day, and to also strengthen specific important connections, which has been supported by evidence from *drosophila* (Tononi and Cirelli, 2003) . In either case, regulating the occurrence of sleep then can be considered to be very important for animals.

What is the neural circuitry that regulates sleep? Studies show that the hypothalamic circuitry plays a major role in the control of sleep-wake behavior. There are two neural systems in the brain that control sleep-wake behavior. The first is the ascending arousal system situated in the brainstem/hypothalamus region. It includes a group of nuclei such as the locus coeruleus (Noradrenergic), Dorsal Raphae nucleus (Serotonergic), laterodorsal tegmental nucleus (Cholinergic), Pedunctulopontine nucleus (cholinergic) and Tuberomamillary nucleus (Histaminergic). The ascending arousal system also includes the orexinergic and melanin concentrating hormone neurons in the lateral hypothalamus, the cholinergic neurons in the basal forebrain and the dopaminergic neurons in the ventral periaqueductal gray region. The second system is the sleep active neurons in the ventero-lateral pre optic area (VLPO). The projections from these neurons inhibit the wake active areas, according to the flip-flop model of sleep regulation (Saper et al., 2001, 2005). At any one time, either the ascending arousal system is active or the sleep system is active since they inhibit each others, which results in sleep state and the wake state being mutually exclusive. The molecules that bring about this flip are unclear

however Adenosine has been proposed to be a candidate. (Basheer et al., 2000; Radulovacki et al., 1984; Saper et al., 2005).

What then controls the timing of the two states? According to the two-process model proposed by (Borbély, 1982), sleep is controlled by two processes, process C and process S. Process C is the circadian process, whose levels vary under the control of the circadian clock. It is regulated by external factors such as light. Process S is the homeostatic process, which responds to internal cues of sleep need. Its levels rise with wakefulness and decrease with sleep. While the molecular workings of process C have been well characterized, its output molecule was unknown. In contrast, for process S, there have been several candidates for the output molecule, including Adenosine, AMP, NOS (Brown et al., 2012), however where in the brain they are produced and how their release is regulated is quite unclear. Melatonin however is a good candidate for the process C output molecule. Its levels are under the control of the circadian clock in humans, rodents as well as in zebrafish (Klein, 2007). Also exogenous melatonin induces sleep in vertebrates (Cajochen et al., 1997, 2003; Zhdanova et al., 1996).

We decided to use zebrafish to study the role played by melatonin and adenosine in sleep wake behavior. Why use zebrafish? Zebrafish is a comparatively new model system developed in the 1970s. Zebrafish can be housed compactly; moreover they produce many hundreds of embryos at a time. They are amenable to genetic manipulations and they are transparent, making them ideal for imaging. In addition to all these characteristics, they also exhibit the hallmarks of sleep behavior by the age of 4dpf (Prober et al., 2006; Zhdanova, 2006). Zebrafish are vertebrates, but they possess a simpler nervous system than mammals. They possess the components of the sleep wake

circuitry in higher vertebrates (Chiu and Prober, 2013), but they have lesser numbers of neurons, for example only 10-12 orexin neurons as compared to the over 100 neurons in rats (Liu et al., 2015; Prober et al., 2006). They also exhibit similar responses to agonists and antagonists as higher vertebrates (Rihel et al., 2010). The zebrafish blood brain barrier is formed during 3-10 days post fertilization. There is therefore, good brain penetrance of sleep-wake affecting drugs added directly to the fish-water, during this period (Fleming et al., 2013). The small size of zebrafish larvae allows for high-throughput behavioral assays to be performed, which allows for statistically solid behavioral experiments (Kokel and Peterson, 2008; Rihel et al., 2010). Moreover they have a fully functional circadian system at 5dpf (Hirayama et al., 2005; Kazimi and Cahill, 1999). Also zebrafish are amenable to the ZFN, TALEN and CRISPR technologies, making it possible to generate desired gene mutants (Chen et al., 2013; Hwang et al., 2013; Sander et al., 2011a, 2011b). All these advantages result in the zebrafish model system being well poised to answer many questions relating to sleep-wake regulation.

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