The Science of Waking Up

Mr. Jervis Fernandes*

Email: jervisferns15@iisertvm.ac.in

Growing up, one's earliest memories include that of the *ladla* betas and *betis* (pampered sons and daughters) accompanying their mothers on trips to vegetable markets. As our young eyes, bearing wonder and curiosity, would look around we would inevitably notice the swarms of persistent flies dodging the *bhajiwalas'* (vegetable sellers) desperate swats as expertly as Vijendra Singh, the boxing champion, would elude an opponent's punches in the ring.

We would return home with the produce only to find that the pesky flies have magically conjured up in our houses, marking their presence around fruits and vegetables. To our mild annoyance, we would find them hovering around any food item left outside, designated to be consumed at a later time-point. But come nightfall they would disappear into nothingness, just to resurface once again, like a phoenix rising from the ashes, at the crack of dawn.

As one grows older, one realizes that these creatures are not adept at the secret art of fairy magic (contrary to what my grandmother led me to believe) but are inherently following the same sleep-wake rhythm that we do. This helps them wake up at dawn and rest come nightfall. In flies, the neuropeptide PDF (Pigment Dispersing Factor), which is a functional homolog of vasoactive intestinal peptide (produced in the suprachiasmatic nucleus of the hypothalamus, the gut), has been implicated to be the intrinsic "grandmother" neuron, critical in ensuring their rhythmic behaviour.

* Mr. Jervis Fernandes, PhD Scholar from Indian Institute of Science Education and Research, Thiruvananthapuram, Kerala, is pursuing his research on "The Role of miR-184 in Neuronal Excitability in *Drosophila*". His popular science story entitled "The Science of Waking up" has been selected for AWSAR Award.

With the proposal of a Negative Feedback Loop of the clock gene, *period*, by Rosbash *etal* in 1990 (for which they received the 2018 Nobel Prize in Physiology and Medicine), the molecular underpinnings that govern this process began to get unravelled. Subsequent, discoveries in the field helped identify a total of four core clock genes and a number of additional supplementary proteins, which help maintain this rhythm.

Consequently, the discovery of microRNAs (by Lee *et. al.* in 1993), which are a set of non-coding RNAs whose function is to regulate the levels of other mRNAs, added on to the possibility that another group of factors could also assist in the governance of this strict rhythm.

At the lab of Dr Jishy Varghese at IISER (Indian Institutes of Science Research), Thiruvananthapuram, I use fruit flies to further understand the factors (micro-RNAs) regulating this simple, yet complex rhythm. Having started out by systematically decreasing the levels of micro-RNAs in the fly's brain and monitoring their activity-rest rhythm, we discovered that decreasing the levels of one particular micro-RNA (miR-184) leads to a disruption in the time-point at which the fly wakes up and the time it decides to go to sleep. We have discovered that it does so by regulating the levels of the genes involved in governing neuronal excitability.

> The struggle of a honeybee throughout her life for food and survival is very inspirational for me. How hardworking they are! I was curious to know more about honeybees, then, started to research on honeybee's life, honey preparation, and beekeeping practices.

A neuron is what my grandma would akin to a child full of *nakhras* (tantrums), negative until they are engaged, upon which they turn positive and smile, which in the case of a neuron is exhibited by the release of neurotransmitter (or as us researchers would call it, the neuron fires). One of the targets of miR-184 is a part of the potassium channel complex, which regulates the number of positively charged ions leaving the neuron. As such when it is present on the neuron's surface, more positive ions leave the neuron, thus, decreasing the possibility of the neurons firing or releasing the neurotransmitter.

The PDF neuron contributes to the maintenance of the sleep-wake cycle by releasing its neurotransmitter in a rhythmic fashion during the course of the day in response to light. Recently, the levels of miR-184 was observed to be cycling in these neurons, which would help it control the daily cyclic release of its neurotransmitter. This rhythmic control of the level of certain targets by miR-184 would lend the neuron as well the organisms control over the time-points at which the neurons are more likely to fire, thereby leading to the organism arousing from its slumber.

Sleep is defined as a state of mind, which is characterized by reduced sensory and muscle activity and surprisingly, increased brain activity. Till date, the role of sleep in organisms has not been understood completely. While we do know that both chronic and acute loss of sleep lead to deterioration in the quality of life, with the latter even causing death, we are yet to grasp the exact reason behind this ancient behaviour essential for the survival of the organism. Chronic sleep loss or alteration in the sleep cycle have been shown to lead to metabolic changes, one of the factors implicated in the great obesity epidemic of the West. Moreover, sleep loss is also known to alter the state of an individual's mind, thereby contributing to the mental health crisis that is slowly engulfing our nation and the world on a large scale. In addition, increasing

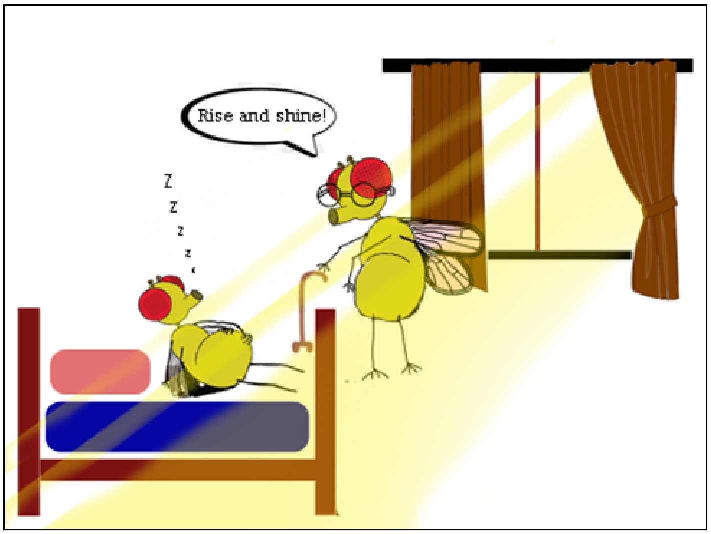

Fig 1.A cartoon emphasizing the role assayed by PDF neurons (depicted as the grandmother) in controlling the sleep-wake pattern in Drosophila melanogaster

dependency on technology and the constant use of artificial light have emerged as major players in the disruption of the sleep-wake cycle of the modern society.

Along with harbouring among the largest populations of individuals in their peak working age, and steadily increasing economic development, India is staring down the barrel of a looming sleep epidemic. The catastrophic sleep loss and irregular sleep patterns, which are so characteristic of this modern society, are the "kryptonites" that we have all turned a blind eye to. Recently, sleep has been linked to not only mental health but also cancer, diabetes, heart diseases and stroke among other such diseases. This would lead to an increasing percentage of the Indian as well the world's GDP as a whole being redirected towards mitigating healthcare costs.

> The struggle of a honeybee throughout her life for food and survival is very inspirational for me. How hardworking they are! I was curious to know more about honeybees, then, started to research on honeybee's life, honey preparation, and beekeeping practices.

The PDF neurons are known to fire in response to light with little to no firing in the night. Our work demonstrates that the increasing levels of miR-184 during the day help the PDF neurons fire. At night, low levels of the microRNA prevent the aberrant firing of neurons by titrating the levels of its downstream target.

With our work on fruit flies, we hope to shed some light on the intricacies of the processes that contribute towards regulating sleep, a behaviour that is deeply embedded in our genetic make-up. This would aid us in obtaining a detailed understanding of the health implications resulting from sleep imbalances and hopefully, in due time, will prove beneficial in alleviating its effects on the human population.

■ ■ ■