1 Introduction

Sleep-wake cycle in organisms is thought to have evolved due to evolutionary adaptation to alteration of day and night (Suárez-Grimalt and Raccuglia 2021). Sleep is characterized by increased periods of inactivity, reduced responses to external stimuli, tendency for recovery sleep after sleep deprivation, and rapid reversibility, making it distinct from hibernation or coma (Shafer and Keene 2021; Andreani et al. 2022; Donlea et al. 2018). These behavioural hallmarks of sleep are shared between both vertebrates and invertebrates including files (Shafer and Keene 2021; Andreani et al. 2022), despite the huge variability in the complexity and size of the nervous systems of the organisms.

Although the regulation of sleep at the cellular and molecular levels is not yet fully understood, across species, sleep has been shown to be important for various cellular processes (e.g. regulation of synaptic strength, cellular metabolism, etc.). Furthermore, poor sleep quality has been linked to numerous health conditions (e.g. diabetes, depression), as well as negative effects on cognitive functions, such as learning, memory, selective attention and social behaviour (Shafer and Keene 2021; Dubowy and Sehgal 2017; Suárez-Grimalt and Raccuglia 2021).

Apart from behavioural similarities, the genetic and molecular mechanisms underlying sleep are also similar across species (Dubowy and Sehgal 2017). In vertebrates, sleep and sleepiness are thought to be linked to large-scale synchronizations in the cortex resulting in Slow-Wave Activity (SWA) and reduction of connectivity between brain areas (Suárez-Grimalt and Raccuglia 2021; Raccuglia, Huang, et al. 2019). Interestingly, SWA with similar characteristics have also been observed in *Drosophila* (fruit flies), where a lower degree of synchronization correlates with reduced sleep duration and arousability threshold, as well as rebound sleep after sleep deprivation (Raccuglia, Huang, et al. 2019). Moreover, brain-wide SWA has been shown to be important for filtering sensory information, thus increasing arousability threshold during sleep (Raccuglia, Suárez-Grimalt, et al. 2022). These striking similarities between evolutionary distinct organisms might indicate to existence of fundamental processes governing sleep regulation (Suárez-Grimalt and Raccuglia 2021).

Drosophila is a well-established model to study sleep (Liu et al. 2016; Andreani et al. 2022; Shafer and Keene 2021; Dubowy and Sehgal 2017). Although its brain is relatively simple

(consisting of around 100,000 neurons (Donlea et al. 2018) in comparison to estimated 86 billion in human brain (Herculano-Houzel 2012)), *Drosophila* still shares many similarities in sleep-regulation with vertebrates (Liu et al. 2016), including mammals (Suárez-Grimalt and Raccuglia 2021; Dubowy and Sehgal 2017) despite long evolutionary distance (approximately 800 million years (Williams and Rae 2021)).

A network of approximately 32 neurons has been identified in the central complex of *Drosophila* brain that is important in sleep regulation CITE. This network of neurons is commonly referred to as R5 (originally termed as R2) CITE. Studying R5 neurons has been of central interest as they are considered to encode sleep drive in fruit flies CITE. Furthermore, expression of Bruch-pilot (BRP) protein, which is important for activity-dependent plasticity, has been reported to increase only in R5 following sleep deprivation CITE.

The function of the R5 network has been characterized as analogous to mammalian thalamus (Suárez-Grimalt and Raccuglia 2021; Raccuglia, Huang, et al. 2019). thalamocortical (TC) relay cells integrate sensory information prior to relaying it to the cortex (Sampathkumar et al. 2021). Similarly, R5 neurons are part of Ellipsoid Body (EB), which integrates sensory information to guide locomotion (Yan et al. 2023). Furthermore, both R5 and TC neurons are filtering sensory information and acting as a sensory gate that controls shifts between wakefulness and sleep (Raccuglia, Suárez-Grimalt, et al. 2022; Gent et al. 2018). Both synchronize electrical patterns and switch from tonic to burst firing (defined as slow alternating transitions between steady and spiking states (Rinzel 1987)) with increasing sleep need, as well as promote transition to sleep (Suárez-Grimalt and Raccuglia 2021; Raccuglia, Huang, et al. 2019).

R5 neurons exhibit tonic spiking activity during the daytime and bursting activity at night and following sleep deprivation CITE. Additionally, they show increased synchronization at night, which results in compound SWA [CITE]. Bursting is thought to facilitate neuronal synchronization [CITE], suggesting that the transition from spiking to bursting between day and night may represent a critical hallmark in the generation of SWA and the induction of sleep.

The cellular mechanism underlying the switch from tonic to bursting activity in R5 neurons remains poorly understood. A recent study on gene expressions in *Drosophila* reported that expression of the ether-à-go-go (EAG) channel, which is a voltage-gated potassium channel, is negatively correlated with sleep drive in *Drosophila* [CITE]. Increased expression of genes

encoding these channels may reflect higher channel concentrations during the daytime compared to the night. Since potassium channels are generally thought to suppress neuronal exitability [CITE], EAG mediated modulation of neuronal excitability might be a potential mechanism of tonic-to-bursting transition of the R5 activity between day and night.

It is also unknown which ionic mechanisms are involved in bursting in R5 neurons. Although there are various mechanisms of burst generation, one of the commonly discussed mechanisms involves T-type Ca^{2+} and Hyperpolarization-activated Cyclic Nucleotide-gated (HCN) channels. The HCN channels are activated upon hyperpolarization and mediate depolarizing current [CITE]. On the other hand, activation of T-type Ca^{2+} (Ca_T) channels requires first deinactivation at hyperpolarized potentials, followed by activation at more depolarized membrane potentials. Interplay between HCN-Ca_T channels has been hyperpolarized to underline bursting in many bursting neurons across phila [CITE].

A recent unpublished study by David Owald, Anatoli Ender and colleagues investigated the role of the T-type Ca^{2+} channels in the activity of the R5 neurons. Interestingly, animals which were deprived of these channels still exhibited bursting behaviour, however, their resting membrane potential was found to be at more depolarized levels. Moreover, slow oscillations in membrane potential following blockade of Na^{+} channels were diminished in flies with T-type channel knockdown.

The slow oscillations observed following sodium channel blockade have been hypothesized to be mediated by T-type calcium channels and, by analogy with other models, were thought to facilitate bursting. However, the observation that flies with T-type channel knockdown still exhibit bursting remains unexplained. It is unclear whether this is due to incomplete removal of the channels or whether other ionic mechanisms are responsible for bursting in R5 neurons.

Furthermore, more depolarized membrane potentials following T-type Ca^{2+} channel knockdown may appear counterintuitive, as calcium is depolarizing current, and one might therefore expect hyperpolarizing effect following its reduction. A potential mechanism underlying this observation could involve calcium-activated potassium channels, which mediate hyperpolarizing current. A reduction in calcium influx may lead to decreased activation of these channels, thereby reducing hyperpolarizing drive and effectively depolarizing the membrane potential, instead of hyperpolarization.

This thesis aims to investigate whether (1) the EAG channels can underline spiking-to-bursing transision in R5 activity, (2) the slow oscillations following Na^+ channel blockade are mediated by T-type channels, and (3) calcium activated potassium channels can explain the increase in resting membrane potential following T-type channel knockdown. Within the scope of this thesis, these questions are explored using several existing conductance-based bursting models, and the generality of the findings is assessed with respect to the model implementations.