CHAPTER 2:

Delving into the Sleep State

INTRODUCTION

Sleep is essential for human and animal health[1] and is behaviorally characterized by its circadian timing, behavioral quiescence, homeostasis, increased arousal threshold, and rapid reversibility.[2] Sleep is a complex process involving many facets: hardwired circadian regulation, context and activity-dependent regulation (homeostasis), global neuronal activity, specific centers for sleep, specific centers for wakefulness, and numerous regulators or sleep factors. Specific areas of the brain such as the hippocampus, cortex, and hypothalamus have been studied in detail across various species, both vertebrates and invertebrates. These studies have been illuminating in understanding the purpose of sleep and some of the processes that occur during sleep. However, integrating all this information as an overview can be a nightmare.

Additional factors such as stress, health, and the effect of genetic variation in the response of individuals to sleep regulation, both circadian and homeostatic, complicate matters further. Studying these processes in the simplest systems can help tie in the disparate pieces of information and provide a more dynamic understanding of these processes and their interactions with each other. Conveniently, sleep is conserved and present in essentially all animal species[3,4], and *C. elegans* exhibits a sleep-like state called lethargus.

Behavioral evidence suggests that our perception is dramatically dampened during sleep, and that there are physiological changes within individual neurons and their connections to each other.[1] However, there are many ways to dampen arousal[5], and despite studies spanning mammalian and non-mammalian species, the circuit modifications that promote sleep behavior are largely unknown. The conservation of neurotransmitters, several sleep regulating molecules, and sleep behaviors make anything found in *C. elegans* of potential interest in mammals, and the ease of genetics and a small nervous system allow for larger scale gene and circuit manipulations than previously possible.[5]

2.1 AN OVERVIEW OF SLEEP

Wakefulness depends on a network of cell groups in the brainstem, hypothalamus, and basal forebrain that activate the thalamus and the cerebral cortex.[1] A switch in the hypothalamus promotes γ-aminobutyric acid (GABA)-containing neurons of the ventrolateral preoptic nucleus (VLPO) to shut off this arousal system during sleep. The monoaminergic cell groups in the

arousal system fire fastest during wakefulness.[6,7] Therefore, sleep can simply be thought of as an interplay between excitatory and inhibitory centers in the central nervous system.

The VLPO projects neuronal processes to the monoaminergic cell groups that show stereotyped and coordinated changes in firing patterns associated with sleep.[8] The VLPO neurons are primarily active during sleep and contain the inhibitory neurotransmitters, galanin and GABA.[9,10,11] Cell-specific lesions showed that neuronal subpopulations in the VLPO regulate REM and NREM sleep[12] by inhibiting the monoaminergic cell groups. These cells in turn project to the VLPO and can inhibit the VLPO using both noradrenaline and serotonin.[13]

A circuit containing mutually inhibitory elements sets up a self-reinforcing loop, resulting in a "flip-flop switch", which produces discrete states with sharp transitions. Hypocretin neurons in the lateral hypothalamus stabilize this switch, and loss of these neurons cause instability of and inappropriate switching between behavioral states.[14] These neurons are mainly active during wakefulness, and especially during motor activity.[14,15]

The two-process model states that sleep results from the combination of both circadian and homeostatic processes.[16] The circadian regulation of sleep ensures that an organism rests at the appropriate and evolutionarily beneficial time, whereas homeostatic regulation is driven by the need for rest induced by the duration and intensity of activity. The earliest circadian sleep studies depended on activity patterns.[17] Now, sleep in humans and other mammals are characterized by electroencephalogram (EEG) as well as behavioral criteria to distinguish between wakefulness and sleep.[18] However, the oscillatory patterns of the EEG data are a result of underlying neural architecture that cannot be reproduced in species with different neuroanatomy. Therefore, behavioral criteria (circadian timing, behavioral quiescence, homeostasis, and increased arousal threshold) are used to identify sleep or sleep-like states in non-mammalian systems, and sleep is conserved and present in essentially all animal species tested.[3,4] Furthermore, these periods of quiescence have been shown to be associated with both circadian and homeostatic regulators of sleep.

2.2 CIRCADIAN REGULATION OF SLEEP

The circadian regulation of sleep ensures that an organism rests at the appropriate and evolutionarily beneficial time. The suprachiasmic nucleus (SCN) is the brain's "master clock", which fires in a 24-hour cycle that is driven by a transcriptional-translational loop.[19] In normal circumstances, SCN is kept in synchrony with the day-light cycle and is reset daily in response to

light detected by retina during the day, and by melatonin secretion from the pineal gland at night.[20] The SCN has some projections to the VLPO and hypocretin neurons[21], but the majority of its output is directed to the subparaventricular zone (SPZ) and the dorsomedial nucleus of the hypothalamus (DMH). Cell-specific lesions of the ventral and dorsal SPZ disrupt circadian rhythms of wakefulness and body temperature.[22] The SPZ projects to the DMH, which in turn inhibits the sleep promoting cells in the VLPO and excites wake promoting cells in the lateral hypothalamic area (LHA).[23] DMH integrates circadian input with physiological state, and DMH activity can be shifted by altered wake-sleep, activity, feeding, body temperature, and corticosteroid rhythms.[24]

The Period gene (*per*) drives the circadian clock, and PER, as well as its downstream components, has been shown to be consistent with sleep behavior. Disruption of this clock and lack of light-dark cues creates anachronistic sleep. However, a functioning circadian clock is not essential, and sleep still occurs even when circadian rhythms are disrupted or abolished.[25]

2.3 HOMEOSTATIC REGULATION OF SLEEP

Homeostatic regulation is driven by the need for rest induced by the duration and intensity of activity. Sleep homeostasis refers to the maintenance of sleep amount or depth following sleep deprivation, and is a reflection of the essential nature of sleep. The cause of this homeostasis is under debate, and there are three contending hypotheses: energy depletion hypothesis, neural plasticity hypothesis, and immune defense hypothesis.

The concept of energy metabolism follows the idea that the neuronal activity during waking consumes energy, and the energy is restored during sleep. According to experimental evidence, the degree of arousal is decreased after prolonged wakefulness, and the propensity to sleep and the intensity of delta EEG waves during sleep are proportional to the duration of prior wakefulness.[26] One possible explanation for accumulation of sleep propensity is the accumulation of substances, sleep factors, during wakefulness. Then, logically, sleep factors should steadily increase during the waking period and decrease during sleep, their concentrations should be greater during waking than sleeping, and they should inhibit neuronal activity. Adenosine, interleukin-1, TNF-alpha, prostoglandin D2 (PDG2), and growth hormone-releasing factor (GnRH) fit these criteria.[27]

Adenosine triphosphate is the primary energy carrier in the cell. Local energy depletion through prevention of ATP synthesis and increases in the dephosphorylated products of ATP

were shown to promote sleep.[27] Extracellular adenosine in the basal forebrain (BF) increases during prolonged wakefulness and decreases during recovery sleep.[28] Neuronal activity and consumption of ATP during waking is the cause of elevated adenosine concentration[29], which in turn feeds back as an inhibitory neuromodulator to decrease activity[30]. It has been suggested that this is a self-controlling neuroprotective mechanism to preclude cell damage.[31] Adenosine is formed from AMP, catalyzed by the enzyme 5'-nucleotidase, and inactivated either back to AMP by the enzyme adenosine kinase (AK) or further to inosine by the enzyme adenosine deaminase (ADA). The enzymes that metabolize adenosine undergo circadian variation, with maximal enzymatic activity during the night and minimal activity during the day.[32]

Four types of adenosine receptors are known: A1, A2, A3, and A4. Adenosine is generally an inhibitory neuromodulator that inhibits both excitatory and inhibitory neurons. A1 and A3 receptors decrease adenylyl cyclase and cAMP whereas A2 increases cAMP.[27] The presynaptic action of adenosine is mediated by the G_{i3} subtype of G-proteins that are coupled to inhibition of N-type Ca^{2+} channels and stimulation of K^{+} channels. G_{i3} either inhibits adenylate cyclase or activates phospholipase C (PLC) as effector pathways[33]. A1 receptors are widely distributed, whereas A2 receptors are restricted to the striatum, nucleus accumbens, and olfactory bulb. The main targets for vigilance state-modulating effects of adenosine are A1 receptors[34] in the cholinergic cells in the BF, hypothalamus and cortex. Selective activation of cholinergic BF neurons using neurotensin promotes EEG gamma activity and state of wakefulness.[35]

2.4 MOLECULAR MECHANISMS OF SLEEP

Study of circadian rhythms in model organisms have helped find and understand several molecular mechanisms of sleep. Analysis of sleep in the fruit fly *Drosophila* showed that increased cAMP promotes wakefulness. Adenylate cyclase mutant rutabaga has reduced cAMP and exhibits increased sleep, whereas the phosphodiesterase mutant dunce increases cAMP and shows decreased sleep.[36] cAMP targets CREB, and CREB activity has been correlated with activity in both flies and mice.[36,37] However, there is not much mechanistic insight into how cAMP promotes wakefulness.

Epidermal growth factor (EGF) was first discovered to promote sleep through intracerebroventricular injections in rabbits.[38] These results were supported by its effects in other mammals as well as in *Drosophila*, where increased release of EGFR ligands increase sleep, while inhibition of ligand release decreases sleep bout durations.[39] The site of action in the fly is

localized to the pars intercerebralis, an area that is developmentally and functionally analogous to the hypothalamus.[39]

Several other conserved molecular components of neuronal activity and signaling have been shown to affect sleep. In Drosophila, increased PKG activity is associated with more sleep.[40] Additionally, preliminary reports suggest that PKG inhibition in the basal forebrain reduces subsequent sleep[41]. Dopamine, hypocretin, GABA, and 5'-hydroxytryptamine (5-HT) all affect the sleep wake cycle[5]. In addition, the activity of potassium channels has been shown to modulate neuronal excitability and sleep in both flies[42] and mammals[43].

2.5 LETHARGUS OR *C. ELEGANS* SLEEP

C. elegans exhibits four larval stages before it matures into the adult (Figure 1). The transition between each larval stage is marked by the molt, a process in which the worm synthesizes a new cuticle, then sheds its old one. *C. elegans* exhibits sleep-like behaviors immediately before the molt during a period called lethargus.[40,44] Lethargus is a quiescent state during which locomotion and feeding are suppressed[44] and sensory arousal is decreased[40]. Furthermore, this state shows a type of homeostasis that is similar to that seen in sleep homeostasis. Inducing spontaneous activity by increased sensory stimuli during lethargus results in anachronistic rebound quiescence.[40]

Lethargus invariably occurs during development after each of the four larval stages, and the timing of lethargus corresponds to upregulation of LIN-42, homolog of circadian regulator PER[45]. Anachronistic quiescence is induced by expression of EGF[44], a function conserved in mammals[5], and sensory arousal can be depressed by PKG[40], another well-conserved signaling protein. This conservation of molecular drivers, as well as ties to circadian and homeostatic processes, suggests that the lethargus state in *C. elegans* could prove insightful in understanding sleep regulation.

FIGURES

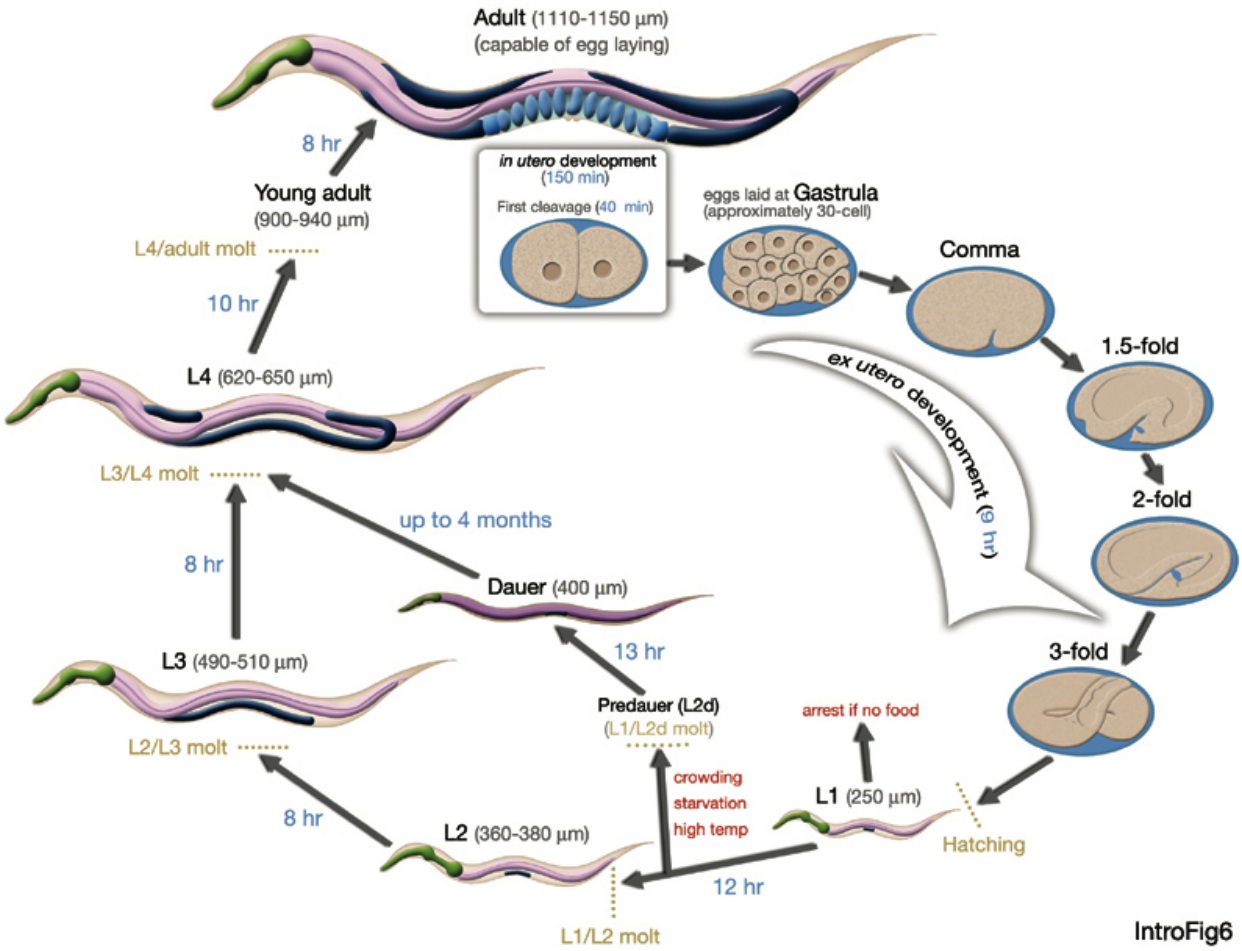

Figure 1. Life cycle of *C. elegans* at 22°C. 0 min is fertilization. Numbers in blue along the arrows indicate the length of time the animal spends at a certain stage. First cleavage occurs at about 40 minutes. post-fertilization. Eggs are laid outside at about 150 minutes post-fertilization and during the gastrula stage. The length of the animal at each stage is marked next to the stage name in micrometers. (Unmodified figure from Wormatlas.[46])

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